

# Changes in direct CO<sub>2</sub> and N<sub>2</sub>O emissions from a loam Haplic Luvisol under conventional moldboard and reduced tillage during growing season and post-harvest period of red clover

Ján Horák<sup>1\*</sup>, Dušan Igaz<sup>1</sup>, Elena Aydin<sup>1</sup>, Vladimír Šimanský<sup>2</sup>, Natalya Buchkina<sup>3</sup>, Eugene Balashov<sup>3</sup>

<sup>1</sup> Department of Biometeorology and Hydrology, Faculty of Horticulture and Landscape Engineering, Slovak University of Agriculture, Hospodárska 7, 94976 Nitra, Slovakia.

<sup>2</sup> Department of Soil Science, Faculty of Agrobiological and Food Resources, Slovak University of Agriculture, Tr. A. Hlinku 2, 94976 Nitra, Slovakia.

<sup>3</sup> Department of Soil Physics, Physical Chemistry and Biophysics, Agrophysical Research Institute, Grazhdansky pr. 14, 195220 St. Petersburg, Russia.

\* Corresponding author. Tel.: +421376415244. E-mail: jan.horak@uniag.sk

**Abstract:** The objectives of the study were to: (1) assess the strength of associations of direct CO<sub>2</sub> and N<sub>2</sub>O emissions with the seasonal variations in the relevant soil properties under both tillage systems; 2) evaluate how CT and RT affect magnitudes of seasonal CO<sub>2</sub> and N<sub>2</sub>O fluxes from soil. Field studies were carried out on plots for conventional tillage (up to 0.22–0.25 m) and reduced tillage (up to 0.10–0.12 m) during the growing season and post-harvest period of red clover. The results showed that daily CO<sub>2</sub> emissions significantly correlated only with soil temperature during the growing season under conventional and reduced tillage. Soil temperature demonstrated its highest influence on daily N<sub>2</sub>O emissions only at the beginning of the growing season in both tillage systems. There were no significant inter-system differences in daily CO<sub>2</sub> and N<sub>2</sub>O emissions from soil during the entire period of observations. Over the duration of post-harvest period, water-filled pore space was a better predictor of daily CO<sub>2</sub> emissions from soils under CT and RT. The conventional and reduced tillage did not cause significant differences in cumulative N<sub>2</sub>O and CO<sub>2</sub> fluxes from soil.

**Keywords:** Tillage systems; Soil organic matter; Temperature; Moisture content; Water-filled pore space; CO<sub>2</sub> and N<sub>2</sub>O emissions.

## INTRODUCTION

Rational use of land-use, soil tillage and fertilization systems are effective tools for maintaining soil quality and crop production because they control soil water, nutrient and temperature regimes, cycles of C and N. Among current approaches to controlling soil quality, a crucial attention is now given to interdisciplinary studies on relationships of greenhouse gas fluxes with soil physical, microbial and chemical quality indicators. Agricultural soils strongly affect global atmospheric concentrations of major greenhouse gases – CO<sub>2</sub>, N<sub>2</sub>O and CH<sub>4</sub>. The potential of global carbon sequestration was calculated as  $0.12 \pm 0.03 \text{ Pg C yr}^{-1}$ , which would compensate for 8% of the direct annual greenhouse gas emissions from agriculture (Poeplau and Don, 2015). Annual increase in global soil organic carbon (SOC) stocks by 0.4% in the upper 0.30–0.40 m soil layers can significantly reduce annual CO<sub>2</sub> fluxes from soils to the atmosphere (Soussana et al., 2019). Therefore, there is a need for a further use of traditional rational technologies and for a development of new measures focused on increasing carbon sequestration in soils. Tillage systems affect CO<sub>2</sub> and N<sub>2</sub>O emissions due to their effects on the soil structural, air, hydrological, and microbiological status (Soon et al., 2007; Zhang et al., 2007).

N<sub>2</sub>O emissions are a key concern in agriculture. N<sub>2</sub>O emissions are mainly induced by the increased application of nitrogen fertilizers. According to Syakila and Kroeze (2011), estimated N<sub>2</sub>O agricultural emissions ( $4.3\text{--}5.8 \text{ Tg N}_2\text{O-N yr}^{-1}$ ) due to nitrogen fertilizer application and manure management represented 23–31% of all global N<sub>2</sub>O sources ( $19 \text{ Tg N yr}^{-1}$  in 2006).

The rates of N<sub>2</sub>O production by processes of nitrification and denitrification in soils are controlled by several factors: temperature, water-filled pore space (WFPS), O<sub>2</sub> concentration, pH, mineral nitrogen (NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>) and available SOC content (Buchkina et al., 2012; Dobbie and Smith, 2003; Lee et al., 2009; Rizhiya et al., 2008; Šimanský et al., 2016; Wrage et al., 2001).

There were differences in a stratification of soil mineral particles, soil porosity, and SOC within depths of plough layers of conventional and reduced tillage (Castellini and Ventrella, 2012; de Oliveira Silva et al., 2019). A uniform distribution of transmission meso- (0.06–0.5 mm) and macropores (> 0.5 mm) is usually observed within a whole plough layer of 0.20–0.22 m after conventional moldboard tillage (CT). Reduced tillage (RT) can lead to the uniform distribution of meso- and macropores within the plough layer of 0–0.10 m while the 0.10–0.22 m layers are mainly presented by micropores of < 0.06 mm in diameter (Dimassi et al., 2014; Głab and Kulig, 2008; Lipiec et al., 2006). In the CT system, the soil macro- and mesopores, compared to the micropores, demonstrate higher preferential flows of water after rainfall (Alaoui et al., 2011; Lipiec et al., 2006; Orfanus et al., 2017). As a result, the 0–0.22 m soil layers in the CT and RT systems show temporal differences in favorable and unfavorable moisture and soil air conditions for carbon- and nitrogen-transforming soil microorganisms.

Different behavior of CO<sub>2</sub> and N<sub>2</sub>O emissions from arable soils is driven by wet and dry weather conditions. Soil total porosity and pore size can be stronger predictors of CO<sub>2</sub> and N<sub>2</sub>O fluxes than content of SOC, microbial biomass carbon and

available mineral nitrogen. The uniform porosity of the 0–0.22 m soil layer under CT favors the respiration of aerobic microorganisms (Mangalassery et al., 2014). The depth and time stratification of soil pore systems can result in the depth and time variations of the soil parameters controlling production of CO<sub>2</sub> and N<sub>2</sub>O. After a heavy rainfall, simultaneous sharp peaks of CO<sub>2</sub> and N<sub>2</sub>O emissions from the soils were observed at water-field pore space (WFPS of > 60%) in their entire plough layers under CT (Forte et al., 2017; Horák and Mukhina, 2016; Horák et al., 2019). The non-uniform porosity of the 0–0.22 m soil layer under RT may prevent N<sub>2</sub>O diffusivity through this layer. Therefore, postponed bursts of N<sub>2</sub>O emissions from the soils under RT could occur only several days after heavy rainfall because of slower water saturation of the entire 0–0.22 m layers to the WFPS values exceeding 60% (Horák et al., 2019). The slower water saturation of these layers can be explained by differences in the profile distribution of soil physical and microbiological properties between 0–0.10 m and 0.10–0.22 m layers of RT (Fuß et al., 2011; Głąb and Kulig, 2008; Nan et al., 2016).

Higher CO<sub>2</sub> and N<sub>2</sub>O emissions can be observed from soils under CT than under RT as a result of a greater potential to a formation of favorable conditions for CO<sub>2</sub> and N<sub>2</sub>O fluxes in the plough layers of CT (Buragienė et al., 2019; Forte et al., 2017; Groenigen et al., 2005; Jabro et al., 2010; Ussiri et al., 2009; Wang et al., 2013).

The greater CO<sub>2</sub> and N<sub>2</sub>O emissions were recorded under RT than under CT due to a formation of higher amount of decomposable crop residues and available mineral nitrogen, and higher WFPS (> 60%) near the soil surface of the plough layers of RT (Guardia et al., 2016; Sheehy et al., 2013). According to Fuß et al. (2011), strong short-term N<sub>2</sub>O emission pulses can be observed from relatively dry soils in the RT system because of: (1) a high accumulation of mineral nitrogen close the soil surface, and (2) a strong input of readily decomposable organic substances enhancing activity of denitrifying microorganisms.

Several studies also showed that there were no effects of CT and RT on CO<sub>2</sub> and N<sub>2</sub>O emissions from soils (Elmi et al., 2003; Kong et al., 2009; Lee et al., 2009; Mutegei et al., 2010).

Nevertheless, there is a trend for the adoption of RT around the world because this system mitigates soil degradation, improves the SOC status and soil structural state (Abdalla et al., 2010; Krauss et al., 2017; Salem et al., 2015).

The objectives of the study were to: (1) assess the strength of associations of direct CO<sub>2</sub> and N<sub>2</sub>O emissions with the seasonal variations in the key soil properties in CT and RT; (2) evaluate how CT and RT affect magnitudes of seasonal CO<sub>2</sub> and N<sub>2</sub>O fluxes from soil.

## MATERIALS AND METHODS

Field studies were carried out from April 4 to August 30 of 2013 at the agricultural experimental station of the Department of Plant Production of the Slovak University of Agriculture in Nitra region of Slovakia (48°19'N; 18°09'E). Average annual air temperature was +10.3 °C and total amount of annual precipitation was 613.8 mm. Over the entire period of the field studies, mean daily temperature was +18.0 °C and total amount of precipitation reached 205.4 mm. The highest daily air temperature was recorded on August 28 (+30.5 °C) and the highest amount of daily precipitation reached 17.4 mm on June 24. The soil was classified as loam Haplic Luvisol containing 360.4 g kg<sup>-1</sup> of sand, 488.3 g kg<sup>-1</sup> of silt and 151.3 g kg<sup>-1</sup> of clay (Šimanský et al., 2008).

The research site was established in 1994 and was split into two blocks. The blocks were arranged into 9 randomized plots

(4 x 10 m) for conventional moldboard tillage (CT) at a depth of 0.22–0.25 m and 9 randomized plots (4 x 10 m) for reduced tillage (RT, disking) at the depth of 0.10–0.12 m. The treatments were replicated three times. The CT and RT plots included treatments: N0 (without nitrogen fertilizer), N1 (with nitrogen fertilizer) and N2 (with nitrogen fertilizer and crop residues). In the present study the only N0 and N1 treatments of CT and RT are reported on.

Soil tillage operations and planting red clover (*Trifolium pratense* L. cv. Vulkan) were finished by April 15. During the growing season, the measurements of greenhouse gas emissions from the soil were finished on July 8. Red clover was made harvested on July 10. The nitrogen fertilizer (NH<sub>4</sub>NO<sub>3</sub>) was spread in a rate of 25 kg N ha<sup>-1</sup> on May 7 after measurements of direct CO<sub>2</sub> and N<sub>2</sub>O emissions. During the post-harvest (dormant) period, the measurements of greenhouse gas emissions from the soils were performed from July 11 to August 30.

A closed chamber method was used for measurements of direct CO<sub>2</sub> and N<sub>2</sub>O emissions from the soil in all the treatments one time a week between 9 a.m. and 12 a.m. to decrease the variability in CO<sub>2</sub> and N<sub>2</sub>O fluxes due to diurnal changes in temperature. One metal collar frame was incorporated in the soil surface to a depth of 0.10 m of each treatment (Horák et al., 2014). The metal collar frames were removed only during tillage, fertilization and harvesting operations, and were reinserted when these operations were finished. PVC chambers (30 cm in diameter and 25 cm in height) were fixed on a water-filled rim of the metal collar frame. Surface area of covered soil was 615 cm<sup>2</sup>. Gas samples (20 mL) were collected from the PVC chambers through tube fittings (sealed with a rubber septum) at regular intervals of 0, 30 and 60 min using airtight glass syringe (Hamilton) and transferred to pre-evacuated 12-mL glass vials (Labco Exetainer). Daily CO<sub>2</sub> and N<sub>2</sub>O emissions were calculated as linear relationships of their concentrations (in ppm and ppb) with time of three measurements using a total chamber volume of 17663 cm<sup>3</sup>. The CO<sub>2</sub> and N<sub>2</sub>O emissions were converted into convenient units: g ha<sup>-1</sup> day<sup>-1</sup> for N<sub>2</sub>O and kg ha<sup>-1</sup> day<sup>-1</sup> for CO<sub>2</sub>. Cumulative N<sub>2</sub>O and CO<sub>2</sub> fluxes were calculated by interpolating the N<sub>2</sub>O and CO<sub>2</sub> emissions between each sampling day.

A gas chromatograph (GC-2010 Plus Shimadzu) equipped with a <sup>63</sup>Ni electron capture detector and a thermal conductivity detector was used for measurements of N<sub>2</sub>O and CO<sub>2</sub> concentrations the collected gas samples. Three certified standard gas mixtures (CO<sub>2</sub>, N<sub>2</sub>O, and N<sub>2</sub>) were used for a calibration of the GC in the expected concentration ranges. Analytical conditions of the GC were: carrier gas Helium (30 ml min<sup>-1</sup>) and Argon-Methane as a make-up gas), detector temperatures 330 °C for the electron capture detector and 120 °C for the thermal conductivity detector.

Soil samples were collected once a month from April to August. Seven soil samples were taken from 0–0.10 m layer of each plot using a 2 cm stainless steel auger. The soil samples were combined into one representative subsample for each treatment. Soil water content at the 0–0.10 m depth and soil temperature at the 0–0.05 m depth was measured by a gravimetric method and by a Volcraft DET3R thermometer at each gas sampling. Volumetric soil moisture content (SMC) was calculated using data on soil bulk density measured monthly by a core method. The values of WFPS were calculated using the data on SMC.

Mineral nitrogen forms (NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>) were extracted with 1% K<sub>2</sub>SO<sub>4</sub> solution at 1:5 ratio of soil to K<sub>2</sub>SO<sub>4</sub> (Yuen and Pollard, 1954). The concentrations of soil NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> were measured using a SP-870 PLUS spectrometer (Metertech Inc.)

at wavelengths of 410 and 420 nm, respectively. Soil pH (at 1:2.5 ratio of soil to 1M KCl solution) was measured using a HI 9017 Microprocessor Bench – top pH Meter (HANNA instruments). A pressure-plate apparatus was used for measurements of water-retention curves of undisturbed soil samples at water potentials of –5, –20, –50, –100 and –300 kPa (Soil Survey Division Staff, 1996).

Strength of associations between sets of soil parameters in all the treatment were assessed with Spearman's rank correlation coefficients at  $P \leq 0.05$  (Wessa, 2017). One-way ANOVA analysis of variance was applied to evaluate the differences between means of normally distributed data: soil temperature, SMC, bulk density, WFPS, pH, SOC content, soil NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N concentrations at  $P \leq 0.05$ . Mann-Whitney U test and Kruskal-Wallis test were applied to compare the differences in means (at  $P \leq 0.05$ ), if the normal distribution of data (CO<sub>2</sub> and N<sub>2</sub>O emissions) was not found.

## RESULTS AND DISCUSSION

### Soil temperature, moisture content, water-filled pore space and bulk density

Soil temperature and moisture content are important factors controlling microbial processes of nitrogen and carbon transformation (Dobbie and Smith, 2003; Nkongolo et al., 2010). During the entire period of our studies, soil temperature showed pronounced dynamics and was strongly induced by the air temperature. Daily mean air temperatures varied from 3.6 °C to 30.5 °C. The Spearman's rank correlations coefficients for relationships between soil temperature and air temperature were in narrow ranges of 0.88–0.89 (at  $P < 0.001$ ) and 0.90–0.91 (at  $P < 0.001$ ) for the CT and RT treatments. Over this period of the study, daily mean soil temperatures in the upper 0.05-m layer did not differ between CT and RT (Table 1).

**Table 1.** Soil temperature of a loam Haplic Luvisol during the entire period of the study, growing season and post-harvest period in 2013 (mean ± standard deviations).

| Treatment | Entire period of study | Growing season | Post-harvest period |
|-----------|------------------------|----------------|---------------------|
| CT N0     | 20.5±6.1               | 18.3±5.5       | 25.8±4.1            |
| CT N1     | 20.5±6.2               | 18.2±5.5       | 25.8±4.3            |
| RT N0     | 20.7±6.2               | 18.3±5.4       | 26.2±4.1            |
| RT N1     | 20.5±6.0               | 18.3±5.5       | 25.7±4.0            |

During the growing season, CT and RT also did not cause distinct differences in soil temperature at the 0–0.05-m soil depth (Table 1). For this period of observations, the Spearman's rank correlations coefficients for relationships between soil temperature and air temperature were equal: 0.86–0.88 (at  $P < 0.001$ ) and 0.89–0.90 (at  $P < 0.001$ ) for all the CT and RT treatments.

After harvesting red clover, solar radiation and air temperatures had stronger impacts on soil temperature. Therefore, during the post-harvest period, mean daily soil temperature was higher than during the growing period. The Spearman's rank correlation coefficients between soil temperature of bare soil and air temperature increased up to 0.95–0.98 (at  $P < 0.001$ ) for CT and RT.

CT, as compared to RT, had a greater contribution to increasing macroporosity, air circulation and soil drying in the whole plough layers (Aletto et al., 2011; Głab and Kulig, 2008; Salem et al., 2015). Therefore, the soils under CT, in contrast to those under RT, often showed higher temperatures of 0–0.22-m layers because of lower SMC and thermal conductivity

(Muñoz-Romero et al., 2015; Ochsner et al., 2007; Salem et al., 2015). Nevertheless, our data demonstrated that CT, compared to RT, had not contributed to any significantly higher increase in soil temperature during the growing season and post-harvest period.

Over the entire period of the study, soil temperature had significant inverse relationships ( $r = -0.85$  and  $r = -0.86$  at  $P < 0.001$ ) with SMC in all the treatments of CT and RT. Data of other studies supported results of our research (Aletto et al., 2011). Over its growing period, red clover possibly contributed to (1) a greater reflecting the solar radiation and insulating the soil surface, and (2) a lower heat flux in soil. As a result, a decrease in soil temperature and weakening of its relationships with SMC was observed in both soil tillage systems. The Spearman's rank correlation coefficients were equal to: –0.70 at  $P < 0.01$  (CT N0), –0.78 at  $P < 0.001$  (CT N1), and –0.75 at  $P < 0.001$  (RT N0 and RT N1). The results of statistical analysis (one-way ANOVA) showed that during the growing period mean values of SMC in the 0–0.10 m soil layers did not differ between CT and RT: 21.8–22.3% (CT) and 22.0–22.0% (RT). The weak differences in soil temperature and SMC between CT and RT were due to the presence of red clover canopy that could limit (1) penetration of solar radiation and consequent soil heating, (2) evaporation from soil surface with consequent its cooling.

During the post-harvest period, a greater soil heating in the treatments of CT and RT without red clover also accelerated a fast decrease of SMC up to its mean values of: 10.8–12.1% (CT) and 10.9–11.7% (RT). Therefore, the Spearman's rank correlation coefficients showed stronger inverse relationships between SMC and soil temperature: –0.98 at  $P < 0.001$  (CT N0), –0.91 at  $P < 0.001$  (RT N0) and –0.90 at  $P < 0.001$  (RT N1). The exception was a lower Spearman's rank correlation coefficient of –0.79 (at  $P < 0.05$ ) for CT N1.

WFPS is often used as one of the important indicators of soil aerobic (< 60%) and anaerobic (> 60%) conditions (Dobbie and Smith, 2003; Wrage et al., 2001). The results of our research showed that during most of the growing season soil conditions were aerobic in the treatments of CT and RT. During the entire period of the study, the precipitation of 8.2 mm (on May 19) caused a distinct increase in WFPS. However, magnitudes of WFPS did not exceed 60% after this rain event: 56.8–57.8% (CT), 53.7–57.2% (RT). In both tillage systems the seasonal values of WFPS were recorded in the same ranges of: 27.1–69.4% (CT N0), 29.9–75.4% (CT N1), 27.7–69.9% (RT N0) and 29.0–67.7% (RT N1). Maximum values of WFPS were observed on April 4.

Over the duration of post-harvest period, the magnitudes of WFPS in both tillage systems were less than those during the growing season. Mean values of the WFPS were equal to: 23.6–26.7% (CT) and 23.9–25.2% (RT). In general, our data on dynamics of WFPS demonstrate that (1) nitrification was a dominant microbial process of mineral nitrogen transformation, and (2) both tillage systems did not cause significant differences in WFPS during the growing and post-harvest period.

During the entire period of the study, CT and RT had the same effects on the soil bulk density in the upper 0.05-m soil layer: on average, 1.44 g cm<sup>-3</sup> (CT) and 1.47 g cm<sup>-3</sup> (for RT).

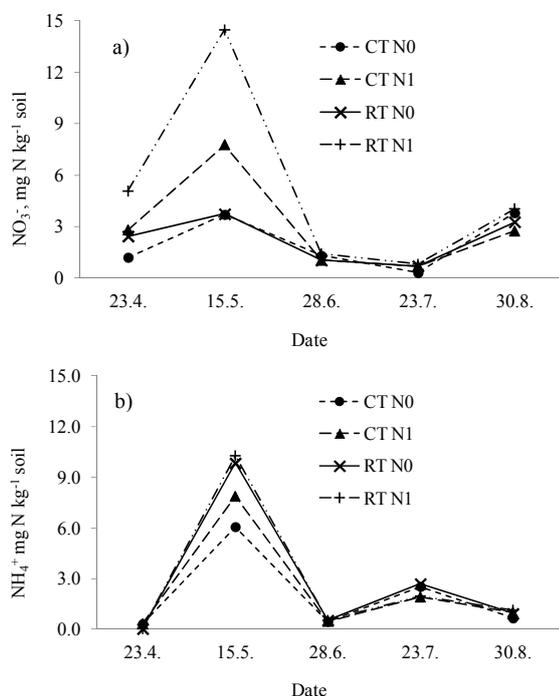
### Soil mineral nitrogen content and pH

Both tillage systems redistribute SOC, plant residues and accelerate the microbial mineralization of protected organic nitrogen increasing the NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> availability in the relevant plough layers (Elder and Lal, 2008). Application of CT, as

compared to RT, leads to a greater mineralization of organic nitrogen originated from easily available plant residues or SOC (Drinkwater et al., 2000). As a result, the concentration of soil NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> can be higher in the plough layers of CT than RT.

Over the duration of the entire study period, the concentrations of soil NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> showed distinct changes at the 0–0.10 m depth of plough layers of CT and RT (Figure 1). The highest increase in concentrations of soil NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> on May 15 was induced by the application of ammonium nitrate fertilizer at the rate of 25 kg ha<sup>-1</sup> on May 7. The results of one-way ANOVA analysis of variance demonstrated significant (*P* < 0.05) differences in mean soil NO<sub>3</sub><sup>-</sup> concentrations (mg N kg<sup>-1</sup> soil) between treatments of RT N0 (2.2 ± 0.5) and RT N1 (5.2 ± 1.1). The concentrations of NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> were only slightly higher in soils from relevant treatments of RT than CT.

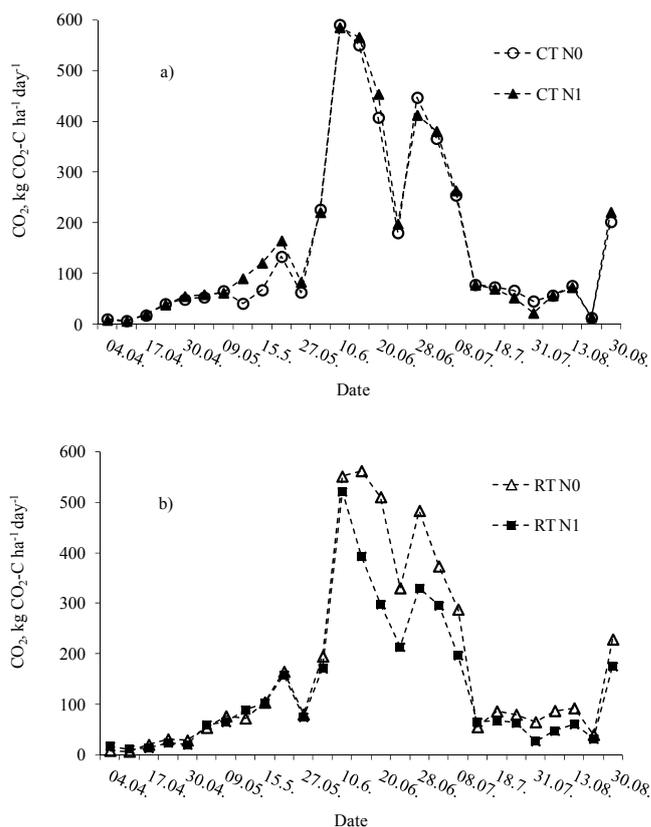
During the entire period of the study, mean values of soil pH were 6.8–6.9 in both tillage systems.



**Fig. 1.** Dynamics of concentrations of NO<sub>3</sub><sup>-</sup> (a) and NH<sub>4</sub><sup>+</sup> (b) in a loam Haplic Luvisol under conventional (CT) and reduced (RT) tillage in treatments without (N0) and with (N1) application of ammonium nitrate fertilizer at a rate of 25 kg N ha<sup>-1</sup> during the entire period of study in 2013.

### Daily CO<sub>2</sub> emissions from soil

Over the duration of the entire period of observations, daily CO<sub>2</sub> emissions from soils showed great variations in all the treatments of both tillage systems (Figure 2). The variations in daily CO<sub>2</sub> emissions were affected by changes in soil properties and by harvesting of crops (Fuß et al., 2011). At the beginning of the study (in April), daily CO<sub>2</sub> emissions were low and did not depend on changes in soil temperature (from 6.5 °C to 11 °C) and WFPS (from 75% to 48%) in all the treatments of CT and RT. These daily CO<sub>2</sub> emissions resulted from heterotrophic respiration. Lee et al. (2009) also reported that low soil temperature did not affect greenhouse gas emissions. An almost two-fold increase in the daily CO<sub>2</sub> emissions was recorded at a soil temperature of more than 17 °C in all the treatments of CT and RT. Both heterotrophic (microbial) and autotrophic (root)



**Fig. 2.** Dynamics of daily CO<sub>2</sub> emission from a loam Haplic Luvisol under conventional (CT, a) and reduced (RT, b) tillage in the treatments without (N0) and with (N1) application of ammonium nitrate fertilizer at a rate of 25 kg N ha<sup>-1</sup> during the entire period of study in 2013.

respiration became factors of CO<sub>2</sub> emissions from soil during the growing season. During the entire period of the study, mean daily CO<sub>2</sub> emissions (kg CO<sub>2</sub>-C ha<sup>-1</sup> day<sup>-1</sup>) were equal to: 188.0 (N0), 199.4 (N1) for CT and 207.6 (N0), 160.7 (N1) for RT. The application of CT and RT did not cause any significant differences in daily CO<sub>2</sub> emissions. The incorporation of ammonium nitrate fertilizers on May 7 did not affect daily CO<sub>2</sub> emissions from soils in CT and RT. However, the daily CO<sub>2</sub> emissions from soils in both tillage systems distinctly increased on May 20 after rainfall event of 8.2 mm on May 19. The magnitudes of mean daily CO<sub>2</sub> emissions (kg CO<sub>2</sub>-C ha<sup>-1</sup> day<sup>-1</sup>) from the soil under red clover reached on May 20: 133.4 (N0), 164.8 (N1) for CT and 165.0 (N0), 158.1 (N1) for RT. On May 20, there were almost anaerobic conditions for soil microorganisms and plant roots. Values of WFPS varied from 56.8% to 57.8% in CT and from 53.7% to 57.2% in RT.

A joint effect of soil aerobic conditions and soil temperature (> 17 °C) could explain the rapid and short-term increase in daily CO<sub>2</sub> emissions induced by elevated autotrophic and heterotrophic respiration after rainfall event (Chou et al., 2008). The high respiration activity of microorganisms can occur from several hours to two days (Kieft et al., 1987).

Over the duration of the growing season, the highest daily CO<sub>2</sub> emissions (kg CO<sub>2</sub>-C ha<sup>-1</sup> day<sup>-1</sup>) from soils reached: 590.8 (N0), 585.3 (N1) for CT and 562.2 (N0), 521.2 (N1) for RT. Both tillage systems did not cause any significant differences in these daily CO<sub>2</sub> emissions. Soil temperature was the most important predictor of daily CO<sub>2</sub> emissions from soil in all the treatments of CT and RT (Lee et al. 2009). The Spearman's

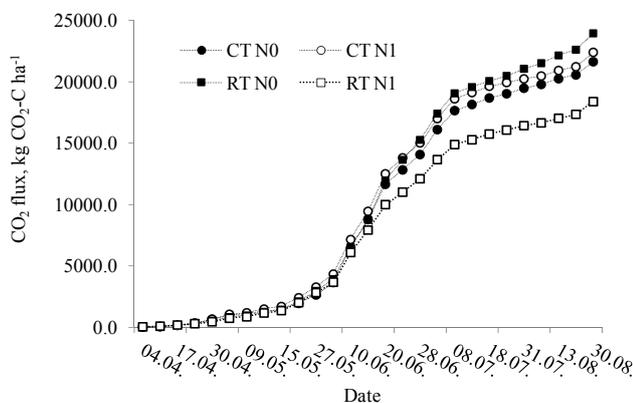
rank correlation coefficients for these parameters varied from 0.61 (N1 treatment, at  $P < 0.05$ ) to 0.66 (N0 treatment, at  $P < 0.01$ ) for CT and from 0.54 (N1 treatment, at  $P < 0.05$ ) to 0.61 (N0 treatment, at  $P < 0.05$ ) for RT. Our data on strong positive relationships of daily CO<sub>2</sub> emissions with daily soil temperature are in accord with results of other studies (Chou et al., 2008; Forte et al., 2017).

After harvesting red clover, the heterotrophic microorganisms have again become main sources of soil respiration. Therefore, mean daily CO<sub>2</sub> emissions from soils decreased in all the treatments of CT and RT. During the post-harvest period, CT and RT led only to slight differences in mean daily CO<sub>2</sub> emissions (kg CO<sub>2</sub>-C ha<sup>-1</sup> day<sup>-1</sup>): 76.5 (N0), 73.2 (N1) in CT and 92.0 (N0), 67.2 (N1) in RT. Over this study period, the highest daily CO<sub>2</sub> emissions (kg CO<sub>2</sub>-C ha<sup>-1</sup> day<sup>-1</sup>) from soils were recorded at WFPS of 55.5–66.5% (CT) and 61.6–61.9% (RT) on August 30 after rain event: 202.1–221.1 (CT) and 175.2–228.9 (RT). The WFPS was a better predictor of daily CO<sub>2</sub> emissions in the treatments of CT and RT during the post-harvest period. Even changes in soil temperature in the ranges of 22.0–30.6 °C (CT) and 22.2–32.1 °C (RT) did not affect the soil heterotrophic respiration and consequent daily CO<sub>2</sub> emissions.

### Cumulative CO<sub>2</sub> fluxes from soil

The CT and RT were permanent sources of CO<sub>2</sub> emissions from soils during the entire period of observations. High magnitudes of soil temperature (21–28 °C) and WFPS (44–60%) in June were the main predictors of increasing cumulative CO<sub>2</sub> fluxes in both tillage systems. These aerobic soil conditions were expressed in the maximum daily CO<sub>2</sub> emissions of more than 400 kg CO<sub>2</sub>-C ha<sup>-1</sup> day<sup>-1</sup> from all the treatments of both tillage systems. CT and RT in the treatments without and with nitrogen fertilizers did not affect total cumulative CO<sub>2</sub> fluxes from soil during the entire period of the study (Figure 3). Both tillage systems did not cause significant differences in key soil properties that would have a crucial effect on soil respiration as a sensitive indicator of status of microbial community (Elbl et al., 2014). Besides, high variations in cumulative CO<sub>2</sub> fluxes did not allow identifying statistically significant differences between CT and RT.

Over the duration of the growing season, cumulative CO<sub>2</sub> fluxes, as fraction of total cumulative fluxes for the entire period of the study, accounted for 81.6% (N0), 83.1% (N1) for CT and

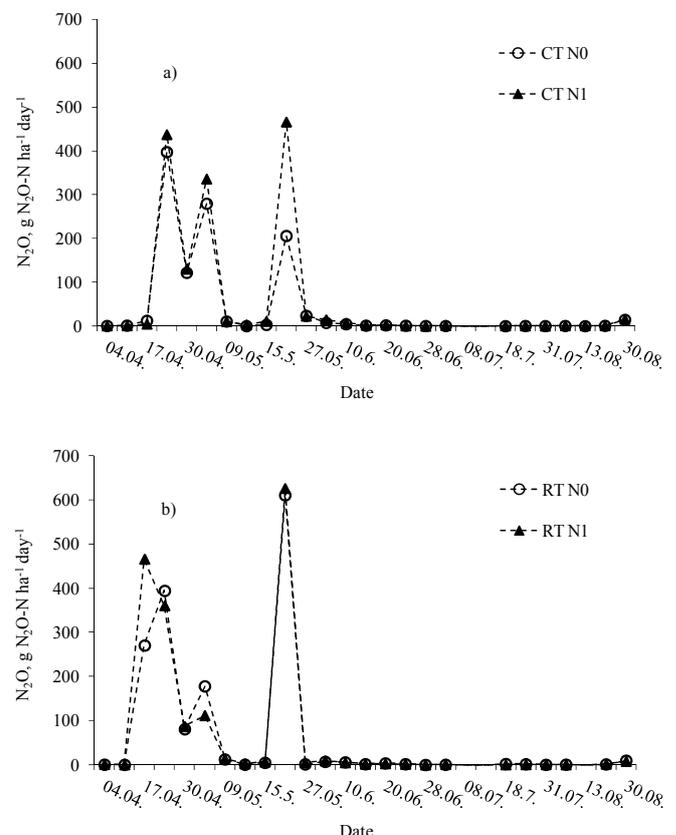


**Fig. 3.** Cumulative CO<sub>2</sub> fluxes from a loam Haplic Luvisol under conventional (CT) and reduced tillage (RT) in treatments without (N0) and with (N1) application of ammonium nitrate fertilizer at a rate of 25 kg N ha<sup>-1</sup> during the entire period of the study in 2013.

79.5% (N0), 81.0% (N1) for RT. During the post-harvest period, the fractions of the cumulative CO<sub>2</sub> fluxes of total ones were much less and accounted only for 18.4% (N0), 16.9% (N1) for CT and 20.5% (N0), 19.0% (N1) for RT. The coupled heterotrophic and autotrophic respiration during the growing season was a more powerful factor of influence on cumulative CO<sub>2</sub> fluxes than the only heterotrophic respiration during the post-harvest period.

### Daily N<sub>2</sub>O emissions from soil

At the beginning of growing season the daily N<sub>2</sub>O emissions from soils were affected by variations in soil temperature (Figure 4). Until mid-April, the daily N<sub>2</sub>O emissions were small because soil temperatures (from 6.5 °C to 11 °C) were unfavorable for nitrifying and denitrifying microorganisms (Lee et al. 2009). The relatively high WFPS (> 65%) did not affect the activity of denitrifying microorganisms at these soil temperatures in all the treatments of CT and RT. These data supported the results of our recent studies (Horák et al., 2019). Only when the soil temperature increased above 17 °C a peak of the daily N<sub>2</sub>O emission occurred at aerobic soil conditions, i.e. at WFPS of 46.9% – 53.1% in CT and 48.3% – 53.3% in RT. Daily N<sub>2</sub>O emissions (g N<sub>2</sub>O-N ha<sup>-1</sup> day<sup>-1</sup>) reached: 398.0 ± 51.6 (N0), 437.9 ± 58.3 (N1) for CT and 394.4 ± 11.2 (N0), 466.0 ± 44.6 (N1) for RT. CT and RT, apart from nitrogen fertilizers, caused only slight differences in the mean magnitudes of the peaks in daily N<sub>2</sub>O emissions. Similar results were reported by Lee et al. (2009) and Ussiri et al. (2009).



**Fig. 4.** Dynamics of daily N<sub>2</sub>O emission from a loam Haplic Luvisol under conventional (CT, a) and reduced (RT, b) tillage in treatments without (N0) and with (N1) application of ammonium nitrate fertilizer at a rate of 25 kg N ha<sup>-1</sup> during the entire period of studies in 2013.

The application of ammonium nitrate fertilizer on May 7 could increase the availability of organic substrates for nitrifying microorganisms in the tillage zone of CT and RT. However, the 0–0.10 m plough layer of RT, as compared with the 0–0.20 m plough layer of CT, demonstrated a lower microbial potential to increasing nitrification and N<sub>2</sub>O emissions after application of nitrogen fertilizer. As a result, there were significant ( $P = 0.05$ ) differences in mean magnitudes of daily N<sub>2</sub>O emissions ( $\text{g N}_2\text{O-N ha}^{-1} \text{ day}^{-1}$ ) from soils in the N1 treatments of CT (336.5) and RT (112.0).

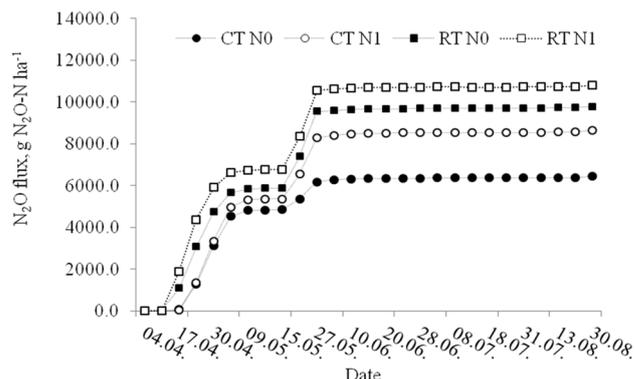
On May 20, the burst of daily N<sub>2</sub>O emissions was observed after a rain event of 8.2 mm recorded on May 19 (Figure 4). This rain event was relatively small and therefore did not lead to immediate formation of anaerobic soil conditions which are usually observed at WFPS > 60% (Dobbie and Smith, 2003). On May 20, WFPS ranged from 56.8% to 57.8% for CT and from 53.7% to 57.2% for RT. However, even at these values of WFPS processes of nitrification and denitrification can occur simultaneously because soil aggregates can be anaerobic microsites of denitrification (Smith, 1980). Additionally, nitrogen fertilizers also stimulate these microbial processes. On May 20, the magnitudes in daily N<sub>2</sub>O emissions ( $\text{g N}_2\text{O-N ha}^{-1} \text{ day}^{-1}$ ) from soils reached:  $206.5 \pm 33.6$  (N0),  $466.6 \pm 39.7$  (N1) for CT and  $610.6 \pm 160.7$  (N0),  $625.6 \pm 43.5$  (N1) for RT.

Despite the possible differences in soil porosity and hydraulic conductivity of plough layers of CT and RT (Głab and Kulig, 2008; Lipiec et al., 2006), postponed peaks of N<sub>2</sub>O production in the plough layer of RT were not observed. Statistical results of Kruskal-Wallis test confirmed that the mean magnitudes of peaks in daily N<sub>2</sub>O emissions were significantly (at  $P = 0.05$ ) higher from soil in the above-mentioned treatments of RT than in those of CT. Higher concentrations of available mineral nitrogen and SOC could be accumulated near the soil surface in the plough layer of RT. Therefore, rain event of 8.2 mm could result in a formation of very short-term hotspots of denitrification in the plough layers of RT (Fuß et al., 2011).

During the post-harvest period, soil temperature, soil moisture content and water-filled pore space did not affect significantly the daily N<sub>2</sub>O fluxes from soil under both tillage systems. According to our studies, at the end of growing season (from May 27 to July 8) and throughout the post-harvest period (from July 11 to August 30) daily N<sub>2</sub>O emissions were low in all the treatments of CT and RT. Over the duration of the post-harvest period, the low daily N<sub>2</sub>O emissions can be possibly caused by unfavorably low SMC for nitrification: on average, from 10.8% to 12.7% for CT and from 10.9% to 11.7% for RT. Even favorable soil temperatures of 25 °C – 27 °C (Lee et al., 2009) were unable to unleash the remaining nitrification potential of soil nitrifying bacteria regardless of soil tillage system.

### Cumulative N<sub>2</sub>O fluxes from soil

CT and RT, apart the nitrogen fertilizer, did not cause significant differences in cumulative N<sub>2</sub>O fluxes from soil (Figure 5). Minor inter-tillage and inter-treatment differences in the above-mentioned soil properties resulted in the little relevant differences in the cumulative N<sub>2</sub>O fluxes. Besides, on the one hand, the high variations in the magnitudes of cumulative N<sub>2</sub>O fluxes from soils also did not allow evaluating possibly significant differences between CT and RT. On the other hand, the relatively dry entire period of the study with dominant process of nitrification did not contribute to the formation of a statistically greater effect of CT or RT on cumulative N<sub>2</sub>O fluxes from soils. Nevertheless, slightly higher N<sub>2</sub>O fluxes from soils under RT than under CT are in accordance with results of other studies



**Fig. 5.** Cumulative N<sub>2</sub>O fluxes from a loam Haplic Luvisol under conventional (CT) and reduced tillage (RT) in treatments without (N0) and with (N1) application of ammonium nitrate fertilizer at a rate of 25 kg N ha<sup>-1</sup> during the entire period of the study in 2013.

(Beheydt et al., 2008; Fuß et al., 2011; Horák et al., 2019; Ussiri et al., 2009).

During the growing season, cumulative N<sub>2</sub>O fluxes, as fractions of their total ones, accounted for 98.4% (N0), 98.6% (N1) for CT and 99.0% (N0), 99.4% (N1) for RT. The coupled autotrophic and heterotrophic respiration was strongly related with the processes of nitrification and denitrification in soils under red clover. The results of our recent studies in the same location in 2012 showed that seasonal cumulative N<sub>2</sub>O fluxes from the same soil under spring barley were much smaller: 12.8% (both N0 and N1) for CT and 15.9% (N0), 13.2% (N1) for RT (Horák et al., 2019). Authors reported that during the spring barley's post-harvest (dormant) period, huge fractions of cumulative N<sub>2</sub>O fluxes as fractions of their total fluxes had been recorded. However, the mitigation of these cumulative N<sub>2</sub>O fluxes can be achieved by growing non-legume cover crops if nitrogen fertilizers are not applied (Mitchell et al., 2013).

During the post-harvest period of red clover, the only heterotrophic respiration was more or less pronouncedly linked to the processes of nitrification and denitrification which did not have a large contribution to the formation of N<sub>2</sub>O and its cumulative fluxes from soils under CT and RT. These results showed that microbial mineralization of red clover roots enriched with a high content of organic nitrogen had a negligible effect on nitrification and denitrification at low SMC during the post-harvest period.

### CONCLUSIONS

Our results show that soil temperature was a better predictor of changes in daily CO<sub>2</sub> emissions from soils under CT and RT at the beginning of growing season of red clover. Over the duration of post-harvest period, water-filled pore space was the better predictor of daily CO<sub>2</sub> emissions from soils under CT and RT. Soil temperature demonstrated its highest influence on daily N<sub>2</sub>O emissions only at the very beginning of the growing season in both tillage systems.

The conventional and reduced tillage did not cause significant differences in cumulative N<sub>2</sub>O and CO<sub>2</sub> fluxes from soil in the treatments with and without nitrogen fertilizer.

There was a risk of higher cumulative N<sub>2</sub>O fluxes from soils after application of ammonium nitrate fertilizer in the RT system compared to those in the CT system. The cumulative CO<sub>2</sub> fluxes tended to be higher in the treatment with nitrogen fertilizers of soil under CT. The highest gaseous cumulative losses of nitrogen (N<sub>2</sub>O) and carbon (CO<sub>2</sub>) from soils under CT and RT were recorded during the growing season of red clover.

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