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ORIGINAL ARTICLE

Greenhouse gas emissions from soil under maize–soybean intercrop in the North China Plain

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Abstract Intercrop systems can exhibit unique soil properties compared to monocultures, which influences the microbially-mediated processes leading to greenhouse gas emissions. Fertilized intercrops and monocultures produce different amounts of N_2O, CO_2 and CH_4 depending on their nutrient and water use efficiencies. The objective of this study was to compare the fluxes and seasonal emissions of N_2O, CO_2, and CH_4 from a maize–soybean intercrop compared to maize and soybean monocultures, in relation to crop effects on soil properties. The experiment was conducted during 2012, 2013 and 2014 at the WuQiao Experimental Station in the North China Plain. All cropping systems received urea-N fertilizer (240 kg N ha^{-1} applied in two split applications). The cropping systems were a net source of CO_2 and a net sink of CH_4, with significantly ($P < 0.05$ in 2012) and numerically (2013 and 2014) lower N_2O flux and smaller seasonal N_2O emissions from the maize–soybean intercrop than the maize monoculture. The proportion of urea-N lost as N_2O was lower in the maize–soybean intercrop (1.6% during the 3-year study) and soybean monoculture (1.7%), compared to maize monoculture (2.3%). Soybean reduced the soil NO_3^-–N concentration and created a cooler, drier environment that was less favorable for denitrification, although we cannot rule out the possibility of N_2O reduction to N_2 and other N compounds by soybean and its associated N_2-fixing prokaryotes. We conclude that maize–soybean intercrop has potential to reduce N_2O emissions in fertilized agroecosystems and should be considered in developing climate-smart cropping systems in the North China Plain.

Keywords Denitrification · Maize–soybean intercropping · Monoculture · Nitrous oxide · Soil environment

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Introduction

The North China Plain is the most important grain-producing region in China. For example, 35% of the country's maize is produced on almost 8 million ha of arable land in the North China Plain (Feng et al. 2012). Maize-wheat rotation is the dominant cropping system on more than 80% of the arable land. Due to policies that encourage grain production to meet domestic demands (Broughton and Walker 2010), coupled with lower profits from soybean production, a maize–soybean rotation is less common. Only about 2.7 million ha of land (11.7% of the cultivated land in this area) in this region is under soybean production (Cheng and Zhang 2010), despite the known benefits of legumes for soil fertility and pest control in the subsequent maize crop (Jensen et al. 2012). As farmers are reluctant to substitute soybean for wheat in their crop rotation, it is proposed that a maize–soybean intercrop system could be beneficial on farms in the North China Plain.

Intercropping with legumes presents several advantages over monoculture with regards to soil fertility and water use efficiency. Intercrops of cereals and legumes such as wheat-chickpea and maize-cowpea had greater phosphate acquisition than cereal crops alone due to their ability to modulate soil pH conditions (Li and Rengel 2012; Latati et al. 2014). Legumes that are actively fixing N_2 take up more cations than anions through the root system, and hence release H^+ ions that acidify the rhizosphere (Tang et al. 2013), which contributes to phosphate solubilization. Intercrops have greater capacity for resource acquisition, compared to monoculture, because the root systems of cereal and legume crops growing together can exploit more soil microenvironments, leading to higher nutrient use and water use efficiencies (Zhang and Li 2003). Consequently, a faba bean-maize intercrop was more efficient at acquiring mineral N (NH_4^+–N plus NO_3^-–N) and reduced the mineral N concentration by 24–31%, compared to maize monoculture (Li et al. 2011). In addition, the canopy of intercropped maize-legume was more effective in capturing radiant energy due to the distribution of different canopy layers: maize occupied the higher layers while soybean occupied the lower layers. This vertical distribution of these crops makes good use of the incoming radiant energy and lowers the soil temperature under the intercrop compared to maize monoculture (Ghanbari et al. 2010).

Intercrop-induced changes in soil pH, nutrient concentrations, moisture and temperature are expected to affect biological processes in soil, such as the microbially-mediated reactions that produce greenhouse gas (GHG). Lowering the soil pH increases total N_2O emissions, probably due to incomplete denitrification caused by the inhibition of the N_2O reductase enzyme under acidic conditions (Robinson et al. 2014). As mineral N is a substrate for nitrification and denitrification processes, greater mineral N concentrations will increase the N_2O emissions from soil (Siciliano 2014; Huang et al. 2014). Soil moisture affects aeration and redox potential, which control nitrification and denitrification processes, while soil temperature is positively related to N_2O, as well as CO_2 and CH_4 emission (Castaldi 2000; Marhan et al. 2015). Although acidic pH conditions are expected to increase N_2O production, a maize–soybean intercrop is hypothesized to have lower N_2O fluxes than monoculture due to the lower mineral N concentration, drier soil conditions and lower temperature in the intercropping system. The CO_2 and CH_4 fluxes from soil under maize–soybean intercrop will be influenced by the soil properties described above, as well as the dissolved organic carbon (DOC) concentration, which was significantly correlated with total CO_2 emissions in Mollisols cultivated with maize (Miao et al. 2015) and CH_4 emissions in peatland (Luan and Wu 2015). Roots and root-associated microorganisms metabolize carbon compounds, including DOC, to produce CO_2 under aerobic conditions and CH_4 under anaerobic conditions. Consequently, CO_2 fluxes are predicted to be lower in maize–soybean intercrop than maize monoculture due to less DOC and lower soil temperature (Ghanbari et al. 2010), whereas CH_4 fluxes are expected to be lower in maize–soybean intercrop than monocultures due to higher water use efficiency of the intercrop.

Field experiments that report differences in N_2O, CO_2 and CH_4 fluxes from maize and soybean monocultures are typically designed to mimic farm practices. For example, Chen et al. (2002) and Dyer et al. (2012) reported lower N_2O and CO_2 emissions in soybean monoculture than maize monoculture, but did not consider that the crops received unequal N fertilizer inputs (maize was fertilized with up to 150 kg N ha^{-1} and the soybean did not receive any fertilizer since the N requirements were met from N_2 fixation and the soil N supply). While such findings can be extrapolated to estimate farm-level GHG emissions, understanding of the underlying microbially-mediated processes in an intercrop compared to monocultures require that all experimental field plots receive an equivalent amount of N fertilizer to make an unbiased comparison of N_2O, CO_2 and CH_4 fluxes in maize–soybean intercrop, maize monoculture and soybean monoculture systems.

The objectives of this field study were (1) to evaluate N_2O, CO_2, and CH_4 emissions from a maize–soybean intercrop compared to maize and soybean monocultures, and (2) to determine the relationship between GHG emissions and soil properties (pH, mineral N and DOC concentrations, soil temperature, soil moisture content) in these cropping systems. The field study was conducted for three growing seasons (2012–2014) in maize and soybean monocultures and maize–soybean intercrops in the North China Plain.

Materials and methods

Site description

The field experiment was carried out from 2012 to 2014 at the Wu Qiao Experimental Station (37°41′N, 116°37′E) of China Agricultural University, Cangzhou, China. The annual mean temperature is 12.9 °C, although the average monthly temperature reaches 26.5 °C in July. Sunshine duration is 2724 h year^{-1} and annual precipitation is 562 mm, with 60–80% of annual rainfall during June to August. The soil at experimental site is an Aquic Cambisol developed on alluvial plain with a loamy texture (166 g sand kg^{-1} and 145 clay kg^{-1}). Prior to establishing the experiment, the soil contained 16.1 g kg^{-1} of organic matter, 1.02 g kg^{-1} of total N, with 20.3 mg kg^{-1} of Olsen-extractable P, 87.5 mg ammonium acetate-extractable K kg^{-1}, and pH 8.05. In the North China Plain, farmers plant two grain crops per year. Maize (or soybean) are grown from mid-June to early October. Following maize harvest, the field is planted with winter wheat in mid-October and the wheat is harvested in early June. We followed the traditional crop sequence, which meant that winter wheat was harvested from the site before this experiment began. Wheat residues (including roots and stubble) were cut into small pieces (< 10 cm fragments) with a mechanical shredder. The site was uniformly fertilized with a broadcast application with calcium superphosphate (75 kg P_2O_5 ha^{-1}) and potassium sulphate (90 kg K_2O ha^{-1}). Wheat residues and fertilizers were incorporated to a depth of 15 cm with a rototiller.

Experimental design

The experiment was established in June 2012 as a randomized complete block design with three treatments, each replicated three times. The treatments were: (1) monoculture maize (*Zea mays* cv. Zhengdan 958), (2) monoculture soybean (*Glycine max* cv. Zhonghuang 13), and (3) intercropped maize–soybean. Plot size was 9 m × 10 m and planted rows were oriented in a south-north direction. Maize monoculture was planted with 60 cm row spacing at a seeding rate equivalent to 54,000 plants ha^{-1}, while soybean monoculture had a row spacing of 40 cm and planting density of 250,000 plants ha^{-1}. The maize–soybean intercrop consisted of two rows of maize (60 cm row spacing) alternating with two rows of soybean (40 cm row spacing) and the gap between adjacent maize and soybean rows was 40 cm, giving a population of 36,000 maize plants ha^{-1} and 111,111 soybean plants ha^{-1}. Crops were sown on 15 June 2012, 18 June 2013 and 18 June 2014.

Each year, all plots received a broadcast application of 75 kg P_2O_5 ha^{-1} (calcium superphosphate) and 90 kg K_2O ha^{-1} (potassium sulphate) that was incorporated with a rototiller before seeding. All plots were fertilized with N fertilizer (urea) in two split applications, timed to occur after rainfall (> 10 mm) or prior to irrigation. We broadcast 120 kg N ha^{-1} on the soil surface of all plots at the 5-leaf stage of maize (9 July 2012, 10 July 2013 and 10 July 2014), with an additional surface broadcast application of 120 kg N ha^{-1} in all plots at the 12-leaf stage of maize (30 July 2012, 7 August 2013 and 3 August 2014). During the 2012 and 2013 seasons, the split N fertilizer applications were made after substantial rainfall (> 10 mm). A prolonged drought in the 2014 growing season necessitated irrigation (75 mm of water applied to all plots at 5-leaf and 12-leaf stages) to dissolve the urea granules. Insecticide and herbicide application, weeding, and other management during the season followed with local farming practices. Maize and soybean were harvested from all plots on 1

October 2012, 2 October 2013 and 2 October 2014 (Table 1). Maize grain was harvested with a plot-scale combine and the maize residues (cobs, stover and roots) were incorporated into the soil with a roto-tiller. Soybean plants were pulled by hand and the grain was separated manually, and soybean residues were not returned to the plot. Then, all plots were fertilized with 250 kg N ha^{-1} and planted with winter wheat, which grew in the field from mid-October to early June.

Greenhouse gas emissions

Greenhouse gases were collected using non-steady state static chambers, as described previously (Ju et al. 2011; Huang et al. 2014). One or two open-ended base collars (polyvinyl chloride pipe, 24 cm inner diameter and 5 cm tall) were installed per plot (n = 3 in 2012, n = 6 in 2013 and n = 6 in 2014). Collars were placed in random locations, between crop rows in the monocultures, or between maize and soybean rows in the intercropped system, and remained in the field from seedling emergence until harvesting. Frequency of measurement was once per week, and more frequently after rainfall or soil preparation. For instance, gas emissions were measured every 3 d for 2 weeks after each split-application of N fertilizer, resulting in 16, 18 and 18 sampling days in 2012, 2013 and 2014, respectively. Fluxes were measured in the morning between 09:00 and 11:00 by attaching the chamber covers (25 cm diameter and 20 cm tall) to the base collars for 30 min and taking headspace gas samples at 10, 20 and 30 min intervals using a polypropylene syringe (which was 50 mL and we removed 35 mL of gas) through a three-way stopcock at the top of chambers, then transferring the gas into a pre-evacuated 12 mL glass exetainer. The N_2O, CO_2 and CH_4 concentrations in headspace gas samples were quantified with a gas chromatograph (Shimadzu GC-2014C) equipped with an electron capture ^{63}Ni detector for N_2O and a flame ionization detector for CH_4 and CO_2 (following conversion with a methanizer containing a Ni catalyst) The CH_4 and CO_2 peaks were distinguishable due to differences in the retention time (3 min for CH_4; 6 min for CO_2). The detector temperature was set at 200 °C, the oven temperature was 50 °C and the carrier gas was ultra-high purity N_2 (99.999%).

Gas flux rates F (mg m^{-2} min^{-1}) were calculated following (Iqbal et al. 2008)

$$F = \frac{M}{V_0}\frac{V}{A}\frac{dc}{dt}\frac{T_0}{T}a$$

where M is the relative molecular mass of N_2O (44 g mol^{-1}), CO_2 (44 g mol^{-1}) and CH_4 (16 g mol^{-1}), V_o is the volume of an ideal gas, V (m^3) and A (m^2) are the volume and bottom area of the chamber, respectively, dc/dt (ppm min^{-1}) is the slope change of gas concentration in the chamber, T is the temperature (K) in the chamber, T_o is the temperature of an ideal gas, and a is the molar ratio of N per molecule of N_2O (28/44), and of C per molecule of CO_2 (12/44) and CH_4 (12/16). We assumed that the gas pressure was equivalent to atmospheric pressure (101.325 kPa), which was reasonable because 0.35% of the headspace gas was sampled at each event, but it would be more accurate to use a pressure transducer to measure gas pressure in the chambers during each sampling event.

Seasonal GHG emissions (t CO_2^{-eq} ha^{-1} $season^{-1}$) from the beginning to end of the sampling period were estimated by linear interpolation between successive sampling days, as described by (Zhai et al. 2011):

Table 1 Dates of the crop management operations done during this study at the Wu Qiao Experimental Station, Cangzhou, China (2012–2014)

Management	Date of the crop management operations		
	2012	2013	2014
Irrigation before sowing	9 June 2012	11 June 2013	N/A[a]
Sowing	15 June 2012	18 June 2013	18 June 2014
First N application	10 July 2012	10 July 2013	10 July 2014
First irrigation	N/A	N/A	10 July 2014
Second N application	30 July 2012	3 August 2013	3 August 2014
Second irrigation	N/A	N/A	3 August 2014
Harvest	1 October 2012	2 October 2013	2 October 2014

[a] N/A = not applicable, as the management was not done

$$\text{Seasonal GHG emission} = \sum\left[\frac{(F_{i+1}+F_i)}{2}\times(t_{i+1}-t_i)\right]\times 60\times 24\times\frac{1}{100}\times\frac{b}{a}$$

where F_i and F_{i+1} are the fluxes of gases at the previous and current sampling dates, expressed in mg N_2O–N m^{-2} min^{-1}, mg CO_2–C m^{-2} min^{-1} and mg CH_4–C m^{-2} min^{-1}, and t_i and $t_{i+1}$are the previous and the current sampling dates. The numerical constants of 60 and 24 in the equation are used to convert fluxes from a min^{-1} to d^{-1} basis, and the area is converted from m^{-2} to ha (10,000 m^2) with the value 1/100. The constant a is the molar ratio of N–N_2O, and C to CO_2 and CH_4 described above, and b is the global warming potential coefficient of N_2O (298), CO_2 (1) and CH_4 (25).

Finally, we calculated the fertilizer-induced N_2O emission each year, based on the peak fertilizer-induced N_2O emission (kg N_2O–N ha^{-1}) from early July when the first split application of N fertilizer occurred, to mid-August when the N_2O emissions were equivalent to pre-fertilization levels. Peak N_2O emissions occurred from 11 July 2012 to 11 August 2012, 12 July 2013 to 19 August 2013, and 11 July 2014 to 16 August 2014.

$$\text{N fertilizer lost}\,(\%) = \frac{\text{Peak fertilizer} - \text{induced}\,N_2O\text{–N emission}}{\text{Applied N fertilizer}}\times 100\%$$

Applied N fertilizer was 240 kg N ha^{-1} in plots with intercropped maize–soybean, maize monoculture and soybean monoculture.

Soil properties and environmental factors

Soil properties were evaluated periodically during the study period (2012–2014) by collecting soil samples (0–10 cm depth) with a soil auger (2 cm diameter) from five random locations per plot, then mixing and sieving (< 2 mm) the soil to generate one composite field-moist sample for each plot, which was stored at -15 °C until analysis. The mineral N (NH_4^+–N and NO_3^-–N) concentration was determined in 2 M $CaCl_2$ extracts (20 g field-moist soil: 50 mL extractant, shaken for 30 min and filtered through Q5 filter paper) on a continuous flow analyzer (AA3, SEAL, Germany). The dissolved organic carbon (DOC) concentration was determined in 0.5 M K_2SO_4 extracts (10 g field-moist soil: 50 mL extractant, shaken for 1 h and filtered through Q5 filter paper) with a dissolved carbon analyzer (TOC-L, Shimadzu, Japan). Soil pH was determined in soil: water slurries (1:5 air-dry soil: distilled water).At every gas sampling event, the ambient air temperature and soil temperature (5 cm depth) in each plot were measured with a hand-held glass thermometer. Soil moisture content was the gravimetric soil water content determined on every sampling date by mass loss after drying soil subsamples at 105 °C for 48 h.

Statistical analysis

Data were checked for normality and the N_2O flux rate, CH_4 flux rate and soil NH_4^+–N concentrations were log transformed to achieve normal distribution. Then, the effect of cropping system on the seasonal N_2O, CO_2 and CH_4 emissions and the global warming potential in each year (2012, 2013 and 2014) was evaluated with analysis of variance (ANOVA), and significant ($P < 0.05$) effects were evaluated with a post hoc least significant difference (LSD) test. The effect of cropping system on soil properties and environment factors, pooled among three growing seasons, was evaluated by comparing multiple dependent values with the Friedman test ($P < 0.05$). Relationships between N_2O, CO_2 and CH_4 fluxes, soil properties (pH, NH_4^+–N, NO_3^-–N and DOC concentrations) and environmental factors (soil moisture content and temperature) were evaluated with Pearson correlation coefficient using data from three study years. The proportion of variance in N_2O, CO_2 and CH_4 fluxes that was related to soil properties and environmental factors was assessed with stepwise multiple regression analysis. Statistical analyses were done with SPSS 17.0 software.

Results

Weather conditions

Weather conditions during the study period were consistent with long-term climatic conditions in this area. In all growing seasons, the months of July and August had higher air temperatures, and received more rainfall and irrigation (2014 only) than the autumn months (September). Air temperature was 20–30 °C from June to August, and between 12 and 20 °C in September and October (Fig. 1). The rainfall pattern was more erratic. The 2012 growing season was the wettest (512 mm), followed by the 2013 growing season with 476 mm of rainfall, while only 288 mm of rainfall occurred during the growing season in 2014 (Fig. 1).

Seasonal variation in N_2O, CO_2 and CH_4 fluxes, in relation to soil properties

Agricultural soils were generally a net source of N_2O. Fluxes were from 0.10 to 72.4 μg N_2O–N m^{-2} min^{-1} in 2012, from − 0.10 to 31.6 μg N_2O–N m^{-2} min^{-1} in 2013 and between − 0.55 and 93.10 μg N_2O–N m^{-2} min^{-1} in 2014 (Fig. 2). Peak N_2O fluxes occurred after N fertilizer application and coincided with rainfall and irrigation events that occurred in the 1–2 week period after N fertilization, from early July to mid-August (Fig. 2).

During the period of peak N_2O fluxes, the minimum air temperature was 22 °C and reached 32 °C in some growing seasons, so the N_2O flux was positively correlated with higher soil temperatures during the study ($r = 0.21$, $P < 0.05$, Table 2). There was a negative correlation between N_2O flux and soil pH, which was related to the decline in pH with increasing NH_4^+–N concentration ($r = -0.74$, $P < 0.01$, Table 2). As soil pH was not retained as a predictor of N_2O flux in the stepwise multiple regression model (Table 3), it is assumed to have an indirect relationship to N_2O flux. Throughout this study, the N_2O flux was strongly correlated with soil moisture content ($r = 0.62$, $P < 0.01$, Table 2) and the soil mineral N concentration (for NH_4^+–N, $r = 0.54$, $P < 0.01$; for NO_3^-–N, $r = 0.64$, $P < 0.01$, Table 2), and these variables were significant predictors of N_2O flux in the stepwise multiple regression analysis (Table 3).

Agricultural soils were a net source of CO_2 that exhibited more peak flux events in July–August (Fig. 3), a period of vegetative growth for maize and soybean. The CO_2 fluxes were positively correlated with N_2O fluxes ($r = 0.69$, $P < 0.01$), which were also higher during July–August than at other times in the three growing seasons, but not related to CH_4 fluxes (Table 2). Soil temperature, moisture, mineral N and DOC concentrations were positively correlated to CO_2 fluxes throughout the study, whereas soil pH was negatively correlated with CO_2 fluxes (Table 2). Multiple regression analysis indicated that soil temperature, soil moisture, the NO_3^-–N and DOC concentrations were predictors of CO_2 fluxes (Table 3).

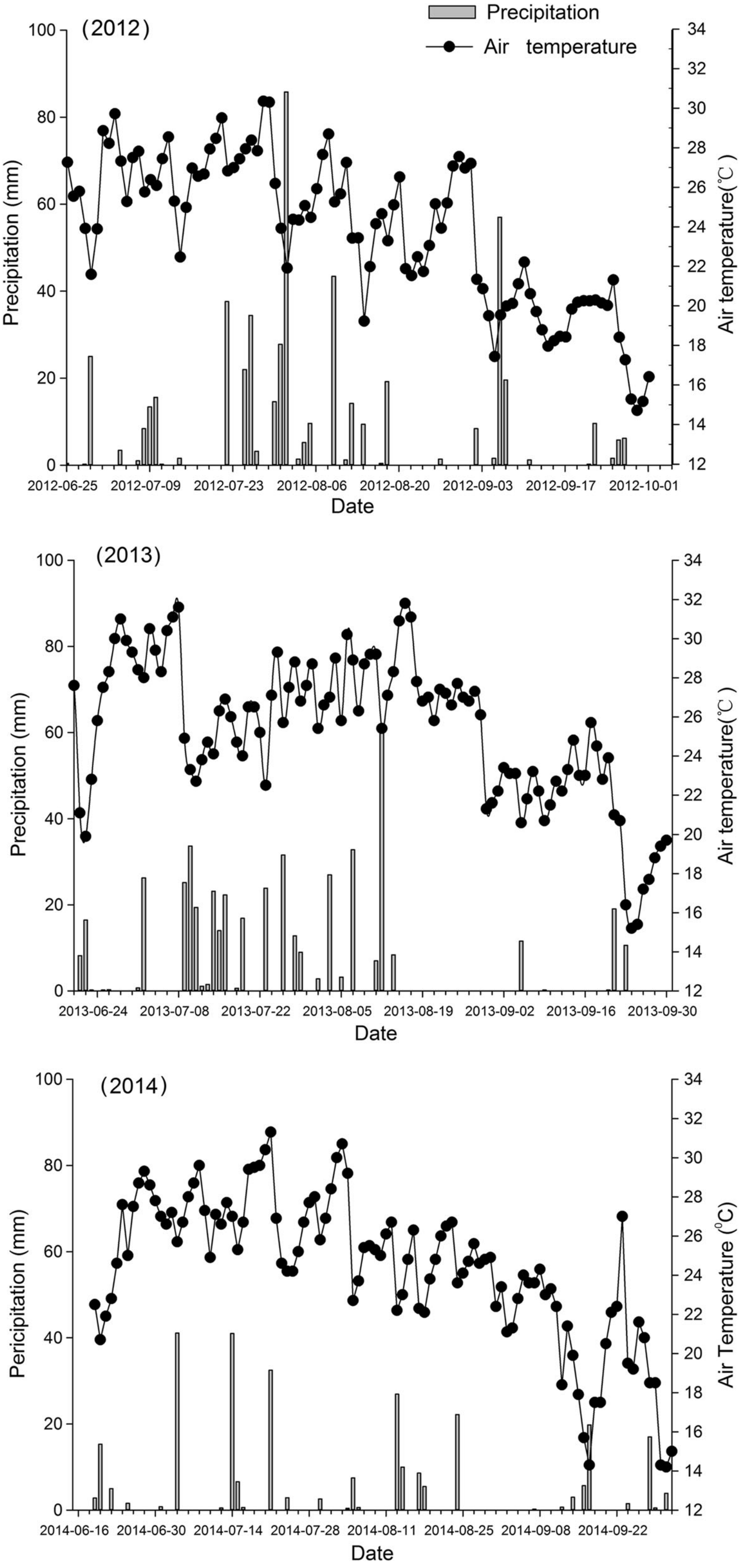
From 2012 to 2014, the CH_4 fluxes were − 3.26 to 0.51 μg CH_4 m^{-2} min^{-1}. On 75% of the sampling dates, we measured negative CH_4 fluxes, indicating that agricultural soils were a net sink for CH_4 (Fig. 4). The CH_4 fluxes were positively correlated with soil moisture and the DOC concentration (Table 2), suggesting that wetter soil conditions and abundant carbon-based substrates favored CH_4 production.

Seasonal GHG emissions and N_2O lost from urea-N in intercropped and monoculture systems

There was no difference ($P > 0.05$, ANOVA, Table 4) in the seasonal GHG emissions between the maize–soybean intercrop, maize monoculture and soybean monoculture during this 3-year field study. These cropping systems had similar global warming potential (GWP) during the growing season, with numerically higher CO_2^{-eq} emissions from the maize monoculture (3-year average of 17.9 t CO_2^{-eq} ha^{-1} $season^{-1}$) than the maize–soybean intercrop with 16.6 t CO_2^{-eq} ha^{-1} $season^{-1}$ and the soybean monoculture with 16.0 t CO_2^{-eq} ha^{-1} $season^{-1}$ (Table 4).

Seasonal N_2O emission from the intercrop was significantly ($P < 0.05$) lower in the first season, with a 37% reduction than the maize monoculture. While not significantly different in the second and third seasons, there was a trend of lower seasonal N_2O emissions by 16 and 18%, respectively, during these growing seasons (Table 4). From 59 to 78% of the seasonal N_2O emissions occurred from early July when the first split application of urea occurred to mid-August (Table 5). Urea-N lost through fertilizer-induced N_2O emissions was significantly ($P < 0.05$) greater from the maize monoculture than the maize–soybean intercrop in 2012, but no other significant differences occurred during the 3-year field study (Table 5). The proportion of urea-N lost as N_2O was smallest for the maize–soybean intercrop (3-year average of 1.6%) and soybean monoculture (3-year average of 1.7%) and larger for the maize monoculture (3-year average of 2.3%). The maize monoculture had greater NO_3^-–N concentration, higher soil temperature, and greater soil moisture content than the soybean monoculture and maize–soybean intercrop during the study period (Fig. 5).

Fig. 1 Daily precipitation (mm) and mean air temperature (^{o}C) during three growing seasons (2012–2014) at the Wu Qiao Experimental Station, Cangzhou, China



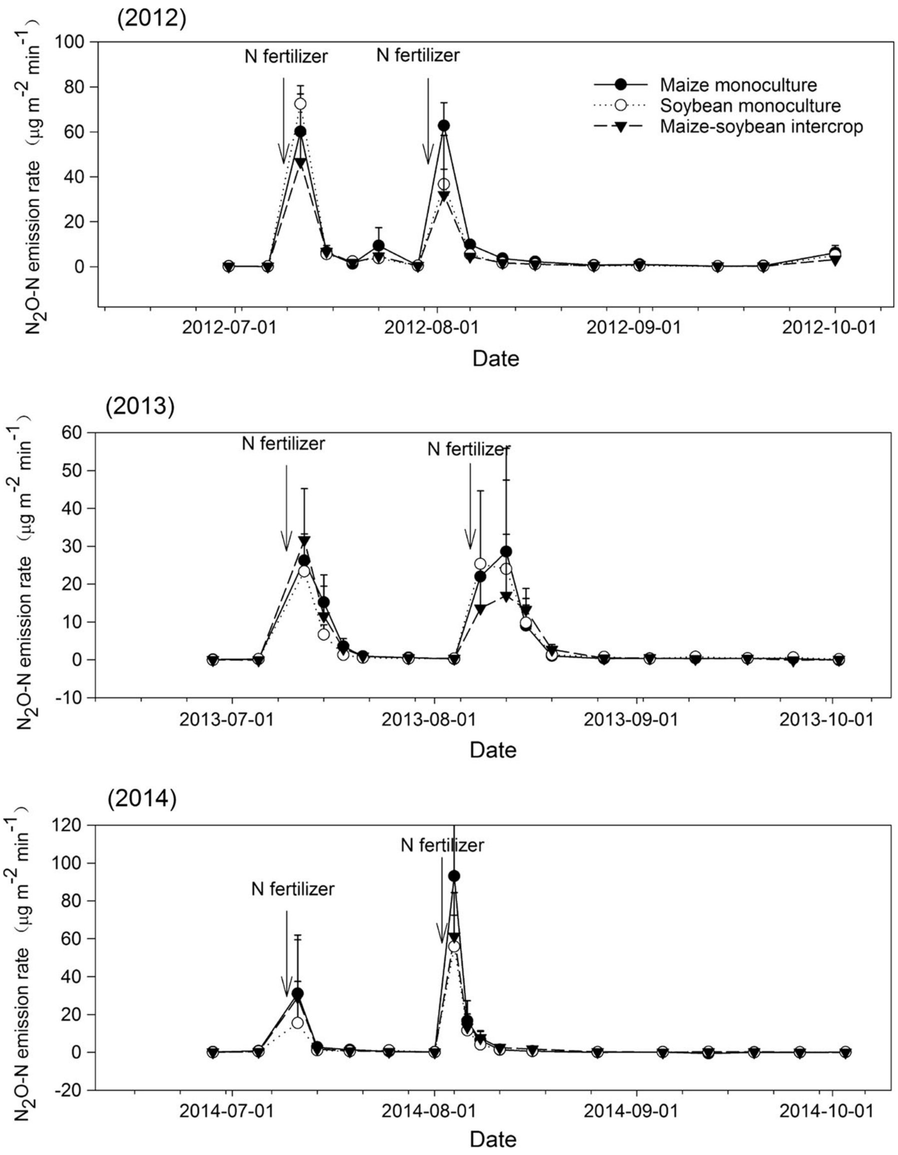


Fig. 2 Seasonal variation in soil N_2O fluxes from maize, soybean and maize–soybean intercrop from 2012 to 2014. Arrows indicate the time of urea application

Discussion

Nitrous oxide emissions in maize–soybean intercropping systems

The hypothesis that maize–soybean intercrop would reduce GHG emissions, compared to maize and soybean monocultures, was partially confirmed for the N_2O component of the GHG emissions. The N_2O fluxes were either statistically ($P < 0.05$ in 2012) or numerically (2013, 2014) lower in the maize–soybean intercrop than the maize monoculture, resulting in less seasonal N_2O emissions. At our field site, the NH_4^+ and NO_3^- concentrations and soil moisture content had the greatest influence on N_2O fluxes. The positive association between pH and NH_4^+ content may be explained by the fact that ammonia oxidation/nitrification and crop N uptake of NH_4^+ release H^+ ions into soil solution to maintain electrical neutrality (Havlin et al. 2014), leading us to conclude that the change in soil pH was associated with NH_4^+ dynamics. Soil temperature was positively correlated with N_2O fluxes, but was not retained in the stepwise multiple regression analysis, probably because the peak N_2O fluxes occurred during July–August when soil temperature varied by less than 11.5 °C. The temperature

Table 2 Pearson correlation coefficients of the relationship between N_2O, CO_2 and CH_4 fluxes, soil properties and environmental factors in maize, soybean and maize–soybean intercrop at the Wu Qiao Experimental Station, Cangzhou, China

Parameter	log N_2O (n = 150)	CO_2 (n = 156)	log (1 + CH_4) (n = 152)	log NH_4^+–N (n = 132)	NO_3^-–N (n = 132)	Moisture content (n = 153)	Temperature (n = 150)	DOC (n = 80)	pH (n = 78)
log N_2O^a	1								
CO_2	0.690^{**}	1							
log (1 + CH_4)	0.191^{*}	0.157	1						
log NH_4^+–N	0.537^{**}	0.432^{**}	0.005	1					
NO_3^-–N	0.636^{**}	0.400^{**}	0.070	0.265^{**}	1				
Moisture content	0.620^{**}	0.563^{**}	0.291^{**}	0.348^{**}	0.280^{**}	1			
Temperature	0.207^{*}	0.614^{**}	0.106	0.244^{**}	0.113	0.214^{**}	1		
DOC^c	0.091	0.480^{**}	0.343^{**}	0.274^{**}	− 0.023	0.323^{**}	0.409^{**}	1	
pH	-0.373^{**}	-0.426^{**}	− 0.158	-0.736^{**}	0.011	-0.359^{**}	-0.325^{**}	-0.425^{**}	1

Data were pooled among three growing seasons (2012 to 2014), and the number of observations (n) used to calculate the correlation coefficients are indicated

[a] Data were log transformed to achieve normal distribution

[b] Correlation is significant at the 0.05 level (*) or the 0.01 level (**) based on a 2-tailed test

[c] *DOC* dissolved organic carbon

Table 3 Variance in CO_2 and N_2O fluxes attributable to soil properties and environmental factors in maize, soybean and maize–soybean intercrop at the Wu Qiao Experimental Station, Cangzhou, China

Greenhouse gas	Regression coefficient							R^2	*P*
	log NH_4^+–N (n = 132)	NO_3^-–N (n = 132)	DOC^b (n = 80)	Temperature (n = 150)	Moisture content (n = 153)	pH (n = 78)	Constant		
CO_2 (n = 156)	–^{a}	0.045	0.025	0.430	0.420	–	− 12.220	0.690	< 0.001
log N_2O (n = 150)	0.576	0.017	–	–^{a}	0.050	–	− 1.668	0.710	< 0.001

Data were pooled among three growing seasons (2012–2014), and the number of observations (n) used in the stepwise multiple regression analysis are indicated

[a] Independent variables not entered in the stepwise regression analysis

[b] *DOC* dissolved organic carbon

range during this period ranged from 20.6 to 29.6 °C in 2012, 25.1 to 34.7 °C in 2013 and 23.6 to 35.1 °C in 2014, as illustrated in the supplementary materials (A3).

Our findings suggest that lower N_2O fluxes from the maize–soybean intercrop were related to its greater efficiency in acquiring NH_4^+, NO_3^- and water than monocultures. However, the NH_4^+ and NO_3^- concentrations and soil moisture content were similar in the maize–soybean intercrop and the soybean monoculture, suggesting that the soybean crop was instrumental in altering these soil properties and thereby affecting N_2O fluxes. There are two lines of evidence to support this argument. First, the NO_3^-–N and water uptake could be achieved more efficiently in the maize–soybean intercrop because the soybean

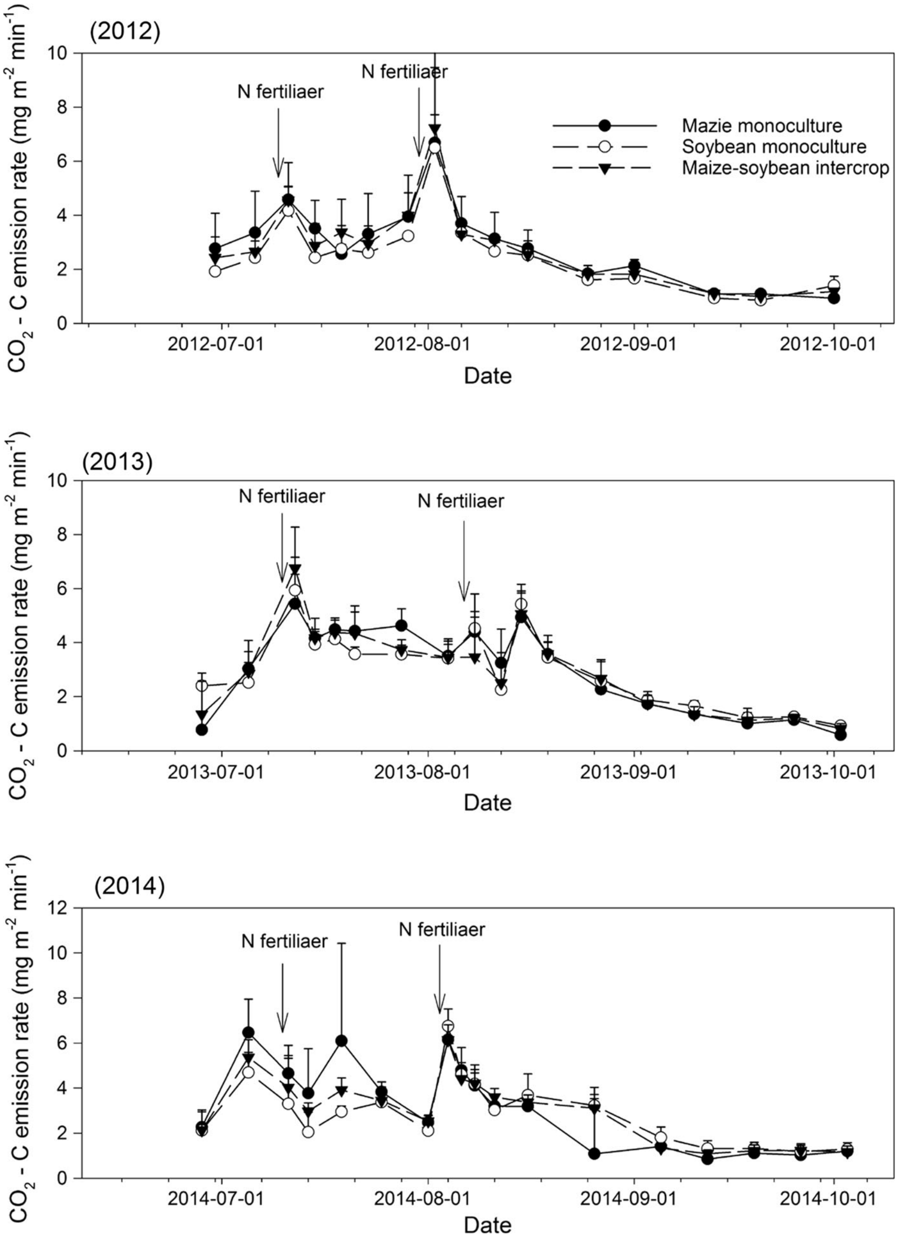


Fig. 3 Seasonal variation in soil CO_2 fluxes from maize, soybean and maize–soybean intercrop from 2012 to 2014. Arrows indicate the time of urea application

roots have a shallow root system, relative to maize roots (Gao et al. 2010). Second, soybean growing in a semi-arid climate has 90% or more of its root system in the upper 15 cm soil (Mitchell and Russell 1971) and is likely to remove nutrients (NH_4^+ and NO_3^-) and water from the surface soil layers. If soybean roots reduce the NO_3^-–N concentration and absorb more water, they will remove the substrate and anaerobic conditions needed for denitrification, the dominant pathway for N_2O fluxes in agroecosystems (Gaillard et al. 2016; Mahmood et al. 2005). As the N_2O produced near the soil surface is susceptible to diffuse to the atmosphere, the possibility that soybean roots can inhibit the N_2O production in the surface soil layers merits further investigation, particularly to distinguish how the root system of soybean grown in monoculture differs from a soybean intercrop. In conclusion, we posit that emergent soil properties resulting from soybean root growth may reduce N_2O emissions.

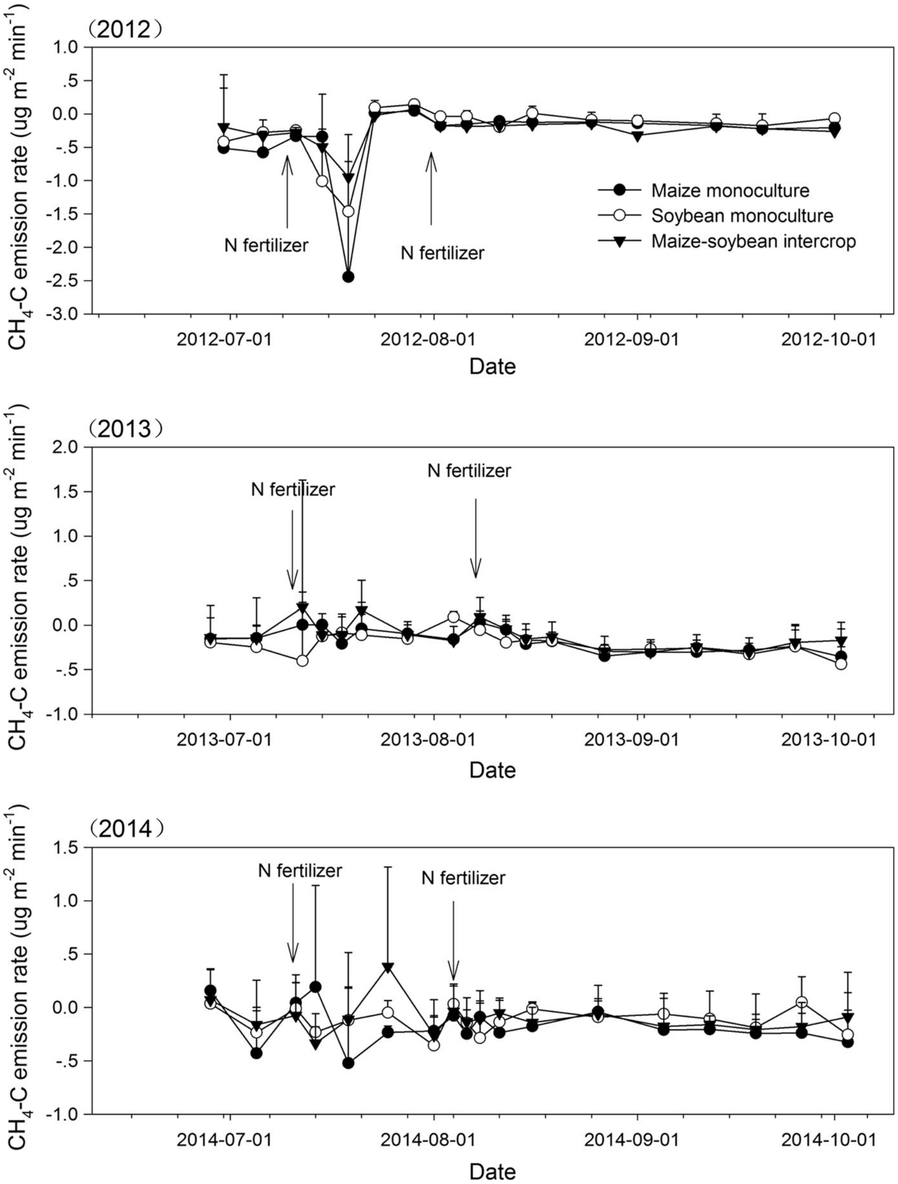


Fig. 4 Seasonal variation of soil CH_4 in maize, soybean or maize–soybean intercrop systems from 2012 to 2014. Arrows indicate the time of urea application

Another intriguing observation of this study is that the urea-N input of 240 kg N ha^{-1} produced lower N_2O fluxes, less seasonal N_2O emissions and smaller N fertilizer losses from the maize–soybean intercrop and soybean monoculture than the maize monoculture. One explanation for this observation is that soybeans have greater N use efficiency from urea fertilizer than maize, regardless of whether soybeans are included as an intercrop or grown as a monoculture. An alternative explanation is that soybean stimulates microbially-mediated processes that reduce N_2O–N_2 or other compounds. Soybean supports the activity of N_2-fixing rhizobacteria that over-express the *nosZ* gene, resulting in N_2O reduction to N_2 (Itakura et al. 2013). In addition, the nitrogenase enzyme can reduce N_2O–NH_4^+ during the process of N_2 fixation (Yamazaki et al. 1987; Vieten et al. 2007). We still need to determine whether soybean, when grown alone or intercropped with maize is (1) more efficient in using urea-N fertilizer, or (2) if N_2O is converted to other forms such as N_2 and NH_4^+, rather than being emitted as N_2O, in fertilized agroecosystems where soybean are grown.

Table 4 Seasonal N_2O, CO_2 and CH_4 emissions (expressed on a kg CO_2^{-eq} ha^{-1} $season^{-1}$ basis) from soils cultivated with maize, soybean and maize–soybean intercrop at the Wu Qiao Experimental Station, Cangzhou, China from 2012 to 2014

Years	Treatment	Seasonal N_2O (kg CO_2^{-eq} ha^{-1} $season^{-1}$)	Difference from maize (%)	Seasonal CO_2 (kg CO_2^{-eq} ha^{-1} $season^{-1}$)	Difference from maize (%)	Seasonal CH_4 (kg CO_2^{-eq} ha^{-1} $season^{-1}$)	GWP[a] (t CO_2^{-eq} ha^{-1} $season^{-1}$)
2012	Maize	5031a	–	13 135a	–	− 13.2a	18.2a
	Soybean	4110ab	− 18	11 343a	− 14	− 9.20a	15.4a
	Intercrop	3188b	− 37	12 608a	− 4.0	− 10.7a	15.8a
2013	Maize	2929a	–	14 749a	–	− 7.08a	17.7a
	Soybean	2666a	− 9.0	14 138a	− 4.1	− 9.66a	16.8a
	Intercrop	2461a	− 16	14 481a	− 1.8	− 6.09a	16.9a
2014	Maize	3121a	–	14 697a	–	− 8.72a	17.8a
	Soybean	1885a	− 40	14 058a	− 4.4	− 5.21a	15.9a
	Intercrop	2551a	− 18	14 574a	− 0.8	− 4.82a	17.1a

Difference from maize is the percent reduction in N_2O and CO_2 emissions in soybean monoculture and maize–soybean intercrop, relative to the maize monoculture. Within each year, seasonal emissions and global warming potential (GWP) values followed by different letters are significantly different ($P < 0.05$, LSD test)

[a] $GWP = (N_2O\text{–}N \times 44/28 \times 298) + (CO_2\text{–}C \times 44/12) + (CH_4\text{–}C \times 16/12 \times 25)$

Table 5 Nitrogen fertilizer-induced N_2O emissions (kg N_2O–N ha^{-1}) from soils cultivated with maize, soybean and maize–soybean intercrop at the Wu Qiao Experimental Station, Cangzhou, China from 2012 to 2014

Years	Treatment	Seasonal N_2O emission (kg N_2O–N ha^{-1})	N fertilizer induced N_2O[a] (kg N_2O–N ha^{-1})	Urea application rate (kg N ha^{-1})	N fertilizer lost[b] (%)
2012	Maize	10.7	7.62a	240	3.2a
	Soybean	8.78	5.85ab	240	2.4ab
	Intercrop	6.81	4.67b	240	1.9b
2013	Maize	6.26	4.89a	240	2.0a
	Soybean	5.69	4.24a	240	1.8a
	Intercrop	5.26	3.99a	240	1.7a
2014	Maize	6.66	4.03a	240	1.7a
	Soybean	4.02	2.42a	240	1.0a
	Intercrop	5.45	3.22a	240	1.3a

Within a year, values within a column followed by different letters are significantly different ($P < 0.05$, LSD test)

[a] Nitrogen fertilizer-induced N_2O emissions occurred from early July when the first split application of N fertilizer occurred, to mid-August when the N_2O emissions were equivalent to pre-fertilization levels (also see Fig. 1)

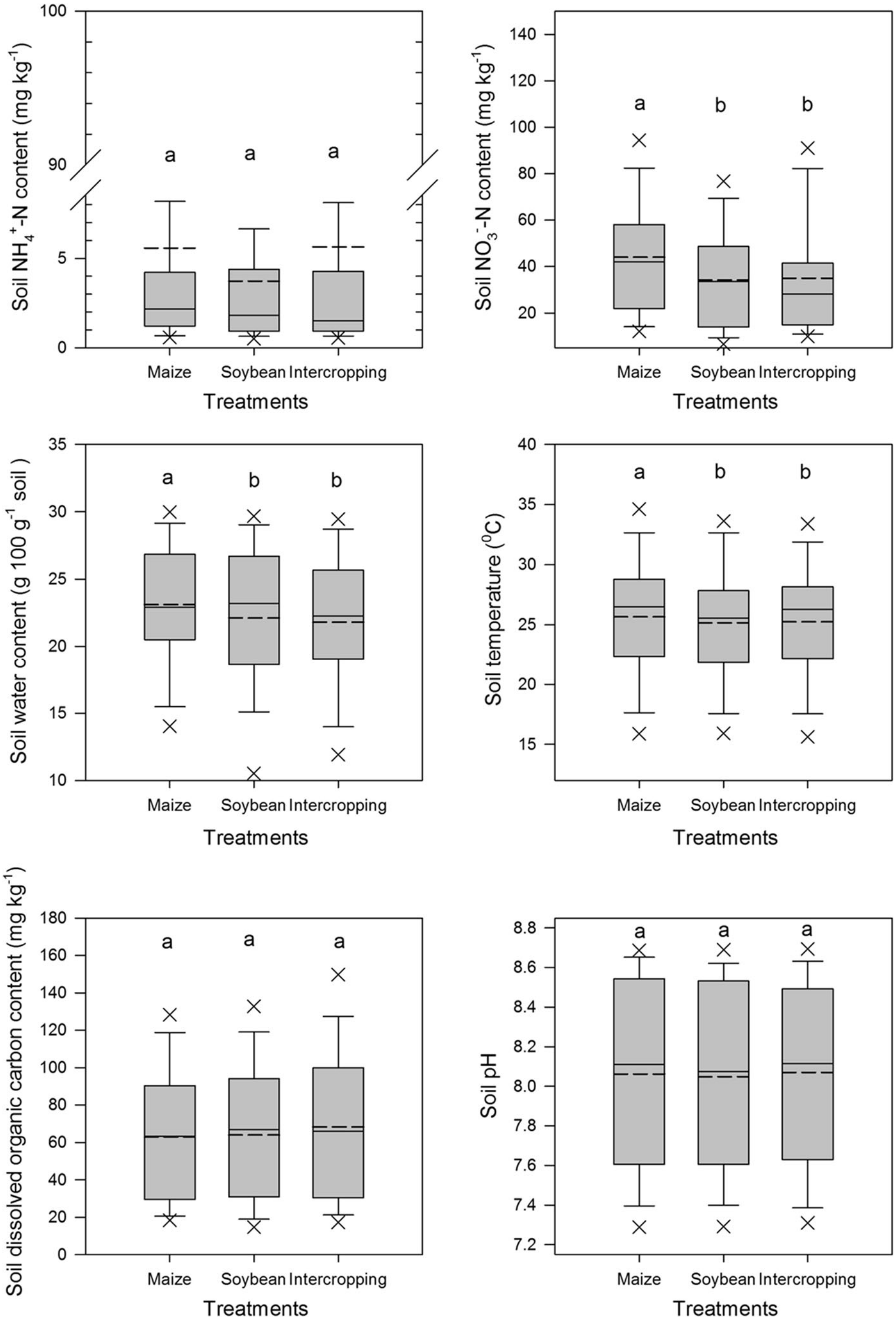
[b] Proportion of applied N fertilizer that was lost as gaseous N_2O–N

Carbon dioxide and methane emissions in maize–soybean intercropping systems

The hypothesis that maize–soybean intercrop would reduce GHG emissions, compared to maize and soybean monocultures, was not confirmed for the CO_2 and CH_4 components of the GHG emissions. Since crop production in this semi-arid region is generally rainfed with minimal inputs of irrigation water, the GHG emissions were dominated by CO_2 and soils were a net sink for CH_4 throughout the study period.

At our field site, the NO_3^{-}–N and DOC concentrations, soil temperature and soil moisture content had the greatest influence on CO_2 fluxes. This is consistent with the trend of greater CO_2 emissions from the

Fig. 5 Soil properties in plots cultivated with maize, soybean and maize–soybean intercrop, averaged across three growing seasons (2012–2014) at the Wu Qiao Experimental Station, Cangzhou, China. Data are box and whisker plots, where boxes represent data between the 25th and 75th percentiles and show the median (solid line) and mean (dotted line) values for each soil parameter. The whiskers are error bars representing values falling in the 10th and 90th percentiles of the distribution



maize monoculture, which had slightly lower NO_3^- and water use efficiency, greater photosynthesis, and higher soil temperature than the maize–soybean intercrop. Although NO_3^- and water are not used directly for respiration, all biological systems require N and water for metabolic processes that lead to CO_2 production. The DOC concentration was considered to be an indicator of the soluble C substrate originating from microbial decomposition and root exudates. Maize produces relatively more above- and below-ground biomass, compared to soybean, that can support greater CO_2 fluxes from the heterotrophic (microbes, soil fauna) and autotrophic (root) respiration (Hanson et al. 2000). For example, maize root respiration contributed 40% of the total soil respiration in a maize monoculture (Kuzyakov and Larionova 2006). In addition, soil temperature was slightly warmer in the maize monoculture than maize–soybean intercrop and soybean monoculture (on average, 0.4–0.5 °C warmer during the study). Soil temperature is often found to affect CO_2 emissions, with a twofold increase in microbial respiration as temperature increases from 0 to 30 °C (Koch et al. 2007). Therefore, the tendency for higher CO_2 emissions from maize monoculture than the maize–soybean intercrop and soybean monoculture were related to the soil properties under these crops.

Conclusions

Maize monoculture in the North China Plain has adverse effects on soil quality and crop yield, which may be alleviated by including a leguminous crop like soybean in the rotation or as an intercrop. Soybean grown as a monoculture or intercrop is effective in reducing the soil NH_4^+ and NO_3^-–N concentrations and maintaining drier soil conditions than a maize monoculture. Consequently, the maize–soybean intercrop tended to have lower N_2O flux, less N_2O emission during the growing season and smaller N loss from urea-N fertilizer than maize monoculture. Soybean root morphology or interactions with N_2-fixing prokaryotes may alter the microbially-mediated reactions that reduce N_2O–N_2 and other N compounds, thereby affecting the N_2O fluxes from soil, but this remains to be confirmed through mechanistic studies involving ^{15}N stable isotopes or $\delta^{15}N$ isotopomers. Soybean is already grown in the North China Plain, although not on as large a scale as cereal crops. If soybean production were expanded, such that the 2.7 million ha of soybean grown in the North China Plain were intercropped with maize in a similar manner as described in this experiment, this would result in maize–soybean intercrop on approximately 6.08 million ha of agricultural land with an estimated capacity to reduce N_2O emissions by 0.012 million t N_2O–N year^{-1} and 1.86 million t CO_2^{-eq} year^{-1}. This could make a substantial reduction in the annual GHG budget for the North China Plain, which according to our estimates is currently about 7.69 million t CO_2^{-eq} year^{-1}. We conclude that intercropping with soybean has potential to reduce GHG emissions in the North China Plain, and merits consideration in developing climate-smart cropping systems for this region.

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