Experimental Agriculture (2020), **56**, 239–254
doi:10.1017/S0014479719000334

RESEARCH ARTICLE

Winter wheat yield and nitrous oxide emissions in response to cowpea-based green manure and nitrogen fertilization

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(Received 06 December 2018; revised 25 June 2019; accepted 29 August 2019; first published online 11 October 2019)

Abstract

The aim of this study was to compare the effects of cowpea green manure and inorganic nitrogen (N) fertilizers on yields of winter wheat and soil emissions of nitrous oxide (N_2O). The comparisons included cowpea grown solely as green manure where all biomass was terminated at maturity by tillage, summer fallow treatments with 90 kg N ha^{-1} as urea (90-N), and no fertilization (control) at planting of winter wheat. Fluxes of N_2O were measured by closed chamber methods after soil incorporation of cowpea in autumn (October–November) and harvesting of winter wheat in summer (June–August). Growth and yields of winter wheat and N concentrations in grain and straw were also measured. Cowpea produced 9.5 Mg ha^{-1} shoot biomass with 253 kg N ha^{-1} at termination. Although soil moisture was favorable for denitrification after soil incorporation of cowpea biomass, low concentrations of soil mineral N restricted emissions of N_2O from cowpea treatment. However, increased concentrations of soil mineral N and large rainfall-induced emissions were recorded from the cowpea treatment during summer. Growth of winter wheat, yield, and grain N concentrations were lowest in response to cowpea treatment and highest in 90-N treatment. In conclusion, late terminated cowpea may reduce yield of winter wheat and increase emissions of N_2O outside of wheat growing seasons due to poor synchronization of N mineralization from cowpea biomass with N-demand of winter wheat.

Keywords: Cover crops; Green manure; Legumes; N mineralization; Nitrous oxide

Introduction

Winter wheat (*Triticum aestivum* L. em Thell.) is the major grain crop in the US Southern Great Plains (SGP) and is mostly cultivated as a monoculture (Patrignani *et al.*, 2014). Although inorganic nitrogen (N) fertilizers are widely applied to winter wheat in the region, interest in cultivating legumes as green N manures is increasing in recent decades (Northup and Rao, 2015; Rao and Northup, 2011). In the SGP, winter wheat is planted between mid-September and mid-October based on its usage as forage during fall through spring, grain, or a dual-purpose crop (November through February grazing and grain harvest), with grain harvested in June (Redmon *et al.*, 1995). There is a possibility of including summer legumes as green N sources into wheat monocultures (Northup and Rao, 2016).

Cowpea [*Vigna unguiculata* (L.) Walp] is a summer-grown legume with potential to act as a green N manure in the SGP. It is easy to establish, thrives in water-deficit environments, and produces high amounts of biomass (Rivas *et al.*, 2016). A well-established cowpea crop can provide enough N to meet the requirements for winter wheat in the SGP (Mahama *et al.*, 2016; Schroeder *et al.*, 1998). However, to be an effective green manure, the N in the legumes should be transferred effectively to the recipient crops, which largely depends on synchronization of N mineralization from legume biomass with N-demand by the recipient crops (Berry *et al.*, 2002). Apart from environmental conditions in soil, such as temperature and moisture at termination, carbon (C) and N mineralization mostly depends on level of biomass maturity at termination of green manures (Kumar and Goh, 2000; Nicolardot *et al.*, 2001). Early terminated green manures have low C/N ratio, which is a conducive trait for rapid C and N mineralization (Aulakh *et al.*, 1991; Nicolardot *et al.*, 2001).

Summer legumes grown as green manures (including cowpea) in the SGP are often terminated in August (within 2 months of planting), which creates fallow periods of 1–2 months between termination and planting of winter wheat (Kandel *et al.*, 2019a; Northup and Rao, 2015; Rao and Northup, 2011). Early termination may help to reduce transpirational loss of soil moisture by the green manure, which is the critical factor for production of winter wheat in the region (Nielsen *et al.*, 2015). However, early termination may limit biomass yield and N fixation by the green manure and limit other environmental benefits expected from cover crops such as weed suppression, and reduced soil erosion, nutrient leaching and runoff. Early termination also exposes highly decomposable legume biomass in fallow soil for an extended warm period which can be conducive for denitrification loss of N as nitrous oxide (N_2O), a highly potent greenhouse gas, during rainfall events. Thus, environmental and agronomical benefits provided by legume green manures may be offset by elevated emissions of N_2O.

Alternatively, green manures can be terminated just before planting of winter wheat. Late termination may increase biomass and N yields, and slow mineralization of C and N post-incorporation due to higher C/N ratios and lignification of biomass at crop maturity (Kumar and Goh, 2000; Nicolardot *et al.*, 2001). However, biomass with higher C/N ratios may immobilize soil N and reduce growth and yield of winter wheat. If the N remains unutilized by a recipient winter wheat, the risk of N_2O emissions can be high outside the growing period of winter wheat. Winter wheat cultivated for grain production in the US SGP reaches maturity in late-April to early-May, a period when demand for soil N by the crop declines. May and June are among the wetter months of the year in the SGP, when the potential for denitrification loss of the unutilized N in cowpea biomass as N_2O is high, since denitrification is favored by high moisture conditions (Kandel *et al.*, 2018; Pimentel *et al.*, 2015; Rosecrance *et al.*, 2000).

Although the effectiveness of cowpea as a green manure for winter wheat, and other winter crops, has been tested in the US SGP (Rao and Northup, 2009; Schroeder *et al.*, 1998), there is limited information on mineralization of biomass N and N_2O emissions after termination of cowpea. Also, most previous studies terminated green manures at early stages during flowering, to plant wheat for use as a forage or dual-purpose crop (MacKown *et al.*, 2007; Northup and Rao, 2015). Therefore, in this study, we tested late terminated cowpea (planted in June and terminated by soil incorporation at maturity in early-October) grown solely as a green N source for a continuous rotation of winter wheat. The comparisons included different treatments that included summer fallow and fertilization (90 kg N ha^{-1} as urea; unfertilized control) at planting of winter wheat. The aim of this study was to investigate the effects of cowpea green manure and inorganic N fertilizers on growth and yield of winter wheat, and emissions of N_2O. We hypothesized that growth and yield of winter wheat and emissions of N_2O would not differ among the cowpea, control, and inorganic fertilizer treatments.

Materials and Methods

Study site and soil properties

This field study was conducted at the USDA-ARS Grazinglands Research Laboratory near El Reno, OK, USA (35°34′21″ N, 98°02′12″ W; 411 m elevation). The site was defined as components of bottomland areas of the drainage basin of the North Canadian River (Goodman, 1977). The predominant soil series was a Dale silt loam (fine-silty, mixed, thermic, Pachic Haplusttols) with 0−1% slope. Soils of the site had average C content of 1.31%, total N content of 0.10%, and C/N ratio of 13.10 (USDA-NRCS, 1999).

Experimental design and agronomic managements

The study consisted of three replicate blocks, with seven plots ($8 \times 8\,m^2$) per block. Cowpea (*cv.* Red Ripper) as green manure was randomly assigned to four plots in each block, while the other three plots were left fallow during growing periods of cowpea and randomly assigned to control and inorganic N treatments at wheat planting. Cowpea seed inoculated with *Bradyrhizobium* spp. was planted at a rate of 40 kg ha^{-1} at 0.025 m depth and in 0.40 m spaced rows in June 2017. Cowpea biomass was terminated by tillage (disked once and rototilled once) on 3 October 2017. Winter wheat (*cv.* Jagger) was planted (seed rate 90 kg ha^{-1}; row spacing 0.20 m) in all plots on 13 October 2017. Two of the three plots without cowpea in each block were randomly assigned to inorganic N treatment, which were broadcasted with urea (46–0–0 N–P–K) at the rate of 90 kg N ha^{-1} (referred as 90-N treatment hereafter) after the emergence of winter wheat on 22 October. One fallow plot per block was left unfertilized as a control. Thus, each block consisted of four cowpea plots, two 90-N plots, and one control plot. Multiple plots in each block were assigned to cowpea and 90-N treatments, since large spot-specific heterogeneity on N_2O fluxes was anticipated due to micro-patch-specific differences in cowpea biomass production and incorporation in cowpea treatment, and distribution of inorganic fertilizer in 90-N treatment (Kandel *et al.*, 2018). All measurements from replicate plots of cowpea and 90-N treatments within a block were subsequently averaged prior to further data analysis. Winter wheat was harvested on 6 June 2018. Thereafter, cowpea was planted on 27 July 2018 by no-till management.

Yield and properties of cowpea biomass at termination

Total aboveground biomass of cowpea was determined by drying biomass harvested from 1 m^2 quadrats from each block ($n = 3$). The biomass was dried at 60 °C to constant weight in a forced-draft oven. To estimate root biomass, five cowpea plants were excavated (0–0.25 m depth) with a shovel. The plants were partitioned to shoot and root biomass, and root biomass was cleaned thoroughly. Thereafter, both parts from each plant were oven-dried, and dry weights were recorded separately. Total root yield was determined from shoot biomass yield determined at three 1 m^2 quadrats and the average root/shoot ratio. The root and shoot biomass was milled separately to pass through a 1 mm sieve to determine biochemical composition on three replicates of both root and shoot biomass; averages are presented.

Total C and N concentrations in cowpea biomass were assessed by a flash combustion method (Model VarioMacro; Elementar Americas, Inc., Mt. Laurel, NJ, USA). Amounts of N per hectare in cowpea biomass were obtained as a product of biomass yield and N concentrations in biomass. Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were determined by the method described by Van Soest and Wine (1967). Concentration of cellulose was estimated as the difference between ADF and ADL and hemicellulose as the difference between NDF and ADF. The ADL fraction was considered as lignin concentration.

Measurement of winter wheat growth, yield, and yield attributing characters

Growth of winter wheat was monitored non-destructively by canopy reflectance using a full range PSR-3500 portable spectroradiometer (Spectral Evolution, Lawrence, MA, USA) with a spectral range of 350–2500 nm. The measurements were taken from three fixed spots in each plot with a sensor held at a height of 1.2 m. Ratio vegetation index (RVI) was calculated as the ratio of reflectance at near-infrared (778 nm) and red (656 nm) wavelengths (Christensen *et al*., 1992).

Total aboveground dry matter (DM) and grain yields of winter wheat was determined at the end of growing season by manually harvesting the crop close to the soil surface from three quadrats (0.25 m^2) per plot. Biomass from the quadrats was pooled as one sample for further measurements. Numbers of tillers enumerated in the pooled sample were counted to estimate tiller numbers per square meter. Thereafter, 10 plants from each plot were randomly selected to determine plant height, length of spikes, and number of grains per spike. All biomass was dried to a constant weight in a forced-draft oven (60 °C) and weighed to calculate aboveground biomass. The harvested plants were separated into grain and straw components and weight of 1000 grains was determined. Harvest index was calculated as ratio of total grain weight to total DM weight.

An aliquot of dried grain and straw biomass were milled separately to pass through a 1 mm sieve to determine concentrations of total C and N. Total amounts of N per hectare in the grain and straw biomass were assessed separately as product of yields and N concentrations.

Measurement and calculation of N_2O fluxes

Fluxes of N_2O during the study were measured using closed chamber methods. A PVC collar (0.65×0.65 m^2) was inserted to 0.10 m depth in all plots on 3 October 2017, immediately after termination of cowpea. The collars had a 0.04 m wide outer flange which was kept parallel to the soil surface to support the chamber used for flux measurement. Fluxes were measured by placing a white PVC chamber ($0.70 \times 0.70 \times 0.21$ m^3) on the flange.

The collars were removed briefly for planting of winter wheat on 13 October 2017 and cowpea on 27 July 2018 but reinstated on the same place. Prior to fertilization, no measurements were taken from 90-N treatment but the fluxes were assumed to be similar to control treatment. Fluxes were measured on 10 dates within 40 days after termination of cowpea. Fluxes were also measured on 19 dates between 25 June and 19 August following the harvest of winter wheat.

During the period of 5 October to 15 November 2017 (during planting of winter wheat), N_2O fluxes were measured by taking gas samples in pre-evacuated vials and analyzing samples with a gas chromatograph. Gas samples were taken between 10:00 and 12:00. The chambers were enclosed for about 60 minutes. During chamber enclosure, headspace air was mixed with two small battery-driven fans. Four gas samples (40 ml) were withdrawn from the chamber headspace at 20-minute intervals (i.e., 0, 20, 40, and 60 minutes) with a polypropylene syringe and collected in 20 ml evacuated glass vials. Gas concentrations were later determined on a Shimadzu gas chromatograph (GC-2014; Shimadzu Corp., Kyoto, Japan), which was equipped with a Shimadzu AOC-5000 auto sampler with a 2.5 ml gastight syringe. Flux calculations were undertaken with the HMR method (Pedersen *et al*., 2010).

During 25 June to 19 August 2018 (after harvest of winter wheat), N_2O fluxes were measured using a chamber as previously described for gas sampling. However, gas concentration was determined in-line by a portable Fourier transform infrared (FTIR)-based analyzer (DX4040; Gasmet Technology Oy, Helsinki, Finland) as previously described by Kandel *et al.* (2018). The chamber headspace air was circulated through 3 mm inlet and outlet tubing to the FTIR analyzer. Concentrations of N_2O were measured at 20 second intervals. The chamber was enclosed for 6–8 minutes during each measurement, and air was mixed by two small fans which ran continuously during the entire enclosure period. Fluxes were calculated by linear regression using the MATLAB® (MathWorks, Inc., Natick, MA, USA) routine of Kutzbach *et al.* (2007) since the linear method provided the robust flux calculation during short periods of chamber enclosure (Kandel *et al*., 2016). Total cumulative emissions of N_2O during the periods of winter wheat planting and harvesting were separately calculated by linear interpolation of measured fluxes between the measured dates.

Measurement of CO_2 fluxes

Although the main interest of this study was emissions of N_2O, simultaneously measured fluxes of CO_2 were also reported to define decomposition of cowpea residue and soil organic matter. Although emissions of CO_2 represent respiration from both living plants and soils (i.e., ecosystem respiration), the study plots were free of any green plants for most of the measurements in October 2017, prior to germination of winter wheat. Similarly, during measurements in June–August 2018, the plots were mostly bare since cowpea was planted during late-July. Therefore, emissions of CO_2 were presented as an indicator of decomposition of cowpea residue and availability of mineralizable C.

Analyses of soil samples

Soil samples (0–0.15 m) were taken from all plots on all 10 dates of flux measurements during October–November 2017 to define concentrations of mineral N [nitrate ($NO_3{}^-$) and ammonium ($NH_4{}^+$)]. During June–August 2018, soil samples were taken on 7 of 19 dates of flux measurements. Two soil cores (diameter, 0.02 m) were taken at about 0.10 m distance from opposite sides of the collars at each soil sampling. The cores were pooled to form a composite sample for analysis. Aliquots of samples were extracted in 1.0 M KCl and analyzed by flow injection (Timberline Instruments, Boulder, CO, USA) to determine concentrations of $NO_3{}^-$ and $NH_4{}^+$.

Total soil organic carbon (SOC) and total N concentrations in soil were determined from samples collected on three dates after planting (10 October, 26 October, and 15 November) and two dates after harvesting (27 June and 8 August) of winter wheat. Samples were assessed by an auto-analyzer by combusting samples at 900 °C for 10 minutes (Model VarioMacro; Elementar Americas, Inc.) and analyzing gases evolved from the samples (Nelson and Sommers 1996).

Measurements of environmental variables

Volumetric water content (VWC) and temperature at 0–0.05 m soil depth were measured outside each collar on dates of flux measurement using Stevens® Hydra Probe® soil moisture sensors (Stevens Water Monitoring Systems, Inc., Portland, OR, USA). The VWC was expressed in terms of normalized VWC contents to the maximum VWC recorded at flooded field conditions, which typically represented water-filled pore space (WFPS) equal to 100%. Air temperature during chamber enclosure and precipitation measurements during the study periods were obtained from a weather station (Oklahoma Mesonet, Oklahoma Climatological Survey) which was roughly 1 km from the study site.

Statistical analysis

All measurements are presented as averages and standard errors of three blocks from each treatment unless stated otherwise. The difference in responses among three treatments was determined using a mixed model in SAS (SAS Inc., Cary, NC, USA) considering blocks as a random variable. The effect of sampling dates was included in the model and treated as repeated measurements for the measured dynamic variables. The compound symmetry covariance structure was applied to account for autocorrelation among dates of repeated measurements. Fisher's LSD method was used for pairwise comparisons of means at 5% level.

Results

Yield and chemical composition of cowpea biomass

At termination, cowpea produced 9.5 Mg ha^{-1} shoot biomass which contained 253 kg N ha^{-1} (Table 1). Root biomass comprised 4% of total biomass, or about 0.4 Mg ha^{-1} biomass, containing 4 kg N ha^{-1}. Concentration of N in cowpea shoots was 2.7% of biomass, while root biomass had N concentrations of 1.1%. The C/N ratio in cowpea shoot and root biomass was 15.8 and 39.3,

Table 1. Yield and chemical composition of cowpea biomass at termination

Parameters	Shoot	Root
Yield (Mg ha^{-1})	9.5	0.4
N concentrations (% of DM)	2.7	1.1
C concentrations (% of DM)	42.0	43.6
C/N	15.8	39.3
Total biomass N (kg ha^{-1})	253	4
Cellulose (% of DM)	24.8	46.2
Hemicellulose (% of DM)	16.0	15.7
Lignin (% of DM)	5.9	11.2

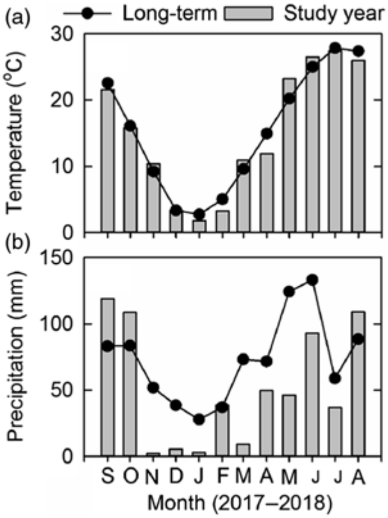

Figure 1. Mean monthly (a) air temperature and (b) total precipitation during the study year (September 2017 to August 2018; gray bars) compared to the long-term means in the study area (1981–2010; black circles).

respectively. Concentrations of cellulose and lignin in root biomass were almost double that of shoot biomass, while concentrations of hemicellulose were similar.

Climate and soil conditions

Average air temperature during the growing season of winter wheat (October–May) of the study year (10.2 °C) was similar to the long-term average (10.1 °C) (Figure 1a). Air temperature during the dormancy period of winter wheat (January–February) was slightly lower than the long-term averages, while temperatures in May were slightly higher.

Total precipitation during late growth of cowpea and planting of winter wheat (September–October) was higher than the long-term average (Figure 1b). However, total precipitation (155 mm) during the remaining growing period of winter wheat (November–May) was 270 mm less than long-term average of 425 mm. After harvesting of winter wheat, June and July received less rainfall, while August received more precipitation than the long-term average.

Soil organic carbon (SOC) and total nitrogen

Concentrations of SOC in 0–0.15 m depth of soils in response to the cowpea and fallow treatments were similar at the first sampling, 1 week after termination of cowpea (Figure 2a). However, the

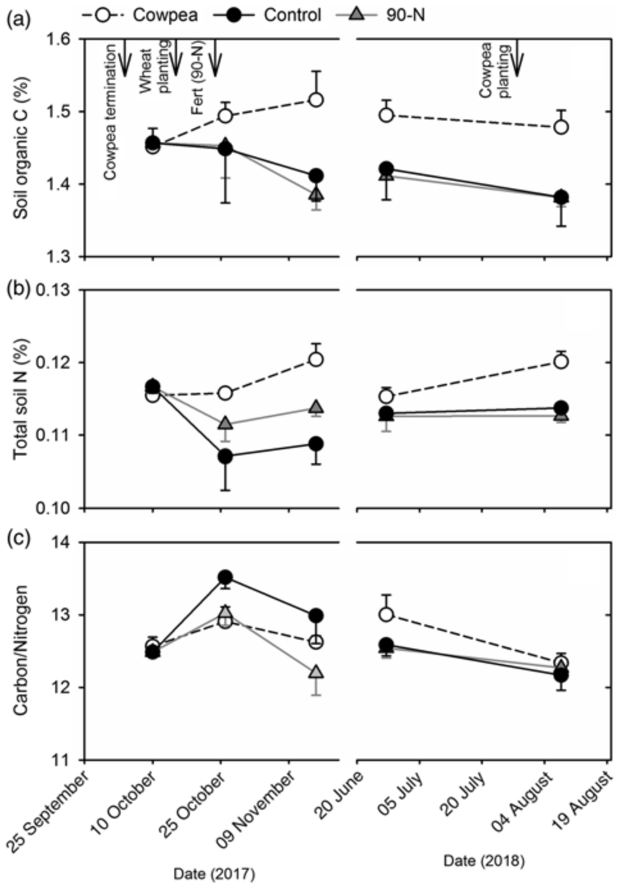

Figure 2. Dynamics of (a) soil organic carbon (C), (b) total nitrogen (N), and (c) C:N ratio in the 0–0.15 m soil depth in response to N treatments. Error bars represent the spatial variations at plot scale (standard error, $n = 3$). Unidirectional error bars are shown for clarity. Note the break in *x*-axis between the periods of planting and harvesting of winter wheat.

concentration of SOC remained slightly higher in cowpea treatment in the subsequent measurements. Average concentrations of SOC measured on five dates in the cowpea treatment (1.49%) were higher ($p < 0.05$) than the control and 90-N treatments (1.42% in both). Effects of sampling date and interaction effects between N treatments and sampling dates on SOC concentrations were not significant.

Concentrations of total N in 0–0.15 m soil depth in cowpea and fallow treatments were similar on the first sampling date, 1 week after termination of cowpea (Figure 2b). Thereafter, the concentration of total N remained slightly higher in cowpea-treated plots than in control and 90-N-treated plots during the subsequent measurements. The average concentrations of soil total N measured on five dates in the cowpea treatment (0.12%) were higher ($p = 0.03$) than the control and 90-N treatments (0.11% in both). Effects of sampling date and interaction effects between N treatments and sampling dates on total soil N concentrations were not significant.

Ratios of C/N in the 0–0.15 m soil depth of cowpea- and fallow-treated plots were similar at the first sampling (Figure 2c). However, the ratio increased slightly in control treatment thereafter. After harvesting of winter wheat, ratios of soil C/N ratios in cowpea-treated plots remained slightly higher. Average C/N ratios measured among the treatments across five dates of measurements were not statistically different. Effect of sampling date was significant with the greatest C/N observed on 26 October, although the ratio fell within a small range of 12.2–13.5.

Environmental and soil conditions during flux measurements

Soil temperatures during flux measurements ranged from 10 to 28 °C during October–November and 24 to 36 °C during June–August (Figure 3a). The plots had standing water on the surface at the first flux measurement on 6 October due to 61 mm rainfall within 48 hours prior to measurements (Figure 3b). Soil moisture (presented as WFPS) declined on the following measurements but increased again with rainfall events. A total of 16 mm rainfall was received between fertilization on 22 October and flux measurement on 24 October. The site was frequently flooded for brief periods after rainfall events in late-June through August.

Dynamics of soil mineral N

Soil concentrations of NO_3^- during October–November were low in response to both cowpea (average, 4.0 mg kg^{-1} soil) and control (average, 2.5 mg kg^{-1} soil) treatments (Figure 3c). However, N fertilization in 90-N treatment increased concentrations to 35 mg kg^{-1} soil on the day following fertilization. Thereafter, soil concentrations of NO_3^- in the 90-N treatment declined gradually. After harvesting of winter wheat, soil concentrations of NO_3^- remained low in all treatments (average, 4–5 mg kg^{-1} soil). Overall, soil concentrations of NO_3^- in 90-N treatment were higher due to elevated concentrations after fertilization events.

Soil concentrations of NH_4^+ during October–November were low in response to both cowpea (average, 1.2 mg kg^{-1} soil) and control (average, 2.5 mg kg^{-1} soil) treatments (Figure 3d). However, N fertilization in 90-N treatment increased concentrations to 14.1 mg kg^{-1} soil 1 day after fertilization. Thereafter, soil concentrations of NH_4^+ in 90-N treatment remained above 8.5 mg kg^{-1} on the subsequent dates. After harvesting of winter wheat, soil concentrations of NH_4^+ were higher in cowpea treatment (average, 10.7 mg kg^{-1} soil) than the control (average, 5.2 mg kg^{-1} soil) and 90-N (average, 4.6 mg kg^{-1} soil) treatments.

Dynamics of CO_2 emissions

Emissions of CO_2 from the cowpea treatment were higher than from the other treatments between incorporation of cowpea and emergence of winter wheat in late-October (Figure 3e). Emissions of CO_2 from control plots approximated zero prior to emergence of winter wheat. After harvest of winter wheat, emissions of CO_2 from cowpea treatments were similar to control and 90-N treatments during measurements taken in late-June, but were higher in late-July and early-August. Emergence of cowpea and contribution of plant respiration to total emissions of CO_2 further widened the gap between cowpea and the fallow treatments during subsequent measurements in mid-August.

Dynamics N_2O emissions

Emissions of N_2O were low from all treatments during October–November (Figure 3f). The maximum rates of N_2O emissions (11 g N_2O–N ha^{-1} d^{-1} from both cowpea and control treatments) during this period were recorded on 16 October. No large rates of N_2O emissions were recorded from 90-N treatment after fertilization, although a total of 16 mm rainfall was received immediately after fertilization and mineral-N concentrations were elevated (Figure 3b–d).

The first measurement after harvesting of winter wheat was taken after a large rainfall event, when a large peak of N_2O emissions (207 g N_2O–N ha^{-1} d^{-1}) was recorded from cowpea treatment. Emissions from 90-N treatment were also elevated slightly by rainfall events. However, the rainfall-induced peaks declined with declining soil moisture and reached close to zero within 1 week. Further rainfall-induced peaks were observed in late-July. Frequent rainfall events occurred in August, which induced frequent peaks of N_2O emissions from cowpea treatment. Few peaks were also observed from the 90-N treatment during this period, but their magnitudes were relatively smaller.

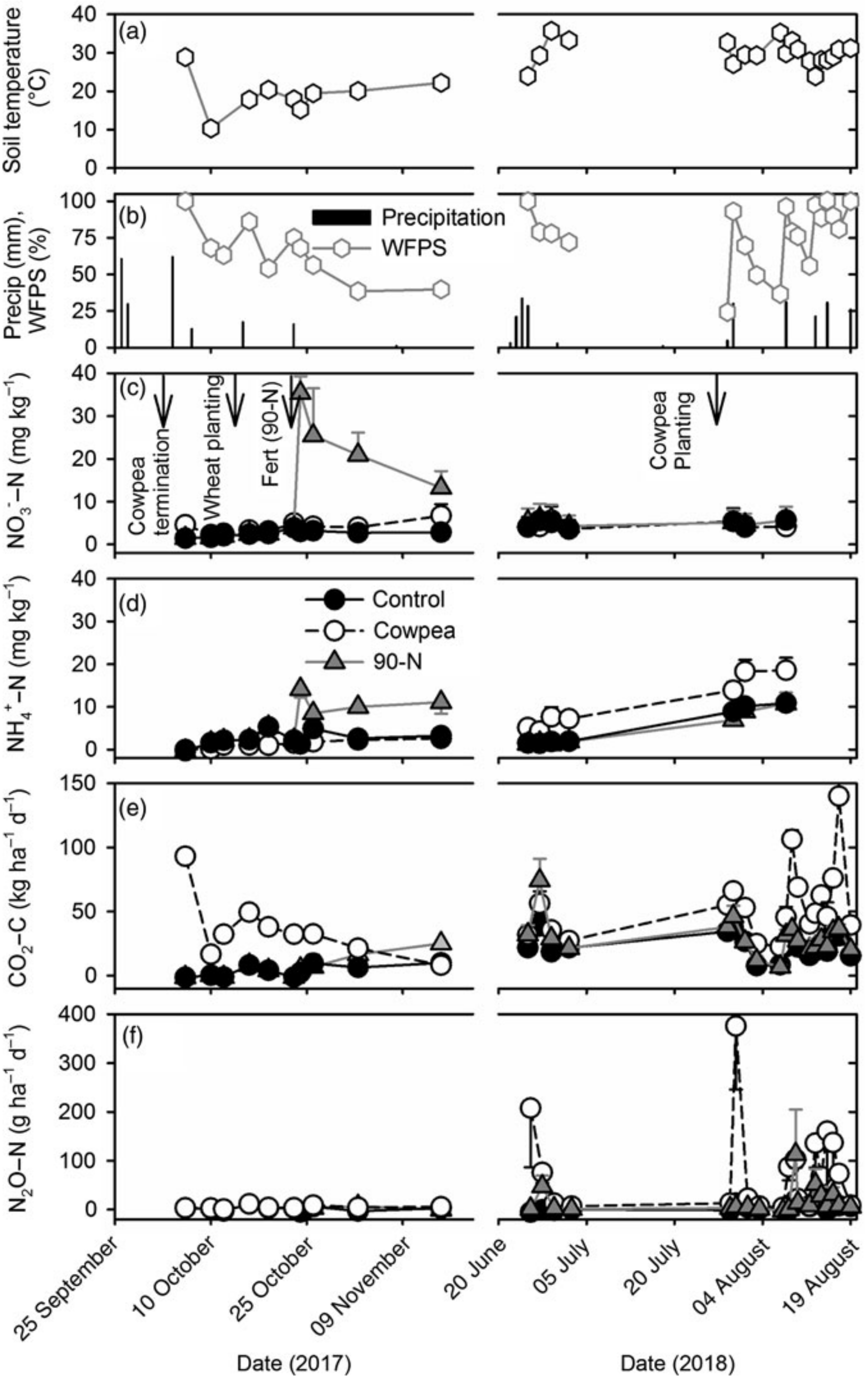

Figure 3. (a) Average soil temperatures at 0–0.05 m depth during flux measurement, (b) dynamics of daily precipitation and water-filled pore space (WFPS) measured at 0–0.05 cm soil depth during flux measurement. Dynamics of soil (c) nitrate (NO_3^-) and (d) ammonium (NH_4^+) N in the 0–0.15 m soil depth. Dynamics of (e) CO_2 and (f) N_2O fluxes. Error bars (c–f) represent the spatial variations at plot scale (standard error, $n = 3$). Unidirectional error bars are shown for clarity. Note the break in *x*-axis between the periods of planting and harvesting of winter wheat.

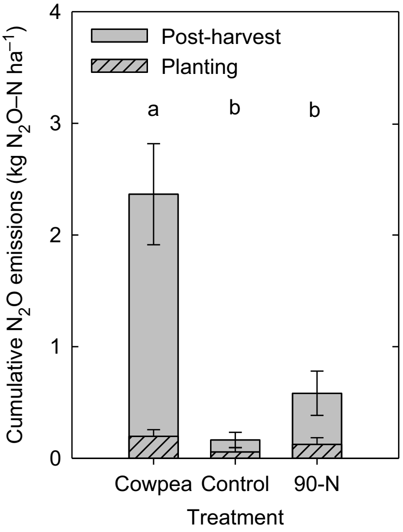

Figure 4. Cumulative estimates of N_2O emissions during planting (40 days from 6 October to 15 November 2017) and after harvesting (55 days from 25 June to 19 August 2018) of winter wheat in response to N treatments. Error bars represent the spatial variations at plot scale (standard error, $n = 3$). The statistical differences ($p < 0.05$) of total cumulative emissions among the treatments are indicated by different letters on the top of bars.

Average rates of N_2O emissions across the measurement dates from cowpea treatment (56 g N_2O–N ha^{-1} d^{-1}) were significantly higher than control (4 g N_2O–N ha^{-1} d^{-1}) and 90-N (12 g N_2O–N ha^{-1} d^{-1}) treatments. Effects of soil sampling dates and interaction effects between treatments and the dates were also significant.

Cumulative N_2O emissions

Total cumulative (40 days from 6 October to 15 November 2017 and 55 days from 25 June to 19 August) emission of N_2O from cowpea treatment (2.4 ± 0.5 kg N_2O–N ha^{-1}) was significantly higher than emissions from control (0.2 ± 0.1 kg N_2O–N ha^{-1}) and 90-N (0.6 ± 0.2 kg N_2O–N ha^{-1}) treatments (Figure 4). Total contribution of cumulative fluxes during October–November in cowpea treatment was 8%, since the large emissions were recorded only after harvesting of winter wheat. However, emissions during October–November accounted for 38 and 22% of the total emissions from control and 90-N treatments, respectively.

Biomass growth, yield, and yield attributing characters of winter wheat

Winter wheat grew more rapidly after planting in response to both control and 90-N treatments than the cowpea treatment, as evidenced by higher RVI growth from November to January (Figure 5). The RVI remained higher in control and 90-N than in cowpea-treated plots after dormancy and reached close to zero at senescence in mid-May.

Total DM and grain produced by winter wheat was significantly different ($p < 0.05$) among N treatments (Figure 6a, b). The highest amount of DM and grain was produced by 90-N treatment and the lowest amount was produced by the cowpea treatment (i.e., 90-N > control > cowpea). Thus, the difference in DM and grain yields among the treatments followed similar patterns of biomass growth measured as RVI.

Plant height, spike length, and number of tillers per m^{-2} were greatest in 90-N treatment and lowest in cowpea treatment (Figure 7a–c). Number of grains per spike under cowpea treatment

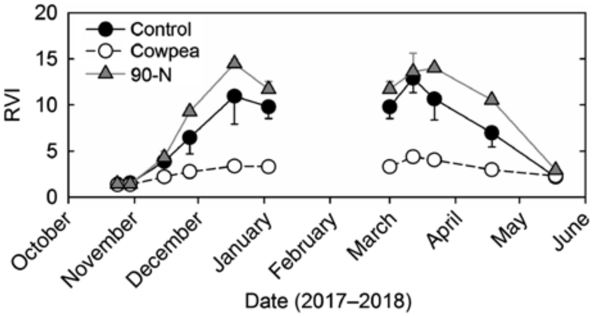

Figure 5. Dynamics of ratio vegetation index (RVI) as a proxy for green biomass of winter wheat in response to N treatments. Error bars represent the spatial variations at plots (standard error, $n = 3$). Unidirectional error bars are shown for clarity.

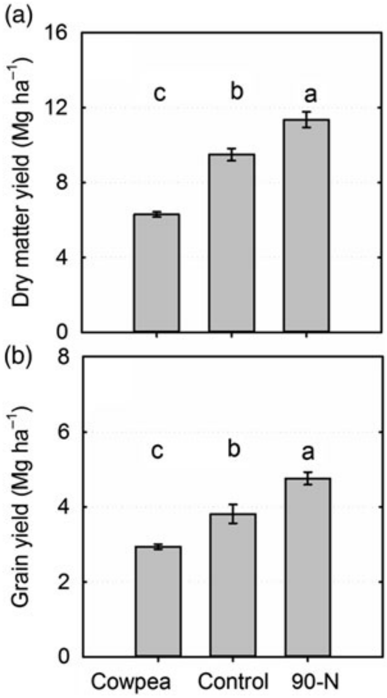

Figure 6. (a) Aboveground dry matter and (b) grain yields of winter wheat in response to N treatments. Error bars represent the spatial variations at plots (standard error, $n = 3$). The statistical differences ($p < 0.05$) among the treatments are indicated by different letters on the top of bars.

was similar to 90-N treatment (Figure 7d). Weight of winter wheat grain under cowpea treatment was similar to 90-N and control treatments, while harvest index was the greatest (Figure 7e, f).

Nitrogen concentrations and uptake of winter wheat

Concentrations of N in grain of winter wheat were lower in response to the cowpea treatment than the 90-N treatment (Figure 8a, b). However, concentrations of N in straw of winter wheat were similar among all three N treatments ($p > 0.05$). Similar to biomass yield, the highest amount of N

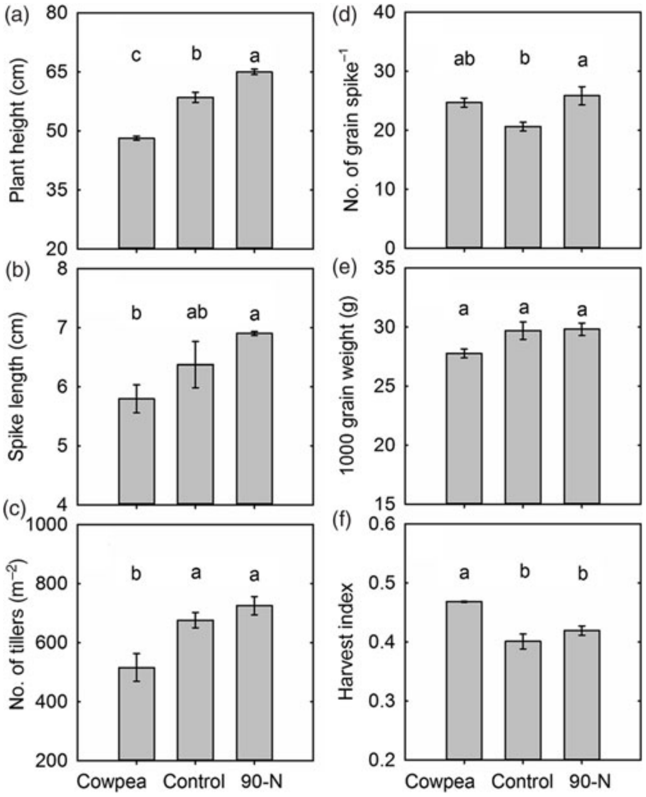

Figure 7. Yield attributing characters of winter wheat in resposne to N treatments. Error bars represent the spatial variations at plots (standard error, $n = 3$). The statistical differences ($p < 0.05$) among the treatments are indicated by different letters on the top of bars.

in winter wheat was recorded in 90-N-treated plots and the lowest amount was noted in cowpea-treated plots (Figure 8c).

Discussion

Emissions of N_2O

Contrary to the results in the current study, previous studies have mostly demonstrated rapid N mineralization from cowpea biomass after termination and soil incorporation which are conducive biomass traits for large emissions of N_2O after soil incorporation of legumes (Frimpong *et al.*, 2011; O'Connell *et al.*, 2015). In the current study, although frequent rainfall events occurred after soil incorporation of cowpea biomass, lack of N mineralization from cowpea biomass, as evidenced by low levels of soil NO_3^- and NH_4^+, resulted in low emissions of N_2O during measurements in October–November. A possible reason for low N mineralization of cowpea after termination was relatively higher C/N ratios (16) compared to cowpea biomass in previous studies (11–12). However, lignin concentrations in cowpea biomass, which is an important factor for biomass decomposition, were similar to concentrations reported in previous studies (Frimpong *et al.*, 2011; O'Connell *et al.*, 2015). The higher emissions of CO_2 from cowpea treatment than control treatment in October 2017, when contribution of plant respiration from winter wheat was mostly absent, indicate decomposition of cowpea biomass occurred after termination.

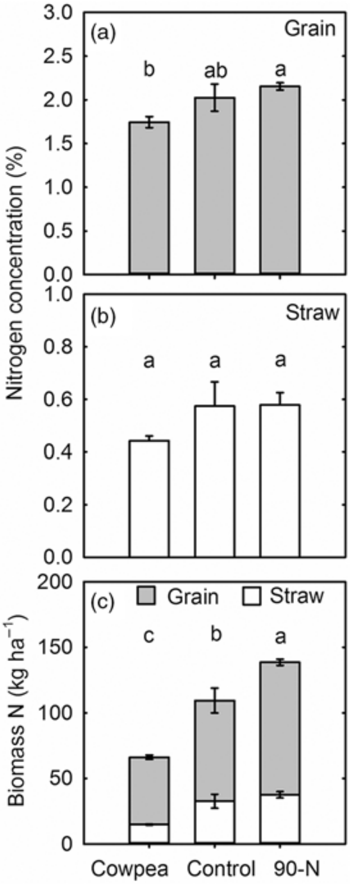

Figure 8. Nitrogen concentrations in the (a) grain and (b) straw of winter wheat. (c) Total amount of nitrogen in the grain and straw of winter wheat in response to N treatments. Standard error (SE) bars represent spatial variations at plot scale ($n = 3$). The statistical differences ($p < 0.05$) among the treatments are indicated by different letters on the top of bars.

In an incubation study, Franzluebbers *et al.* (1994) found significantly lower rates of N mineralization of cowpea biomass after soil incorporation under repeated drying and wetting cycles than under the continuous moist conditions. In addition to N mineralization, repeated drying and wetting severely inhibited growth and/or activity of nitrifiers (Franzluebbers *et al.*, 1994). Such periodic dry–wet cycles prevailed at the current field site during October–November after incorporation of cowpea. Such a lack of N mineralization, and inhibition of nitrifiers, at the current study site might have contributed to low concentrations of mineral N and emissions of N_2O. The low N_2O emissions from 90-N treatment despite elevated soil concentrations of NO_3^- and NH_4^+ are due to declining soil moisture in the absence of rainfall events after late-October. Since no major rainfall events were recorded during November 2018–January 2019 (Figure 1b), no measurements of N_2O fluxes were taken during this period. Although few rainfall events were recorded thereafter prior to maturity of winter wheat, N mineralization from soil incorporated cowpea biomass was possibly restricted as growth of winter wheat was not improved even after winter dormancy (Figure 5).

Large peaks of N_2O emissions observed from cowpea-treated plots after harvesting of winter wheat can be related to delayed mineralization of biomass N during summer. In particular, soil concentrations of NH_4^+ in cowpea-treated plots during summer were significantly higher (Figure 3d). Additionally, higher levels of mineralizable C contributed by incorporated cowpea biomass may have contributed for larger emissions of N_2O from cowpea-treated plots (Mitchell *et al.*, 2013). The peaks of N_2O emissions observed following rainfall events indicated synergistic effects of high moisture and availability of mineralized N on N_2O emissions (Kandel *et al.*, 2018; 2019b). During summer measurements until mid-August, there was no strong stand of cowpea to compete for available mineral N with soil microbes responsible for N_2O production. Thus, high rates of N mineralization, lack of crop N uptake, and frequent rainfall events contributed for such large emissions of N_2O outside the growing period of winter wheat.

Winter wheat growth, yield, and N uptake

Poor growth of winter wheat in cowpea-treated plots may be particularly related to crop uptake of mineralized N during growing periods of cowpea and lower rates of biomass N mineralization after incorporation of cowpea biomass into soil. The lower rate of N mineralization and possible immobilization of soil N by cowpea biomass was evidenced as lower concentrations of NH_4^+ in cowpea treatment, although soil concentration of NO_3^- was mostly similar in cowpea and control treatments. Additionally, possible immobilization of phosphorous and other soil nutrients in cowpea biomass might have contributed for poor growth by winter wheat (Espinosa *et al.*, 2017).

Although depletion of soil moisture by cowpea cultivation may reduce the growth of following winter crops (Nielsen *et al.*, 2015), high amounts of rainfall in September and October had replenished the soil moisture at planting of winter wheat. Although a long period of drought persisted in winter, the drought had a minor role in poor growth of winter wheat in cowpea-treated plots since the crop was dormant during winter. After dormancy, growth of winter wheat was much greater in the control and 90-N than cowpea-treated plots, which indicates lack of complete recovery by wheat from poor pre-winter growth.

To be an effective N source, legumes must fix enough N in their plant tissues during growth to meet the needs of the recipient crop, which in turn must be capable of effectively being transferred to the following crop (Northup and Rao, 2016; Rao and Northup, 2011). The high yield and amounts of N in cowpea biomass indicated that a productive stands of cowpea can provide sufficient N for successful cultivation of following crops of winter wheat in the SGP. Higher concentrations of total soil N in cowpea-treated plots also indicated an increased N pool. Although cowpea biomass contained sufficient N to meet requirements of following winter wheat, lack of mineralization of the biomass N after termination limited effective transfer of the N to following crops of winter wheat. This lack of rapid biomass N mineralization contributed for low emissions of N_2O at planting of winter wheat, and wheat yields were also significantly affected.

Our results indicated that late terminated cowpea might not be an effective source of N for winter wheat. Future studies are needed to synchronize N-mineralization from cowpea-based green manures after incorporation into soil, with N-demand of winter wheat. A possible management tool to increase availability of mineralized N for winter wheat during early growth can be grazing of cowpea prior to termination (Francis *et al.*, 1998). Utilization of the biomass for direct economic returns can be an attractive option to increase adoption of legumes as green manures. Further, testing of other summer legumes which produce high levels of biomass and N and mineralize N during the early period of growth by winter wheat is needed. For example, a recent study in the US SGP has demonstrated high levels of biomass production (up to 18.1 Mg ha^{-1}) and biomass N (up to 316 kg ha^{-1}) by moth bean (*Vigna aconitifolia* L.) which is a novel crop in the region (Baath *et al.*, 2018). Further studies are required for synchronization potential between biomass N mineralization from such high yielding novel legumes and crop N-demand of winter wheat in the region.

Conclusions

The present study showed late terminated cowpea was not an effective source of green N for winter wheat. Despite producing 9.5 Mg ha^{-1} shoot biomass with 253 kg N ha^{-1} at termination, N mineralization from cowpea biomass did not synchronize with N-demand of winter wheat. The lack of N mineralization from soil incorporated cowpea residue contributed for poor growth and yields of winter wheat compared to fallow treatments with and without inorganic N fertilizer. Concentration of mineral N in cowpea treatment remained higher in summer which contributed for large rainfall-induced peaks of N_2O outside growing season of winter wheat. Overall, the results of this study indicate that late terminated cowpea as green manure can be a lose–lose option as it not only reduced yield and grain quality of winter wheat but also increased emissions of N_2O significantly. However, cowpea appears to improve soil quality in long run due to increased pool of C and N, which may benefit production of winter wheat if synchronization between N mineralization from cowpea biomass and demand of following winter wheat is achieved.

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Financial support. None.

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Cite this article: Kandel TP, Gowda PH, Northup BK, and Rocateli AC (2020). Winter wheat yield and nitrous oxide emissions in response to cowpea-based green manure and nitrogen fertilization. *Experimental Agriculture* **56**, 239–254. https://doi.org/10.1017/S0014479719000334