Impacts of crop rotational diversity and grazing under integrated crop-livestock system on soil surface greenhouse gas fluxes

Gandura Omar Abagandura1, Songül Şentürklu2,3, Navdeep Singh1, Sandeep Kumar1*, Douglas G. Landblom2, Kris Ringwall2

1 Department of Agronomy, Horticulture and Plant Science, South Dakota State University, Brookings, South Dakota, United States of America, 2 Dickinson Research Extension Center, North Dakota State University, Dickinson, North Dakota, United States of America, 3 Animal Science Department, Canakkale Onsekiz Mart Universitesi, Canakkale, Turkey

* sandeep.kumar@sdstate.edu

Abstract

Integrated crop-livestock (ICL) system is beneficial in enhancing soil organic carbon and nutrient cycling. However, the benefits of the ICL system on mitigation of GHG emissions are poorly understood. Thus, the present study was initiated in 2011 to assess the effect of crop rotation diversity and grazing managed under the ICL system on GHG emissions. The cropping system investigated here included spring wheat grown continuously for five years and a 5-yr crop rotation (spring wheat-cover crops-corn-pea/barley-sunflower). Each phase was present each year. Yearling steers grazed only the pea/barley, corn and cover crops plots in 2016 and 2017. Exclusion areas avoided the grazing in these crops to compare the GHG fluxes under grazed vs. non-grazed areas. The GHG fluxes were measured weekly from all crop phases during the growing season for both years using a static chamber. Cumulative CO2 and CH4 fluxes were similar from all crop phases over the study period. However, continuous spring wheat recorded higher cumulative N2O fluxes (671 g N ha⁻¹) than that under spring wheat in rotation (571 g N ha⁻¹). Grazing decreased cumulative CO2 fluxes (359 kg C ha⁻¹) compared to ungrazed (409 kg C ha⁻¹), however, no effect from grazing on cumulative CH4 and N2O fluxes over the study period were found. The present study shows that grazing and crop rotational diversity affected carbon and nitrogen inputs, which in turn affected soil CO2 and N2O fluxes. Long-term monitoring is needed to evaluate the response of soil GHG emissions to grazing and crop rotation interactions under the ICL system.

Introduction

Increasing atmospheric concentrations of greenhouse gases [GHG, particularly carbon dioxide (CO2), methane (CH4), and nitrous oxide (N2O)] are contributing to increasing trends in global warming [1]. Agriculture is one of the sources of worldwide GHG emissions, accounting for 9% of total U.S. GHG emissions [2]. Integrated crop-livestock (ICL) system can play a
prominent role in GHG emissions mitigation [3–5]. Few examples of commonly implemented ICL system in the U.S. include: animal grazing of cover crops within cash crop rotations, crop residue grazing, silvopasture and agroforestry, sod based crop rotation (perennial forage for grazing with crops), dual purpose cereal crops (harvesting for grains followed by grazing e.g. corn) [6]. Crop rotation and grazing under the ICL system can impact soil organic carbon (SOC), crop residue mineralization, and root and microbial respiration [7–8], thus play a major role in regulating soil surface GHG emissions [9].

Crop residues returned to the soil are the main input in maintaining SOC, which generally seems to increase with the diversified crop rotation compared to the mono-cropping [10–11], which if employed continuously increases the need for off-farm inputs due to increased infestation of weeds, diseases, and pests [12–13]. Crop rotation affects numerous soil properties, including water holding capacity, nutrient availability, and soil structure [14–15, 9], all of those can influence soil GHG emissions. For example, crop type can impact soil temperature and water content by affecting shade intensity and evapotranspiration [16, 11].

There are few studies investigating the effect of crop rotational diversity on GHG emissions from different plant species within the rotation. Wegner et al. [17], who monitored GHG fluxes under corn and soybean rotation (both phases were present each year) for three years, found that corn and soybean emitted similar soil CH$_4$ fluxes each year. In addition, Lehman et al. [18] reported no significant differences on N$_2$O fluxes from different plant species (corn-pea-winter wheat-soybean, each phase was present every year) within the rotation for four years. However, Halvorson and Del Grosso [19] reported that CO$_2$ emissions were influenced by plant species within the rotation in Northeastern Colorado, with barley (corn-barley rotation) emitting higher cumulative CO$_2$ flux compared to corn (continuous corn) and dry beans (corn-dry beans rotation) in the same year. These differences on cumulative CO$_2$ flux were attributed to the amount and quality of decomposing residue from the previous crops.

Other studies have compared soil GHG fluxes from a crop grown in rotation with a similar crop grown in mono-cropping, and their results were contradictory. For example, corn in rotation has shown to reduce total N$_2$O as well as CO$_2$ emissions compared with continuous corn [20–22] due to the increased fertilizer input from the mono-cropping. Sainju et al. [16] reported higher CH$_4$ uptake by barley from a barley–pea rotation than from continuous barley. However, other studies have reported no effect on soil GHG from crop rotation compared to mono-cropping. According to Barton et al. [23], wheat in rotation (lupin–wheat) has been found to emit similar soil N$_2$O fluxes compared with continuous wheat after two years. Behnke et al. [24] reported that corn emitted similar soil CO$_2$ fluxes from continuous corn, soybean-corn and soybean-wheat-corn rotation. Such discrepancies suggest that the response of GHG emissions to crop rotational diversity may vary with different crop diversity, climate and soil type.

Crop rotation, especially forage crops, can offer livestock mixed grazing pastures. Grazing, which is an important component in the ICL system, is used to control weeds and pests, reduce feed costs, and increase nutrient turnover rate [25]. Grazing can impact soil GHG by modifying canopy structure and residue accumulation [26–27]. In addition, grazing alters soil temperature and water content by consuming crop residues [28–29], both of which in turn affect soil microbial processes, the primary processes responsible for soil GHG fluxes [30–32]. The material added to the soil from animal excreta deposition (dung and urine) as well as the soil compaction increased by the movement of the animals can create anaerobic environments suitable for denitrification, resulting in the acceleration of soil GHG fluxes [33–34].

Several studies in the past have reported that grazing can enhance soil GHG fluxes [35–38], however, Zhong et al. [34] found that five years of grazing did not affect N$_2$O emissions compared to ungrazed plots. Other studies have reported that grazing decreased soil CO$_2$ fluxes.
compared to ungrazed soil [39–41]. Such a reduction was attributed to various, but presently uncertain, issues that include a decrease of (1) the diffusion of CO$_2$ in soil (due to the increase in soil bulk density), (2) the SOC (grazing can remove the biomass, thus reducing C input), and (3) activities of soil organisms (grazing would deplete SOC and depress microbial activities). Wolf et al. [42] reported that grazing decreased the emissions of N$_2$O due to the reduction in soil organic matter and soil moisture, especially in arid and semi-arid regions. No significant difference in CH$_4$ flux between grazed and ungrazed plots was reported by Wang et al. [43]. Typically, upland agricultural soils are minor emitters or minor sinks for CH$_4$ [17], therefore, grazing usually has minor effects on CH$_4$ flux in these systems. These results emphasize the need to explain the mechanistic reasons for differences in the impact of grazing on soil GHG fluxes.

Current knowledge cannot explain the mechanisms responsible for the crop rotational diversity and grazing effects under the ICL system on soil GHG emissions in the semi-arid region of the Northern Great Plains. The specific objectives of this study were to study the mechanisms affecting soil GHG emission from (i) different plant species within the rotation, (ii) crop rotation and mono-cropping, and (iii) grazing during the growing season.

**Materials and methods**

**Site description**

A field experiment to investigate the effect of crop rotation and grazing under the ICL system on soil GHG fluxes was initiated in 2011 at the Dickinson Research Extension Center in Dunn County, North Dakota (46°53′N, 102°49′W). The present study was conducted for 2 yr: 2016 and 2017 on a Vebar Series (coarse-loamy, mixed, superactive, frigid Typic Haplustolls) and on a Savage Series (fine, smectitic, frigid Vertic Argiustolls). To minimize the impact of soil on the analysis of the ICL system effects, two replicates were assigned to one soil type and the third replicate was assigned to the other soil for each treatment. This study area is representative of a typical rainfed farming system in North Dakota. The experimental site was characterized as a continental climate with moist springs (April, May, and June), relatively dry summers (August and September), and cold and snowy winters (November through March). Before the initiation of the experiment, basic soil characterization data were collected and represented in Table 1.

**Crop rotation treatments**

The cropping system investigated here included mono-cropping system (spring wheat [*Triticum aestivum* L.]) grown continuously for five years and a 5-yr crop rotation (spring wheat-cover crop-corn [*Zea mays* L.]-pea [*Pisum sativum* L.]/barley [*Hordeum vulgare* L.]-sunflower.

| Crop                | pH | EC$^a$ (dS m$^{-1}$) | OM (% ) | N (kg ha$^{-1}$) | P (ppm) |
|---------------------|----|----------------------|---------|------------------|---------|
| Continuous wheat    | 5.43 | 0.33                 | 3.37    | 27.24            | 25.70   |
| Wheat               | 5.93 | 0.29                 | 2.78    | 20.74            | 26.70   |
| Pea/barley          | 5.60 | 0.38                 | 3.83    | 14.23            | 28.30   |
| Sunflower           | 6.27 | 0.34                 | 3.60    | 18.27            | 31.70   |
| Cover crops         | 5.43 | 0.27                 | 3.30    | 15.13            | 23.00   |
| Corn                | 5.55 | 0.26                 | 3.31    | 16.23            | 26.32   |

**Analysis of variance $P > F$**

|                     | $P > F$ |
|---------------------|---------|
|                     | 0.640   |
| 0.260               | 0.340   |
| 0.220               | 0.800   |

$^a$EC, electrical conductivity; OM, organic matter; N, nitrogen; P, phosphorus.

https://doi.org/10.1371/journal.pone.0217069.t001
Helianthus annuus L.), a total of 6 main plots, with three replicates. Species of cover crops used in this study are listed in S1 Table. Every phase of the crop was present each year. The pea/barley, corn and cover crops plots were split into two areas in 2016 and 2017 (grazed and ungrazed), where grazing occurred only for these three species. Treatments were arranged in a randomized complete block in 18 uniform rectangular 1.74 ha (31.3 ha in total) plots. All crops were seeded with a John Deere 1590 no-till drill (Deere & Company, Moline, IL). For the pea/barley field pea (Arvika, var.) mix, seeding rate was 67.2 kg ha\(^{-1}\) and the forage barley (Stockford var.) was seeded at the rate of 44.8 kg ha\(^{-1}\) resulting in a combined 3,087,500 estimated plants ha\(^{-1}\). Crop management decisions, based on soil test results and recommendations from the NDSU Soil Testing Laboratory, indicated that no nitrogen fertilizer needed to be applied to the crops in 2016 and 2017. However, herbicides for grass and broadleaf weed control were applied, as needed for weed control. All crops were grown under rain fed condition, and no irrigation was applied. Crop managements are included in Table 2.

### Grazing treatments

Spring wheat and sunflower were cash crops in the cropping system, and yearling crossbred beef steers grazed the pea/barley, corn and cover crops plots in 2016 and 2017. When grazing started, the experimental design was modified to incomplete split-plot with pea/barley, corn and cover crops plots split in two grazing treatments (grazed and ungrazed). Protocols were reviewed and approved for animal use in this investigation by the North Dakota State University Institutional Animal Care and Use Committee (Protocol Approval #A16015). Before grazing started in the experimental plots in 2016 and 2017, the yearling beef cattle steers grazed native range from May 4 to July 11 (68 d) in both years to allow adequate time for the annual forages that were to be grazed to attain sufficient growth before grazing began. Based on crop growth, grazing in both years began in the pea/barley plot first before moving to the corn and finally to the cover crops plots. Based on best grazing management practices, the harvest efficiency goal was to utilize approximately one-half or the available forage and leave one-half for soil armor, resulting in the

| Crop                  | Planting Date | Population (plants ha\(^{-1}\)) | Seeding depth (cm) | Hybrid | Row spacing (cm) | Harvest date | Grazing start date | Grazing end date |
|-----------------------|---------------|---------------------------------|--------------------|--------|------------------|--------------|-------------------|------------------|
| Continuous wheat      | 9-May         | 3,087,500                       | 2.54               | Barlow | 19.10            | 16-Aug.      |                   |                  |
| Wheat                 | 9-May         | 3,087,500                       | 2.54               | Barlow | 19.10            | 16-Aug.      |                   |                  |
| Sunflower             | 9-May         | 49,400                          | 5.08               | 60ME80 | 76.20            | 9-Nov.       |                   |                  |
| Pea/barley            | 9-May         | 3,087,500                       | 3.81               | Mixed  | 19.10            | 20-July      | 16-Aug.           |                  |
| Corn                  | 21-May        | 49,400                          | 5.08               | Master graze BMR | 76.20      | 16-Aug.           | 5-Oct.           |
| Cover crops           | 13-July       | 3,730,170                       | 2.54               | 13 species | 19.10      | 5-Oct.           | 11-Nov.          |
| Continuous wheat      | 3-May         | 3,087,500                       | 2.54               | Barlow | 19.10            | 18-Aug.      |                   |                  |
| Wheat                 | 3-May         | 3,087,500                       | 2.54               | Barlow | 19.10            | 18-Aug.      |                   |                  |
| Sunflower             | 15-May        | 49,400                          | 5.08               | 60ME80 | 76.20            | 19-Oct.      |                   |                  |
| Pea/barley            | 1-May         | 3,087,500                       | 2.54               | Mixed  | 19.10            | 11-July      | 16-Aug.           |                  |
| Corn                  | 9-May         | 49,400                          | 5.08               | Master graze BMR | 76.20      | 16-Aug.           | 23-Sep.          |
| Cover crops           | 15-June       | 3,730,170                       | 2.54               | 13 species | 19.10      | 23-Sep.          | 23-Oct.          |

https://doi.org/10.1371/journal.pone.0217069.t002
number of hectares grazed per steer per month of 0.211, 0.143, and 0.25 for pea/barley, corn, and cover crops, respectively. Grazing information including the beginning and end of grazing are included in Table 2. The yearling steers grazed in the appropriate fields at all times from the beginning of the grazing until they were moved to the next plot.

Soil sampling and analysis
At planting in 2016 and 2017, four samples from random spots in each plot, including grazed and ungrazed plots from 2016 grazing, were collected at 0–5 cm depth using a push probe auger (3.2-cm diam.), composited and air-dried at ambient temperature, and ground to pass through a 2-mm screen for determining SOC and total nitrogen (TN) using the method outlined by Nelson and Sommers [44]. In addition, soil bulk density from all plots at planting in 2016 and 2017 were measured from 0–5 cm depth using the core method [45]. No soil samples were collected after 2017 grazing.

Measurements and analysis of GHG fluxes
Soil GHG fluxes from all the plots were measured for 2016 and 2017 using the static chamber technique described by Parkin and Venterea [46]. The chambers, which were made of polyvinyl chloride (PVC) pipe anchors (20.3 internal diameter and 15 cm height), were installed in each plot at the beginning of the study and remained undisturbed during the entire monitoring period except for temporary removal when mechanized farm operations were performed. A minimum of 24 h elapsed before resumption of sampling activities following chamber installation. The gas samples were collected once a week depending on weather conditions from June through October of 2016 and 2017. The GHG fluxes were measured only during the growing seasons, and the sampling was discontinued during periods when the installation of the chambers on the collars would have necessitated disturbance of the snow cover causing a non-representative sampling location (≈ four months each year). Sampling was typically done between 8:00 am and noon, with gas samplings being taken during the same period each day to reduce the diurnal effect of temperature on GHG fluxes. A PVC cap with a vent tube and sampling port were placed on anchors prior to taking the gas samples. A lid was used to keep these chambers closed during the gas sample collection. The gas samples from the chamber were collected with a syringe (10 mL) at 0, 20 and 40 minutes and transferred into 10 mL glass vials sealed with butyl rubber septa pre-charged inert argon gas that was removed by needle puncture prior to transfer of collected gas samples from the static chambers. Two chambers were installed per plot to reduce spatial variability in GHG measurement, and the average value was used for each treatment for data analysis.

Air chamber temperature during the time of sampling from each chamber was measured for calculating the GHG fluxes. The gas samples were analyzed using a gas chromatograph (CombiPAL; CTCAnalytics, Zwingen, Switzerland), which was fully automated with thermocconductivity, flame ionization, and electron capture detectors for analysis of CO$_2$, CH$_4$, and N$_2$O concentrations in one gas sample. Daily GHG flux was calculated as:

$$F = \rho \left( \frac{V}{A} \right) \left( \frac{\Delta c}{\Delta t} \right) \frac{273}{(273 + T)}$$

where $F$ is gas flux, $\rho$ is gas density under normal conditions (mg m$^{-3}$), $V$ is the volume of the static chamber (m$^3$), $A$ is the area that the static chamber covered, $\Delta c/\Delta t$ is changes in gas concentration (A$c$) during a certain time (At), and $T$ is mean temperature inside the chamber. Cumulative fluxes for each crop growing season in a year was calculated using linear interpolation.
As a supplement to gas flux measurements, soil water content and temperature at the 0 to 5 cm depth were measured using a HH2 moisture sensor (Delta-T-Devices, Cambridge, England) and a thermometer (Acurite Digital Meat Thermometer, 00641W, AcuRite Company, Geneva, WI), respectively. Average daily minimum and maximum air temperature and precipitation (mm) for each year (2016 and 2017) were collected from a weather station located approximately 25 km from the field.

**Statistical analysis**

Data were normal (Skewness and Kurtosis’s tests) and homogeneous (Levene’s test) for all the variables. To determine the effect of crop rotational diversity and grazing, data for GHG fluxes were analyzed using the GLIMMIX procedure in SAS (SAS Institute Inc., North Carolina, U. S.). Sampling date, year, soil, crop and their replicates were defined as random variables. Fixed effects in the model included crop phase ‘nested’ within grazing. Mean values were separated using pairwise differences method (adjusted by Tukey). Analysis of variance (ANOVA) was conducted to investigate the effect of crop rotational diversity and grazing on average soil temperature and soil water content. Linear ($Y = B_0 + B_1X_1$) and multiple linear regression analysis ($Y = B_0 + B_1X_1 + B_2X_2$) were conducted to examine the relationship between soil temperature and soil water content with CO$_2$ and N$_2$O fluxes using SIGMA PLOT 14.0. Differences were considered significant at 0.05 probability.

**Results**

**Climate, soil temperature and water content**

The mean values of precipitation and air temperature for 2016 and 2017 are shown in Fig 1. Average (30-yr) air temperature at the site was 6.2˚C, and mean annual precipitation was 610
mm. Total precipitation in 2016 (740.7 mm) was 29.7% higher than in 2017 (520.3 mm) (Fig 1). Generally, the spring and summer of 2016 were unusually wet with higher precipitation throughout the growing season. The precipitation during the growing season (May–October) was greater in 2016 than in 2017. Air temperature was similar during the growing season in the two years. The air temperature values at the beginning of the growing seasons for both years rose before falling later in the season (Fig 1). Soil temperature and water content (S1 and S2 Figs) were not affected by crop rotational diversity and grazing over the study period (averaged across measurements dates, \( p > 0.05 \)). The linear regressions for the CO\(_2\) and N\(_2\)O fluxes and soil temperature showed a significant positive correlation between soil temperature and the CO\(_2\) fluxes (\( R^2 = 0.63 \) and \( p = 0.021 \)) and N\(_2\)O fluxes (\( R^2 = 0.71 \) and \( p = 0.011 \)) over the two years of this study. However, non-significant correlations between soil water content and CO\(_2\) fluxes (\( R^2 = 0.16 \) and \( p = 0.121 \)) and N\(_2\)O fluxes (\( R^2 = 0.21 \) and \( p = 0.834 \)) were found in this study. The CO\(_2\) and N\(_2\)O fluxes plotted against soil temperature and water content over the study period are shown in Fig 2. The results from multiple regression analysis showed significant positive correlations between the combination of soil water content and soil temperature with CO\(_2\) and N\(_2\)O fluxes (\( p < 0.001 \) for CO\(_2\) fluxes and \( p = 0.0051 \) for N\(_2\)O fluxes). This combination (soil temperature and water content) can explain up to 26% of the variation in CO\(_2\) fluxes and 64% of the variation in N\(_2\)O fluxes. Maximum CO\(_2\) and N\(_2\)O fluxes appeared generally at soil water content >26% and soil temperatures warmer than 27°C (Fig 2).

Effect of crop rotational diversity on GHG fluxes

Daily GHG fluxes based on crop phases in 2016 and 2017 are shown in Fig 3. Regardless of crop phases, soil GHG fluxes were lower during the 2016 than the 2017 season (Fig 3). Cumulative GHG fluxes from all crop phases were higher in 2017 compared to the 2016 (Table 3). The trend in soil CO\(_2\) fluxes under all the crop phases exhibited the same pattern for all sampling dates (Fig 3). The highest peak of CO\(_2\) flux over the two years from crop phases was recorded on August 14, 2017 (Fig 3) under pea/barley (56.8 kg C ha\(^{-1}\) d\(^{-1}\)), corn (52.1 kg C ha\(^{-1}\) d\(^{-1}\),

![Fig 2. Effects of soil temperature (°C) and soil water content (%) on CO\(_2\) and N\(_2\)O fluxes over the study period (2016 and 2017).](https://doi.org/10.1371/journal.pone.0217069.g002)
d\(^{-1}\), continuous spring wheat (40.7 kg C ha\(^{-1}\) d\(^{-1}\)), spring wheat (37.8 kg C ha\(^{-1}\) d\(^{-1}\)), sunflower (31.3 kg C ha\(^{-1}\) d\(^{-1}\)), and cover crops (28.7 kg C ha\(^{-1}\) d\(^{-1}\)). No significant differences in the daily CO\(_2\) flux was observed among all the crop phases during this peak (\(p = 0.5341\), Fig 3). For other sampling dates, no significant effect of crop rotational diversity (\(p \geq 0.05\) for each sampling date for each year) on CO\(_2\) fluxes was found. Cumulative CO\(_2\) fluxes exhibited no significant differences among all phases over the study period (Table 4). Comparing the effect of mono-cropping with rotation on CO\(_2\) flux revealed that continuous spring wheat resulted in similar cumulative CO\(_2\) flux compared to spring wheat over the study period (Table 4).

The trend of CH\(_4\) fluxes under crop phases varied on the sampling dates over the two years (Fig 3). Generally, peaks of CH\(_4\) release were observed after rainfall events, while peaks

![Graph](https://doi.org/10.1371/journal.pone.0217069.g003)

**Fig 3. Trend of CO\(_2\), CH\(_4\) and N\(_2\)O fluxes in the 2016 and 2017 growing seasons as influenced by crop rotational diversity.**

**Table 3. Significance of F values for year for GHG cumulative fluxes.**

| Nparm | DF | Sum of squares | F ratio | Prob > F |
|-------|----|----------------|---------|----------|
| CO\(_2\) | 1 | 459272.7 | 2.18 | 0.0148 |
| CH\(_4\) | 1 | 5550524.0 | 13.49 | 0.0008 |
| N\(_2\)O | 1 | 1749469.2 | 10.53 | 0.0026 |

[https://doi.org/10.1371/journal.pone.0217069.t003](https://doi.org/10.1371/journal.pone.0217069.t003)
of CH₄ uptake corresponded with an increase in soil temperature. No significant effects were observed for all phases on the CH₄ fluxes during these peaks and other sampling dates for both years (p > 0.05 for all sampling dates). Although not significant, CH₄ effluxes have negative peaks very much pronounced for corn and wheat compared to other crops. In addition, no significant effects from crop rotational diversity on cumulative CH₄ fluxes were observed (Table 4).

Similar to the trend of the CO₂ fluxes, the variation in N₂O fluxes exhibited the same pattern under all phases on all sampling dates for both years (Fig 3). The highest peak of N₂O flux over the two years was recorded on August 14, 2017, the same date as the highest peak of CO₂ flux (Fig 3). This N₂O peak occurred under pea/barley (51.85 g N ha⁻¹ d⁻¹), a peak that was significantly higher (p = 0.001) than for the other phases (27.1 g N ha⁻¹ d⁻¹ for corn, 26.7 g N ha⁻¹ d⁻¹ for continuous spring wheat, 19.6 g N ha⁻¹ d⁻¹ for spring wheat, 11.3 g N ha⁻¹ d⁻¹ for sunflower, and 23.2 g N ha⁻¹ d⁻¹ for cover crops). Except for the peak in N₂O fluxes, no significant effect of crop rotational diversity (p > 0.05 for each sampling date) on cumulative CH₄ fluxes was observed (Table 4).

Similar to the trend of the CO₂ fluxes, the variation in N₂O fluxes exhibited the same pattern under all phases on all sampling dates for both years (Fig 3). The highest peak of N₂O flux over the two years was recorded on August 14, 2017, the same date as the highest peak of CO₂ flux (Fig 3). This N₂O peak occurred under pea/barley (51.85 g N ha⁻¹ d⁻¹), a peak that was significantly higher (p = 0.001) than for the other phases (27.1 g N ha⁻¹ d⁻¹ for corn, 26.7 g N ha⁻¹ d⁻¹ for continuous spring wheat, 19.6 g N ha⁻¹ d⁻¹ for spring wheat, 11.3 g N ha⁻¹ d⁻¹ for sunflower, and 23.2 g N ha⁻¹ d⁻¹ for cover crops). Except for the peak in N₂O fluxes, no significant effect of crop rotational diversity (p > 0.05 for each sampling date) on cumulative CH₄ fluxes was observed (Table 4).

Effect of grazing on GHG fluxes

The GHG fluxes based on grazing in 2016 and 2017 are shown in Fig 4. Cumulative GHG fluxes from grazing are listed in Table 4. Similar to the trend in daily soil CO₂ fluxes under

Table 4. Cumulative soil surface CO₂, CH₄ and N₂O fluxes influenced by crop rotational diversity and grazing over the study period.

| Rotation                | CO₂ (kg C ha⁻¹) | CH₄ (g C ha⁻¹) | N₂O (g N ha⁻¹) |
|-------------------------|-----------------|----------------|---------------|
| Continuous wheat        | 1570.17a        | 520.54a        | 671.46b       |
| Wheat                   | 1583.73a        | 128.18a        | 571.57c       |
| Pea/barley              | 1889.85a        | 369.32a        | 1071.40a      |
| Sunflower               | 1420.87a        | 416.91a        | 500.89c       |
| Cover crops             | 1454.96a        | 528.74a        | 746.94b       |
| Corn                    | 1777.02a        | 609.42a        | 722.74b       |

| Grazingb | CO₂ (kg C ha⁻¹) | CH₄ (g C ha⁻¹) | N₂O (g N ha⁻¹) |
|----------|-----------------|----------------|---------------|
| Pea/barley Grazed | 642.91a | -45.46a | 443.81a       |
| Ungrazed | 642.08a         | 53.07a        | 444.51a       |
| Corn     Grazed | 349.18b | 214.54a | 252.41a       |
| Ungrazed | 459.17a         | 122.38a       | 187.46a       |
| Cover crops Grazed | 85.78b | 118.87a | 51.38a        |
| Ungrazed | 126.18a         | -87.50a       | 148.80a       |
| Average  Grazed | 359.29b | 95.90a | 249.20a       |
| Ungrazed | 409.14a         | 29.31a        | 260.29a       |

aWithin a column, values followed by the same letters are not significantly different at a = 0.05.
bCumulative GHG was only for the period when grazing occurred.

https://doi.org/10.1371/journal.pone.0217069.t004
crop phases in rotation, the trend in daily soil CO$_2$ fluxes under grazing treatments appeared to be similar on all sampling dates (Fig 4). On August 14, 2017, the same date when the highest peak was recorded under crop phases in rotation, the highest peak of CO$_2$ flux over the two years was recorded for the pea/barley plots (Figs 3 and 4). No significant differences ($p = 0.543$) on the daily CO$_2$ fluxes were recorded during this peak (53.3 kg C ha$^{-1}$ d$^{-1}$ for
grazed plots and 56.6 kg C ha\(^{-1}\) d\(^{-1}\) for ungrazed plots, Fig 4). For other sampling dates, no significant effect of grazing (\(p \geq 0.05\) for each sampling date for each year) on CO\(_2\) fluxes was found. Regardless of the grazed crop, cumulative CO\(_2\) flux was significantly lower under grazed plots than under ungrazed plots (Table 4). The trend and the peaks of the CH\(_4\) fluxes under grazing treatments over the two years (Fig 4) were similar to the trend and the peaks of the CH\(_4\) fluxes under crop phases in rotation (Fig 4). No significant effects were observed for grazing on the cumulative CH\(_4\) fluxes (Table 4).

The highest peak of N\(_2\)O flux over the two years was recorded on August 14, 2017 from the pea/barley plots (Fig 4), with ungrazed plots emitting similar N\(_2\)O flux (51.9 g N ha\(^{-1}\) d\(^{-1}\)) compared to the grazed plots (50.3 g N ha\(^{-1}\) d\(^{-1}\)). For other sampling dates, no significant effect of grazing (\(p \geq 0.05\) for each sampling date for each year) on N\(_2\)O fluxes was observed. Grazing did not affect cumulative N\(_2\)O flux as seen in Table 4.

**Discussion**

**Soil temperature and water content**

While the daily trend of soil temperature was similar to that of the maximum and minimum air temperature under crop phases in rotation and grazing during the growing season for both years, the daily trend of soil water content was highly influenced by precipitation events and varied with the measurement dates. Soil temperature and water content were not affected by crop rotational diversity over the study period. Soil organic carbon for mono-cropping and rotation indicated that both practices have similar SOC, perhaps explaining why continuous spring wheat recorded similar soil temperature and soil moisture compared to spring wheat in this reported study. Significant changes in SOC may need long-term study to be detected. King and Blesh [10] reported that enhancing SOC can improve soil water content and temperature. The continuous cropping of a similar crop leads to retarded plant growth, serious pest and disease damage, and low crop productivity [47], therefore, it was expected that continuous spring wheat would record lower SOC compared to spring wheat, however, both had similar SOC. Long-term crop rotation study (>5 years) might show the negative effects of mono-cropping on plant yield. Fu et al. [48], who investigated the 30-yr effect of crop rotation (alfalfa–potato and winter wheat) compared with mono-cropping (continuous winter wheat), reported that wheat in rotation increased SOC compared to continuous wheat in the semiarid climate. This difference in the effect of crop rotation on SOC between our study and Fu et al. [48] can be attributed to the differences in crops used in the rotation and the length of the rotation study.

Soil temperature and water content were not affected by grazing. Grazing can alter soil temperature by increasing the radiant energy reaching it, leading to higher soil temperature. Risch et al. [49] also reported that grazing grass for five years did not affect the temperature of the soil (Mollisols, like the studied soil) compared to ungrazed plots in Wyoming. In addition, soil water content was not affected by grazing, confirming the results found by Barsotti et al. [25], who conducted a study in Montana (dryland cropping systems) on silt loam soil, reported that grazing spring wheat, pea/barley hay, and alfalfa for 11 years did not affect soil water content compared to ungrazed soil. Conversely, soil water content in an ungrazed pasture was higher than in a grazed one (grazing for more than 13 years) in other studies [50–51], which they attributed to the greater accumulation of litter in the ungrazed plots decreasing evaporation and thus, increasing soil water content in these plots.

**Effect of crop rotational diversity on GHG fluxes**

Soil GHG fluxes under crop phases were lower during the 2016 season than the 2017 season, a result partially attributed to the higher soil temperature in 2017. Higher soil temperature can
stimulate microbial activity and C and N mineralization, causing an increase in GHG fluxes [52–53,16]. The positive correlation between soil temperature and the CO$_2$ and N$_2$O fluxes over the two years emphasizes the important role of this parameter in their productions. Higher temperature can enhance the microbial activity needed to breakdown the organic matter and hence increase CO$_2$ production. The emission of N$_2$O from soils is primarily caused by microbial nitrification and denitrification, both of which are controlled by temperature: more specifically, the lower the temperature, the lower the soil microbial activity, resulting in reduced N$_2$O emissions [17]. Our previous study [54] conducted in South Dakota on fine silty soil reported that GHG emissions increased with the increase in soil temperature due to the increase in the microbial activity and C and N mineralization, causing an increase in GHG emissions, consistent with our results.

The variation in soil CO$_2$ fluxes under all crop phases appeared to be related to the trends in soil temperature or water content for both years. The peak of CO$_2$ that observed in 2017 and not in 2016 was a response to an increase in soil temperature after precipitation events. Soil CO$_2$ from root respiration is a primary contributor to total soil respiration rates in most soils, it is likely that higher soil temperature increased root respiration and microbial activities which increased CO$_2$ emission in this year compared to 2016. These results were supported by Lee et al. [55], who reported that a higher soil temperature corresponded with a higher soil respiration rate due to the increase of the biological process. However, no significant effects of crop rotational diversity on the daily CO$_2$ fluxes for each sampling date for each year in this study were found. In addition, no significant differences between all phases on cumulative CO$_2$ fluxes were found, results that may be due to the absence effect of crop rotational diversity on soil temperature over the study period. However, Carvalho et al. [56], Rochette and Janzen [57] and Brock et al [58] reported that the decomposition of the previous crop residues in the crop rotation can influence GHG fluxes. Other parameter that could influence soil respiration included SOC; however, there was no significant effect on SOC by crop rotational diversity.

The variation in daily CH$_4$ fluxes under crop phases were not similar over the two years, varying both positively (atmospheric source) and negatively (atmospheric sink). Generally, peaks of CH$_4$ release were observed after rainfall events, while peaks of uptake corresponded with an increase in soil temperature. Ozlu and Kumar [54] and Lee et al. [55] reported that whether a soil is a source or a sink for CH$_4$ depends on the activity of the CH$_4$ release microorganisms (increases under anaerobic conditions) and the CH$_4$ uptake microorganisms (increases under aerobic conditions). Although not significant, CH$_4$ effluxes have negative peaks very much pronounced for corn and wheat compared to other crops. The reasons for higher soil CH$_4$ uptake for corn and wheat than in other phases in this study were not known. No significant effects of crop rotational diversity on daily and cumulative CH$_4$ fluxes were observed in this study, perhaps because of the non-significant effect of crop rotational diversity on soil water content. Similarly, Behnke et al. [20] conducted an experiment in Northwestern Illinois (cropping system) reported that a corn-soybean rotation had similar CH$_4$ fluxes from silty clay loam soil compared to continuous corn or continuous soybean systems alone after 17 years of rotation. In addition, crop rotational diversity did not affect soil bulk density (data not shown), which may explain the lack of effect from crop rotational diversity on CH$_4$ fluxes in this study. In well-drained mineral soils, diffusion of CH$_4$ flux into the soil is the main factor limiting the CH$_4$ flux. Any changes in soil aeration can significantly affect the CH$_4$ flux.

Similar to the trend of the daily CO$_2$ fluxes, the variation in N$_2$O fluxes showed the same pattern under crop phases on all sampling dates for both years. The highest peak of N$_2$O flux was attributed to the increase in soil temperature that occurred after heavy rainfall [59]. The N$_2$O flux was correlated with soil temperature and water content, suggesting N mineralization, and nitrification/denitrification processes may occur simultaneously, resulting in greater N$_2$O
flux. A significant effect during this peak was observed, with pea/barley recording higher $N_2O$ flux than the other phases. Legumes can increase $N_2O$ emission through atmospheric $N_2$ fixation by the rhizobia living in the root nodules. This fixed $N_2$ can be mineralized to release inorganic $N$ producing $N_2O$ when nitrified [60–62]. However, no significant effect of crop rotational diversity on other daily $N_2O$ fluxes was found. Although daily $N_2O$ fluxes were higher in continuous spring wheat compared to spring wheat for many days during 2016 and 2017, no significant differences in daily $N_2O$ fluxes between these two phases were observed. Unlike cumulative CO$_2$ fluxes, pea/barley recorded higher cumulative $N_2O$ flux than other phases, perhaps due to the ability of pea/barley to fix $N_2$, increasing $N_2O$ emissions compared to other phases as mentioned earlier. Leguminous crops can be a source of $N_2O$ emissions during residue decomposition because of their greater $N$ concentration than non-leguminous crops. The fact that leguminous plots had higher TN than other phases may explain the significant increase in cumulative $N_2O$ fluxes from leguminous plots compared to other plots. Sainju et al. [9] reported increased accumulation of total organic C from legumes compared to non-legumes. Comparing the effect of mono-cropping with crop rotation, continuous spring wheat resulted in greater cumulative $N_2O$ fluxes compared to spring wheat. Soil grown with continuous corn emitted higher $N_2O$ emission than soil grown with corn in rotation [30, 24]. Other soil parameters (like soil aggregates and enzyme activities) that affect GHG flux were not measured in this study. These properties may have been improved by crop rotation compared to mono-cropping as suggested by [63, 12], which can decrease soil $N_2O$ emissions.

Effect of grazing on GHG fluxes

The trend of soil CO$_2$ fluxes under grazing treatments (grazed and ungrazed) appeared to be related to trends in soil temperature or water content for both years as the CO$_2$ peaks under grazing were in response to an increase in the soil temperature that occurred after precipitation events. Wegner et al. [17], who conducted a study on fine silty soil in South Dakota from 2013 to 2015, reported that higher soil temperature can cause an increase in CO$_2$ fluxes due to the increase in microbial activity, C mineralization and root respiration, causing an increase in CO$_2$ production. No significant effects of grazing on the daily CO$_2$ fluxes for each sampling date for each year in this study were found. However, grazed plots recorded lower cumulative CO$_2$ fluxes than ungrazed plots. Cumulative CO$_2$ flux was reduced by grazing as a result of reduced C input since this biomass was removed by the grazing event, consistent with SOC being lower in grazed plot in 2016 ($p = 0.0210$). Cao et al. [64] reported that seasonal CO$_2$ flux was significantly higher in a low grazed site (grazing for 13 years) in China than in a high grazed one, a difference they attributed to the lower SOC in the high grazed site. Tang et al. [40] reported that heavy-grazing for ten years decreased soil CO$_2$ flux during spring-thaw period in desert steppe in China compared to ungrazed soils. It is possible that grazing in this study reduced the diffusion of CO$_2$ fluxes in soil, resulting in lower cumulative CO$_2$ fluxes from grazed plots compared to the ungrazed plots. The fact that grazed plots had higher soil bulk density than ungrazed plots in 2016 ($p = 0.0022$) supports this conclusion. On the other hand, grazing did not affect daily and cumulative CH$_4$, perhaps because of the similar soil water content between the grazing treatments. Paz-Ferreiro et al. [65] reported that sheep grazing for 14 years in Northern England (temperate weather) had no effect on CH$_4$ fluxes compared to ungrazed site.

The variation in $N_2O$ fluxes under grazing treatments showed the same pattern on all sampling dates for both years. Denef et al. [66] and Sainju et al. [16] reported that yearling steers feces and urine returned to the soil from grazing can enhance microbial activity and N mineralization. However, grazed plots emitted similar cumulative $N_2O$ flux compared to ungrazed
plots in this reported study. This result can be attributed to the lack effect from grazing on soil TN in 2016 ($p = 0.341$). Shaaban et al. [62] reported that soil TN content is critical in controlling soil N$_2$O emissions. On the other hand, Wolf et al. [42] reported that grazing decreased annual N$_2$O fluxes compared to ungrazed plots, which was attributed to the increase in the soil water content in the ungrazed plots, stimulating denitrifying microbial activity, causing the increase in the N$_2$O fluxes compared to grazed plots in this cited study.

Because only three crops were grazed in this reported study, these findings are not applicable to all other plant species within the rotation. In addition, spring wheat in mono-cropping and rotation systems was used as a cash crop in this study. Therefore, comparing the effects of grazing a crop grown in rotation with a similar crop grown in mono-cropping on GHG fluxes was not evaluated. More research is required to evaluate the response of GHG emissions to grazing with different crops and soil types under different climatic conditions.

Limitations of the study

Very limited studies are conducted across the world to evaluate the impacts of ICL system on soil surface GHG emissions. Nonetheless, like many other studies, this study had two limitations. First, the GHG fluxes were measured only during the growing season; therefore, the effect of ICL system on GHG emission is not quite conclusive. Measurements of GHG emissions over the entire year may be required to evaluate the effect of ICL system on GHG emissions. Second, this study did not include the emissions from the livestock (enteric), which can be a critical component in addressing the overall goal of the ICL system. Despite these limitations, however, we believe that this study provides important observational data assessing the effects of ICL system on soil GHG emissions.

Conclusions

This study was conducted to assess the impact of crop rotational diversity and grazing under an ICL system on soil surface GHG emissions, and to compare the effects of crop rotation and mono-cropping on soil GHG emissions. Differences in weather conditions between the two studied years influenced soil temperature, which, in turn, affected GHG emissions. The findings indicated that crop rotational diversity did not affect cumulative CO$_2$ and CH$_4$ emissions. Mono-cropping (continuous spring wheat) recorded higher cumulative N$_2$O fluxes than crop rotation (spring wheat). Grazing decreased cumulative CO$_2$ flux compared to ungrazed, however, cumulative N$_2$O and CH$_4$ fluxes were not affected by grazing. Further, long-term monitoring of GHG fluxes from plots under crop rotation and grazing with different crops under different climatic conditions is required to explore sustainable strategies for mitigating the agricultural emissions.

Supporting information

S1 Table. Species of cover crops used in this study for 2016 and 2017. (PDF)

S1 Fig. Soil temperature and water content as influenced by crop rotational diversity for the 2016 and 2017 growing seasons. (PDF)

S2 Fig. Soil temperature and water content as influenced by grazing during the grazing period for 2016 and 2017. (PDF)
S1 File. Daily means for greenhouse gas fluxes in 2016 and 2017 under different crop rotational diversity. (PDF)

S2 File. Daily means for greenhouse gas fluxes in 2016 and 2017 under grazing. (PDF)

Acknowledgments

This work was supported by the USDA National Institute of Food and Agriculture, Coordinated Agricultural Projects (CAP) (Award No. 2016-68004-24768) and the project entitled "Back to the Future: Enhancing food security and farm production with integrated crop-livestock production systems".

Author Contributions

Conceptualization: Sandeep Kumar.

Data curation: Douglas G. Landblom.

Formal analysis: Gandura Omar Abagandura.

Funding acquisition: Sandeep Kumar.

Investigation: Songül Şentürklu, Sandeep Kumar, Douglas G. Landblom.

Methodology: Douglas G. Landblom.

Project administration: Sandeep Kumar.

Software: Gandura Omar Abagandura, Navdeep Singh.

Supervision: Kris Ringwall.

Validation: Gandura Omar Abagandura, Sandeep Kumar.

Writing – original draft: Gandura Omar Abagandura, Navdeep Singh.

Writing – review & editing: Songül Şentürklu, Sandeep Kumar, Douglas G. Landblom.

References

1. IPCC. 2007. Climate change 2007. The physical science basis. 4th assessment report. IPCC Secretariat, Geneva.

2. USEPA. 2018. Sources of greenhouse gas emissions. Accessed October 1, 2018 United States Environmental Protection Agency.

3. Buller LS, Bergier I, Ortega E, Moraes A, Bayma-Silva G, Zanetti MR. Soil improvement and mitigation of greenhouse gas emissions for integrated crop–livestock systems: Case study assessment in the Pantanal savanna highland, Brazil. Agricultural Systems. 2015; 137: 206–219.

4. Cai Y, and Akiyama H. Nitrogen loss factors of nitrogen trace gas emissions and leaching from excreta patches in grassland ecosystems: A summary of available data. Sci. Total Environ. 2016; 572: 185–195. https://doi.org/10.1016/j.scitotenv.2016.07.222 PMID: 27498390

5. Salton JC, Mercante FM, Tomazzi M, Zanatta JA, Concenco G, Silva WM, et al. Integrated crop-livestock system in tropical Brazil: Toward a sustainable production system. Agric., Ecosyst. Environ. 2014; 190: 70–79.

6. Sulc RM, and Franzluebbers AJ. Exploring integrated crop–livestock systems in different ecoregions of the United States. Eur. J. Agron. 2014; 57: 21–30.

7. Hafner S, Unteregelsbacher S, Seeber E, Lena B, Xu X, Li X, et al. Effect of grazing on carbon stocks and assimilate partitioning in a Tibetan montane pasture revealed by 13 CO2 pulse labeling. GCB Bioenergy. 2012; 18: 528–538.
8. Hewins DB, Lyseng MP, Schoderbek DF, Alexander M, Willms WD, Carlyle CN, et al. Grazing and climate effects on soil organic carbon concentration and particle-size association in northern grasslands. Sci. Rep. 2018; 8: 1336. https://doi.org/10.1038/s41598-018-19785-1 PMID: 29358591

9. Sainju UM, Stevens WB, Caesar-TonThat T, Liebig MA, Wang J. Net global warming potential and greenhouse gas intensity influenced by irrigation, tillage, crop rotation, and nitrogen fertilization. J. Environ. Qual. 2014; 43: 777–788. https://doi.org/10.2134/jeq2013.10.0405 PMID: 25628807

10. King AE, and Blesh J. Crop rotations for increased soil carbon: perenniality as a guiding principle. Ecological Applications. 2018; 28: 249–261. https://doi.org/10.1002/eap.1648 PMID: 29112790

11. Sainju UM. A global meta-analysis on the impact of management practices on net global warming potential and greenhouse gas intensity from cropland soils. PLoS one. 2016; 11.

12. Kremen C, and Miles A. Ecosystem services in biologically diversified versus conventional farming systems: benefits, externalities, and trade-offs. Ecology and Society. 2012; 17.

13. Sainju UM. Can novel management practice improve soil and environmental quality and sustain crop yield simultaneously? PLoS one. 2016; 11.

14. Benitez MS, Osborne SL, Lehman RM. Previous crop and rotation history effects on maize seedling health and associated rhizosphere microbiome. Sci. Rep. 2017; 7: 15709. https://doi.org/10.1038/s41598-015-0311-z PMID: 29146930

15. West TO, and Post WM. Soil organic carbon sequestration rates by tillage and crop rotation. Soil Sci. Soc. Am. J. 2002; 66: 1930–1946.

16. Sainju UM, Stevens WB, Caesar-TonThat T, Liebig MA. Soil greenhouse gas emissions affected by irrigation, tillage, crop rotation, and nitrogen fertilization. J. Environ. Qual. 2012; 41: 1774–1786. https://doi.org/10.2134/jeq2012.0176 PMID: 23128735

17. Wegner BR, Chalise KS, Singh S, Lai L, Abagundura GO, Kumar S, et al. Response of soil surface greenhouse gas fluxes to crop residue removal and cover crops a corn–soybean rotation. J. Environ. Qual. 2018; 47: 1146–1154. https://doi.org/10.2134/jeq2018.03.0093 PMID: 30272788

18. Lehman RM, Osborne SL, Duke SE. Diversified no-till crop rotation reduces nitrous oxide emissions, increases soybean yields, and promotes soil carbon accrual. Soil Sci. Soc. Am. J. 2017; 81: 76–83.

19. Halvorson AD, Del Grosso, SJ. Nitrogen, tillage, and crop rotation effects on carbon dioxide and methane fluxes from irrigated cropping systems. J. Environ. Qual. 2009; 38: 023–2033.

20. Behnke GD, Zuber SM, Pittelkow CM, Nafziger ED, Villamil MB. Long-term crop rotation and tillage effects on soil greenhouse gas emissions and crop production in Illinois, USA. Agric., Ecosyst. Environ. 2018; 261: 62–70.

21. Omonode RA, Smith DR, Gál A, Vyn TJ. Soil nitrous oxide emissions in corn following three decades of tillage and rotation treatments. Soil Sci. Soc. Am. J. 2011; 75: 152–163.

22. Wilson HM, and Al-Kaisi MM. Crop rotation and nitrogen fertilization effect on soil CO2 emissions in central Iowa. Applied soil ecology. 2008; 39: 264–270.

23. Barton L, Murphy DV, Butterbach-Bahl K. Influence of crop rotation and liming on greenhouse gas emissions from a semi-arid soil. Agric., Ecosyst. Environ. 2013; 167: 23–32.

24. Behnke GD, Pittelkow CM, Nafziger ED, Villamil MB. Exploring the relationships between greenhouse gas emissions, yields, and soil properties in cropping systems. Agriculture. 2018; 8: 62.

25. Barsotti JL, Sainju UM, Lenssen AW, Montagne C, Hatfield PG. Net greenhouse gas emissions affected by sheep grazing in dryland cropping systems. Soil Sci. Soc. Am. J. 2013; 77: 1012–1025.

26. Drewry JJ, Cameron KC, Buchanan GD. Pasture yield and soil physical property responses to soil compaction from treading and grazing—a review. Soil Res. 2008; 46: 237–256.

27. Nawaz MF, Bourrie G, Trolard F. Soil compaction impact and modelling. A review. Agron. Sustainable Dev. 2013; 33: 291–309.

28. Wei D, Wang Y, Wang Y, Liu Y, Yao T. Responses of CO2, CH4 and N2O fluxes to livestock exclosure in an alpine steppe on the Tibetan Plateau, China. Plant and Soil. 2012; 359: 45–55.

29. Zhou X, Wan S, Luo Y. Source components and interannual variability of soil CO2 efflux under experimental warming and clipping in a grassland ecosystem. GCB Bioenergy. 2007; 13: 761–775.

30. Campbell B, Chen L, Dygert C, Dick W. Tillage and crop rotation impacts on greenhouse gas fluxes from soil at two long-term agronomic experimental sites in Ohio. J. Soil Water Conserv. 2014; 69: 543–552.

31. Chiavegato MB, Powers WJ, Carmichael D, Rowntree JE. Pasture-derived greenhouse gas emissions in cow-calf production systems 1. Journal of animal science. 2015; 93: 1350–64. https://doi.org/10.2273/jas.2014-8134 PMID: 26020912
32. Jia B, Zhou G, Wang Y, Wang F, Wang X. Effects of temperature and soil water-content on soil respiration of grazed and ungrazed Leymus chinensis steppes, Inner Mongolia. Journal of Arid Environments. 2006; 67: 60–76.

33. Liu Y, Yan C, Matthew C, Wood B, Hou F. Key sources and seasonal dynamics of greenhouse gas fluxes from yak grazing systems on the Qinghai-Tibetan Plateau. Sci. Rep. 2017; 7: 40857. https://doi.org/10.1038/srep40857 PMID: 28106070

34. Zhong L, Du R, Ding K, Kang X, Li FY, Bowatte S, et al. Effects of grazing on N2O production potential and abundance of nitrifying and denitrifying microbial communities in meadow-steppe grassland in northern China. Soil Biol. Biochem. 2014; 69: 1–10.

35. Boon A, Robinson S, Chadwick D, Cardenas L. Effect of cattle urine addition on the surface emissions and subsurface concentrations of greenhouse gases from a UK lowland peatland. In:EGU General Assembly Conference Abstracts. 2014.

36. da Silva Cardoso A, de Figueiredo Brito L, Januszkiewicz ER, da Silva Morgado E, Barbero RP, Koscheck JFW, et al. Impact of grazing intensity and seasons on greenhouse gas emissions in tropical grassland. Ecosystems. 2017; 20: 845–859.

37. Kebreab E, Clark K, Wagner-Riddle C, France J. Methane and nitrous oxide emissions from Canadian animal agriculture: A review. Can. J. Anim. Sci. 2006; 86: 135–157.

38. Rafique R, Hennessy D, Kiely G. Nitrous oxide emission from grazed grassland under different management systems. Ecosystems. 2011; 14: 563–582.

39. Chen W, Wolf B, Zhang Y, Yao Z, Butterbach-Bahl K, Brüggemann N, et al. Carbon dioxide emission from temperate semi-arid steppe during the non-growing season. Atmos. Environ. 2013; 64: 141–149.

40. Wu X, Yao Z, Brüggemann N, Shen ZY, Wolf B, Dannenmann M, et al. Effects of soil moisture and temperature on CO2 and CH4 soil-atmosphere exchange of various land use/cover types in a semi-arid grassland in Inner Mongolia, China. Soil Biol. Biochem. 2010; 42: 773–787.

41. Wolf B, Zhang Y, Brüggemann N, Chen W, Dannenmann M, Han X, et al. Grazing-induced reduction of natural nitrous oxide release from continental steppe. Nature. 2010; 464: 881. https://doi.org/10.1038/nature08931 PMID: 20376147

42. Wang X, Huang D, Zhang Y, Chen W, Wang C, Yang X, et al. Dynamic changes of CH4 and CO2 emission from grazing sheep urine and dung patches in typical steppe. Atmos. Environ. 2013; 79: 576–581.

43. Nelson DW, and Sommers LE. Total carbon, organic carbon, and organic matter. Methods of soil analysis part 4 physical methods. 2002: 201–228.

44. Parkin TB, and Venterea RT. USDA-ARS GRACEnet project protocols chapter 3. Chamber-based trace gas flux measurements. 2010; 4: 1–39.

45. Fu H, Zhang G, Zhang F, Sun Z, Geng G, Li T. Effects of continuous tomato monoculture on soil microbial properties and enzyme activities in a solar greenhouse. Sustainability. 2017; 9: 317.

46. Fu X, Wang J, Sainju UM, Liu W. Soil carbon fractions in response to long-term crop rotations in the Loess Plateau of China. Soil Sci. Soc. Am. J. 2017; 81: 503–513.

47. Risch AC, Jurgensen MF, Frank DA. Effects of grazing and soil micro-climate on decomposition rates in a spatio-temporally heterogeneous grassland. Plant and Soil. 2007; 298: 191–201.

48. Lecain DR, Morgan JA, Schuman GE, Reeder JD, Hart RH. Carbon exchange rates in grazed and ungrazed pastures of Wyoming. J. Range Manage. 2000: 199–206.

49. Zhao Y, Peth S, Reszkowska A, Gan L, Krümmelbein J, Peng X, et al. Response of soil moisture and temperature to grazing intensity in a Leymus chinensis steppe, Inner Mongolia. Plant and soil. 2011; 340: 89–102.

50. Drury CF, Reynolds WD, Tan CS, McLaughlin NB, Yang XM, Calder W, et al. Impacts of 49–51 years of fertilization and crop rotation on growing season nitrous oxide emissions, nitrogen uptake and corn yields. Can. J. Soil Sci. 2014; 94: 421–433.

51. Hegyegi P, Gáli A, Czinkota I, Vyn T. Soil gas emission measurements in long term tillage experiments. Cereal Res. Commun. 2005; 33: 373–376.

52. Ozlu E, and Kumar S. Response of surface GHG fluxes to long-term manure and inorganic fertilizer application in corn and soybean rotation. Sci. Total Environ. 2018; 626: 817–825. https://doi.org/10.1016/j.scitotenv.2018.01.120 PMID: 29898556
55. Lee J, McKnight J, Skinner LS, Sherfy A, Tyler D, English B. Soil carbon dioxide respiration in switchgrass fields: assessing annual, seasonal and daily flux patterns. Soil Systems. 2018; 2: 13.

56. Carvalho AM, Bustamante MM, Coser TR, Marchão RL, Malaquias JV. Nitrogen oxides and CO2 from an Oxisol cultivated with corn in succession to cover crops. Pesquisa Agropecuária Brasileira. 2016; 51: 1213–1222.

57. Rochette P, and Janzen HH. Towards a revised coefficient for estimating N2O emissions from legumes. Nutr. Cycling Agroecosyst. 2005; 73: 171–179.

58. Brock PM, Muir S, Herridge DF, Simmons A. Cradle-to-farmgate greenhouse gas emissions for 2-year wheat monoculture and break crop–wheat sequences in south-eastern Australia. Crop Pasture Sci. 2016; 67: 812–822.

59. Chatskikh D, and Olesen JE. Soil tillage enhanced CO2 and N2O emissions from leamy sand soil under spring barley. Soil Tillage Res. 2007; 97: 5–18.

60. Ghosh S, Majumdar D, Jain M. Nitrous oxide emissions from kharif and rabi legumes grown on an alluvial soil. Biol. Fertil. Soils. 2002; 35: 473–478.

61. Schwenke GD, Herridge DF, Scheer C, Rowlings DW, Haigh BM, McMullen KG. Soil N2O emissions under N2-fixing legumes and N-fertilised canola: a reappraisal of emissions factor calculations. Agric., Ecosyst. Environ. 2015; 202: 232–242.

62. Shaaban M, Peng Q, Hu R, Lin S, Zhao J. Soil nitrous oxide and carbon dioxide emissions following incorporation of above-and below-ground biomass of green bean. Int. J. Environ. Sci. Technol. 2016; 13: 179–186.

63. Tiemann L, Grandy A, Atkinson E, Marin-Spiotta E, McDaniel M. Crop rotational diversity enhances belowground communities and functions in an agroecosystem. Ecol. Lett. 2015; 18: 761–771. https://doi.org/10.1111/ele.12453 PMID: 26011743

64. Cao G, Tang Y, Mo W, Wang Y, Li Y, Zhao X. Grazing intensity alters soil respiration in an alpine meadow on the Tibetan plateau. Soil Biol. Biochem. 2004; 36: 237–243.

65. Paz-Ferreiro J, Medina-Roldán E, Ostle NJ, McNamara NP, Bardgett RD. Grazing increases the temperature sensitivity of soil organic matter decomposition in a temperate grassland. Environ. Res. Lett. 2012; 7: 014027.

66. Denef K, Archibeque S, Paustian K. Greenhouse gas emissions from US agriculture and forestry: A review of emission sources, controlling factors, and mitigation potential. Interim report to USDA under Contract# GS23F8182H. URL http://www.usda.gov/oce/climate_change/techguide/Denef_et_al_2011_Review_of_reviews_v1.0.pdf. 2011.