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# Organic and inorganic fertilizer effect on soil $CO_2$ flux, microbial biomass, and growth of *Nigella sativa* L.

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A b s t r a c t. Cattle manure has a high carbon/nitrogen ratio and may not decompose; therefore, full-dose application of urea fertilizer might improve biological properties by increasing manure decomposition. This study aimed to investigate the effect of combining cattle manure and urea fertilizer on soil $CO_2$ flux, microbial biomass carbon, and dry matter accumulation during *Nigella sativa* L. (black cumin) growth under field conditions. The treatments were control, cattle manure, urea, different levels of split and full-dose integrated fertilizer. The results showed that integrated application of cattle manure and chemical fertilizer significantly increased microbial biomass carbon by 10%, soil organic carbon by 2.45%, total N by 3.27%, mineral N at the flowering stage by 7.57%, and $CO_2$ flux by 9% over solitary urea application. Integrated application increased microbial biomass carbon by 10% over the solitary application and the full-dose application by 5% over the split application. The soil properties and growth parameters of *N. sativa* L. benefited more from the full-dose application than the split application of urea. Cattle manure combined with chemical fertilizer and the full-dose application of urea increased fertilizer efficiency and improved biological soil parameters and plant growth. This method decreased the cost of top dressing urea fertilizer and proved beneficial for the environment and medicinal plant health.

K e y w o r d: carbon flux, inorganic fertilizer, integrated application, organic manure; soil respiration

## INTRODUCTION

The rapid increase in the global population is consuming natural resources and threatens the sustainability of farming systems in many parts of the world, especially in arid and semiarid regions (Abbasi and Khizar, 2012). Intensification without suitable management and the use of chemical fertilizers decreases organic matter and soil fertility and increases soil erosion and environmental degradation (Tiwari *et al.,* 2008). Agricultural intensification using high yield modern varieties and agrochemicals, including chemical fertilizers, has negative implications for the ecosystem and environment (Abbasi and Khizar, 2012).

The use of organic fertilizers is increasing to offset pollution by chemical fertilizers of the aerial and soil environment and the gradual decline in soil fertility. Continuous use of synthetic fertilizers can lead to a decline in soil health. Most N fertilizers leach down into the root zone or pollute the groundwater, causing disease in plants and humans (Azraf-ul-Haq *et al.,* 2007). Organic amendments in the form of compost, manure, and cover crops are a source of plant nutrients that also improve the quality of the soil (Shrestha *et al.,* 2013) through chemical (electrical conductivity, soil pH, and soil organic carbon), physical (bulk density and aggregate stability), and biological activity (Fereidooni *et al.,* 2013).

Improvement in soil quality increases crop yield and can help mitigate climate change by sequestration of carbon (Shrestha *et al.,* 2013). The addition of organic amendments is essential for sustainable soil fertility management and crop production, but can increase greenhouse gas (GHG) emissions. Understanding the effects of organic soil amendments on gaseous emissions is relevant to minimizing agricultural effects on net emissions of GHGs (Shrestha *et al.,* 2013).

Agricultural emissions account for 13.5% of the total anthropogenic gaseous ($CO_2$, $CH_4$, and $N_2O$) emissions (IPCC, 2007). Agricultural emissions of $CO_2$ include microbial respiration in the rhizosphere and bulk soil (Rochette

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*et al.,* 1999). Soil respiration is an important component of global carbon (C) cycling and has received considerable attention in recent years with the discovery of large amounts of soil organic carbon (SOC) stored in terrestrial ecosystems (Schlesinger and Andrews, 2000; Wang *et al.,* 2011). Global $CO_2$ emissions from soil are the source of one of the largest fluxes in the global C cycle. Small changes in the magnitude of soil $CO_2$ flux could have a large effect on $CO_2$ concentrations in the atmosphere (Schlesinger and Andrews, 2000). Knowledge of the factors contributing to soil $CO_2$ flux and controlling soil C sequestration is essential for understanding the changing global C cycle.

Agricultural management practices that include changing the soil environment (C/N ratio of substances, soil temperature, and soil moisture) affect soil $CO_2$ flux. These soil characteristics affect microbial activity in the soil and decomposition that transforms plant-derived C into $CO_2$ and soil organic matter (SOM) (Franzluebbers *et al.,* 1995). Studies have shown that SOC accumulation increases in annual cropping systems under fertilization, particularly the addition of N (Grant *et al.,* 2001).

Animal manure is a readily-available source of C for microbial activity that can increase $CO_2$ emissions (Rochette *et al.,* 2004). The application of N fertilizer and urea has been shown to influence most biological processes in the soil (Yan *et al.,* 2007), which are important in mineralization, carbon sequestration, and nutrient cycling (Bastida *et al.,* 2006). Manure application has been shown to have a significant positive effect on soil $CO_2$ flux in annual cropping systems (Rochette *et al.,* 2004). The presence of SOM buffers change soil pH and temperature. Organic and inorganic amendments and other types of manure can positively influence biochemical properties such as soil microbial biomass (SMB), enzymes, and activity (Vineela *et al.,* 2008; Azeez and Averbeke, 2010). Studies have found that manure amendment to the soil increases the rate of SMB enzyme activity and nutrient mineralization (Fereidooni *et al.,* 2013). Mandal *et al.* (2007) reported that microbial biomass can be controlled long-term by application of fertilizer and manure.

Integrated application of organic and inorganic fertilizer improves soil fertility and crop yield long-term (Alizadeh *et al.,* 2012; Fallah *et al.,* 2013; Fereidooni *et al.,* 2013). The application of organic fertilizers has been shown to affect the structure of the microbial community differently than application of solely mineral fertilizers (Wu *et al.,* 2012). The application of organic fertilizers like cattle manure increase the accumulation of organic C in the soil, which in turn induces change in the microbial community structure and stimulates the microbial biomass (Peacock *et al.,* 2001). Intelligent usage of organic manure and inorganic fertilizers is essential to augment productivity and input use efficiency and safeguard soil health (Bandyopadhyay *et al.,* 2010).

The positive effect of integrated use of inorganic fertilizer and farmyard manure on the productivity of soybean was demonstrated by Hati *et al.* (2006). Lee *et al*. (2007) reported that the average soil $CO_2$ flux during the growing periods of switchgrass were 472, 488, and 706 g $CO_2$ C $m^{-2}$ for the control, $NH_4NO_3$-N, and manure-N plots, respectively. Alsafar and Al-Hassan (2009) applied 75 kg N $ha^{-1}$ and significantly increased the total dry matter in mint (*Menthe longifolia* L.).

Although the effect of fertilizers and manures on crop growth and soil properties has been studied, there is little information on holistic study of the effect of integrated use of chemical fertilizers and cattle manure on medicinal plant growth and soil properties (soil $CO_2$ flux, microbial biomass carbon, and dry matter accumulation). Cattle manure has a high C/N ratio and may not decompose; the full-dose application of urea fertilizer can aid decomposition. It has been hypothesized that improvement in the physical properties of soil after manure application influences growth characteristics like dry matter accumulation (DMA), which ultimately influence the productivity and input use efficiency of *N. sativa* L. (black cumin). The present investigation evaluated the effect of solitary and integrated application of cattle manure and urea fertilizer on soil $CO_2$ flux, microbial biomass, and the growth of *N. sativa* L. in a semi-arid region of Iran. The study investigated the effects of cattle manure and chemical fertilizer on soil $CO_2$ efflux and microbial biomass, determined the relationship between soil $CO_2$ efflux and DMA, and investigated N mineralization under integrated fertilizer application.

## MATERIALS AND METHODS

This experiment was carried out in 2011-12 on the research farm of Shahrekord University (50°49′ E, 32°21′ N; 2050 m in elevation). The experimental field had not been fertilized for five years prior to testing with any type of inorganic fertilizer or organic manure. Soil samples were taken at depths of 0 to 30 cm and analyzed before the onset of the experiment (Table 1). Cattle manure was obtained from cattle farms and analyzed to determine its characteristics before use (Table 1).

The experiment design was a randomized complete block with three replications and nine treatments (Table 2). The treatments were control (no N amendment), cattle manure (CM), urea (U), three levels of split integrated fertilizer (CM:U (2:1), CM:U (1:1), CM:U (1:2)), and three levels of full-dose integrated fertilizer (CM:U (2:1), CM:U (1:1), CM:U (1:2)). The amount of nitrogen applied was 80 kg N $ha^{-1}$ in cattle manure, which released approximately 50% of the total N (Alizadeh *et al.,* 2012).

After soil tillage with a mouldboard plough, the cattle manure was applied in a subsurface band and then 10-13 cm stacks were formed over each manure band. In the full-dose urea fertilizer treatments, urea-N was applied at

**T a b l e 1.** Chemical characteristics of the soil (Clay loam) and the cattle manure used in the present study

| Property | Unit | Soil | Cattle manure |
|---|---|---|---|
| EC | (dS $m^{-1}$) | 1.011 | 6.71 |
| pH | - | 7.96 | 7.48 |
| N | (g $kg^{-1}$) | 0.82 | 4.1 |
| OC | (g $kg^{-1}$) | 9.55 | 275 |
| P | (mg $kg^{-1}$) | 10.8 | 1 672 |
| K | | 391 | 10 043 |
| Zn | | 0.68 | 8.41 |
| Mn | | 8.73 | 74.5 |
| Fe | | 8.09 | 98.4 |
| Cu | | 0.91 | 5.9 |
| C:N | - | 11.64 | 67.1 |

a depth similar to that for cattle manure. In the split fertilizer treatments, one-third of the urea was applied at planting and two-thirds at 30 days after planting. Phosphorus fertilizer, a source of triple superphosphate, was applied to the urea-fertilized plots at a rate equivalent to the total P added by the cattle manure treatments.

The experimental design was 27 plots each 2 × 3 m in area. Each plot had eight rows spaced 25 cm apart. To avoid mixing of water from the plots, the distance between the plots and between the blocks was 1.5 m. Seeds of *N. sativa* L. were provided by Pakan Bazr (Iran) and sown on 14 April 2012 at a depth of 2-3 cm. To obtain 200 plants $m^{-2}$, the *N. sativa* L. seedlings were thinned at the 3-4 leaf stage. Irrigation was done by automatic sprinklers at 6-day intervals. Soil $CO_2$ (flux) production and DMA were measured periodically during the growing season beginning at 46 days after planting until harvest time, and for $CO_2$ flux at 7 days after harvest. Microbial biomass carbon (*MBC*), SOC, total N, and mineral N were measured only at the flowering stage (95 days after planting).

SOC was determined at the flowering stage with the method recommended by Walkley and Black (Nelson and Sommers, 1982).

Soil nitrogen was determined at the flowering stage in soil samples taken randomly from depths of 0 to 25 cm. The total N was determined with the method suggested by Kjeldahl (Hesse, 1971). Soil mineral N was measured in an extract taken by KCl. After analysis of $NO_3^-$-N and $NH_4^+$-N concentrations (Alef and Nannipieri, 1995), the net N mineralization was calculated (Keeney and Nelson, 1982).

Five 50 g soil samples were randomly collected by auger from each plot at a depth of 25 cm. The soil samples were transported to the laboratory, mixed to obtain composite samples and sieved (2 mm mesh size) to determine the soil *MBC*. *MBC* was measured using the chloroform-fumigation incubation method (Alef and Nannipieri, 1995). Two 40 g subsamples were put in 100 ml glass beakers. The one containing ethanol-free chloroform in a vacuum desiccator was fumigated for 24 h at room temperature. The chloroform vapour was eliminated by repeated evacuation of the desiccator. Plastic jars that included a vial containing 15 ml of 0.5 M NaOH for the $CO_2$ trap were placed in

**T a b l e 2.** Treatment details in the present study

| Treatment | | Description |
|---|---|---|
| C | Control | Unfertilized plots |
| U | Urea fertilizer | 80 kg N $ha^{-1}$ in the form of urea |
| CM | Cattle manure | 40 ton cattle manure/ha |
| CM:U (1:1) SA | Split application (SA) of urea; 33 and 67% of urea was applied at plantingand 30 days after planting | 116 kg N $ha^{-1}$ in the form of urea + 13.33 t cattle manure $ha^{-1}$ |
| CM:U (2:1) SA | | 86.7 kg N $ha^{-1}$ in the form of urea + 20 t cattle manure $ha^{-1}$ |
| CM:U (1:2) SA | | 58.3 kg N $ha^{-1}$ in the form of urea + 26.6 t cattle manure $ha^{-1}$ |
| CM:U (1:1) FDA | Full dose application (FDA) of urea at planting stage | 116 kg N $ha^{-1}$ in the form of urea + 13.33 t cattle manure $ha^{-1}$ |
| CM:U (2:1) FDA | | 86.7 kg N $ha^{-1}$ in the form of urea + 20 t cattle manure $ha^{-1}$ |
| CM:U (1:2) FDA | | 58.3 kg N $ha^{-1}$ in the form of urea + 26.6 t cattle manure $ha^{-1}$ |

all beakers. After closing the jars tightly, they were incubated for 10 days at 25 ± 1°C and 70% of soil water-holding capacity. At the end, the evolved $CO_2$ was measured. The *MBC* content was calculated as:

$$MBC = \frac{F_c}{K_c},$$

where: *MBC* is microbial biomass *C* (mg kg$^{-1}$ soil) expressed as oven-dry (105°C) weight, $F_c$ is the flash point of $CO_2$, and $K_c$ is the recovery factor of 0.45 (Jenkinson and Ladd, 1981).

For determination of soil respiration ($CO_2$ production), three 1.8 l plastic jars were randomly inserted 3 cm into the surface soil of the rows in each plot. The jars had an open bottom and sealed top and were left in the soil for the course of the study. For collection and determination of $CO_2$ absorption, each jar contained a plastic vial containing 20 ml 1 M NaOH.

The rate of $CO_2$ evolved was measured from the soil using 0.25 N HCl after precipitating the carbonate with a $BaCl_2$ solution by back-titrating the alkali (Alef and Nannipieri, 1995). $CO_2$ evolution was expressed as mg $CO_2$-C m$^{-2}$ soil.

Above-ground biomass was harvested by cutting above the soil surface during the growing season at 36, 50, 64, 78, 92, 106, 120, 134, and 144 days (every 14 days) after planting. The samples were then brought to the laboratory and dried at 80°C until they reached a constant weight.

One-way ANOVA was conducted to determine significant differences in the effect of fertilizer on SOC, total N, mineral N, and *MBC* using SAS software. Two-way ANOVA was performed to determine the effect of fertilizer on soil $CO_2$-C flux and DMA. Fisher least significant difference (LSD) was used for comparison of means. The significance of relationships between soil $CO_2$ flux and *N. sativa* L. dry matter accumulation (DMA) was determined using Pearson correlation coefficient.

## RESULTS

The results of ANOVA showed that at the flowering stage, the fertilizer had a significant effect ($p \leq 0.001$) on SOC (Table 3). The SOC content was the lowest in the check plot and the highest (9.63 g kg$^{-1}$) in the cattle manure plot. There was no significant difference between CM:U (2:1) SA and CM:U (1:1) FDA treatments (Fig. 1). The results indicate that the SOC in the fertilizer was significantly greater (15.47%; $p \leq 0.001$) than in the unfertilized plot. Integrated application of the fertilizer increased the SOC by 2.5% over the solitary application and the SOC was greater for the full-dose application than the split application (Table 3, Fig. 1).

The N fertilizer had a significant effect ($p \leq 0.001$) on total N at the flowering stage (Table 3). The total soil N was significantly greater (36.84%) for the fertilizer application than for the unfertilized control (Table 3, Fig. 2). Total soil N was 0.57 g kg$^{-1}$ in the check plot and 0.85 g kg$^{-1}$ in the cattle manure. There were no significant differences between CM, and CM:U (2:1) SA and FDA. The integrated application of fertilizer increased total soil N 3.27% over the solitary application of N (Table 3, Fig. 2).

The results indicate that, at the flowering stage (95 days after planting), the mineral N content of CM:U (1:1) FDA was 49.4, CM:U (1:1) SA was 46.66, CM:U (2:1) SA was 44.05, cattle manure was 43.07, urea was 36.69, and the check plot was 32.17 mg kg$^{-1}$, (Fig. 3). Mineral N was by 37% greater in the fertilizer application than in the unfertilized control (Table 3, Fig. 3). Integrated application of the fertilizer increased mineral N by 7.57% over the solitary application of the fertilizer (Table 3, Fig. 3).

**T a b l e 3.** ANOVA analysis for soil organic carbon (SOC), total N, mineral N and microbial biomass carbon (*MBC*) under urea fertilizer combined with cattle manure at the flowering stage of *N. sativa* L.

| Source of variation | SOC | Total N | Mineral N | *MBC* |
|---|---|---|---|---|
| Replication | 0.14ns | 0.0004ns | 2.559ns | 1 115ns |
| N fertilizer | 0.94** | 0.224** | 65.35** | 11 104** |
| Error | 0.037 | 0.0005 | 3.99 | 649.7 |
| CV (%) | 2.14 | 3.06 | 5.33 | 9.82 |
| | | Groups mean contrasts | | |
| | (g kg$^{-1}$) | (g kg$^{-1}$) | (mg kg$^{-1}$) | (mg kg$^{-1}$) |
| N fertilizer *vs* control | 9.13 *vs* 7.91** | 0.78 *vs* 0.57** | 42.15 *vs* 32.17** | 274.2 *vs* 141.2** |
| Integration *vs* solitary | 9.18 *vs* 8.69** | 0.79 *vs* 0.76** | 42.9 *vs* 39.88* | 280.6 *vs* 225.1* |
| FDA *vs* SA | 9.22 *vs* 9.14** | 0.78 *vs* 0.8 ns | 42.83 *vs* 42.97ns | 287.2 *vs* 273.9ns |

ns – not significant, *p < 0.05, **p < 0.01. See Table 1 for abbreviations.

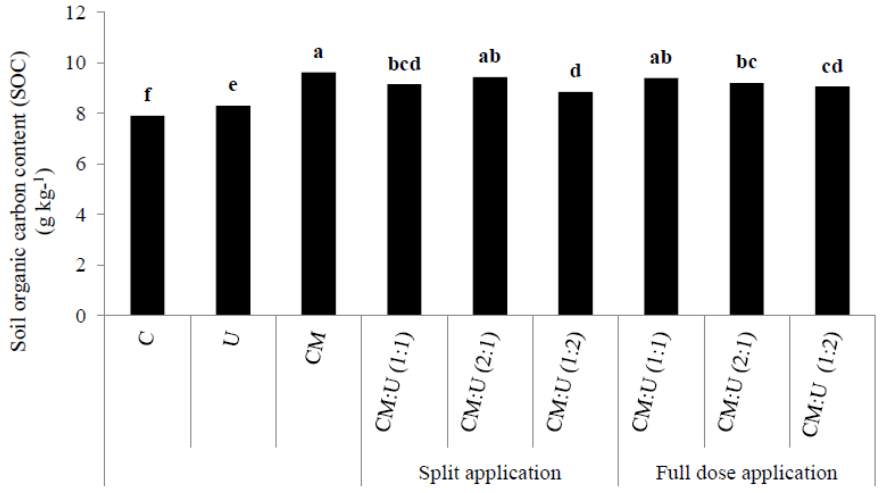


**Fig. 1.** Effect of urea fertilizer combined with cattle manure on soil organic carbon content at the flowering stage of *N. sativa* L. plants. See Table 1 for abbreviations.

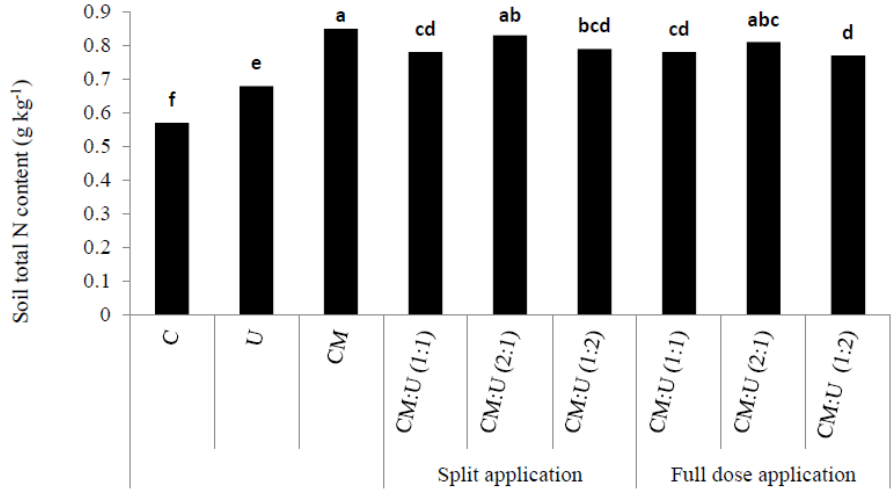


**Fig. 2.** Effect of urea fertilizer combined with cattle manure on soil total N content at the flowering stage of *N. sativa* L. plants. See Table 1 for abbreviations.

The results demonstrated that, at the flowering stage, the fertilizer had a significant effect ($p \leq 0.001$) on soil *MBC* (Table 3). The *MBC* content was higher in fertilized than in unfertilized soil. The maximum *MBC* was 339.81 mg $kg^{-1}$ for CM:U (1:1) FDA and 306.03 mg $kg^{-1}$ for cattle manure. The lowest value was observed at 141.22 mg $kg^{-1}$ in the control plot (Fig. 4). Soil *MBC* was significantly greater (94.32%; $p \leq 0.001$) for the fertilized plots than for the unfertilized plots (Table 3, Fig 4).

Integrated application of the fertilizer increased soil *MBC* by 10% over the solitary application of the fertilizer and soil *MBC* was by 5% greater for the FDA than the SA treatment (Table 3, Fig 4).

The results of ANOVA indicated a significant effect of the N fertilizer treatment (urea and cattle manure) and sampling time on soil $CO_2$ flux (Table 4). The interaction of the N fertilizer and sampling time was also significant for soil $CO_2$ flux. During the experimental period (46-144 days), the lowest (3.47 g $CO_2$-C $m^{-2}$ $day^{-1}$) and the highest (5.61 g $CO_2$-C $m^{-2}$ $day^{-1}$) mean soil $CO_2$ flux were recorded for the unfertilized control and CM:U (1:1) FDA treatments, respectively (Table 5).

The results also indicated no significant difference between the results for CM:U (2:1) FDA (5.41 g $m^{-2}$ $day^{-1}$) and SA (5.41 g $m^{-2}$ $day^{-1}$), CM:U (1:1) SA (5.43 g $m^{-2}$ $day^{-1}$) and CM (5.44 g $m^{-2}$ $day^{-1}$; Table 5). The minimum daily soil $CO_2$ flux was 4.98 g $CO_2$-C $m^{-2}$ $day^{-1}$ at 46 days after planting

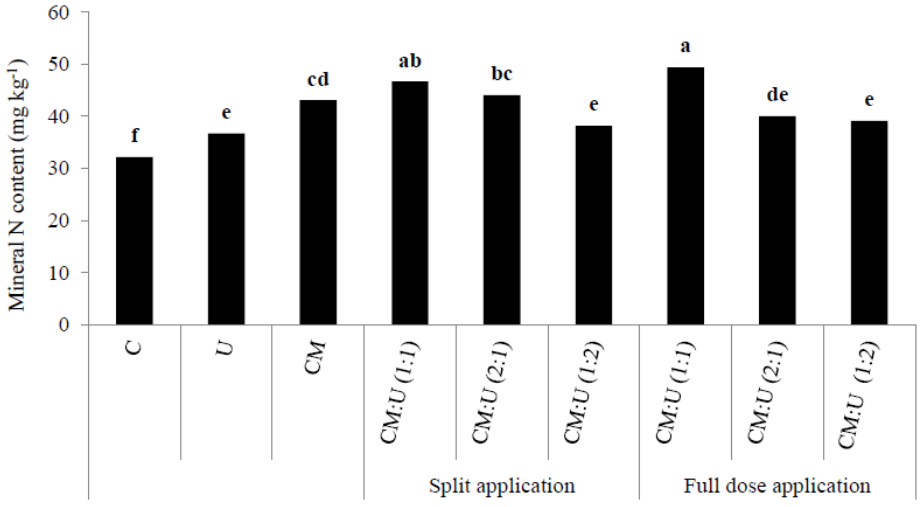


**Fig. 3.** Effect of urea fertilizer combined with cattle manure on mineral N content at the flowering stage of *N. sativa* L. plants. See Table 1 for abbreviations.

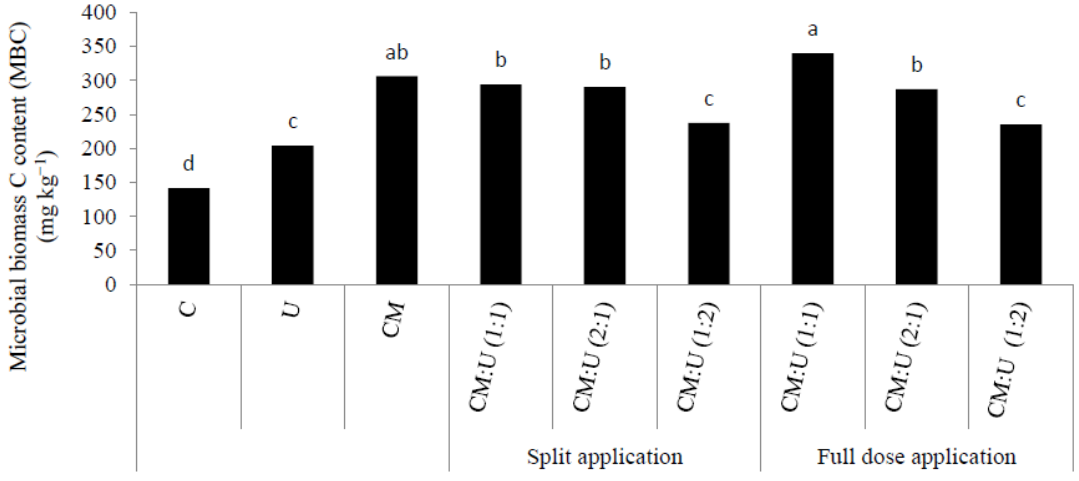


**Fig. 4.** Effect of urea fertilizer combined with cattle manure on microbial biomass carbon content at the flowering stage of *N. sativa* L. plants. See Table 1 for abbreviations.

**T a b l e 4.** ANOVA analysis for soil $CO_2$-C flux with two main factors, N fertilizer and sampling time under black cumin plant

| Source of variation | df | $CO_2$-C | Dry matter |
|---|---|---|---|
| Replication | 2 | 0.222** | 1508** |
| N fertilizer (N) | 8 | 18.153** | 78070** |
| Sampling time (T) | 12 | 0.197** | 642905** |
| N×T | 96 | 0.253** | 8686** |
| Error | 232 | 0.016 | 285.9 |
| C.V (%) | - | 2.51 | 8.09 |

**p < 0.01.

and 5.01 g $CO_2$-C $m^{-2}$ $day^{-1}$ at 53 days after planting. The maximum daily soil $CO_2$ flux was 5.22, 5.18, and 5.22 g $CO_2$-C $m^{-2}$ $day^{-1}$ for 102, 109 and 130 days after planting, respectively (Table 5). At all sampling dates (13 stages), soil $CO_2$ flux for the manure treatment rapidly increased compared to the check plots. At 46 days after planting for the fertilized plots, cattle manure recorded the minimum soil $CO_2$ flux and CM:U (2:1) SA treatment recorded the maximum soil $CO_2$ flux (Table 5). At early stages of growth, soil $CO_2$ flux was similar for the treatments with easily available nitrogen and the combined treatments of CM:U (1:1) SA and FDA, CM:U (1:2) SA and FDA, and CM:U (2:1) FDA (Table 5). At 88 days after planting, soil $CO_2$ flux was greater for the integrated fertilizers (cattle manure + urea) than for the solitary urea fertilizer.

**T a b l e 5**. Soil $CO_2$-C flux (g $m^{-2}$ $d^{-1}$) at nine treatments of N fertilizer at 13 sampling times during *N. sativa* L. growth under field conditions. Values are means (n = 3)± SD

| Day after planting | C | U | CM | CM:U (1:1) SA | CM:U (2:1) SA | CM:U (1:2) SA | CM:U (1:1) FDA | CM:U (2:1) FDA | CM:U (1:2) FDA | Mean |
|---|---|---|---|---|---|---|---|---|---|---|
| 46 | 3.77±0.14i | 5.26±0.10bcd | 4.69±0.07h | 5.12±0.04cdefg | 5.61 ±0.003 a | 5.13 ±0.01 cdef | 5.28 ±0.04 bc | 5.38± 0.06b | 5.24± 0.01bcde | 5.01DEF |
| 53 | 2.53±0.04i | 5.33±0.08bcd | 5.35±0.04bc | 5.12±0.02def | 5.04 ±0.09 h | 5.24 ±0.07 bcde | 5.71 ± 0.02a | 5.43±0.08 b | 5.09±0.17 efg | 4.89 G |
| 60 | 3.27±0.07i | 3.93±0.02h | 5.41±0.06abc | 5.37±0.04bcde | 5.40 ±0.003 abcd | 5.20 ±0.01 cdefg | 5.61 ±0.01 a | 5.44±0.02 ab | 5.32±0.01 bcdef | 5.00FG |
| 67 | 3.13±0.24i | 3.74±0.02h | 5.50±0.07abc | 5.44±0.02bcd | 5.21 ± 0.05 def | 5.18 ±0.12 g | 5.68 ± 0.14 a | 5.55±0.03 ab | 5.35±0.4bcde | 4.98 G |
| 74 | 2.82±0.02i | 3.95±0.03h | 5.49±0.09abc | 5.48±0.03abcd | 5.50 ±0.09 ab | 5.32 ±0.02 cdefg | 5.69 ± 0.10 a | 5.43±0.05 bcdef | 5.44±0.005bcde | 5.01 EFG |
| 81 | 3.1±0.18i | 4.19±0.008h | 5.40±0.04cdef | 5.63±0.10ab | 5.54 ±0.03 abc | 5.34 ±0.03 cdefg | 5.76 ±0.04 a | 5.5±0.04 bcd | 5.45±0.01bcde | 5.10CD |
| 88 | 3.03±0.19i | 4.37±0.01h | 5.59±0.09ab | 5.53±0.02abc | 5.35 ±0.04 cdef | 5.14 ±0.04 fg | 5.72 ±0.01 a | 5.46±0.01 bcd | 5.43±0.01bcdef | 5.07 DE |
| 95 | 2.99±0.16i | 4.47±0.003h | 5.54±0.05abc | 5.56±0.08ab | 5.53 ±0.04 abcd | 5.03 ±0.06 g | 5.66 ±0.03 a | 5.47±0.04 abcde | 5.42±0.02 bcdef | 5.07 DE |
| 102 | 4.28±0.06i | 4.59±0.008h | 5.51±0.05abc | 5.42±0.08bcdef | 5.55 ±0.003 ab | 5.05 ±0.04 g | 5.68 ±0.008 a | 5.47±0.05 abcd | 5.47±0.02 abcde | 5.22A |
| 109 | 4.15±0.05i | 4.58±0.008h | 5.45±0.06abcde | 5.48±0.02ab | 5.47 ±0.01 abcd | 5.08 ±0.03 g | 5.61 ±0.01 a | 5.47±0.02 abc | 5.36± 0.02 bcdef | 5.18AB |
| 116 | 4.08±0.16i | 4.68±0.003h | 5.35±0.05bc | 5.34±0.08bcd | 5.38 ±0.04 b | 5.13 ±.0.02 cde | 5.70 ±0.15 a | 5.13±0.19 def | 5.12±0.09 defg | 5.10CD |
| 130 | 4.07 ±0.06i | 4.77±0.0 h | 5.57±0.01ab | 5.45±0.03abcd | 5.65 ±0.04 a | 5.14 ±0.03 g | 5.41 ±0.02. bcdef | 5.47 ±0.02 abc | 5.45± 0.02 abcde | 5.22A |
| 144 | 3.97±0.05i | 4.76±0.003h | 5.48±0.01ab | 5.37±0.04abcde | 5.54 ±0.02 a | 5.09 ±0.03 fg | 5.43 ±0.03 abcd | 5.46 ±0.03 abc | 5.22± 0.03 def | 5.14BC |
| Mean | 3.47F | 4.51 E | 5.41B | 5.41 B | 5.44 B | 5.16 D | 5.61 A | 5.43 B | 5.33 C | |

Within a row mean values followed by different small letters denote significantly different at $p < 0.05$ among sampling time (ST) by LSD; within a column mean values followed by different capital letters are significantly different at $p < 0.05$ among treatments by LSD. See Table 1 for abbreviations.

**T a b l e 6**. Dry matter accumulation (g $m^{-2}$) at nine treatments of N fertilizer at nine sampling times during *N. sativa* L. growth under field conditions. Values are means (n = 3) ± SD

| Day after planting | C | U | CM | CM:U (1:1) SA | CM:U (2:1) SA | CM:U (1:2) SA | CM:U (1:1) FDA | CM:U (2:1) FDA | CM:U (1:2) FDA | Mean |
|---|---|---|---|---|---|---|---|---|---|---|
| 36 | 8.23 ±0.60 a | 14.58 ±0.36 a | 11.20 ±0.44 a | 13.57 ±0.58 a | 14.57 ±0.64 a | 12.12 ±0.70 a | 14.16 ±36 a | 12 ±0.0 a | 14.39 ±0.64 a | 12.75 H |
| 50 | 26.12 ±1.45 a | 41.03 ±0.44 a | 35.90 ±0.68 a | 52.33 ±9.34 a | 38.5 ±0.17 a | 40.75 ±0.43 a | 39.58 ±0.30 a | 42.18 ±0.34 a | 42.35 ±0.26 a | 39.86 G |
| 64 | 47.25 ±2.98 a | 73.99 ±1.08 a | 59.71 ±1.79 a | 70.40 ±0.34 a | 74.93 ±1.88 a | 73.23 ±1.13 a | 70.58 ±0.30 a | 68.17 ±1.71 a | 72.90 ±0.52 a | 67.90 F |
| 78 | 83.92 ±1.08 h | 118.0 ±10.70 def | 114.90 ±10.80 defg | 151.72 ±3.20 ab | 100.16 ±9.65 h | 142.4 ±17.7 bcd | 171.48 ±1.99a | 149.43 ±2.95 abc | 140.8 ±16.9 bcde | 13.30 E |
| 92 | 97.66 ±0.60 i | 149.23 ±9.99 fg | 162.30 ±10.70 f | 263.33 ±3.33 a | 132.1 ±11.0 gh | 214.23 ±9.71 de | 261.6 ±11.7 ab | 242.90 ±3.57 abc | 234.67 ±9.45 bcd | 195.33 D |
| 106 | 162.38 ±7.53 i | 203.02 ±4.05 h | 267.03 ±3.35 f | 359.74 ±6.53 b | 331.9 ±12.0 bcd | 238.7 ±11.7 g | 404.7 ±10.3 a | 343.81 ±6.65 bc | 318.74 ±8.30 cde | 292.22 C |
| 120 | 205.20 ±10.8 i | 232.80 ±8.10 gh | 339.14 ±9.4 ef | 403.0 ±11.0 cd | 454.7 ±12.60 b | 260.3 ±10.5 g | 484.29 ±3.38 a | 416.8 ±6.65 c | 361.8 ±12.3 e | 350.98 B |
| 134 | 231.33 ±5.46 i | 263.60 ±17.0 gh | 380.44 ±5.17 ef | 478.99 ±3.41 bc | 500.40 ±22.80 b | 274.2 ±12.2 g | 565.4 ±27.8 a | 470 ±17.1 cd | 397.2 ±13.2 e | 395.74 A |
| 144 | 228.22 ±9.70 i | 260.30 ±16.60 gh | 386.63 ±4.90 ef | 480.48 ±3.30 bc | 503.00 ±22.80 b | 273.0 ±12.5 g | 565.7 ±26.4 a | 473 ±18.3 cd | 397.5 ±12.9 e | 396.49 A |
| Mean | 121.21 H | 150.73 G | 195.25 E | 252.61B | 238.91 C | 169.88 F | 286.38 A | 246.48 BC | 220.03 D | |
| 116 | 4.08±0.16i | 4.68±0.003h | 5.35±0.05bc | 5.34±0.08bcd | 5.38 ±0.04 b | 5.13 ±.0.02 cde | 5.70 ±0.15 a | 5.13±0.19 def | 5.12±0.09 defg | 5.10CD |
| 130 | 4.07 ±0.06i | 4.77±0.0 h | 5.57±0.01ab | 5.45±0.03abcd | 5.65 ±0.04 a | 5.14 ±0.03 g | 5.41 ±0.02. bcdef | 5.47 ±0.02 abc | 5.45± 0.02 abcde | 5.22A |
| 144 | 3.97±0.05i | 4.76±0.003h | 5.48±0.01ab | 5.37±0.04abcde | 5.54 ±0.02 a | 5.09 ±0.03 fg | 5.43 ±0.03 abcd | 5.46 ±0.03 abc | 5.22± 0.03 def | 5.14BC |
| Mean | 3.47F | 4.51 E | 5.41B | 5.41 B | 5.44 B | 5.16 D | 5.61 A | 5.43 B | 5.33 C | |

Explanations as in Table 5.

**T a b l e 7.** Pearson correlation coefficients (r) between different soil $CO_2$ flux and *N. sativa* L. dry matter (DM) under urea fertilizer combined with cattle manure (n = 27)

| DM (day) | $CO_2$ flux (day) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 46 | 53 | 67 | 81 | 95 | 102 | 116 | 130 | 144 |
| 36 | 0.83** | 0.71** | 0.45** | 0.58** | 0.65** | 0.52** | 0.56** | 0.60** | 0.58** |
| 50 | 0.86** | 0.86** | 0.65** | 0.73** | 0.77** | 0.59** | 0.61** | 0.68** | 0.70** |
| 64 | 0.92** | 0.76** | 0.52** | 0.63** | 0.68** | 0.49** | 0.55** | 0.61** | 0.61** |
| 78 | 0.48** | 0.55** | 0.62** | 0.61** | 0.56** | 0.51** | 0.55** | 0.45** | 0.48** |
| 92 | 0.50** | 0.60** | 0.71** | 0.71** | 0.66** | 0.61** | 0.60** | 0.54** | 0.57** |
| 106 | 0.63** | 0.63** | 0.82** | 0.83** | 0.81** | 0.88** | 0.80** | 0.78** | 0.80** |
| 120 | 0.60** | 0.56** | 0.77** | 0.78** | 0.78** | 0.88** | 0.79** | 0.78** | 0.80** |
| 134 | 0.56** | 0.54** | 0.74** | 0.75** | 0.75** | 0.85** | 0.74** | 0.74** | 0.77** |
| 144 | 0.56** | 0.54** | 0.75** | 0.75** | 0.76** | 0.85** | 0.74** | 0.75** | 0.77** |

**p< 0.01.

The mean soil $CO_2$ flux for the interaction of fertilizer and time at the flowering stage (95 days after planting) was by 78.43% greater in the fertilized versus unfertilized, integrated versus solitary application (8.79%), and FDA versus SA integrated application of urea (3.73%). After *N. sativa* L. harvesting (144 days after sowing), higher $CO_2$-C flux was seen in the fertilized treatments of CM:U (2:1) SA, CM:U (1:1) SA and FDA, and CM:U (2:1) FDA, respectively, while lower soil $CO_2$-C flux was observed for the urea treatment. At this stage, there were no plants in the soil; thus, $CO_2$-C flux was lower in some treatments than at the previous sampling dates (Table 5).

ANOVA showed a significant effect for N fertilizer treatment (urea and cattle manure) and sampling time on DMA of *N. sativa* L. (Table 4). The interaction of fertilizer and sampling time was also significant ($p \leq 0.001$). The greatest DMA (286.3 g $m^{-2}$) was obtained by CM:U (1:1) FDA followed by CM:U (1:1) SA and CM:U (2:1) FDA with no significant difference between ranks (Table 6). The lowest DMA (121.2 $g^{-2}$) was produced by the control treatment. DMA was by 81.52% greater after application of fertilizer than for the control, 36.25% greater for the integrated application than solitary application, and by 13.83% greater for FDA of urea fertilizer than SA of urea fertilizer. The total oven-dried biomass obtained at the highest rate of 170.60 T of cattle manure $ha^{-1}$ was significantly higher than, but statistically similar to, that obtained for the medium chemical fertilizer treatment. In the present study, at 36 to 46 days after planting, there was no statistical difference between the fertilized and unfertilized plots. The highest and the lowest DMA were recorded for the solitary application of urea and the unfertilized plots, respectively. This reveals the effects of mineral N from the urea source on *N. sativa* L. growth. The results of the current study showed that at 50 to 64 days after planting, DMA was the highest for the CM:U (1:1) SA and CM:U (2:1) SA and the lowest for the unfertilized control (Table 6). At 78 days after planting (vegetative growth of *N. sativa* L.), DMA was higher in the fertilized plots than in the unfertilized plots. The highest DMA was recorded for the CM:U (1:1) FDA treatment and the lowest DMA for the CM:U (2:1) SA treatment.

DMA was higher at the flowering stage (92 days after planting) in the fertilized plots than the unfertilized plots. The highest and the lowest DMA were observed for CM:U (1:1 ) SA and CM:U (2:1 ) SA, respectively (Table 6). At 106 to 120 days after planting, the DMA of the fertilized plots increased significantly in comparison with the unfertilized treatments. The highest DMA was recorded for CM:U (1:1 ) FDA and the lowest DMA was observed for the solitary application of urea. The results also showed that there was no significant difference between the values for CM:U (1:1) SA and CM:U (2:1) SA and FDA treatments. DMA at 134 to 144 days after planting was the same as for 106 to 102 days after planting.

Pearson coefficient of the correlation for soil $CO_2$ flux versus DM is reported in Table 7. *Nigella sativa* L. DMA significantly and positively correlated with soil $CO_2$ flux for the same time intervals. Positive relationships were also observed between DM and *MBC* ($r = 0.55$, $p < 0.002$), and DM and soil $CO_2$ flux ($r = 0.66$; $p < 0.0002$) at the flowering stage. *MBC* was also found to be significantly correlated with soil $CO_2$ flux ($r = 0.83$; $p < 0.0001$) at the flowering stage.

## DISCUSSION

SOC increased at the flowering stage at highest the rate for cattle manure and the integrated levels (Fig. 1) and led to an increase in *MBC* (Fig. 4). Elsgaard *et al.* (2001) reported that the direct effect of the use of organic residues and manure was an increase in SOC and positive effects on soil properties such as water-holding capacity, soil biological activity, and increased soil *MBC* (Tejada and Gonzalez, 2005). Also, Kanchikerimath and Singh (2001) reported that SOC content was greater in manure than in chemical fertilizer. In addition, integrated treatments create better conditions for plant growth and root development could influence the SOC content.

The results of the present study showed that total N and mineral N increased in plots that received more cattle manure (Figs 2 and 3). The higher rates of total N and mineral N in the integrated treatment compared with the urea and unfertilized treatments resulted from the use of cattle manure with urea. This treatment, in addition to the entry of carbon into soil from the cattle manure (Fig. 1), also provided the N required for decomposition microorganisms, which increased root growth. Soil total N, SOM, soil nutrients, microbial activity, and mineral N increase with the use of organic fertilizer (Drinkwater *et al.,* 1995). These results are similar to those from other researchers on the effects of cattle manure (Eneji *et al.,* 2002), poultry manure (Travis *et al.,* 2004), and combined manure and urea fertilizer for increasing in N mineralization and decreasing the need for urea fertilizer (Mallory and Griffin, 2007).

The highest *MBC* was recorded in the fertilized plot because limitation in the N and C content is known to limit *MBC* and microbial growth in calcareous soils. This result was confirmed by the results of soil respiration for limited N and C as well. Factors contributing to the increase in *MBC* appear to be the increase in root biomass and exudates from the increased growth of *N. sativa* L. and the presence of metabolizable N and C in cattle manure. The results of this study are similar to the increase in soil *MBC* following application of urea (Abbasi and Khizar, 2012), horse manure (Jannoura *et al.,* 2014), and poultry manure (Fereidooni *et al.,* 2013; Abbasi and Khizar, 2012) in soils with low soil N and C contents. The results indicate that both urea-treated and CM:U (1:2) SA and FDA plots contained similar soil *MBC* contents that were lower than for the other combined treatments. Fereidooni *et al.* (2013) reported that soil *MBC* increased significantly in fertilized than unfertilized soil. They found the highest *MBC* values for corn with the application of broiler litter at 300 kg N $ha^{-1}$ (100-295 mg $kg^{-1}$) during the growing season (20 to 80 sampling dates). Jannoura *et al.* (2014) found that soil *MBC*, C, N, and P significantly increased at all sampling times with application of organic fertilizers and that the increase from manure was significantly higher than for the compost treatments. This could be the result of the higher *MBC* in the manure itself (Gattinger *et al.,* 2004) or the higher content of more readily decomposable carbon fractions in horse manure (Jannoura *et al.,* 2014).

The lowest *MBC* rates were found in urea-treated soils, probably because of the lowest availability and supply of C. Chemical fertilizers may not refill the soil organic C necessary as a microbial substrate, which will decrease activity and soil microbial biomass compared with organic fertilizers (Fereidooni *et al.,* 2013). The overall increase in soil *MBC* in the integrated treatments occurred because the urea treatment decreased the C/N ratio of cattle manure by 32.56%. The application of cattle manure increased SOC (Fig. 1), total N (Fig. 2), and mineral N (Fig. 3), which increased the release of nutrients and N for soil microbes and plants (Sharma and Bhushn, 2001). Lee *et al.* (2007) reported that soil *MBC* did not change with $NH_4NO_3$-N application, but increased significantly over the unfertilized control for the manure-N treatment. The greatest increase in soil *MBC* and may be related to the high manure application rate. Annual application of manure rapidly increased the soil *MBC* immediately following application (Lee *et al.,* 2007).

In the present study, the soil $CO_2$-C flux was higher in the fertilized plots. At the early stages of *N. sativa* L. growth, soil $CO_2$-C flux caused by the available N in urea fertilizer was not significantly different from the plots fertilized with CM+U; however, the soil $CO_2$-C flux was lower for cattle manure because of the high C/N ratio (Table 2) and slow release of nutrients (Table 5). The increase in soil $CO_2$-C flux at the later stages for the CM and other treatments with higher cattle manure content appears to be the result of the gradual release of nutrients by the manure (Table 5). The increase in soil $CO_2$-C flux in the fertilized treatment could presumably be the result of the entry of P (Table 2), organic C (Fig. 1), and N (Figs 2 and 3) into the soil from cattle manure and the growth of *N. sativa* L. in both the CM and urea treatments. Similar results in other studies were found for cattle manure (Shrestha *et al.,* 2013), horse manure (Jannoura *et al.,* 2014), urea (Abbasi and Khizar, 2012), poultry manure (Abbasi and Khizar, 2012; Fereidooni *et al.,* 2013), and FYM fertilizers (Mahmood *et al.,* 1997).

An annual $CO_2$ emission rate of 9.7 mg $CO_2$-C $ha^{-1}$ $y^{-1}$ has been reported from corn treated with pasteurized chicken manure at a rate of 10 mg $ha^{-1}$ $yr^{-1}$ in sandy loam soil (Heller *et al.,* 2010) and at 13.3-15.3 mg $CO_2$-C $ha^{-1}$ $y^{-1}$ from cattle manure at a rate of 20 mg $ha^{-1}$ (fresh weight) in sandy soil (Matsumoto *et al.,* 2008). Fereidooni *et al.* (2013) concluded that soil $CO_2$-C production for fertilized and unfertilized plots over 20-day sampling times was similar, but was greater after the addition of urea and broiler litter than for the unfertilized plot at other sampling times. This indicates that soil respiration factors (as soil microbial activity) may be co-limited by both N and C availability and that the root activity or plant growth that contribute to

soil respiration may be limited by the limited N in the soil. Shrestha *et al.* (2013) showed that cumulative soil $CO_2$ flux was higher for cattle manure followed by compost, bare fallow, and cover crop treatments. The highest $CO_2$ flux after organic amendment over the non-amended treatment can be attributed to the combined effects of available C substrate, soil temperature, moisture regimes (Smith *et al*., 2003), and higher microbial activity (Rochette *et al.,* 2000).

Soil respiration was higher in integrated treatments (cattle manure + urea) than in the urea and unfertilized treatments. This increase could result from the available C substrate (Fig. 1) and easily mineralizable organic compounds (Fig. 3), and other essential nutrients (P and N) for soil microorganisms available in cattle manure. Shimizu *et al.* (2009) reported that soil respiration was higher in the manure plot (beef cattle) than in the fertilizer and unfertilized plots, probably because of the high organic C content (Fig. 1) in the manure. Lee *et al.* (2007) reported that about 45% of the C added by beef cattle manure was lost as $CO_2$-C over four years in switchgrass in South Dakota.

The present study indicated that soil $CO_2$-C respiration from fertilized soils was dependent on the amount of cattle manure and urea applied initially and the split and non-split urea. Soil respiration was generally greater in plots that received CM:U (50:50) FDA and CM:U (50:50) SA (Table 5). This means that the application of urea in the first stage plus cattle manure enhanced soil $CO_2$-C flux from the soil surface more effectively that for the urea application at later stages. Alizadeh *et al.* (2012) showed that adding urea fertilizer to cow manure can increase the decomposition of the manure. They reported that N availability was greater in CM + UF (47%) than CM (28%) soils.

Overall, the stronger relationship between soil $CO_2$ flux (Table 5) and soil *MBC* (Fig. 4) at the flowering stage may be caused by the combined effects of readily-available C and increased *MBC* with manure application and the greater contribution of microbial respiration to total soil $CO_2$ flux.

The DMA was low at the early stages of *N. sativa* L. growth because of the lower biomass from the plants and lower growth rate; DMA increased in the flowering and reproductive stages (Table 6). This indicates that the increase in DMA is the result of the increase in mineral N (Fig. 3) available for the plants. The higher rates of urea-N fertilizer in the solitary and integrated applications resulted in higher DMA in the early stages. Gheysari *et al.* (2009) found that application of 225 kg N $ha^{-1}$ resulted in the highest aboveground biomass for silage in the arid and semi-arid areas under study. At the flowering stage (92 days after planting), DMA was higher in the split integrated treatments that provided a second portion of urea (top dressing application); the gradual release of cattle manure nutrients improved photosynthesis (Table 5) and plant growth, which continued until the end of sampling. Azeez *et al.,* (2010) reported that the mean total oven-dry aboveground biomass of *Cucurbita maxima* L. increased progressively from 1.99 to 42.96 g $pot^{-1}$ when the application rate of cattle manure increased from 5.33 to 170.60 t $ha^{-1}$. They found that the difference in mean total oven-dry biomass between the control and cattle kraal manure treatments was statistically significant ($p < 0.05$) when cattle kraal manure was applied at a rate of ≥10.66 t $ha^{-1}$.

The results indicate that the integrated application of cattle manure and chemical fertilizer increased total N (Fig. 2), mineral N (Fig. 3), *MBC* (Fig. 4), and consequently $CO_2$ flux (Table 5). Mineral N and *MBC* increased in CM:U (1:1) FDA. The highest soil $CO_2$-C flux was recorded for CM:U (1:1) FDA and SA (Table 5). This shows that soil $CO_2$-C flux and DMA ran in parallel. The role of soil N, *MBC*, and $CO_2$ flux in increasing the DMA of *N. sativa* L. in the present study can be attributed to the higher leaf area that allowed absorption of more light and continuous photosynthesis, which ultimately increased DMA. Similar results were reported by Shoor and Mondani (2012) under greenhouse conditions; they found that increasing the concentration of $CO_2$ and nutrients usually increased photosynthesis, the growth of crops, and the yield. They concluded that the use of animal manure and nitrogen fertilizer appears to increase the $CO_2$, DMA, and yield per plant. Niboyet *et al.* (2010) demonstrated the DMA of *Dactylis* significantly increased after the addition of N.

Somanath *et al*. (2005) reported improved dry matter yield in *Coleus forskohlii* after integrated use of FYM + NPK than for NPK alone. The results of the present study show that the integrated use of cattle manure and urea fertilizer resulted in higher DMA of *N. sativa* L. over the solitary application of cattle manure and urea fertilizer (Table 6). This can be attributed to the better nutrient supply from cattle manure over time and the timely availability of N-urea fertilizer needed for plant growth.

The current study shows that the positive effects of the integrated CM:U (50:50 ) treatment on DMA can be attributed to the stimulation of microbial activity (Fig. 4) and acceleration of decomposition. Other studies have reported that the integration of 50% organic manure can improve soil quality (Agbede *et al.,* 2008), the gradual release of nutrients (Sharma and Mittra, 1991), and the increase in DMA. Alizadeh *et al.* (2012) reported that at 110 days of field incubation, there was no statistical difference in maize dry weight among CM, UF, and CM+UF treatments. They reported higher maize dry weight in CM+UF (14900 kg $ha^{-1}$) than in CM (14000 kg $ha^{-1}$) and UF (12900 kg $ha^{-1}$) treatments.

Studies have found that the highest DMA at the end of sampling occurred for the 50% chemical fertilizer + 50% broiler litter application and the lowest DMA was observed in the unfertilized control (Ghosh *et al.,* 2003).

Pearson correlation results indicate that the increase in *N. sativa* L. DMA is connected with the increase in soil $CO_2$ flux following the application of organic and inorganic

fertilizers. This is probably caused by the addition of urea fertilizer and cattle manure that stimulate *N. sativa* L. performance. Fereidooni *et al.* (2013) observed a significant and positive correlation between maize aboveground biomass dry weight and $CO_2$ production. They indicated that the addition of urea fertilizer and poultry manure appeared to stimulate maize performance (Fereidooni *et al.,* 2013).

Enhancement of the soil physiochemical properties after the addition of mineral and organic fertilizer could influence the soil properties and *N. sativa* L. DMA. The present study shows that cattle manure combined with chemical fertilizer increased *N. sativa* L. growth and microbial activity in the soil.

## CONCLUSIONS

1. Cattle manure with a high C/N ratio with addition of chemical fertilizer significantly improved the biological properties of soil under *N. sativa* L. and resulted in higher microbial biomass C, soil organic carbon, soil total N, soil mineral N, and soil $CO_2$-C flux. This method significantly increased, dry matter accumulation over that for the solitary application of urea fertilizer and cattle manure.

2. The results showed that, at the early stages of *N. sativa* L. growth, the positive effect of solitary application of urea fertilizer on soil $CO_2$-C flux and dry matter accumulation was greater than the solitary cattle manure application and at the later flowering stage, the effect of solitary cattle manure application over urea application.

3. Addition of urea fertilizer to cattle manure with high C/N increased decomposition of organic N and nutrient mineralization and availability.

4. The combined application of cattle manure and chemical fertilizer and full-dose application of urea increased fertilizer efficiency, maintained soil biological conditions, decreased the need for chemical fertilizers, and decreased the cost of top dressing fertilizer. This decreases environmental problems and the knock-on effects of cattle manure produced in the surrounding villages.

5. Dry matter increased as the soil $CO_2$ flux increased; thus, this method can help reduce global warming and climate change. The use of carbon-14 is recommended for determination of the ratio of soil $CO_2$ flux in plant photosynthesis under organic and conventional systems.

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## REFERENCES

**Abbasi M.K. and Khizar A., 2012.** Microbial biomass carbon and nitrogen transformations in a loam soil amended with organic-inorganic N sources and their effect on growth and N-uptake in maize. Ecol. Eng., 39, 123-132.

**Agbede T.M., Ojeniyi S.O., and Adeyemo A.J., 2008.** Effect of poultry manure on soil physical and chemical properties, growth and grain yield of sorghum in southwest Nigeria. Am. Eurasian J. Sustain. Agric., 2, 72-77.

**Alef K. and Nannipier A., 1995.** Methods in Applied Soil Microbiology and Biochemistry. Academic Press, London.

**Alizadeh P., Fallah S., and Raiesi F., 2012.** Potential N mineralization and availability to irrigated maize in a calcareous soil amended with organic manures and urea under field conditions. Int. J. Plant Prod., 6, 493-512.

**Alsafar M.S. and Al-Hassan Y.M., 2009.** Effect of nitrogen and phosphorus fertilizers on growth and oil yield of indigenous mint (*Mentha longifolia* L.). Biotech., 8, 380-384.

**Azeez J.O. and Averbeke W.V., 2010.** Nitrogen mineralization potential of three animal manures applied on a sandy clay loam soil. Bioresour. Technol., 101, 5645-5651.

**Azeez J.O., Averbeke W.V., and Okorogbona A.O.M., 2010.** Differential responses in yield of pumpkin (*Cucurbita maxima* L.) and nightshade (*Solanum retroflexum* Dun.) to the application of three animal manures. Bioresour. Technol., 101, 2499-2505.

**Azraf-ul-Haq A., Ahmad R., and Naeem M., 2007.** Production potential and quality of mixed sorghum forage under different intercropping systems and planting patterns. Pak. J. Agric. Sci., 44, 203-207.

**Bandyopadhyay K.K., Misra A.K., Ghosh P.K., Hati K.M., and Mandal K.G., 2010.** Effect of integrated use of farmyard manure and chemical fertilizers on soil physical properties and productivity of soybean. Soil Till. Res., 110, 115-125.

**Bastida F., Moreno J.L., Hernandez T., and Garcıa C., 2006.** Microbiological degradation index of soils in a semiarid climate. Soil Biol. Biochem., 38, 3463-3473.

**Drinkwater L.E., Letourneau D.K., Worknesh F., Van Bruggen A.H.C., and Shennan C., 1995.** Fundamental differences between conventional and organic tomato agro ecosystems in California. Ecol. Appl., 5, 1098-1112.

**Elsgaard L., Petersen S.O., and Debosz K., 2001.** Effect and risk assessment $O_2$ linear alkylbenzene sulfonate (LAS) in agricultural soil. 2. Effects on soil microbiology as influenced by sewage sludge and incubation time. Environ. Toxicol. Chem., 20, 1664-1672.

**Eneji A.E., Honna T., Yamamoto S., Satio T., and Masuda T., 2002.** Nitrogen transformation in four Japanese soils following manure+ urea amendment. Commun. Soil. Sci. Plant Anal., 33, 53-66.

**Fallah S., Ghalavand A., and Raisi F., 2013.** Soil chemical properties and growth and nutrient uptake of maize grown with different combinations of broiler litter and chemical fertilizer in a calcareous soil. Commun. Soil Sci. Plant Annal., 44, 3120-3136.

**Fereidooni M., Raiesi F., and Fallah S., 2013.** Ecological restoration of soil respiration, microbial biomass and enzyme activities through broiler litter application in a calcareous soil cropped with silage maize. Ecol. Eng., 58, 266- 277.

**Franzluebbers A.J., Hons F.M., and Zuberer D.A., 1995.** Tillage-induced seasonal changes in soil physical properties affecting soil $CO_2$ evolution under intensive cropping. Soil Till. Res., 34, 41-60.

**Gattinger A., Bausenwein U., and Bruns C., 2004.** Microbial biomass and activity in composts of different composition and age. J. Plant Nutr. Soil Sci., 167, 556-561.

**Gheysari M., Mirlatifi S.M., Bannayan M., Homaee M., and Hoogenboom G., 2009.** Interaction of water and nitrogen on maize grown for silage. Agric. Water Manag., 96, 809-821.

**Ghosh P.K., Bandyopadhyay K.K., Tripathi A.K., Hati K.M., Mandal K.G., and Misra A.K., 2003.** Effect of integrated management of farmyard manure, phosphocompost, poultry manure and inorganic fertilizers for rainfed sorghum (*Sorghum bicolor*) in Verticols of central India. Ind. J. Agron., 48, 48-52.

**Grant R.F., Juma N.G., Robertson J.A., Izaurralde R.C., and McGill W.B., 2001.** Long-term changes in soil carbon under different fertilizer, manure, and rotation: testing the mathematical model ecosystem with data from the Breton plots. Soil Sci. Soc. Am. J., 65, 205-214.

**Hati K.M., Mandal K.G., Misra A.K., Ghosh P.K., Bandyopadhyay K.K., 2006.** Effect of inorganic fertilizer and farmyard manure on soil physical properties, root distribution, and water-use efficiency of soybean in Vertisols of central India. Bioresour. Technol., 97(16), 2182-2188.

**Heller H., Bar-Tal A., Tamir G., Bloom P., Venterea R.T., Chen D., Zhang Y., Clapp C.E., and Fine P., 2010.** Effects of manure and cultivation on carbon dioxide and nitrous oxide emissions from a corn field under Mediterranean conditions. J. Environ. Qual., 39, 437-448.

**Hesse P.R., 1971.** A Textbook of Soil Chemical Analysis. John Murray Press, London, UK.

IPCC, **2007.** Technical summary. In: Climate Change (Eds S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor, and H.L. Miller. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK.

**Jannoura R., Joergensena R.G., and Bruns C., 2014.** Organic fertilizer effects on growth, crop yield, and soil microbial biomass indices in sole and intercropped peas and oats under organic farming conditions. Eur. J. Agron., 52, 259-270.

**Jenkinson D.S. and Ladd J.N., 1981.** Microbial biomass in soil: Measurement and turnover. In: Soil Biochemistry (Eds E.A. Paul and J.N. Ladd). New York, USA.

**Kanchikerimath M. and Singh D., 2001.** Soil organic matter and biological properties after 26 years of maize-wheat-cowpea cropping as affected by manure and fertilization in a combisol India. Agr. Ecosyst. Environ., 86,155-162.

**Keeney D.R. and Nelson D.W., 1982.** Nitrogen-inorganic forms. In: Methods of Soil Analysis, Part 2 (Eds A.L. Page, R.H. Miller, D.R. Keeney,). American Society of Agronomy. Madison, WI, USA. .

**Lee D.K., Doolittle J.J., and Owens V.N., 2007.** Soil carbon dioxide fluxes in established switchgrass land managed for biomass production. Soil Biol. Biochem., 39, 178-186.

**Mahmood T., Azam F., Hussain F., and Malik A., 1997.** Carbon availability and microbial biomass in soil under an irrigated wheat-maize cropping system receiving different fertilizer treatments. Biol. Fert. Soils, 25, 63-68.

**Mallory E.B. and Griffin T.S., 2007.** Impacts of soil amendment history on nitrogen availability from manure and fertilizer. Soil Sci. Soc. Am. J., 71, 964-973.

**Mandal A., Patra A.K., Singh D., Swarup A., and Masto R.E. 2007.** Effect of long term application of manure and fertilizer on biological and biochemical activities in soil during crop development stage. Bioresour. Technol., 98, 3585-3592.

**Matsumoto N., Paisancharoen K., and Hakamata T., 2008.** Carbon balance in maize fields under cattle manure application and no-tillage cultivation in Northeast Thailand. Soil Sci. Plant Nutr., 54, 277-288.

**Nelson D.W. and Sommers L.E., 1982.** Total carbon, organic carbon and organic matter. In: Methods of Soil Analysis (Eds A.L. Page, R.H. Miller, and D.R. Keeney). American Soc. of Agronomy, Madison, Wisconsin, USA.

**Niboyet A., Barthes L., Hungate B.A., Le Roux X., Bloor J.M.G., Ambroise A., Fontaine S., Price, P.M., and Leadley, P.W., 2010.** Responses of soil nitrogen cycling to the interactive effects of elevated $CO_2$ and inorganic N supply. Plant Soil., 327, 35-47.

**Peacock A.D., Mullen M.D., Ringelberg D.B., Tyler D.D., Hedrick D.B., Gale P.M., and White D.C., 2001.** Soil microbial community responses to dairy manure or ammonium nitrate applications. Soil Biol. Biochem., 33, 1011-1019.

**Rochette P., Angers D.A., Chantigny M.H., Bertrand N., and Cote D., 2004.** Carbon dioxide and nitrous oxide emissions following fall and spring applications of pig slurry to an agricultural soil. Soil Sci. Soc. Am. J., 68, 1410-1420.

**Rochette P., Angers D.A., and Cote D., 2000.** Soil carbon and nitrogen dynamics following application of pig slurry for the 19th consecutive year: 1. Carbon dioxide fluxes and microbial biomass carbon. Soil Sci. Soc. Am. J., 64, 1389-1395.

**Rochette P., Flanagen L.B., and Gregorich E.G., 1999.** Separating soil respiration into plant and soil components using analysis of the natural abundance of carbon-13. Soil Sci. Soc. Am. J., 63, 1207-1213.

**Schlesinger W.H., and Andrews J.A., 2000.** Soil respiration and the global carbon cycle. Biogeochemistry, 48, 7-20.

**Sharma A.R. and Mittra B.N., 1991.** Effect of different rates of application of organic and nitrogen fertilizers in a rice-based cropping system. J. Agr. Sci. (Cambridge), 117, 313-318.

**Sharma P.K. and Bhushn L., 2001.** Physical characterization of a soil amended with organic residues in a rice-wheat cropping system using a single value soil physical index. Soil Till. Res., 60, 143-152.

**Shimizu M., Marutani S., Desyatkin A.R., Jin J., Hata H., and Hatano R., 2009.** The effect of manure application on carbon dynamics and budgets in a managed grassland of Southern Hokkaido, Japan. Agric. Ecosyst. Environ., 130, 31-40.

**Shoor F. and Mondani F., 2012.** Effect of elevated CO2 in different fertilizer conditions on physiological traits in Lemon balm (*Melissa officinalis*) at greenhouse (in Persian). Iran. J. Field Crop Res., 1, 133-142.

**Shrestha R.K., Lal R., and Rimal B., 2013.** Soil carbon fluxes and balances and soil properties of organically amended no-till corn production systems. Geoderma, 197-198, 177-185.

**Smith K.A., Ball T., Conen F., Dobbie K.E., Massheder J., and Rey A., 2003**. Exchange of greenhouse gases between soil and atmosphere: interactions of soil physical factors and biological processes. Eur. J. Soil Sci., 54, 779-791.

**Somanath S., Bhaskar S., and Sreenivasmurthy C.A., 2005.** Influence of FYM and inorganic fertilizer (NPK) and sources of potassium on the yield of *Coleus forskohlii*. J. Med. Arom. Plants Sci., 27, 16-19.

**Tejada M. and Gonzalez J.L., 2005.** Beet vinasse applied to wheat under dry land conditions affects soil properties and yield. Eur. J. Agron., 23, 336-347.

**Tiwari K.R., Nyborg I.L.P., Sitaula B.K., and Paudel G.S., 2008.** Analysis of the sustainability of upland farming systems in the Middle Mountain region of Nepal. Int. J. Agr. Sustain., 6, 289-306.

**Travis A.H., Donald A.G., and Thomas A.O., 2004.** A comparison of in situ methods for measuring net nitrogen mineralization rates of organic soil amendments. Agron. J., 33, 1098-1105.

**Vineela C., Wani S.P., Srinivasarao C., Padmaja B., and Vittal, K.P.R., 2008.** Microbial properties of soils as affected by cropping and nutrient management practices in several long-term manurial experiments in the semi-arid tropics of India. Appl. Soil Ecol., 40, 165-173.

**Wang X., Zhu B., Li C., Gao M., Wang Y., Zhou Z., and Yuan H., 2011.** Dissecting soil $CO_2$ fluxes from a subtropical forest in China by integrating field measurements with a modeling approach. Geoderm, 161, 88-94

**Wu F., Gai Y., Jiao Z., Liu Y., Ma X., An L., and Wang W., 2012.** The community structure of microbial in arable soil under different long-term fertilization regimes in the Loess Plateau of China. Afr. J. Microbial. Res., 6, 6152-6164.

**Yan D., Wang D., and Yang L., 2007.** Long-term effect of chemical fertilizer, straw, and manure on labile organic matter fractions in a paddy soil. Biol. Fertil. Soils., 44, 93-101.