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# Effect of injection depth of digestate liquid fraction on soil carbon dioxide emission and maize biomass production

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## Abstract

The aim of this study was to evaluate, in open field conditions, the effect of injection depth of digestate liquid fraction (10 cm, 25 cm and 35 cm) in clay loam soil, on CO<sub>2</sub> emission. An un-amended soil was considered as control. The study was performed in 2014 on a farm located in Terrasa Padovana, Veneto region (Italy) distributing digestate before maize sowing.

Digestate injection determined a high soil CO<sub>2</sub> emission in the first hour after application, followed by a progressive reduction in as early as 24 h, reaching significantly lower values, similar to those measured in the un-amended control, after 48 h. Gas emissions measured 1 h after digestate application decreased as injection depth increased with significantly higher emission values in the 10 cm treatment (median value 23.7 g CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup>) than in the 35 cm one (median value 2.5 g CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup>). In the 3 days between digestate distribution and maize sowing, soil CO<sub>2</sub> emission was significantly higher in the amended treatments than un-amended one, with median values of 1.53 g CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup> and 0.46 g CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup> respectively. During maize growing season, no significant soil CO<sub>2</sub> emission difference was monitored among

treatments, with a median value of 0.33 g CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup>.

Digestate application significantly improved maize aboveground dry biomass with an average yield of 22.0 Mg ha<sup>-1</sup> and 16.2 Mg ha<sup>-1</sup> in amended and un-amended plots, respectively, due to the different amount of nutrients supplied.

## Introduction

Intensive soil fertilisation with mineral fertilisers has led to several issues, like loss of soil carbon (C), and nitrogen (N) leaching (Borin *et al.*, 1997; Nardi *et al.*, 2004; Morari *et al.*, 2006). Fertilisation with organic wastes therefore represents an alternative for sustainable agriculture (Casacchia *et al.*, 2012; Marchetti *et al.*, 2012; Morra *et al.*, 2013; Barbera *et al.*, 2013; Nkoa, 2014). In this context the agricultural reuse of digestate, organic waste product of biogas plants, should be considered. Furthermore, the sustainability of biogas production may depend on an appropriate end-use of the downstream effluents of anaerobic digestion, which should be treated, disposed of, or re-used in a proper way, avoiding any environmental impact (de la Fuente *et al.*, 2013). Digested waste materials present some advantages for their use as soil amendments in comparison with untreated wastes, such as greater microbial stability and hygiene and a higher NH<sub>4</sub><sup>+</sup>-N amount (Holm-Nielsen *et al.*, 2009; Alburquerque *et al.*, 2012b; Möller and Müller, 2012). Therefore, digestate can be considered as organic amendment or organic fertiliser when properly handled and managed (Nkoa, 2014). In fact, the application of organic matter to agricultural soils stimulates microbial activity, increasing greenhouse gases emission (Bol *et al.*, 2003; Fanguero *et al.*, 2010), thus requiring the application of appropriate agronomic techniques for greenhouse gases emission mitigation (Pezzolla *et al.*, 2012).

Several laboratory scale studies investigated the effect of soil amendment with digestate on CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O emissions (Cayuela *et al.*, 2010; Grigatti *et al.*, 2011; Sängner *et al.*, 2011; Alburquerque *et al.*, 2012a; de la Fuente *et al.*, 2013; Johansen *et al.*, 2013). A limited number of studies reported results obtained in open field conditions, mainly focusing on CH<sub>4</sub>, N<sub>2</sub>O and NH<sub>3</sub> emissions, comparing the effect of anaerobically digested and undigested slurries or different digestate soil distribution techniques (Rubæk *et al.*, 1996; Petersen, 1999; Wulf *et al.*, 2002; Dieterich *et al.*, 2012). Only a few open field studies investigated soil CO<sub>2</sub> emission after digestate application, spreading it on grassland (Pezzolla *et al.*, 2012) or maize (Bachmann *et al.*, 2014). To our knowledge, no field experiment has been conducted to evaluate soil CO<sub>2</sub> emission after digestate injection at different soil depths.

Given the current knowledge, the aim of this work was to evaluate, in clay loamy soil, the effect of digestate liquid fraction (DLF) injection depth on CO<sub>2</sub> emission and maize biomass production.

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Key words: Soil CO<sub>2</sub> emission; digestate use; soil digestate injection; maize biomass yield.

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## Materials and methods

### Site description and experimental design

The study was performed in 2014 on a farm located in Terrasa Padovana (45°15'N 11°55'E, 1 m a.s.l.), Veneto Region, Italy, on a clay loamy soil (USDA classification) after winter wheat (*Triticum aestivum* L.) aerial biomass harvested at dough stage. The effect of DLF injection depth on soil CO<sub>2</sub> emission was studied through four treatments: no digestate distribution (ND), digestate injection with 1 m width between two injection nozzles at 10 cm depth (10 cm), 25 cm depth (25 cm) and 35 cm depth (35 cm). A randomised block design with three replicates and experimental plots of 500 m<sup>2</sup> was used. DLF, obtained from anaerobic digestion of cattle slurry and manure, maize silage and flour, was distributed in the soil by injection technique on June 3 in a volume to obtain a total nitrogen supply of 170 kg ha<sup>-1</sup>. The main chemical DLF characteristics, determined in three samples before the spreading operation, are reported in Table 1. N fertilisation was integrated adding 50 kg N ha<sup>-1</sup> as urea during mechanical weed control, at fifth leaf phenological stage. The same urea dose was distributed in the un-amended plots to highlight the DLF effect. The DLF distribution added also 32.6 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, 170.1 kg K<sub>2</sub>O ha<sup>-1</sup>.

Distribution was carried out in undisturbed soil (June 3); after 23 h and 45 h, respectively, a cultivation (25 cm depth) and harrowing (power harrow, 20 cm depth) were carried out to prepare the seedbed, and on June 6 maize (*Zea mays* L., Hybrid Pioneer P0837; FAO 400) was sown as second crop after winter wheat at a density of 7.5 seeds m<sup>-2</sup>.

### Soil CO<sub>2</sub> flux measurement

CO<sub>2</sub> flux was measured with the static non-stationary chamber technique (Maucieri *et al.*, 2014) using a chamber with a volume of 5 L and 10 cm square base.

CO<sub>2</sub> emissions were detected in three points of each experimental plot in order to replicate the measures in the space with 9 measures for each studied treatment. After DLF distribution, soil CO<sub>2</sub> emission was measured 3 times before maize sowing (after 1 h in undisturbed soil and after 24 and 48 h, soon after the two tillage interventions), and 9 times after this (from 1 to 104 days) at regular intervals of about 13 days. Soil CO<sub>2</sub> flux was determined by measuring the temporal change in CO<sub>2</sub> concentration inside the chamber using a portable infrared instrument (Geotech G150; Geotechnical Instruments Ltd., Royal Leamington Spa, UK), detecting CO<sub>2</sub> concentrations at levels of parts per million.

**Table 1. Digestate chemical characteristics (mean value ± standard deviation).**

Parameters	Values
Dry matter %	6.4±0.3
C/N ratio	9.2±0.2
TKN (mg kg <sup>-1</sup> FM)	2936.7±8.7
NH <sub>4</sub> -N (mg kg <sup>-1</sup> FM)	28.0±0.9
NO <sub>3</sub> -N (mg kg <sup>-1</sup> FM)	3.0±0.6
P (mg kg <sup>-1</sup> FM)	246.1±9.2
K (mg kg <sup>-1</sup> FM)	2438.9±14.3
Ca (mg kg <sup>-1</sup> FM)	621.0±8.5
Na (mg kg <sup>-1</sup> FM)	268.7±2.2
Mg (mg kg <sup>-1</sup> FM)	235.0±7.5

C/N, carbon/nitrogen ratio; TKN, total Kjeldahl nitrogen; FM, fresh matter; NH<sub>4</sub>-N, ammonium nitrogen; NO<sub>3</sub>-N, nitrate nitrogen; P, phosphorus; K, potassium; Ca, calcium; Na, sodium; Mg, magnesium.

CO<sub>2</sub> flux was calculated using the following formula:

$$CO_2 = \frac{V}{A} \cdot \frac{dc}{dt} \quad (1)$$

where CO<sub>2</sub> flux is expressed in mg CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>; V (m<sup>3</sup>) is the volume and A (m<sup>2</sup>) the footprint of the flux chamber; *c* is the CO<sub>2</sub> concentration (mg CO<sub>2</sub> m<sup>-3</sup>) and *t* the time step (s).

In each CO<sub>2</sub> measurement point, soil temperature and moisture (TDR 100 Soil Moisture Meter; Spectrum Technologies, Aurora, IL, USA) in the first 7.5 cm were also detected.

In maize-grown soil, CO<sub>2</sub> fluxes measured between 9:00 and 12:00 a.m. can represent the mean CO<sub>2</sub> daily emissions (Rochette and Flanagan, 1997; Lou *et al.*, 2003; Ding *et al.*, 2006). In view of this, in our study soil CO<sub>2</sub> emission measures were carried out between these hours during the whole monitoring. Based on soil CO<sub>2</sub>-C fluxes, the mean cumulative soil CO<sub>2</sub>-C emission for each treatment, during both distribution phase and maize growing season, were calculated by summing the products of the average of two neighbouring measurement fluxes by their interval time. To compare cumulative soil CO<sub>2</sub>-C emission with the amount of C supplied to the soil by DLF, the cumulative CO<sub>2</sub>-C emission value monitored in the ND treatment was subtracted from those calculated for each amended treatment.

### Maize biomass measurement

Maize aboveground biomass was harvested on September 26<sup>th</sup> at dough stage. In each experimental plot, fresh biomass production was measured in four points (each 1.5×4 m) for a total 12 replicated production areas per treatment. Areas were randomly selected and maize plants were manually cut at 10 cm from soil. Biomass dry weight was determined by drying plant tissue samples in a thermo-ventilated oven at 65°C until constant weight was reached.

### Statistical analysis

The normality of CO<sub>2</sub> data was checked using the Kolmogorov-Smirnov test; due to the fact that they did not show normal distribution, the Kruskal-Wallis and Mann-Whitney non-parametric tests were used to check the significance of differences. Correlation between soil temperature and moisture with CO<sub>2</sub> emissions were evaluated using Spearman Rank correlation.

Statistical analysis of biomass production and cumulative CO<sub>2</sub>-C emission was conducted by one-way analysis of variance (ANOVA); mean values were compared using Fisher least significant difference test at P<0.05.

## Results and discussion

### Soil CO<sub>2</sub> emissions

The DLF effect on soil CO<sub>2</sub> emission followed the same trend in all three injection depths with a high CO<sub>2</sub> emission in the first hour after application, followed by a rapid significant reduction as early as 24 h, reaching values similar to those measured in the un-amended control after 48 h (Figure 1).

Considering the CO<sub>2</sub> emission trend in the 48 h after injection, our data are in line with studies carried out in laboratory conditions by Sanger *et al.* (2011), who monitored a rapid soil CO<sub>2</sub> production increase after biogas slurry application, and Grigatti *et al.* (2011) who reported, after digestate application, a very intensive CO<sub>2</sub> emission in the first 24 h of soil incubation, followed by a reduction to a value close

to the control. de la Fuente *et al.* (2013), again in a laboratory study, monitored a rapid soil CO<sub>2</sub> emission decrease in the days after liquid digestate application and, after three weeks, CO<sub>2</sub> emission values similar to those measured in the control soil. High CO<sub>2</sub> soil flux in the first hour after distribution was likely due to both the release of CO<sub>2</sub> dissolved in the digestate, and the rapid microorganism respiration of easily degradable C. In fact, as reported by Johansen *et al.* (2013), digested residues from biogas production induced only small and transient changes on the total soil microbial biomass, function and community structure. Focusing on the emissions measured 1 h after DLF injection, CO<sub>2</sub> flux decreased when injection depth increased, with significantly higher emission value in the 10 cm treatment (median value 23.7 g CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup>) and the lowest one in the 35 cm treatment (median value 2.5 g CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup>) (Figure 2).

On the average of the 3 days between DLF distribution and maize sowing, soil CO<sub>2</sub> emission was significantly higher (Mann-Whitney test,  $P < 0.0006$ ) in the three amended treatments than un-amended one with median values of 1.53 g CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup> and 0.46 g CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup> in the two respective cases. Data are in agreement with Pezzolla *et al.* (2012) and Johansen *et al.* (2013), who reported, in an open field and laboratory experiment, respectively, that after digestate application soil CO<sub>2</sub> emission increased. Focusing on treatments, a significantly higher CO<sub>2</sub> emission (Kruskal-Wallis,  $P < 0.05$ ) was detected in the plots where DLF was injected into the soil at a lesser depth (10 cm and 25 cm); no significantly different emission was found between 35 cm and ND treatments (Figure 3).

The soil tillage with cultivator, done 20 h after DLF application in all treatments, did not exert a significant effect on soil CO<sub>2</sub> emission measured 24 h after distribution. Instead, the harrowing (45 h after DLF application) determined a significantly higher CO<sub>2</sub> emission (Kruskal-Wallis test,  $P < 0.05$ ) at 48 h measures in the amended treatments (median value 0.58 g CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup>) than un-amended one (median value 0.22 g CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup>), although absolute median values were lower than those measured in the first two measurements. The significant effect of harrowing on CO<sub>2</sub> emission can be due to both: i) the higher oxygen availability in the first soil layer because of the increase in soil macroporosity, which stimulates aerobic microbial populations; ii) the higher digestate physical accessibility for microorganisms and extracellular enzymes activities (Paustian *et al.*, 2000).

During maize growing season, no significant difference in soil CO<sub>2</sub> emission was monitored among treatments (Figure 4) with a median value of 0.33 g CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup>.

The DLF distribution applied 156.4 g m<sup>-2</sup> of C to soil. A significantly higher cumulative soil CO<sub>2</sub>-C emission during the experimental period was found for 10 cm and 25 cm treatments, with an average value of 411.8 ± 63.6 g CO<sub>2</sub>-C m<sup>-2</sup>; no significant difference was found between 35 cm and ND treatments, with an average value of 301.3 ± 49.0 g CO<sub>2</sub>-C m<sup>-2</sup> (Figure 5).

Comparing cumulative soil CO<sub>2</sub>-C emission with the amount of C supplied to the soil by DLF, until maize sowing the highest percent value was detected in the 10 cm treatment with a 61.4% emission of supplied C, followed by the 25 cm (43.8%) and 35 cm (2.2%) treat-

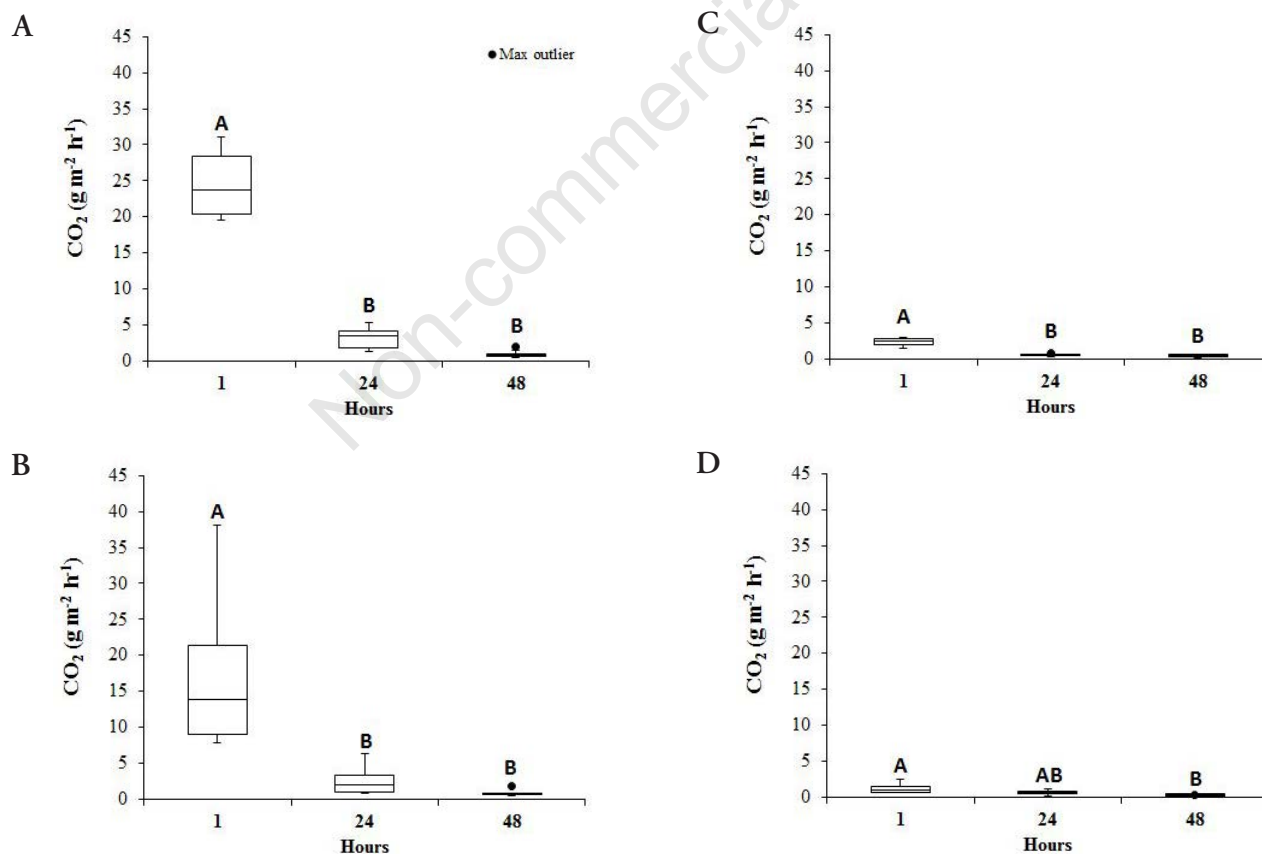


Figure 1. Box-plot diagrams of soil CO<sub>2</sub> emissions in the 48 h after digestate liquid fraction distribution in the experimental treatments. A) Digestate injection at 10 cm depth; B) digestate injection at 25 cm depth; C) digestate injection at 35 cm depth; D) un-amended control. Different letters indicate significant differences at  $P < 0.05$  by Kruskal-Wallis test.



ments. From maize sowing to its harvest, the highest soil CO<sub>2</sub>-C cumulative emission was measured in the 25 cm treatment (43.6%) followed by the 35 cm (36.1%) and 10 cm (25.4%) ones. Data obtained suggest that: i) in the short period (from digestate distribution to maize sowing), the CO<sub>2</sub>-C emission decreases enhancing DLF injection depth; ii) in the long period (from digestate distribution to maize harvest), the lowest CO<sub>2</sub>-C emission was shown by the deepest injection (38.3%), whereas similar values were found for 10 cm (86.8%) and 25 cm (87.4%) which therefore showed the same cumulative CO<sub>2</sub>-C emission but with different proportions between before and after sowing. Considering 10 cm and 25 cm treatments, the data suggest that the injection at 10 cm is preferable to indirectly reduce CO<sub>2</sub>-C release in the atmosphere because lower tractor power is required for digestate distribution. The emission values showed by DLF injection at 35 cm depth are indubitably interesting; however to reduce CO<sub>2</sub> losses in the atmosphere further studies are needed to compare soil CO<sub>2</sub> emission with tractor CO<sub>2</sub> emission to inject digestate at different depth.

During maize growing season in the upper 7.5 cm soil layer, mois-

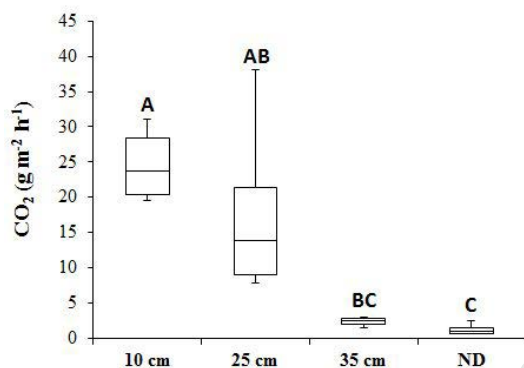


Figure 2. Box-plot diagram of soil CO<sub>2</sub> emissions 1 h after the digestate distribution in the experimental treatments. 10 cm, digestate injection at 10 cm depth; 25 cm, digestate injection at 25 cm depth; 35 cm, digestate injection at 35 cm depth; ND, plots without digestate injection. Different letters indicate significant differences at  $P < 0.05$  by Kruskal-Wallis test.

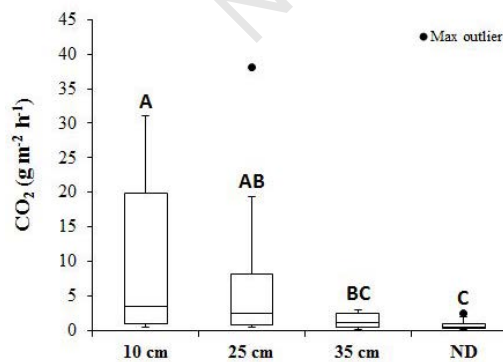


Figure 3. Box-plot diagram of soil CO<sub>2</sub> emissions in the 48 h between digestate distribution and maize sowing in experimental plots. 10 cm, digestate injection at 10 cm depth; 25 cm, digestate injection at 25 cm depth; 35 cm, digestate injection at 35 cm depth; ND, plots without digestate injection. Different letters indicate significant differences at  $P < 0.05$  by Kruskal-Wallis test.

ture ranged from 11.5% to 53.2% and temperature from 19.3°C to 33.9°C. Soil CO<sub>2</sub> emission was positively correlated with both soil moisture and temperature (Table 2), supporting the strong direct and indirect effect on organic material decomposition (Sänger *et al.*, 2011) exerted by soil aerobic metabolism. Considering the simultaneous effect of soil moisture and temperature on soil CO<sub>2</sub> emissions, the highest emission values were monitored when soil temperature ranged from 32°C to 34°C and, at the same time, soil moisture from 21% to 26%. Results are in agreement with Suseela *et al.* (2012), who found that soil respiration proceeded fastest at the warmest temperatures when soil water content ranged from 20% to 30%.

### Maize biomass production

DLF distribution significantly (ANOVA,  $P < 0.05$ ) improved maize aboveground dry biomass with an average production, in amended and un-amended plots, of 22.0 Mg ha<sup>-1</sup> and 16.2 Mg ha<sup>-1</sup>, respectively. The difference may be attributed to the higher nutrients input received by the amended plots. Maize yield obtained in amended plots is in agreement

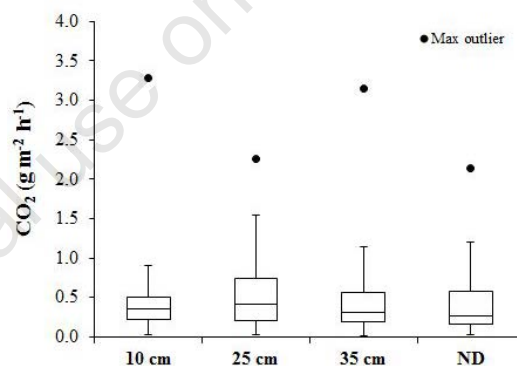


Figure 4. Box-plot diagram of soil CO<sub>2</sub> emissions between maize sowing and harvest in experimental plots. 10 cm, digestate injection at 10 cm depth; 25 cm, digestate injection at 25 cm depth; 35 cm, digestate injection at 35 cm depth; ND, plots without digestate injection. Different letters indicate significant differences at  $P < 0.05$  by Kruskal-Wallis test.

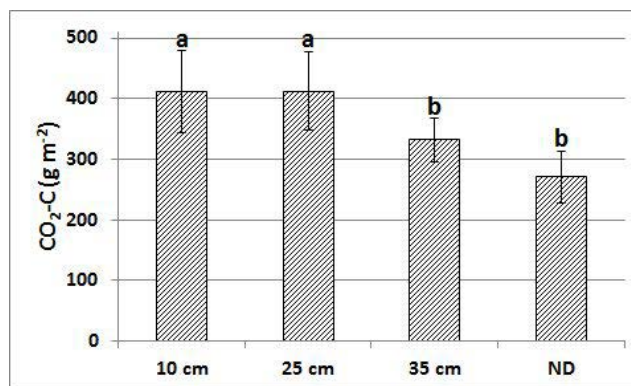
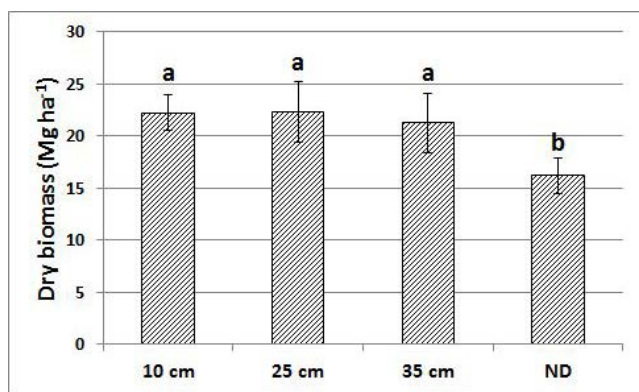


Figure 5. Soil cumulative CO<sub>2</sub>-C emissions during the whole experimental period. 10 cm, digestate injection at 10 cm depth; 25 cm, digestate injection at 25 cm depth; 35 cm, digestate injection at 35 cm depth; ND, plots without digestate injection. Different letters indicate significant differences at  $P < 0.05$  by Fisher least significant difference test.

**Table 2. Spearman rank correlation of soil CO<sub>2</sub> emissions with its temperature and moisture during maize growing season.**

Correlation	10 cm	25 cm	35 cm	ND
CO <sub>2</sub> vs temperature	0.580***	0.487***	0.595***	0.633***
CO <sub>2</sub> vs moisture	0.299*	0.471***	0.410**	0.442***

10 cm, digestate injection at 10 cm depth; 25 cm, digestate injection at 25 cm depth; 35 cm, digestate injection at 35 cm depth; ND, plots without digestate injection. \*P<0.05; \*\*P<0.01; \*\*\*P<0.001.



**Figure 6. Maize dry biomass production at dough stage. 10 cm, digestate injection at 10 cm depth; 25 cm, digestate injection at 25 cm depth; 35 cm, digestate injection at 35 cm depth; ND, plots without digestate injection. Different letters indicate significant differences at P<0.05 by Fisher least significant difference test.**

with our previous data (22.7 Mg ha<sup>-1</sup>) obtained with DLF splash-plate spreading on a clay loam soil. In our research, maize dry biomass yield was not significant influence by digestate injection depth (Figure 6). Obtained results confirmed that anaerobic digestate could be regarded as effective organic fertilisers (Nkoa, 2014). Furthermore, Walsh *et al.* (2012) reported that replacing inorganic fertilisers with liquid digestate could maintain or improve yields from grassland systems, with less impact on the environment. Considering only the DLF macronutrients (N, P<sub>2</sub>O<sub>5</sub>, K<sub>2</sub>O), and using the CO<sub>2(eq)</sub> specific emission factors for mineral fertilisers production (Capponi *et al.*, 2012), the avoided carbon emission in the atmosphere, in this study, was equivalent to 859.6 kg CO<sub>2(eq)</sub> ha<sup>-1</sup>.

## Conclusions

The DLF effect on soil CO<sub>2</sub> emission followed the same trend for all studied digestate soil injection depths with high emission in the first hour after distribution, and a significant reduction already after 24 h, reaching values similar to un-amended plots after 48 h. Comparing the emissions measured 1 h after digestate injection, CO<sub>2</sub> flux decreased when injection depth increased, with significantly higher emission in the 10 cm treatment (median value 23.7 g CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup>) and the lowest one in the 35 cm treatment (median value 2.5 g CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup>). During maize growing season, no significant soil CO<sub>2</sub> emission difference was monitored among treatments, with a median value of 0.33 g CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup>.

A significantly higher cumulative soil CO<sub>2</sub>-C emission during the experimental period was found for 10 cm and 25 cm treatments, with

an average value of 411.8±63.6 g CO<sub>2</sub>-C m<sup>-2</sup>; no significant difference was found between 35 cm and ND treatments, with an average value of 301.3±49.0 g CO<sub>2</sub>-C m<sup>-2</sup>.

Our results clearly showed that increasing DLF injection depth soil CO<sub>2</sub>-C flux decreases. This suggests that for maize sown as second crop in late spring, a potential containment of CO<sub>2</sub> emission can be achieved through deep injection associated with tillage, *i.e.*, one pass strategy with a chisel equipped with tank and nozzles.

Digestate liquid fraction presents fertiliser properties indicating the possibility to reduce the use of mineral fertilisers with a consequent reduction of energy use and CO<sub>2(eq)</sub> emissions for their production.

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