doi: 10.17221/216/2016-PSE

CO_2 efflux and microbial activities in undisturbed soil columns in different nitrogen management

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ABSTRACT

The surface carbon dioxide (CO_2) fluxes together with the soil microbial biomass and activity in undisturbed soil columns were studied in three growing seasons. Soil columns had six treatments: (1) control without plants; (2) mineral fertilized without plants; (3) no fertilizer and maize plants; (4) mineral fertilized and maize plants; (5) manure and maize plants; (6) mineral fertilized plus manure and maize plants. Soil microbial biomass was measured by substrate-induced respiration (SIR) and microbial activity as fluorescein-diacetate hydrolysing activity (FDA). Treatments had a significant effect ($P < 0.001$) on CO_2 fluxes, SIR and FDA. The presence of maize increased CO_2 efflux, SIR and FDA compared to unplanted column. Fertilizer + manure treatment resulted in the greatest plant biomass and the greatest CO_2 efflux. Significant correlation ($r = 0.680$; $r = 0.586$ in two consecutive years) between SIR and FDA was found.

Keywords: farmyard manure; root respiration; soil carbon budget; soil respiration; soil temperature

The soil carbon pool dynamics in cropland that occupies 12 percent of the land surface depends on the input and output of organic matter and is influenced by climate and human activities and the interaction of crop, climate and soil (Vleeshouwers and Verhagen 2002). Tillage and fertilization both have substantial effect on soil carbon dynamics (Schlesinger and Andrews 2000). In recent papers, controversial results have been reported on soil respiration after addition of nitrogen to the soil. The soil respiration increased after nitrogen addition was more related to the increased root respiration and increased microbial respiration by enhanced root-derived organic carbon (C) input came rather from the higher photosynthetic rates than the increased decomposition of soil organic matter (Liljeroth et al. 1990). Organic input alone as manure or in combination with chemical fertilizers resulted in higher soil respiration, microbial biomass and also soil organic carbon (SOC) accumulation compared with unfertilized or inorganic fertilized soils in long-term (Zhang et al. 2013, Šimon and Czakó 2014). Still, there are few reports on the measurement of soil carbon dioxide (CO_2) emission in parallel with soil microbial activity in various nitrogen (N) fertilized systems.

It was hypothesized that different N additions would affect soil CO_2 efflux that partly relates to soil microbial activity during vegetation season. The objective was to compare soil CO_2 efflux and soil microbial activities in unplanted and maize-planted columns treated with mineral or organic fertilizers or both during three growing seasons. Further, the aim of the study was to determine if there is correlation between CO_2 efflux and soil temperature and moisture and whether soil CO_2 efflux is correlated with soil microbial activity and biomass

Supported by the National Scientific Research Fund of Hungary, Grants No. OTKA-Consortium K 72926; K 73326; and K 73768.

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characterized by the fluorescein-diacetate hydrolysing activity and substrate-induced respiration.

MATERIAL AND METHODS

Soil column preparation and treatments. This study was based on the long-term organic-mineral fertilizer experiment field of the University of Pannonia at Keszthely, Hungary (46°40'N; 17°15'E). The soil type was Eutric Cambisol (FAO), the soil organic C content was 1.7%, the pH_{KCl} was 5.4, and texture was sandy loam (Hoffmann et al. 2008).

Undisturbed soil columns (0.9 m height, 0.4 m diameter) were prepared in the spring of 2008 according to Németh et al. (1991); briefly, soil columns were digged around carefully in a desired diameter and depth, then they were curled round by multilayer fiberglass and resin to consolidate, and finally translocated to Őrbottyán Research Station. Treatments were: unplanted and unfertilized soil (SC); unplanted and NPK fertilized soil (SF); maize (*Zea mays* L. Mv Norma) planted and unfertilized soil (PC); planted and NPK fertilized (PF); and planted soil with farmyard manure (PM) and farmyard manure plus NPK (PFM). Each treatment had three replicates. The mineral nitrogen fertilizer was added in spring at the rate of 109 kg N/ha each year while farmyard manure was added at the rate of 52 t/ha (corresponding to 127 g organic C/column) in October of 2008 and 2010. Maize was seeded, four in each column, except SC and SF, at the end of April, but only one plant was left to grow up to maturity. The columns were irrigated (207 mm in 2011 and 175 mm in 2012) from July to September.

Surface CO_2 efflux. A static closed chamber technique was used to study soil surface CO_2 efflux. The chambers made from a PVC tube and inserted into the soil with capped head and with two sampling ports at the middle height on the tube. Gas samples were taken by a gas-tight syringe through the sampling ports at 0 min and 30 min after closure and injected into evacuated tubes. Soil air was sampled at 9:00 a.m., which was proposed to best fit to the daily rate (Ding et al. 2006).

Soil sampling and analyses. Soil samples were taken from the columns during the growing seasons 6 times (7, 14, 23, 51, 86, and 128 days after sowing) in 2010 and 7 times (20, 29, 41, 48, 99, 120, and 162 days after sowing) in 2011 from the 0–20 cm layer for chemical analyses and SIR and FDA measurements. Soil moisture was analysed gravimetrically. Other measurements were made at the beginning and at the end of the experiment, soil pH_{H_2O} and pH_{KCl} were analysed from 1:2.5 soil:solution extract by pH-meter with glass electrode.

Substrate-induced respiration. Substrate-induced respiration (Anderson and Domsch 1978) was measured as modified by Szili-Kovács et al. (2011). 2.0–2.0 g of moist soil samples were weighed into 25 cm^3 vials. 200 µL glucose solutions were added to a sample (8 mg glucose/g soil) and the evolved CO_2 was measured after 3 h at 22°C.

CO_2 measurement. 250 µL gas sample was injected into a gas chromatograph (FISONS GC8000, Milano, Italy) equipped with Porapak Q column and methanizer chamber and the CO_2 was detected with flame ionization detector after methane conversion.

FDA hydrolysing activity. FDA hydrolysing activity was determined according to Schnürer and Rosswall (1982) and Adam and Duncan (2001) with a slight modification: a 1.5-h shaking was applied with glass beads to disrupt aggregates before substrate addition, and the substrate concentration was increased ten-fold.

Meteorological data (solar radiation, daily min., max. and average air temperature, soil temperature at –5 cm, precipitation, and soil moisture) were also collected at the site of Őrbottyán station.

Calculation of the carbon budget. Soil organic C was measured in 2008 at the beginning and in 2012 at the end of the experiment. SOC input from manure was 126.9 g into PM and PFM columns in 2008 and also in 2010. The amount of belowground C deposition was estimated as 29% of shoot biomass C as an average for maize at physiological maturity proposed by Amos and Walters (2006). Cumulative soil CO_2-C efflux during the growing season was calculated by summing the products of the averaged two neighbouring fluxes, multiplied by their interval time (Gong et al. 2012).

Statistical analysis. Statistical analysis was carried out with the statistical package SPSS v 9.0 (SPSS Inc, Chicago, USA), ANOVA and Tukey's *HSD* posthoc test, Spearman's rank correlation between variables was calculated.

RESULTS AND DISCUSSION

Effect of N treatments on soil CO_2 efflux. Surface CO_2 efflux was significant both in sampling

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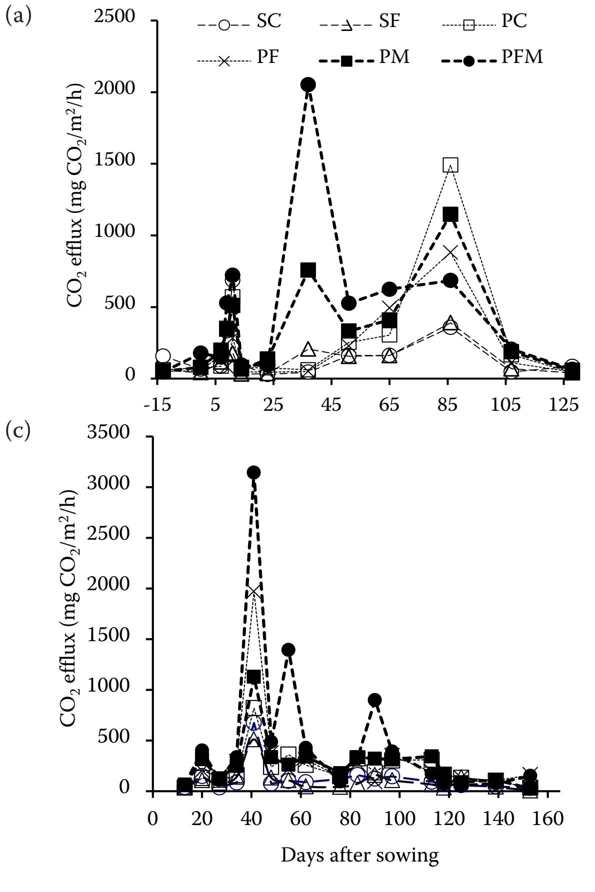
time and treatments in all three years according to ANOVA ($P < 0.001$). Considering the treatments, the daily averaged CO_2 efflux during the growing seasons was significantly higher in SC (0.85, 0.48, and 0.64 g C/m^2/day) than in SF (0.75, 0.37, and 0.55 g C/m^2/day) in 2010, 2011 and 2012 years (Figure 1 and Table 1). In a similar study Ni et al. (2012) suggested that the stimulatory or inhibitory effect of N fertilization on soil respiration may depend on labile organic C concentration in soil. They found also decreased CO_2 evolution in plantless N fertilized soil, while there was no significant change in maize-planted N fertilized soil because the increased rhizosphere respiration was balanced by the reduced native soil organic C decomposition. In contrary, a significant increase in soil respiration was observed in plantless and N fertilized soil compared to the unfertilized soil (Ding et al. 2010), however in the presence of maize they measured lower cumulative CO_2 emission in N fertilized treatment than without N fertilization. The dose dependent effect of N fertilization on soil respiration showed that 150 kg/ha/year increased while 250 kg/ha/year suppressed soil respiration in maize planted soil (Song et al. 2009). Planted columns always resulted in higher respiration than the unplanted ones. The order of CO_2 efflux was PF < PC < PM < PFM in 2010 whereas PC < PM < PF < PFM recorded in 2011 and 2012 year in our experiment. Manure use resulted not only in the increased soil respiration but efficiently elevated soil organic material, especially the fraction linked to clay minerals in a long-term mineral and organic fertilization experiment (Ding et al. 2007). The highest CO_2 efflux data (2.23–3.66 g C/m^2/day averaged from Table 1) were recorded in columns with manure plus NPK additions (FPM), whereas it was only 1.52–1.93 g C/m^2/day in case of PM treatments suggesting that NPK accelerated not only the root respiration but also the decomposition of organic C in manure.

According to the time, the CO_2 efflux was lower at the beginning and end of the growing season and was the highest in June and July (Figure 1). At least three high peaks of surface CO_2 emission were recorded during the growing season (Figure 1), similarly to the studies of Ding et al. (2010) and Gong et al. (2012), partly in pollination stage of maize or periods when soil moisture after precipitation coincides with high temperature optimal for microbial activity.

Correlation between surface CO_2 efflux and temperature and moisture. Surface CO_2 efflux



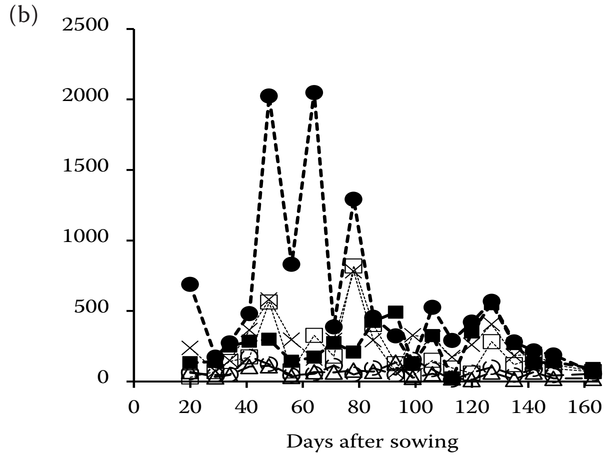


Figure 1. Mean soil surface CO_2 efflux in undisturbed soil columns after maize sown in (a) 2010; (b) 2011 and (c) 2012. SC – without plants; SF – without plants + NPK; PC – maize without fertilization; PF – maize + NPK; PM – maize + manure; PFM – maize + NPK + manure

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Table 1. Carbon budget calculation of the soil columns in 2009–2012 periods, means ± standard error (g C/m^2)

Treatment	2009		2010		2011			2012	
	input Rd.	input FYM	input Rd.	output CO_2 efflux (141 days)	input Rd.	input FYM	output CO_2 efflux (163 days)	input Rd.	output CO_2 efflux (155 days)
SC	0	0	0	121 ± 14	0	0	78.8 ± 3	0	99.9 ± 3
SF	0	0	0	106 ± 12	0	0	59.6 ± 4	0	85 ± 2
PC	101 ± 7	0	81 ± 18	237 ± 10	75 ± 13	0	198 ± 4	81 ± 16	190 ± 8
PF	248 ± 34	0	204 ± 42	200 ± 15	237 ± 19	0	269 ± 5	220 ± 23	231 ± 2
PM	213 ± 55	1010	121 ± 25	273 ± 6	170 ± 21	1010	249 ± 2	162 ± 33	235 ± 8
PFM	273 ± 45	1010	238 ± 28	348 ± 13	293 ± 25	1010	596 ± 4	269 ± 29	346 ± 9

Rd. – roots + rhizodepositions (calculated from shoot biomass); manure treatment was applied in the previous autumn (2008 and 2010) but its effect was accounted in the following year. FYM – farmyard manure; CO_2 efflux was not measured during 2009. SC – without plants; SF – without plants + NPK; PC – maize without fertilization; PF – maize + NPK; PM – maize + manure; PFM – maize + NPK + manure

was significantly correlated with soil temperature at –5 cm depth in all years (r = 0.624 in 2010, r = 0.222 in 2011 and r = 0.414 in 2012). There was no significant correlation between gravimetric water content and CO_2 efflux. Soil moisture is an important factor beside the soil temperature; however, similarly to our results, it does not always have a significant effect on soil respiration (Tortorella and Gelsomino 2011, Ni et al. 2012). If it is significant, it is less strongly influencing factor than temperature (Tóth et al. 2009, Ding et al. 2010, Lellei-Kovács et al. 2011).

Substrate-induced respiration. SIR was significant both according to sampling time and treatments in 2010 and 2011 as shown by ANOVA (P < 0.001). SIR was lower at the beginning and at the end of the season and higher in June and July (Figure 2). Considering the treatments, the following order in SIR was recorded: SF < SC < PC, PF < PM < PFM, however SC was not significantly different from PC and PF in 2010.

Comparing the planted treatments (PC and PF) their SIR values changed in time when the SIR was higher at PF than PC at the beginning of the season, but later it was shifted for the benefit of PC treatment from the middle to the end of the season.

FDA hydrolysing activity. FDA hydrolysing activity was significant both according to sampling time and treatments in 2010 and 2011 year as shown by ANOVA (P < 0.001). FDA was lower at the beginning and at the end of the season and was higher in June and July in 2010, while the FDA was enhanced almost continuously during the

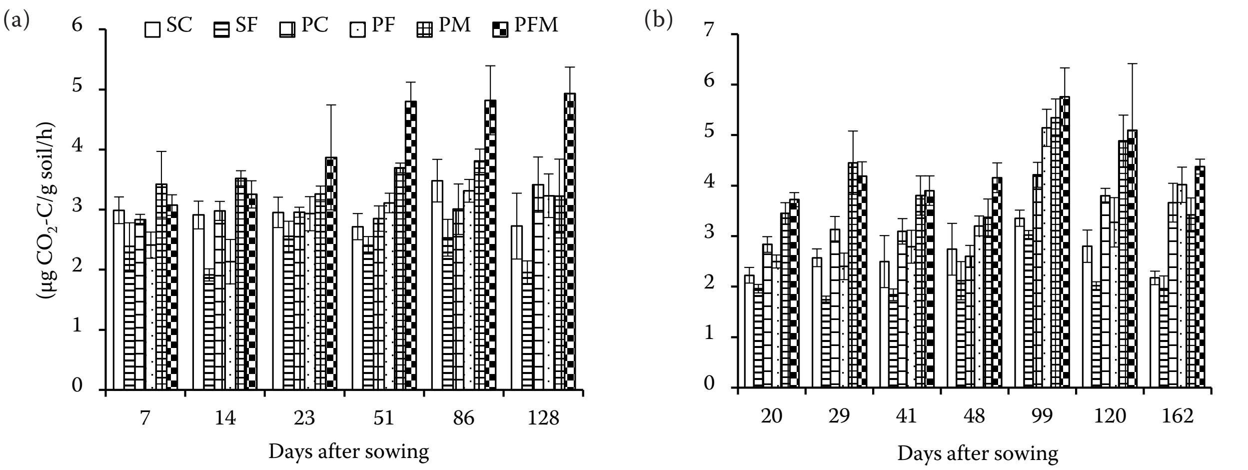

Figure 2. Mean substrate-induced respiration in soil columns after maize sown in (a) 2010 and (b) 2011. SC – without plants; SF – without plants + NPK; PC – maize without fertilization; PF – maize + NPK; PM – maize + manure; PFM – maize + NPK + manure

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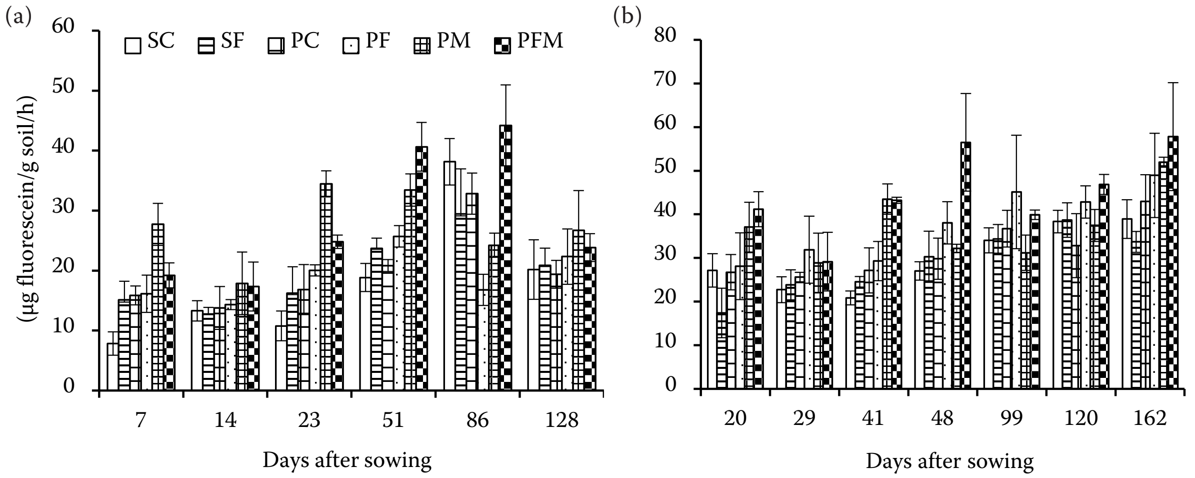

Figure 3. Mean fluorescein-diacetate hydrolysing activity in soil columns after maize sown in (a) 2010 and (b) 2011. SC – without plants; SF – without plants + NPK; PC – maize without fertilization; PF – maize + NPK; PM – maize + manure; PFM – maize + NPK + manure

growing season reaching the highest value at the last sampling on 28 September 2011 (Figure 3). Considering the treatments the following order in FDA was recorded in 2010: SC, SF, PC, PF < PM < PFM. The main difference between the two consecutive years in FDA results was that it was significantly higher at PF than at PC but not different between PF and PM in 2011.

Correlation between surface CO_2 efflux and SIR and FDA. The Spearman's correlation coefficient between FDA hydrolysing activity and SIR was r = 0.680 (P < 0.001) and r = 0.586 (P < 0.001) in 2010 and 2011, respectively. FDA hydrolysing activity was correlated with soil microbial respiration (Schnürer and Rosswall 1982, Sánchez-Monedero et al. 2008, Piotrowska and Długosz 2012), with soil microbial biomass C (Perucci 1992, Sánchez-Monedero et al. 2008) and with substrate-induced respiration (Szili-Kovács et al. 2011). The correlation between SIR and surface CO_2 efflux was significant in 2010 (r = 0.397, P = 0.033) but not in 2011. The correlation between FDA and CO_2 efflux was significant both in 2010 (r = 0.492; P = 0.006) and 2011 (r = 0.620; P < 0.001). In central Korea, at three sites that had been changed from abandoned agricultural lands to natural vegetation, soil respiration was only very weakly correlated with FDA hydrolysis at all sites (Son et al. 2006).

Calculation of the carbon budget. Soil organic C was significantly increased at manure treated columns (PM and PFM), but no significant change could be established at other treatments (data not shown). Carbon input to the soil calculated form the added manure (PM and PFM treatments) and the root deposition of maize which was estimated from the harvested shoot biomass (Table 1). The plant biomass was significantly differed in the order of PC < PM < PF < PFM which was in accordance with the order of CO_2 efflux. The results suggest that mineral fertilizer accelerated not only the root respiration but the manure decomposition rate as well. The soil carbon balance was negative at SF, SC, and PC treatments, neutral or slightly positive at PF and positive at PM and PFM treatment (Table 2). This estimation, however, has a limited validity, but the comparison among treatments give

Table 2. Estimated carbon balance during a four-year period, means (g C/m^2)

Treatment	C-input	C-input	C-efflux	C-balance
	Rd. 2009–2012	FYM 2009–2012	2010–2012	
SC	0	0	299	negative
SF	0	0	251	negative
PC	337	0	625	negative
PF	910	0	699	slight positive
PM	666	2020	756	positive
PFM	1073	2020	1291	positive

Rd. – roots + rhizodepositions (calculated from shoot biomass); FYM – farmyard manure; CO_2 efflux was not measured during 2009. SC – without plants; SF – without plants + NPK; PC – maize without fertilization; PF – maize + NPK; PM – maize + manure; PFM – maize + NPK + manure

doi: 10.17221/216/2016-PSE

strong evidence about the significance of different N management in maize on soil carbon balance.

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Received on March 15, 2016
Accepted on August 8, 2016

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