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Soil Carbon Dioxide Flux and Organic Carbon in Grassland after Manure and Ammonium Nitrate Application

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ABSTRACT: Fertilization effects on changes in soil CO_2 flux and organic C in switchgrass (*Panicum virgatum* L.) land managed for biomass production were investigated. The mean daily soil CO_2 flux in the manure treatment was 5.63 g CO_2-C m^{-2} d^{-1}, and this was significantly higher than the mean value of 3.36 g CO_2-C m^{-2} d^{-1} in the control. The mean daily CO_2 fluxes in N and P fertilizer treatments plots were not different when compared to the value in the control plots. Potentially mineralizable C (PMC), soil microbial biomass C (SMBC), and particulate organic C (POC) were highest at the 0 to10 cm depth of the manure treatment. Potentially mineralizable C had the strongest correlation with SMBC ($r = 0.91$) and POC ($r = 0.84$). There was also a strong correlation between SMBC and POC ($r = 0.90$). Our results indicated that for the N and P levels studied, fertilization had no impact on temporal changes in soil organic C, but manure application had a significant impact on temporal changes in soil CO_2 evolution and active C constituents such as PMC, SMBC, and POC.

Key Words: Soil CO_2 evolution, Potentially mineralizable C, Soil microbial biomass C, Particulate organic C

INTRODUCTION

Grasslands, composing 24% of the earth's vegetation[1], have an important meaning for greenhouse gas emission and potential C sequestration. Switchgrass (*Panicum virgatum* L.) grown for bioenergy production decreases atmospheric CO_2 concentration and increases soil quality by C sequestration[2]. Fast-growing bioenergy crops decrease atmospheric CO_2 concentration from burning fossil fuel by increasing soil organic C (SOC) and enabling the replacement of fossil fuels with plant-derived fuels. Switchgrass sequesters carbon through its deep, productive root system[3] and contributes to soil quality by increasing soil organic matter (SOM) and improving soil structure.

The dynamics of SOC play an important role in long-term agroecosystem conservation and productivity and the emission of greenhouse gases[4]. The SOC pool forms from the net result of C input (crop residue and biomass) and output by C mineralization (CO_2 flux and other losses). Maximizing C sequestration in soil is an important means of reducing net CO_2 levels in the atmosphere[5,6]. Soils play an important role in the global carbon budget, containing 5.4% of the carbon reserves of the earth. This is a considerable amount when compared with 1.7% in the atmosphere, 11.2% in fossil fuels, 1.2% in biota, and 80.5% in the oceans[7,8].

Soil is an important source-sink component in the global C budget. The C sink capacity of soils can be altered by management practices. The adoption of soil management practices that increase C sequestration is needed to maximize the sink capacity of soils[8].

Agricultural management practices affect soil productivity by changing the soil environment (i.e., soil moisture, soil temperature, vegetation, plant root densities and activities, and the availability of C substrates for microorganisms). Soil environmental

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characteristics have a large impact on microbial growth and decomposition processes that transform plant-derived C to SOM and CO_2[9]. Agricultural management practices, such as conservation tillage, fertilizer application, and cropping system, have the potential to reduce the amount of CO_2 emitted from soil[6]. Many studies have shown that fertilization, particularly N additions, enhances SOC accumulation in cropping systems[10-12]. Nitrogen fertilization tends to reduce microbial C in tallgrass prairie[13]. Schlesinger[14] suggested that fertilization, irrigation, and manure treatment are important practices for soil conservation and soil C sequestration. Grant et al.[12] reported that manure application had a positive effect on SOC accumulation in cropping systems. Pig manure slurry applications can increase both soil CO_2 emissions and microbial biomass[15].

There is very little information on the relationships between C and N dynamics, especially soil CO_2 flux, and management practices for established switchgrass. The objectives of this study were to i) determine the effects of fertilization on soil CO_2 flux and various soil chemical and biological C fractions including SOC, potentially mineralizable C (PMC), soil microbial mass C (SMBC), particulate organic C (POC), mineral associated organic C (MOC), and labile C (LC), and ii) determine relationships between these C pools and C mineralization.

MATERIALS AND METHODS

The experimental site was located in Moody County, SD, U.S.A. (96°41′W and 44°10′N). Average (30 yr) annual temperature is 6.3℃ and average annual precipitation is 580 mm[16]. Average daily temperature during the growing season (May-October) is 10.5℃ and maximum daily temperature is 22.2℃ in July. Precipitation during the growing season is about 75% of the annual precipitation. The soil is an Egan silty clay loam (fine-silty, mixed, superactive, mesic Udic Haplustolls). Selected soil chemical and physical properties are shown in Table 1. This site has been established in switchgrass since 1975.

Experimental plots (1.8 m × 6.0 m) were arranged in a randomized complete block design with four replications for each treatment. Manure, N, and P treatments were broadcast on the surface on 16 May 2001. Treatments included the following: 1) 0 kg N ha^{-1} and 0 kg P ha^{-1} (0N0P; control), 2) 224 kg N ha^{-1} and 0 kg P ha^{-1}(N), 3) 0 kg N ha^{-1} and 56 kg P ha^{-1} (P), 4) 224 kg N ha^{-1} and 56 kg P ha^{-1} (NP), and 5) 55 Mg dry wt. manure ha^{-1} (Manure). Ammonium nitrate (33-0-0) and triple-superphosphate (0-45-0) were used for N and P treatments, respectively, and manure was applied as fresh cattle manure.

The soil CO_2 flux was measured using a modified static chamber method[9] with an LI-800 CO_2 analyzer (LI-COR, Lincoln, NE) from May to December 2001 with 5 to 15d intervals. Two PVC chambers (10.16 cm i.d., 4.7 L volume) each with an inlet on the top and an outlet on the bottom were located on a plot. Chambers were placed adjacent to a switchgrass crown and pushed 2 cm into the soil at 07:00 hrs in the morning. Carbon dioxide gas was collected for 2 h. The CO_2 concentration in the headspace was measured with a CO_2 analyzer. Measurements were conducted during the early morning to avoid heating of the chamber[9]. Soil temperature at the depth of 5 cm and volumetric soil water content were measured at the same time as CO_2 was measured. In addition, air temperature, soil temperature at the depth of 5 cm, and precipitation data were collected from an automatic weather station.

Soil samples for baseline information were collected with a hydraulic soil probe (6.6 cm i.d.) in November 2000. Individual soil samples consisted of four composited cores per plot that were divided into 0 to 10, 10 to 20, and 20 to 30 cm depth increments. Soil samples were sieved to pass an 8 mm sieve and dried in a forced air oven at 55℃ until constant mass was attained. Visible plant residues and roots were removed before drying. Dried soil samples were ground to pass a 2 mm sieve and analyzed for selected chemical and physical properties. A portion of the moist soil sample was dried at 105℃ for 24 h to calculate field moisture content and bulk density.

To evaluate the effect of fertilization on the changes of soil organic C pools and the relationship between soil CO_2 flux and soil organic C pools, soil samples were collected from under the static chamber following CO_2 measurement and chamber removal. Soil samples were collected from 0 to 10, 10 to 20, and 20 to 30 cm depth with a hand probe (3.2 cm ID) at monthly interval from May to October 2001. Two cores per each chamber and four cores per plot were composited by depth. Soil samples were sieved to pass a 5.0 mm screen and analyzed for SMBC and PMC on the same day. Two sub samples were taken from each moist sample. One subsample was dried at 105℃ for 24 h for moisture content and bulk density determination. A portion of the soil samples collected on 25 June 2001 were dried at 55℃ for 3 d and ground to pass a 2 mm sieve for chemical analysis, including SOC, TN, POC, MOC, and LC.

Soil bulk density was calculated using the measured moisture content and the volume of the core. Soil pH was determined using a pH meter and a 1:1 soil to water paste[17]. Available P (Olsen P) was determined using buffered (pH 8.5) 0.5 M $NaHCO_3$ extracting solution[18]. Particle size distribution was determined using a modified pipette method[19]. Particulate organic matter and C were determined using a modified wet sieving method[20,21]. Thirty g of air-dried soil was dispersed by shaking overnight in 100 mL of 5 g L^{-1} sodium hexametaphosphate. The dispersed organic matter plus sand (POM fraction) was collected on a 0.053 mm sieve by washing with deionized water and drying at 105℃ for 24 h. The particulate organic matter fraction was ground and 1 g of sub sample was taken to determine POC. Soil organic C, POC, and total N were determined by dry combustion method in a Vario Max CNS elemental analyzer (Elementar Instrument, Mt. Laurel, NJ). Mineral associated organic carbon (MOC) fraction was calculated by subtracting POC from SOC. Labile C (LC) was determined using the oxidation method[22] with 0.2 M $KMnO_4$.

Soil microbial biomass C and N were determined by the modified chloroform fumigation-incubation method[23,24]. Thirty g (air dried basis) of moist-sieved soil sample was placed in 50 mL beakers and fumigated. Water content was adjusted to 55% water-filled pore space by adding deionized water and then incubating it in 1 L air-tight mason jars with 4 mL of 1 *M* NaOH at 25℃ for 10 d. The quantity of CO_2-C trapped in the NaOH solution was determined by titration with HCl after the addition of excess 2 *M* $BaCl_2$ to a phenolphthalein endpoint[25]. Soil microbial biomass C was calculated by dividing the CO_2-C flush of fumigated soil by k_C = 0.41[26]. Potentially mineralizable C and N were estimated from the incubation of unfumigated soil[24].

Differences of soil CO_2 flux and various C fractions among fertilizer treatments were analyzed with a one-way analysis of variance. A least significant difference was used to separate means when the F-test was significant (P=0.05). Correlation analysis was used to test associations among various soil chemical and biological properties[27].

RESULTS AND DISCUSSION

Temporal changes in soil CO_2 flux were similar to that in soil temperature (Figs. 1 and 2). Soil CO_2 flux increased as soil temperature increased and reached a maximum. Volumetric soil water content during the growing season of 2001 (Fig. 1) was below the level of 0.8 cm^3 cm^{-3} and above the level of 0.2 cm^3 cm^{-3}, which are the breakpoints for water limitations on soil microbial activity for CO_2 flux[28]. However, soil CO_2 flux seemed to be influenced by soil water content in September, although soil water content (0.17 cm^3 cm^{-3}) in that time period was not as low as the breakpoint of a water limitation on microbial activity. Mean daily soil CO_2 flux were 3.36, 3.55, 3.89, 3.51, and 5.63 g CO_2-C m^{-2} d^{-1} for the control, P, N, NP, and manure treatment, respectively. The mean rate of soil CO_2 evolution in the control during the growing period (May-October) of switchgrass was 3.38 g CO_2-C m^{-2} d^{-1}. This value is similar to the daily soil CO_2 evolution of 3.5 g CO_2-C m^{-2} d^{-1} measured at the mixed-grass prairie in North Dakota, U.S.A.[29], and was lower

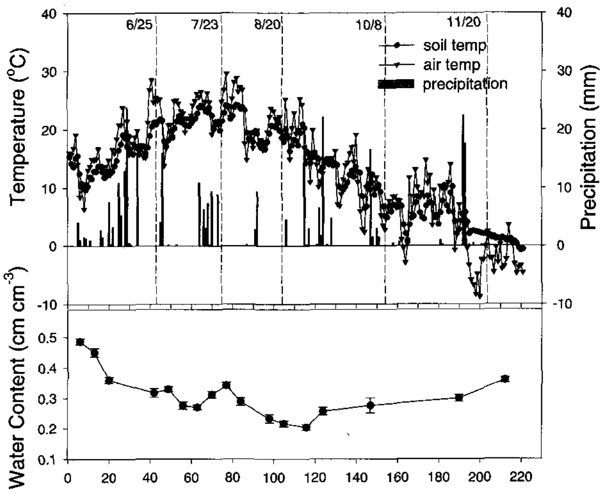

Fig. 1. Air temperature, soil temperatures at the 5 cm depth, precipitation, and volumetric soil water content at the 0 to 10 cm depth in switchgrass land. Error bars for water content denote standard error.

than the 6.73 g CO_2-C $m^{-2}d^{-1}$ at the Konza prairie in Kansas, U.S.A[30]. Daily soil CO_2 fluxes among control, N, and P treatments were not significantly different ($P>0.05$) (Fig. 2). Soil CO_2 flux is a net result of the oxidation of SOM by soil microorganism and root respiration. Chemical N and P fertilization did not affect soil CO_2 flux in this study. This may be due to the high SOM content from switchgrass residue inputs on long-term grass land. Generally, N addition does not result in soil C changes in a high organic matter soil[31].

Mean soil CO_2 flux in the manure treatment during the growing season was significantly higher than that in control, N, and P treatments ($P<0.05$) (Figs. 1 and 2). In agreement with previous studies[15], higher soil CO_2 flux observed in the manure treatment was probably caused by the high content of organic C (Table 1) and high microorganism populations in manure.

Nitrogen and P application did not significantly impact temporal changes in PMC and SMBC, but manure application significantly increased PMC and SMBC at the 0 to 10 cm depth (Figs. 3 and 4). However, there were no differences for PMC and SMBC among fertilizer and manure treatments at the depth of 10 to 20 and 20 to 30 cm (data not shown). Potentially

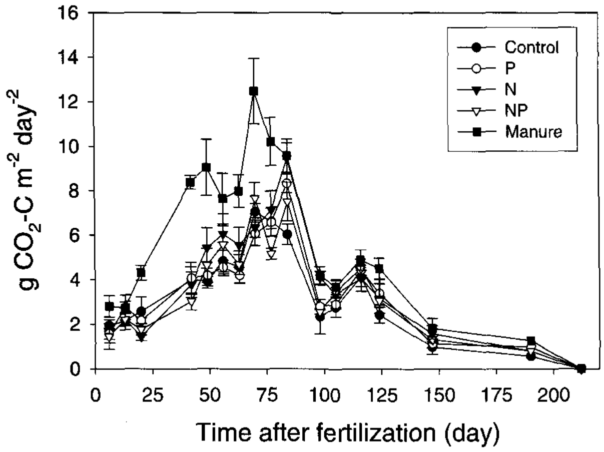

Fig. 2. Soil CO_2 flux (n=4 for each point) affected by phosphorus (P), nitrogen (N), nitrogen and phosphorus (NP), and manure application in switchgrass land. Error bars denote standard error.

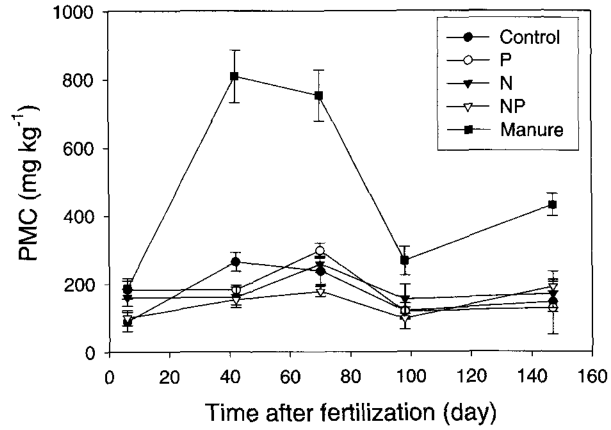

Fig. 3. Potentially mineralizable C (PMC; n=4 for each point) affected by phosphorus (P), nitrogen (N), nitrogen and phosphorus (NP), and manure application at the 0-10 cm depth in switchgrass land. Error bars denote standard error.

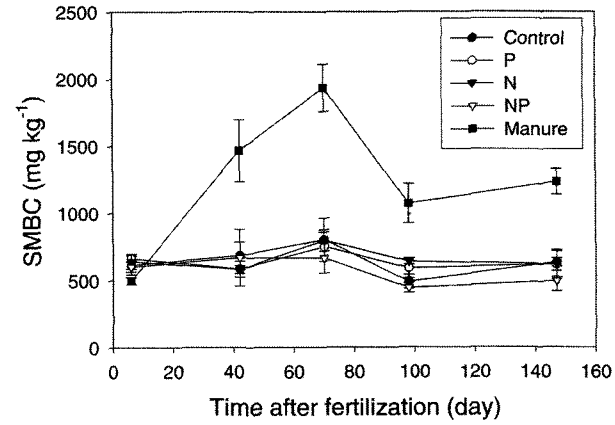

Fig. 4. Soil microbial biomass C (SMBC; n=4 for each point) affected by phosphorus (P), nitrogen (N), nitrogen and phosphorus (NP), and manure application at the 0-10 cm depth in switchgrass land. Error bars denote standard error.

Tablel. Selected chemical and physical properties for switchgrass land soil and cattle manure

Depth	pH	BD*	SOC+	TN†	POC‡	Olsen P	Clay	Silt	Sand
cm		g cm^{-3}	---------g kg^{-1}---------			mg kg^{-1}	------g kg^{-1}------		
0 - 10	6.20	1.20	29.3	2.45	3.49	16.1	305	630	65
10 - 20	6.25	1.23	24.2	2.03	1.14	14.2	320	617	63
20 - 30	6.62	1.17	23.1	1.95	1.23	11.4	327	610	63
Manure	-	-	201	11.9	-	667	-	-	-

*BD: Bulk density; +SOC: Soil organic carbon; †TN: Total nitrogen; ‡POC: Particulate organic carbon mineralizable C and SMBC in the subsurface were 3 times lower than those in the surface and not significantly changed through the growing season. Temporal change in PMC and SMBC in the surface had a similar pattern with that in soil CO_2 flux. Potentially mineralizable C and SMBC increased to increasing soil temperature and significantly decreased when soil water content was 17 cm cm^{-3} in September. This lower microbial activity could be associated with lower soil CO_2 flux in September. Manure induced SMBC and PMC were estimated by subtracting PMC and SMBC in the control from those in the manure treatment. Manure induced SMBC and PMC were 47% and 61% of the total SMBC and PMC, respectively, at the surface (0 to 10 cm depth) of the manure treatment. Manure application affected C mineralization and soil microbial activity at the surface but did not affect them at the subsurface during this study period.

Significant differences for soil carbon fractions among treatments in the surface 0 to 10 cm were found, but there were no significant differences in the subsurface (10 to 20, 20 to 30 cm) layers tested. Particulate organic C was not significantly different among different chemical fertilizer treatments but was significantly higher in the manure treatment at the depth of 0 to 10 cm. The average POC across the control and chemical fertilizer treatments was 3.80 g kg^{-1}, and the mean POC of the manure treatments was 6.77 g kg^{-1} in the surface 0 to 10 cm. There were no differences in POC among treatments in the subsurface. Particulate organic C significantly decreased with increasing the depth and the POC averaged across treatments was 1.13 g kg^{-1} at the 10 to 20 cm depth and 1.15 g kg^{-1} at the 20 to 30 cm depth.

Labile C (LC), which is the C fraction oxidized with 0.02 *M* $KMnO_4$, showed a different trend from other C fractions. There were no differences of LC among treatments and depths. Labile C averaged across treatments and depths was 400 mg kg^{-1}. The lack of differences in LC with depth across treatments suggests that LC is a stable soil C fraction and not changed as fast as other C fractions (PMC, SMBC, and POC) by management practices.

Potentially mineralizable C (PMC) had the strongest correlation with SMBC and POC among the various C and N fractions (Table 2). Potentially mineralizable C was highly correlated with SMBC ($r = 0.91$; $P < 0.001$) and POC ($r = 0.84$; $P < 0.001$). Soil microbial biomass C (SMBC) had a strong relationship with POC ($r = 0.90$; $P < 0.001$). The relationship between SOC and PMC was not as strong as that between PMC and POC. However, SOC had a relatively high correlation with SMBC ($r = 0.73$; $P < 0.001$). Labile C was significantly correlated to MOC ($r = 0.59$; $P < 0.01$). Mineral associated organic C are relatively stable[32)] when compared to active C pools like PMC and SMBC. This result was in agreement with results of the long-term study of Blair et al.[33)]. Labile C and MOC were not significantly associated with C mineralization and soil microbial activity.

Particulate organic C is the most readily decomposable fraction of soil organic carbon. Particulate organic C is intermediate in the decomposition continuum between fresh plant litter or residue and humified, stable organic matter[21)]. Soil microbial biomass C increased with the high levels of POC and soluble C from the manure application. The high SMBC concurrently caused a rapid increase in PMC and CO_2 evolution in the

Table 2. Correlation matrix of chemical and biological properties$^\$$ (n = 60) in switchgrass land

	SOC	TN	PMC	SMBC	LC	POC	MOC
SOC	-	***	***	***	**	***	***
TN	0.986	-	***	***	*	***	***
PMC	0.598	0.537	-	***	NS	***	*
SMBC	0.728	0.691	0.905	-	NS	***	**
LC	0.341	0.284	0.246	0.343	-	NS	**
POC	0.737	0.700	0.840	0.900	0.331	-	**
MOC	0.915	0.918	0.310	0.450	0.592	0.402	-

*, **, and *** Significant at $P \le 0.1$, 0.01, and 0.001, respectively. NS = not significant

$^\$$SOC = Soil organic carbon, TN = Total nitrogen, PMC = Potentially mineralizable carbon, SMBC = Soil microbial biomass carbon, LC = Labile carbon, POC = Particulate organic carbon, and MOC = Mineral associated organic carbon.

early part of the growing season (May-June). However, PMC and SMBC did not contribute significantly in explaining the variability of soil CO_2 flux in the later part of growing season.

CONCLUSIONS

The changes of soil organic matter and C mineralization for the management practices studied in switchgrass land were characterized. Chemical N and P fertilization had no impact on temporal changes of soil CO_2 flux and organic C whereas manure application had a significant impact on soil CO_2 evolution and active C pools such as PMC, SMBC, and POC. Manure application caused a rapid increase in soil microbial biomass that coincided with an increase in C mineralization and soil CO_2 flux.

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