Contribution of soil organic carbon and C_3 sugar to the total CO_2 efflux using ^{13}C abundance

R. Koçyiğit

Agricultural Faculty, Gaziosmanpaşa University, Tokat, Turkey

ABSTRACT

The differences in C isotope ratio of C_3 and C_4 plant species have been used to determine relative contributions of carbon (C) sources to total CO_2 efflux. The objective of this study was to estimate the contribution of soil organic C and C_3 sugar to total CO_2 of corn and wheat monocultures during a short-term incubation. Control soils and soils amended with sugar were incubated at 25°C for 48 hours and total CO_2 concentration and $\delta^{13}C$ values of evolved CO_2 were measured. The proportional contribution of C sources on CO_2 efflux was determined by using isotopic composition of soil organic C and C_3 sugar. $\delta^{13}C$ values of soils are highly affected by the type of vegetation and the soil management. The C_3 sugar addition in soils double the CO_2 efflux in the corn soil, but it did not affect CO_2 efflux in the wheat soil. This indicated a larger turnover of microbial biomass in the corn soil. The greatest significant ($P < 0.05$) difference in $\delta^{13}C$ values between the control and sugar added soils occurred at 12 hours in the corn soil (11.2‰) and at 24 hours in the wheat soil (9.4‰). The estimated relative contribution of sugar to CO_2 efflux was stronger at 12 hours incubation in the corn soil.

Keywords: ^{13}C natural abundance; soil organic carbon; sugar; CO_2 source

Agricultural practices reduce soil organic matter and contribute to an increase of atmospheric CO_2 concentration (Houghton et al. 1983). The tracer studies including both radioactive (^{14}C) and stable (^{13}C and ^{12}C) isotopes are usable for a study of atmospheric CO_2. These studies enable to describe an assimilation of C into plants and a translocation of C into soils through a decomposition of plant materials. Many studies using ^{13}C natural abundance were conducted to measure vegetation shifts (Galimov 1985, Collins et al. 1999), soil respiration (Qian and Doran 1996, Gregorich et al. 1996, Rochette et al. 2000, Bol et al. 2003b), soil organic C turnover (Balesdent et al. 1988, Collins et al. 2000), and microbial biomass (Ryan and Arevena 1994, Gregorich et al. 2000, Bol et al. 2003a).

The differences in the ratios of stable carbon isotopes ^{13}C and ^{12}C are expressed as $\delta^{13}C$. The distinguished difference in the carbon isotopes of the C_3 and C_4 plant species has been used to determine relative proportions of C_3 and C_4 plants to soil organic matter and soil respirations. The soils developed under C_3 or C_4 plant species will have the characteristic composition of soil organic carbon. Therefore, the natural tracer of organic C has been suggested to be a useful technique to study the fate of new and old organic C, microbial activity, and turnover in soils (Balesdent and Balabane 1992, Flach et al. 1997, Paustian et al. 1997).

Biological transformations of C in soils are controlled by microbial availability of soil organic C. Carbon availability in soils determines the growth of soil microbial biomass and C turnover (Mary et al. 1992). A better estimation of C availability in plant residues requires a simultaneous study of C dynamics, particularly with the radioactive ^{14}C and stable ^{13}C. In nature, approximately 98.89% of all carbon is ^{12}C, and 1.11% of all carbon is ^{13}C (Bautton 1991). The principle of ^{13}C natural tracer method is based on the differences in ^{13}C to ^{12}C ratio. The ratio of these stable isotopes in organic materials varies as a result of isotopic fractionation during physical, chemical, and biological processes. The $\delta^{13}C$ composition of C_3 plants is close to –27‰ whereas the $\delta^{13}C$ composition of C_4 plants is close to –12‰. The isotopic composition of soil organic C reflects the plant materials which it is derived from. Therefore, the $\delta^{13}C$ of soil organic matter is related to the proportion of C_3 and C_4 derived carbons. The measurement of CO_2 evolution from soils was used to determine the effects of management systems on the decomposition of soil organic C pools (Collins et al. 1992, Motavalli et al. 1994, Collins et al. 2000) and microbial biomass turnover (Ryan and Aravena 1994). Soil microbes have a little discrimination against ^{13}C during decomposition and humification of organic matter. However, they prefer to use readily available C as an energy source.

The objective of this study was to estimate the contribution of soil organic C and C_3 sugar to total CO_2 efflux of corn and wheat soils during a short-term incubation.

MATERIAL AND METHODS

Soil sampling

The soil samples were taken from two different soil managements, continuous corn and wheat sites, in order to measure the changes in carbon isotope ratios of evolved CO_2 based upon crop type and sugar addition (C_3). The soil taken from the North Agronomy Farm, Manhattan, KS had been under dry land continuous corn (C_4) production for more than 10 years. The other soil had been under continuous winter wheat (*Triticum aestivum* L.) production for more than 20 years. The previous use of the wheat land was native grassland dominated by C_4 vegetation. The physical and chemical properties of both soils are presented in Table 1. The soil samples were taken from 0 to 150 mm depth (four replicates) one year after the harvest of wheat and corn and they were stored at 5°C until analyses. The soil samples were passed through a 4-mm sieve and analyzed for soil water contents.

Table 1. Some physical and chemical properties of wheat and corn soils at 0 to 150 mm depth

Soil properties	Wheat	Corn
pH	5.5	6.5
Clay (g/kg)	278	220
Bulk density (g/cm^3)	1.15	1.42
Organic carbon (g/kg)	11	17
C:N	10.3	10.5
$\delta^{13}C$ (‰)	–16	–19.3

Incubation experiment and analyses

Soil C and N were determined using air-dried sub-samples. Plant materials were removed from the sub-samples and the soil was ground to a fine powder with a mortar and pestle. Samples were then analyzed by direct combustion using a Carlo Erba Elemental Analyzer, Model 1500 CNS Analyzer (Carlo Erba Strumentazione, Milan, Italy). Since the pH was < 7 it was assumed that the carbonates were insignificant and the measured C was organic C.

The carbon isotope composition of the soils and sugar [sugar beet (C_3)] were measured before the experiment. $\delta^{13}C$ values of soil samples and sugar were analyzed by solid combustion technique mass spectrometer (ANCA, Europe Scientific, Crewe, UK).

The soil samples (10 g) were placed in a serum bottle (160 ml) and mixed with 50 mg C_3 sugar ($\delta^{13}C$ = –26.0‰) and 0.17 mg NH_4NO_3 as four replicates and before the serum bottles were sealed with rubber septa all the samples were brought to 55% of water holding capacity (Murayama 1988). Moreover, the same amount of soil samples was prepared as a control with the same treatment except the sugar addition. All the samples were incubated at 35°C and gas samples were collected from the headspace using a syringe after 3, 12, 24, and 48 hours of incubation in order to determine CO_2 evolved from the soil and $\delta^{13}C$ values of the CO_2. In this study, the incubation time was kept so short because of the availability of sugar to soil microorganisms. The CO_2 concentration was measured on a Shimadzu Gas Chromatograph-8A (Shimadzu Inc., Japan). The gas chromatograph was equipped with a thermal conductivity detector (TCD) and 2-m Porapak column.

At each incubation time, $\delta^{13}C$ of CO_2 were determined using a dual-inlet gas isotope ratio mass spectrometer (ANCA, Europe Scientific, Crewe, UK). The ^{13}C value is expressed with parts per thousand (‰) relative to a PeeDee belemnite (PDB) reference standard. Isotope ratios are expressed as $\delta^{13}C$ values:

$$\delta^{13}C(‰) = (R_{sample}/R_{reference} - 1) \times 1000 \quad (1)$$

where: $R = {}^{13}C/{}^{12}C$

To calculate the proportion of organic C derived from C_3 sugar and soil organic matter: proportion (f) of derived organic C ($\delta^{13}C$) from the mixture of sugar (S) and soil organic matter (OM) is equal to δ, the formula can be applied:

$$\delta = (1 - f)\delta_S + f\delta_{OM} \quad (2)$$

where: δ_S and δ_{OM} are the $\delta^{13}C$ of the two sources S and OM

Respective amounts of carbon respired from two sources are calculated as $C_S = (1 - f)C$ and $C_{OM} = fC$. In this equation, C is the total amount of CO_2-C evolved from soil.

Data analysis

The experiments were conducted with four replicates. The statistical differences between the treatments through incubation time were examined using the analysis of variance (ANOVA). The significance level was at $P = 0.05$.

RESULTS AND DISCUSSION

The initial isotopic ratio of soil organic matter and C_3 sugar (sugar beet) is presented in Table 1. $\delta^{13}C$ values of soils ranged from –16.0 to –19.3‰. These observed $\delta^{13}C$ values are typical for topsoil developed on C_4 plant species. After 20 years of continuous dry land wheat production, the old organic material from previous native grassland (C_4) still controls carbon isotope ratio of the soil. It indicates that the old organic material had a greater impact on $\delta^{13}C$ values of soils than the recently added plant materials. It was reported that 84 years of wheat cultivation resulted in a slight decrease of ^{13}C content of –19.3‰ compared to –16.1‰ for an adjacent native vegetation. According to the depletion of ^{13}C in soil CO_2 efflux, 30% of soil organic C derived from wheat residues and remaining 70% was from soil organic C from native vegetation. Similarly, after 10 years of continuous corn production, the $\delta^{13}C$ value of soil is –19.3‰, which indicates mixed (C_3 and C_4) soil organic matter. Soil under continuous C_4 corn production for 25 years on an original C_3 forested site (–28.2‰) in Ontario Canada showed 30% of soil organic C in the depth of 0 to 300 mm coming from corn (Gregorich et al. 1995).

There was an average decrease 2.9‰ and 4.8‰ in the $\delta^{13}C$ values of emitted CO_2 compared to the $\delta^{13}C$ values of the original soils during 48 hours incubation (Figure 1). This may be associated with the initial rewetting of soils that flushed C from decomposition of dead microbial cells (Ryan and Aravena 1994) and disturbance of soil during preparation. Collins et al. (2000) pointed out that $\delta^{13}C$ of CO_2 derived from soil with C_3 and C_4 soil organic matter did not equal to that of all soils until after 1000 days of incubation. Based on the assumption, as regards the decomposition of C_3 and C_4, C-sources are similar in soils (Balesdent and Mariotti 1996). However, the $\delta^{13}C$ values of CO_2 in the control soils reflect ^{13}C enrichment of the soils which were depleted in the CO_2 efflux.

The sugar added corn soil had a significantly ($P < 0.05$) lower $\delta^{13}C$ value of CO_2 compared to the wheat soil (Figure 1). The lowest $\delta^{13}C$ value of CO_2 in the corn field may be the effect of old organic C on C isotope ratio of CO_2 due to a disturbance effect and rewetting of soil to bring 55% of water holding capacity or slight discrimination of heavier isotope ^{13}C by soil microorganisms. The initial $\delta^{13}C$ values of the incubated soil samples showed lower $\delta^{13}C$ values in the sugar added soils compared to the control samples (Figure 1). The response of soil microorganism to C_3 sugar addition was greater in the corn soil. In contrast, the response was lower in the wheat soil, which was

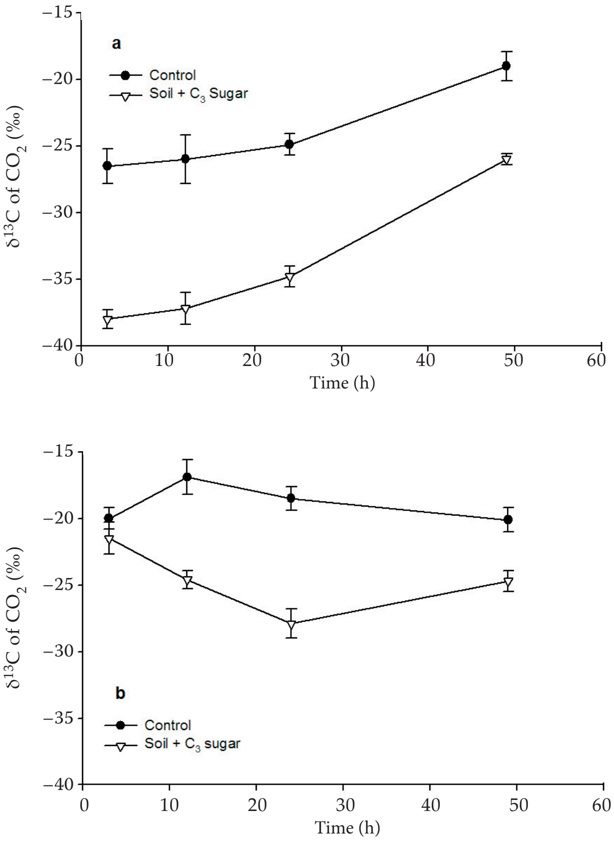

Figure 1. The $\delta^{13}C$ values of soil CO_2 during incubation of corn (a) and wheat (b) soils; the bars represent standard error

Table 2. Total CO_2-C (μg CO_2-C/g soil) and C_3 sugar derived C as estimated ^{13}C measurements; standard errors of the means are given in parenthesis

Time (hour)	Corn soil			Wheat soil		
	control	C_3 sugar	sugar derived C (% fraction C)	control	C_3 sugar	sugar derived C (% fraction C)
3	9.42	12.95*	–	14.98	15.20	55
	(2.21)	(2.72)		(2.35)	(2.42)	(8.7)
12	18.72	25.87*	–	30.02	30.45	86
	(3.24)	(4.01)		(3.89)	(4.52)	(12.7)
24	28.28	38.87*	–	44.98	45.64	81
	(3.47)	(4.43)		(5.26)	(4.76)	(8.4)
49	39.45	53.39*	–	61.37	62.24	87
	(4.32)	(5.47)		(5.88)	(5.97)	(8.3)

*indicates significant difference between two columns at $P = 0.05$ level

closer to the control soil. The most significant ($P < 0.05$) differences between the control and sugar added soils (11.2‰) occurred at 12 hours after sugar addition in the corn soil and at 24 hours after sugar addition in the wheat soil (9.4‰). Higher $\delta^{13}C$ values of CO_2 efflux and substrate availability in the wheat soil could be a consequence of conversion of native grassland to an agricultural field about 20 years ago. In addition, this result indicates that older organic materials can have a greater influence on CO_2 efflux and microbial activity. The $\delta^{13}C$ value of CO_2 in the corn soil showed a generally increasing trend during incubation and the sugar added soil after 48 hours incubation was –26.0‰, which is lower than the $\delta^{13}C$ values of the original soil. The lower values observed during incubation of the sugar added soils explain the presence of sugar after 49 hours incubation. There were emphasized the presence of sugar in a soil up to 4 days of incubation of sugar added soils.

The total CO_2-C production was significantly higher ($P < 0.05$) with sugar addition and the differences between the sugar added soils and control soils were greater in the corn soil as the incubation time increased, while the sugar addition did not affect the cumulative CO_2-C in the wheat soil (Table 2). The significant difference ($P < 0.05$) in the corn soil indicates a greater microbial turnover and substrate limitation compared to the wheat soil. The microbial respiration decreased with incubation time in both soils (Figure 2). Microbial respiration was greater at the beginning of incubation due to the disturbance effect, readily available substrate addition and rewetting of soils. Until the end of the incubation, microbial respiration stayed almost constant.

The amount of soil organic matter derived C was calculated according to the equation (2), which is the same way as in the previous study (Balesdent and Balabane 1992). The estimated soil organic

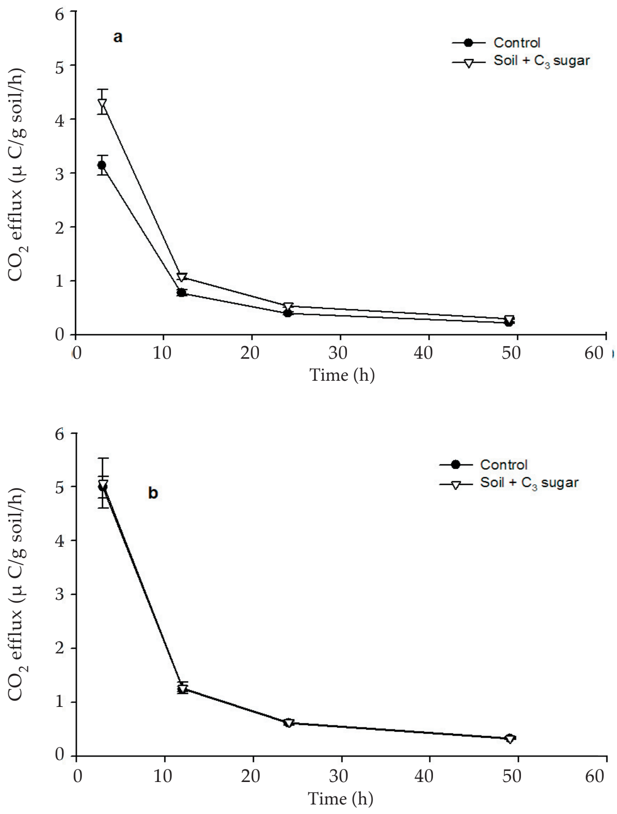

Figure 2. Soil respiration during incubation of corn (a) and wheat (b) soils; the bars represent standard error

matter derived CO_2-C in the wheat soil accounted for 45% of the total CO_2-C over C_3 sugar at 3 hours incubation (Table 2). The contribution of soil organic C to the total CO_2-C decreased through incubation and after 48 hours incubation, the estimated soil organic matter derived C accounted only for 13% of the total CO_2-C, which can be attributed to the microbial use of readily available soil organic C at the beginning of incubation. The estimated contribution of applied sugar to total CO_2 efflux without slurry added soils was the greatest after 17 hours of incubation (95%), and then significantly decreased and finished after 4 days. However, in this study the extensions of incubation time increased the proportional contribution of C_3 sugar to total CO_2 production and after 12 hours stayed almost constant (~ 85%) in the wheat soil. The proportional contribution of C sources to total CO_2 production could not been estimated in the corn soil because of lower $\delta^{13}C$ values in the sugar added samples than the $\delta^{13}C$ value of sugar and marginally lower contribution of soil organic C to the total CO_2 flux during the short-term incubation time. Furthermore, the isotopic ratio of the corn soil showed a mix of organic C (C_3 and C_4). It has been presented that at the beginning of incubation 50 to 70% of the CO_2 evolved from soils with no slurry added, previously frosted (C_3) soil was released from new organic matter (C_4), while the rest of it from old organic matter (Collins et al. 1999).

$\delta^{13}C$ values of soils are highly affected by the type of vegetation and soil management. After 10 years of continuous corn and more than 20 years of wheat cultivation, previous plant materials are still mineralized in soils and affected $\delta^{13}C$ values in both soils. The C_3 sugar addition to soils doubled the CO_2 efflux in the corn soil, but it did not change CO_2 efflux in the wheat soil. Hence, this indicates a greater turnover of microbial biomass in the corn soil. The estimated relative contribution of sugar to CO_2 efflux was higher during a short-term incubation. Generally, soil microbes responded to a readily available C source in a short period of time. Depending on the history of soil managements, the microorganisms at the same time mineralized a small portion of soil organic matter.

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Corresponding author:

Dr. Rasim Koçyiğit, Gaziosmanpaşa University, Agricultural Faculty, Department of Soil Science, Tokat, Turkey
phone: + 90 356 252 1616, e-mail: rkocyigit@gop.edu.tr