Effect of legume intercropping on N_2O emission and CH_4 uptake during maize production in the Ethiopian Rift Valley

Shimelis G Raji[1,2] and Peter Dörsch[1]

[1] Faculty for Environmental Sciences and Resource Management, Norwegian University of Life Sciences (NMBU), 1432 Ås, Norway

[2] College of Agriculture, Hawassa University, P.O, Box 05, Hawassa, Ethiopia

Correspondence to: Peter Dörsch (peter.doersch@nmbu.no)

Abstract

Intercropping with legumes is an important component of climate smart agriculture (CSA) in sub-Saharan Africa, but little is known about its effect on soil greenhouse gas (GHG) exchange. A field experiment was established at Hawassa in the Ethiopian rift valley, comparing nitrous oxide (N_2O) and methane (CH_4) fluxes in minerally fertilized maize (64 kg N ha^{-1}) with and without crotalaria (*C. juncea*) or lablab (*L. purpureus*) as intercrops over two growing seasons. To study the effect of intercropping time, intercrops were sown either three or six weeks after maize. The legumes were harvested at flowering and half of the above-ground biomass was mulched. In the first season, cumulative N_2O emissions were largest in 3-week lablab, with all other treatments being equal or lower than the fertilized maize monocrop. After reducing mineral N input to intercropped systems by 50% in the second season, N_2O emissions were comparable with the fully fertilized control. Maize yield-scaled N_2O emissions in the first season increased linearly with above-ground legume N-yield (P=0.01), but not in the second season when early rains resulted in less legume biomass because of shading by maize. Growing season N_2O-N emission factors varied from 0.02 to 0.25 and 0.11 to 0.20% of the estimated total N input in 2015 and 2016, respectively. Growing season CH_4 uptake ranged from 1.0 to 1.5 kg CH_4-C ha^{-1} with no significant differences between treatments or years, but setting off the N_2O-associated emissions by up to 69%. Our results suggest that leguminous intercrops may increase N_2O emissions when developing large biomass in dry

years, but when mulched, can replace part of the fertilizer N in normal years, thus supporting CSA goals while intensifying crop production in the region.

Key words: yield-scaled N_2O emissions, CH_4 uptake, legume-intercropping, maize, Africa

1. Introduction

With a rapidly increasing population and declining agricultural land in Sub-Saharan Africa (SSA), increasing productivity per area (intensification) is the only viable alternative for producing sufficient food and feed (Hickman et al., 2014a). Intensification entails increased use of inorganic fertilizers, which may cause emissions of nitrous oxide (N_2O). Abundant ammonium (NH_4^+) may also reduce the soil CH_4 sink by competing with CH_4 for the active binding site of methane monooxygenase, the key enzyme of CH_4 oxidation (Bédard and Knowles, 1989). Climate smart agriculture (CSA) has been proposed as a way forward to simultaneously increase agricultural productivity, while increasing climate resilience and reducing greenhouse gas (GHG) emissions (Neufeldt et al., 2013). However, greenhouse gas emission measurements in SSA crop production systems are scarce and proof-of-concept for the mitigation potential of specific CSA practices is missing (Kim et al., 2016, Hickman et al., 2014b). Moreover, modelling studies predict significant negative impacts of climate change on crop productivity in Africa (Blanc and Strobl, 2013) and it is largely unknown how these and the countermeasures taken to maintain agricultural productivity will affect GHG emissions.

Crop production is a major source of N_2O, the third-most important anthropogenic GHG after CH_4 and CO_2 (IPCC, 2014). Emission rates of N_2O reported for SSA crop production so far are low (Kim et al., 2016) owing to low fertilization rates, but may increase with increasing intensification. Inorganic and organic N added to soil provide ammonium (NH_4^+) and nitrate (NO_3^-) for nitrification and denitrification, respectively, which are the two main processes of microbial N_2O production in soil (Khalil et al., 2004). The rate of N_2O formation depends greatly on the extent and distribution of anoxic microsites in soils, which is controlled by moisture, texture and the distribution of decomposable organic matter and NH_4^+ fueling heterotrophic and autotrophic respiration, respectively (Schlüter et al., 2019, Wrage-Mönnig et al., 2018). The magnitude of soil N_2O emissions depends on O_2 availability as controlled by soil moisture and respiration, availability of mineral N and readily decomposable C (Harrison-Kirk et al., 2013) and soil pH (Russenes et al., 2016), all of which are affected by management practices. Other important factors are soil type (Davidson et al., 2000) and temperature (Schaufler et al., 2010). The N_2O yield of nitrification and the production and reduction of N_2O during denitrification are further controlled by soil pH (Bakken et al., 2012, Nadeem et al., 2019) and by the balance between oxidizable carbon and available NO_3^- (Wu et al., 2018). Mulching and incorporation of crop residues leads to increased N mineralization and respiratory O_2 consumption, thus potentially enhancing N_2O emissions both from nitrification and denitrification (Drury et al., 1991), if soil moisture is sufficient to support microbial activity and restrict O_2 diffusion into the soil. Accordingly, N_2O emissions are variable in time, often following rainfall events (Schwenke et al., 2016).

Crop diversification by combining legumes with cereals, both in rotation and intercropping, enhances overall productivity and resource use efficiency, if managed properly (Ehrmann and Ritz, 2014). Intercropping of maize with grain legumes is common in the rift valley of Ethiopia and central component in CSA (Arslan et al., 2015). In low input systems common to the Rift Valley, integration of legumes with cereals diversifies the produce and improves farm income and nutritional diversity for smallholder farmers (Sime and Aune, 2018). Moreover, by partially replacing energy-intensive synthetic N, intercropping with legumes may increase the sustainability of the agroecosystem as a whole (Carranca et al., 2015). However, to make best use of the resource use complementarity of inter and main crop, the planting time of the intercrop has to be optimized so that the maximum nutrient demand of the two components occurs at different times (Carruthers et al., 2000). The timing of intercrops could also affect N_2O emissions if N mineralization from legume residues is poorly synchronized with the N requirement of the cereal crop. This can be counteracted by reducing mineral N additions to intercropping systems, but the timing of the intercrop (sowing date relative to the cereal crop) remains an issue that has, to the best of our knowledge, not been studied with regard to N_2O emissions.

Intercropping and mulching may also affect the soil's capacity to oxidize atmospheric CH_4 as abundant NH_4^+ might inhibit methanotrophs (Laanbroek and Bodelier, 2004). However, field studies with incorporation of leguminous or non-leguminous catch crops have been inconclusive (e.g. Sanz-Cobena et al., 2014). In a meta-study on CH_4 fluxes in non-wetland soils, Aronson and Helliker (2010) concluded that N inhibition of CH_4 uptake is unlikely at fertilization rates below 100 kg N ha^{-1} y^{-1} and that much to the contrary, N addition may stimulate CH_4 uptake in N-limited soils. Ho et al. (2015) found that incorporation of organic residues stimulated CH_4 uptake even in fairly N-rich Dutch soils. Apart from providing reactive nitrogen to the soil, leguminous intercrops may also affect CH_4 uptake by lowering soil moisture and thus increasing the diffusive flux of atmospheric CH_4 into the soil. For instance, Wanyama et al. (2019) found that CH_4 uptake in soil was negatively correlated with mean annual water-filled pore space in a study on different land use intensities in Kenya.

In a review on N_2O fluxes in agricultural legume crops, Rochette and Janzen (2005) concluded that the effect of legumes on N_2O emission is to be attributed to the release of extra N by rhizodeposition of soluble N compounds and decomposition of nodules rather than to the process of nitrogen fixation itself. Intercropped legumes may thus affect N_2O emissions in two ways: by directly providing organic N or by modulating the competition between plants and microbes for soil N, for example by acting as an additional N sink prior to nodulation. Compared to mineral fertilizers, N supply from biological fixation is considered environmentally friendly as it can potentially replace industrially fixed N (Jensen and Hauggaard-Nielsen, 2003), provided that crop yields remain the same. However, combining easily degradable crop residues with synthetic N can lead to elevated N_2O emissions (Baggs et al., 2000), potentially compromising the environmental friendliness of intercropping in CSA. It is well known that the effect of crop residues on N_2O emission depends on a variety of factors such as residue amount and quality (C:N ratio, lignin and cellulose content), soil properties (e.g. texture), placement mode (mulching, incorporation) and soil moisture and temperature regimes (Sanz-Cobena et al., 2014, Li et al., 2016). So far, there is only a limited number of studies addressing the effect of legume intercropping on N_2O emissions and CH_4 uptake in SSA crop production (Baggs et al., 2006; Millar et al., 2004; Dick et al., 2008).

The main objective of the present study was to evaluate the effects of forage legume intercropping with maize on N_2O and CH_4 emissions during maize production in the Ethiopian Rift Valley. We hypothesized that forage legumes increase N_2O emissions and decrease CH_4 uptake depending on above-ground biomass, legume species and sowing date; legumes intercropped three weeks after sowing of maize would result in higher yields than those intercropped six weeks after maize and lead to increased N_2O emissions if used with full-dose mineral fertilization. With late intercropping, legume yields would be suppressed having no or little effect on N_2O emissions. Hence, choosing legume species, sowing date and accounting for potential N inputs from legume intercrops could allow for better management of legume intercropping in SSA with reduced GHG emissions.

2. Materials and methods

2.1 Study area

The field experiment was conducted during two years (2015-2016) at the Hawassa University Research Farm, 07°3’3.4”N and 38°30”20.4’E at an altitude of 1660 m a.s.l.. The mean annual rainfall is 961 mm, with a bimodal pattern. The rainy season between June and October accounts for close to 80% of the annual rainfall. Average maximum and minimum monthly temperatures are 27.4 and 12.9^{o}C, respectively. The soil is clay-loam (46% sand, 26% silt, 28% clay) derived from weathered volcanic rock (Andosols), with a bulk density of 1.25 ± 0.05 g cm^{-3}, a total N content of 0.12%, an organic C content of 1.64%, an available Olsen P content of 175 mg kg^{-1} and a pH_{H2O} of 6.14.

2.2 Experimental design and treatments

Experimental plots (20 m^{2}) with six treatments were laid out in a complete randomized block design (RCBD) with four replicates: unfertilized maize monocrop (M-F), fertilized maize monocrop (M+F), crotalaria intercropping three (M+Cr3w) and six (M+Cr6w) weeks after sowing maize and lablab intercropping three (M+Lb3w) and six (M+Lb6w) weeks after sowing maize (Table 2). Seed bed was prepared in both years by mold board plow to a depth of 0.25 m followed by harrowing by a tractor. A hybrid maize variety, BH-540 (released in 1995) was sown on May 30 and May 7 in 2015 and 2016, respectively. Maize was planted at a density of 53,333 plants ha^{-1}. Following national fertilization recommendations, diammonium phosphate (18 kg N, 20 kg P) was applied manually at planting and urea (46 kg N) four weeks after sowing maize, except for the unfertilized control. The N fertilization rate was halved for the intercropping treatments in the 2016 season to account for carry-over of N from forage legumes grown in the previous year. The forage legumes crotalaria (*C. juncea*) and lablab (*L. purpureus*) were planted between maize rows at a density of 500,000 and 250,000 plants ha^{-1}, respectively.

The above-ground forage legume biomass was harvested at flowering and half of it was removed. The remaining half was spread manually between the maize rows after cutting the fresh biomass into ~10 cm pieces. Three- and 6-week intercrops were mulched on 27 July and 4 September and 2 August and 8 September in 2015 and 2016, respectively. As the mulching was done plot wise, plots within the same treatment received different amounts of mulch depending on the legume yield of each plot. In the 2016 growing season, all treatments were kept on the same plots as in 2015, capitalizing on plot-specific N and C input from previous mulch. Aboveground dry matter yield was determined by drying a subsample at 72ºC for 48 hours and C and N contents were measured by an element analyser.

2.3 N_2O and CH_4 fluxes and ancillary data

GHG exchange was monitored weekly at random spots within the middle maize row by static, non-vented chambers (Rochette et al., 2008). We used custom-made aluminum chambers with an internal volume of 0.144 m^3 and a cross-sectional area of 0.36 m^2. The chambers were pushed gently ~3 cm into the soil including 2 - 5 legume plants before closing the septum and sealing the chambers around their circumference with moist clay.

Sampling was carried out weekly during the period June to September in 2015 and May to September in 2016 on 15 and 17 sampling dates, respectively. Gas samples were collected between 9:00 am and 2:00 pm. For each flux estimate, four gas samples were drawn from the chamber headspace at 15 min intervals, starting immediately after deployment. Samples were taken with a 20 ml polypropylene syringe equipped with a 3-way valve. Before transferring the sample to a pre-evacuated 10 cc serum vial crimp-sealed with butyl septa, the sample was pumped 5 times in and out of the chamber to obtain a representative sample. Overpressure in the septum vials was maintained to protect the sample from atmospheric contamination during storage and shipment to the Norwegian University of Life Sciences, where the samples were analyzed by gas chromatography. Helium-filled blank vials were included to evaluate contamination, which was found to be less than 3% of ambient.

All samples were analyzed on a GC (Model 7890A, Agilent Santa Clara, CA, USA) connected to an auto-sampler (GC-Pal, CTC, Switzerland). Upon piercing the septum with a hypodermic needle, ca. 1 ml sample is transported via a peristaltic pump (Gilson minipuls 3, Middleton, W1, USA) to the GC's injection system, before reverting the pump to backflush the injection system. The GC is configured with a Poraplot U wide-bore capillary column connected to a thermal conductivity, a flame ionization and an electron capture detector to analyze CO_2, CH_4 and N_2O, respectively. Helium 5.0 was used as carrier and Ar/CH_4 (90:10 vol/vol) as makeup gas for the ECD. For calibration, two certified gas mixtures of CO_2, N_2O and CH_4 in Helium 5.0 (Linde-AGA, Oslo, Norway), one at ambient concentrations and one ca. 3 times above ambient were used. A running standard (every tenth sample) was used to evaluate drift of the ECD signal. Emission (CO_2, N_2O) and uptake (CH_4) rates were estimated by fitting linear ($R^2 \geq 0.85$) or quadratic functions to the observed concentration change in the chamber headspace and converting them to area flux according to eq. 1

$$F_{GHG\ (\mu g\ m^{-2} h^{-1})} = \frac{dc}{dt} * \frac{Vc}{A} * \frac{Mn}{Vn} * 60 \qquad \text{Eq. (1)}$$

where, F_{GHG} is the flux (µg N_2O-N m^{-2} h^{-1} in case of N_2O; µg CH_4-C in the case of CH_4), $\frac{dc}{dt}$ the rate of change in concentration over time (ppm min^{-1}), V_c the volume of the chamber (m^3), A the area covered by the chamber (m^2), M_n the molar mass of the element in question (g mol^{-1}) and Vn the molecular volume of gas at chamber temperature (m^3 mol^{-1}). A quadratic fit was only used in cases where N_2O accumulation in the chamber showed a convex downwards and CH_4 uptake a convex upwards trend (i.e. decreasing emission or uptake rates with time) to estimate time-zero rates. Fluxes were cumulated plot-wise by linear interpolation for each growing season.

In 2016, soil moisture and temperature at 5 cm depth were monitored hourly using data loggers (Decagon EM50, Pullman, WA, USA) together with ECH$_2$O sensors (Decagon) for volumetric soil water content (VSWC) and temperature at five points across the experimental field. The sensors were placed in the experimental field at 5 random spots. No data are available for the 2015 season, due to equipment failure.

Soil bulk density was measured at 10 random spots in the experimental field using 100 cm^3 steal cylinders and drying them at 105 °C for 24 hours. To calculate daily water filled pore space values for the 2016 growing season, a particle density of 2.65 g cm^{-3} was assumed:

$$WFPS = VSWC/(1 - \frac{BD}{PD}) * 100 \qquad \text{Eq. (2)}$$

where *WFPS* is the water filled pore space, *VSWC* the volumetric soil water content, *BD* the bulk density and *PD* the particle density. Daily rainfall data were collected using an on-site rain gauge.

2.4 Estimating N inputs and N_2O emission factors

N input from forage legume crop residues was estimated from measured above-ground dry matter yield, its N content and the amount of mulch applied. To account for belowground inputs a shoot to root ratio of two was assumed for both crotalaria and lablab (Fageria et al., 2014). Dry matter yields of forage legumes differed greatly depending on sowing time, with generally larger yields in 3-week than 6-week intercropping. Also, forage legumes sown three weeks after maize grew faster and were harvested and mulched earlier than those sown six weeks after maize. We assumed that 50% of the legume N (mulched and belowground) was released during the growing season but reduced this amount to 30% for the aboveground component (mulch) of the 6-week treatments to account for the later mulching date. The proportions becoming available during the growing seasons are conservative estimates based on Odhiambo (2010), who reported that about 50% of N contained in crotalaria, lablab and mucuna was released during a 16-week incubation experiment at optimal temperature and moisture conditions. Placing litter bags into dry surface soil, Abera et al. (2014) found that legume residues decomposed rapidly under *in situ* conditions in the Ethiopian Rift Valley, releasing up to 89% of the added N within 6 months.

For the second year, 50% of the N left after the growing season (below and aboveground) was assumed to become available, on top of the N-input from the newly sown forage legumes. Dry matter yields of forage legumes and estimated N input for the two years are presented in Table 1.

Treatment-specific, growing-season N_2O emission factors were calculated as:

$$N_2O\ EF = \frac{(N_2O_{treatment} - N_2O_{control})}{N\ input} * 100 \qquad \text{Eq. (3)}$$

where $N_2O\ EF$ is the N_2O emission factor (% of N input lost as N_2O-N), $N_2O_{treatment}$ the cumulative N_2O-N emission (from sowing to harvest) in the fertilized and intercropped treatments, $N_2O_{control}$ the emission from the 0N0P treatment (background emission) and N_{input} the estimated total input of N.

Non-CO_2 GHG emissions were calculated as CO_2 equivalents balancing cumulative seasonal N_2O-N emissions with CH_4 uptake on the plot level and averaging them for treatments (Table 2, Fig. 5).

2.5 Grain yields and yield-scaled N_2O emissions

Maize grain yield was determined by manually harvesting the three middle rows (to avoid border effects) of each plot, and was standardized to 12.5% moisture content using a digital grain moisture meter. All values were extrapolated from the plot to the hectare. To estimate yield-scaled N_2O emissions (g N_2O-N ton^{-1} grain yield), cumulative emissions were divided by grain yield.

2.6 Statistical analysis

Differences in cumulative CH_4 and N_2O emissions between treatments in each cropping season were tested by analysis of variance (ANOVA) with LSD used for mean separation after testing the data for normality and homoscedasticity. Cumulative seasonal N_2O emissions for 2015 were log-transformed. Statistical significance was declared at $P \leq 0.05$.

3. Results

3.1 Weather conditions

The year 2015 was one of the most severe drought years in decades and, as a result, sowing in 2015 was delayed by 3 weeks as compared to 2016. Rain fell late during the growing season and the cumulative rainfall for April to October was about 100 mm lower in 2015 than in 2016 (Fig. 1d, g).

3.2 N_2O fluxes

N_2O emission rates in 2015 (treatment means, n=4) ranged from 1.1 to 13.7 µg N m^{-2} h^{-1} for the control treatment (Fig. 1a). Similarly, for fertilized maize, N_2O emissions ranged from 2 to 23.5 µg N m^{-2} h^{-1}. Emission fluxes were generally larger for the intercropped treatments: crotalaria treatments emitted N_2O at rates of 1.7 - 34.3 and 2.1 – 24.2 µg N m^{-2} h^{-1} when intercropped 3 or 6 weeks after maize, respectively, while maize-lablab emitted 1.9 – 62.7 µg N m^{-2} h^{-1} when sown 3 weeks and 1.5 - 10.7 µg N m^{-2} h^{-1} when sown 6 weeks after maize. The generally low emission rates in the latter system (6-weak lablab intercropping) corresponded to poor growth of lablab due to shading by the maize plants. Irrespective of legume species, the highest emission rates were found for intercrops planted three weeks after maize (Fig. 1b, c). A peak of N_2O emission occurred in the 3-week intercropping systems around mid-August, 2015, which was significantly larger than in the unfertilized control (P=0.013), the fertilized maize monocrop (P=0.001), and the 6 weeks crotalaria (P=0.021) and lablab (P=0.002) intercrops.

During the 2016 season, N_2O emission rates in the 0N-control varied between 2.5 and 22.8 µg N m^{-2} h^{-1}, peaking at the beginning of the season when WFPS was >50%. There were no significant differences in WFPS values between treatments (data not shown). Fertilized maize had similar rates (3.1 - 24.2 µg N m^{-2} h^{-1}) peaking at around four weeks after planting. Maize-forage legume treatments had larger emission rates, ranging from 1.8 to 40.2 and 3.2 to 58.6 for crotalaria planted 3 and 6 weeks after maize, respectively and 3.9 to 38.0 and 1.9 to 45.2 µg N m^{-2} h^{-1} for lablab planted 3 and 6 weeks after maize, respectively. In general, emission rates were higher in the beginning than in the end of the cropping season (Fig. 1d-f). Despite higher fluxes for intercropping treatments than in the unfertilized control in week 1 (P=0.162) and 4 (P=0.061), there were no statistically significant differences in flux rates between the treatments.

3.3 Cumulative N_2O emissions

During the 2015 growing season, all treatments had equal or higher cumulative N_2O emissions than the unfertilized control, with the 3-week lablab intercropping system emitting significantly more N_2O than the unfertilized control (p=0.006) and the 6-week lablab intercrop (Fig. 2a). Comparing intercropping treatments with the fertilized control, lablab sown 3 weeks after maize clearly increased N_2O emissions but not significantly (P=0.35), whereas all other intercropping treatments had cumulative N_2O emissions comparable with fertilized maize control. Regarding sowing date, 3-week lablab had significantly higher N_2O emissions (P<0.01) than its 6-week counterpart, whereas no such effect was seen for crotalaria.

During the 2016 growing season, lablab intercropping 3-weeks after maize showed significantly higher (P<0.01) cumulative N_2O emissions than the unfertilized control, but there was no difference between fully fertilized maize monocrop and intercropped maize treatments fertilized with 50% of the mineral N applied in 2015, nor was there any effect of intercropping date (3 vs. 6 weeks; Fig. 2b).

3.4 Legume and maize yields

Aboveground yields of lablab were generally higher than those of crotalaria (Table 1). Intercropping three weeks after maize resulted in higher biomass yields compared to six weeks for both legume species. Both legumes grew poorly during the second growing season, particularly crotalaria. Maize grain yields differed greatly between the years and were roughly 20% higher in the wetter year of 2016 (Table 2). Better growth conditions for maize in the second year resulted in smaller yields of intercrop legumes.

3.5 N_2O emission factor and intensity

Growing-season emission factors (EF) varied from 0.02 to 0.25 and 0.11 to 0.20% in 2015 and 2016, respectively (Table 2). Of the intercropped treatments, lablab intercropped 3 weeks after maize resulted in a significantly larger emission factor than fertilized maize and other intercropping treatments, whereas there was no significant difference in 2016. Overall, growing-season N_2O emission factors were ~ 40% higher in 2016 than in 2015, which is mainly due to the smaller N input in 2016 which was 25 to 45% lower than in 2015, except for the 3-week lablab system which had an estimated 18% higher N input in 2016 than 2015 (Table 1). The latter was due to the extraordinary high lablab yield in the previous year and its stipulated carryover (Table 1).

Mean yield-scaled N_2O emissions in 2015 varied between 25 to 55 g N_2O ton^{-1} grain yield. In 2015, 3-week lablab had a higher N_2O intensity than 6-week lablab, whereas all other differences were insignificant. In 2016, with mineral N fertilization reduced to 50%, N_2O emission intensities varied from 26 to 37 g N_2O ton^{-1} grain, with no significant effect of legume species, sowing date or N fertilization (Table 2).

To further explore the variability of N_2O emissions, we plotted cumulative N_2O emissions plot-wise against legume N yield, but found no relationship (not shown). However, when plotting yield-scaled N_2O emission over legume N yield, a significant positive relationship (P=0.01) emerged for 2015, but not 2016 (Fig. 3a, b), suggesting that leguminous N input increased N_2O emissions more than maize yields in the dry year of 2015.

3.6 CH_4 fluxes

All treatments acted as net sink for CH_4, with uptake rates ranging from 31 to 93 µg C m^{-2} h^{-1} in 2015 (Fig. 4a-c). Uptake rates in 2015 were rather constant in time with somewhat elevated uptake rates towards the end of the season. There were no obvious treatment effects. By contrast, in the wetter year of 2016, CH_4 uptake showed a pronounced maximum in the beginning of June with uptake rates of up to 140 µg C m^{-1} h^{-1} irrespective of treatment (Fig. 4d-f), when WFPS values declined to values below 25% (Fig. 4g). Methane uptake during this period tended to be greatest in the unfertilized control, while intercropping treatments had smaller uptake rates, which, however, were not significantly different from maize monocrop treatments. Differences between treatments at single sampling dates were insignificant throughout the season. Highest CH_4 uptake in 2016 was recorded with lowest WFPS (~10%).

3.7 Cumulative CH_4 uptake

Cropping season cumulative CH_4 uptake exceeded 1 kg C ha^{-1} in both years with no significant effect of intercropping, legume species or time of intercropping (Fig. S1a, b). Maize intercropped with crotalaria tended to take up less CH_4 but this effect was not statistically significant in 2015 or 2016 (P=0.056). Plotting cumulative CH_4 uptake plot-wise over legume dry matter yield did not result in a significant relationship, but highest seasonal uptake rates occurred in plots with lowest legume dry matter yield (data not shown).

3.8 Total non-CO_2 GHG emissions

The relative contribution of CH_4 to the non-CO_2 GHG emission of the different cropping systems varied between 22 and 69% and was highest in the non-fertilized maize monocrop. Three-week lablab intercropping resulted in significantly higher total emissions compared with 6-week lablab intercropping and maize mono-cropping (Table 2). By contrast, in 2016, legume species but not intercropping time affected the non-CO_2 GHG emission balance ($P<0.05$). Lablab intercropped 3 weeks after maize resulted in significantly higher ($P<0.05$) total GHG emission than the unfertilized control but was indistinctive from the fertilized maize monocrop, or other intercrop treatments (Table 2, Fig. 5a, b).

4. Discussion

4.1 Maize-legume intercropping and N_2O emissions

Background N_2O emissions (in unfertilized maize monocrop) fluctuated between 1.1 and 23.0 µg N_2O-N m^{-2} h^{-1}, which is in the range of previously reported emission rates for soils in SSA with low N fertilizer input (0 – 20 µg N_2O-N m^{-2} h^{-1}; Pelster et al., 2017). Baseline emissions were somewhat higher in the wetter season of 2016, owing to ~100 mm more rainfall in the beginning of the season (Fig. 1d, g). Elevated emission rates >30 µg N_2O-N m^{-2} h^{-1} occurred in 2015 on few occasions in intercrop treatments, notably in mid-August when rain fell right after mulching of the

3-week intercrops. Mulching of the 6-week intercrops did not affect N_2O emissions, probably because the mulched legume biomass was too small to affect the flux (Fig. 1b, c; Table 1). In 2016, mulching of the 3-week legumes was followed by rainfall, increasing the WFPS to 50% (Fig. 1g), however, without resulting in elevated N_2O emission rates (Fig. 1e, f). Together, this suggests that the direct effect of mulching on N_2O emission is highly dependent on soil moisture and the amount of mulch and cannot be generalized, contrary to our hypothesis that legume intercrops would invariably increase N_2O emissions.

Legume dry matter yields varied strongly (100 to 3000 kg ha^{-1}) throughout the two experimental years (Table 1, Fig. 3), depending on species, intercropping time and weather. Lablab grew more vigorously and realized larger dry matter yields than crotalaria (Table 1). Moreover, lablab is known to be a better N_2 fixer than crotalaria (Ojiem et al., 2007), presumably leading to higher N input, which would explain larger N_2O emissions with this intercrop (Fig. 2). Three-week intercrops performed generally better than 6-week intercrops. This was particularly apparent for the low-growing lablab (Table 1). Weather in the beginning of the season played a major role for the growth performance of the intercrops by controlling maize growth, which in turn controlled legume growth by shading. Together, this resulted in a wide range of potential leguminous N-inputs in our experiment, which could be used to examine their overall effect on N_2O emissions on a seasonal basis under the semi-arid conditions of the central Ethiopian rift valley. Surprisingly, we did not find any significant relationship between estimated total N input or legume N yield and cumulative N_2O emission. This may be due to the notoriously high spatial and temporal variability of N_2O emissions rates (Flessa et al., 1995), or reflect the fact that intercropping had no or opposing effects on N_2O forming processes. Cumulative N_2O emissions and legume N yields integrate over the entire season and do not capture seasonal dynamics of soil N cycling and N uptake, which could obscure or cancel out transient legume effects on N_2O emissions. Possibly, N released in intercropping treatments was efficiently absorbed by the main crop, even though intercropping did not lead to significantly higher maize grain yields in our experiment. Alternatively, changes in physicochemical conditions brought about by intercrops, such as potentially lower soil moisture due to more evapotranspiration, may have counteracted the commonly observed stimulating effect of legume N on N_2O emissions (Almaraz et al., 2009, Sant'Anna et al., 2018).

We found a significant positive relationship between N_2O intensity and legume N yields in 2015, suggesting that intercropped legumes indeed increase N_2O emissions relative to maize yields (Fig.

3a). It is impossible to say, however, whether this relationship was driven by the extra N entering the system through biological N fixation, or whether an increasing legume biomass affected physicochemical conditions in the rhizosphere favoring N_2O formation. In 2016, legume dry matter yields were much lower than in 2015, owing to early rains favoring maize growth, and no significant relationship with N_2O intensity was found (Fig. 3b). This illustrates that the effect of legume intercropping on N_2O emissions is highly dependent on sowing date and weather, both of which control the growth of legume and main crops and ultimately the amount and fate of leguminous N in the intercropping system. Our data suggest that excessive accumulation of leguminous biomass in SSA maize cropping enhances the risk for elevated N_2O emissions.

We expected N_2O emissions to respond more strongly to intercropping in the second year (2016), as legume mulches were applied according to their plot-wise aboveground yields in the previous year. Indeed, N_2O emission rates were clearly higher in intercropping treatments on the first sampling date in 2016 (Fig. 1e, f), indicating increased N cycling in mulched plots (Campiglia et al., 2011). This difference vanished quickly, however, suggesting that the effect of intercrop mulches, even at high amounts (Table 1), on N_2O emissions in the subsequent year was negligible. It is noteworthy that our estimates of the fraction of N carried over between the years were based on literature data (Table 1), and that a considerable part of the mulched N may have been lost during abundant rainfalls (300 mm) early in the 2016 season before crops were sown.

Cumulative N_2O emissions from intercrops, with mineral fertilization rate halved, were comparable to those in the fully fertilized maize monocrop in 2016. This may be partly due to the 50% reduction in mineral N application to intercrop treatments, as found by others (Tang et al., 2017). Another reason may be that a considerable proportion of the cumulative emission in 2016 occurred before or shortly after 3-week intercrops were sown, and was thus unaffected by growing legumes. Overall, cumulative N_2O emissions were equal or higher in 2016 than in 2015, despite reduced mineral N addition to intercrops and lower legume biomass. Ultimately, the lack of a clear emission response to legume intercropping in the second year calls for studies tracing cumulative mulching effects over multiple years and exploring their driving factors in more detail. In our study, amount and timing of rainfall appeared to be more important for N_2O emissions in the second year than amount and carryover of legume N.

Given our finding that N_2O intensity responded positively to legume biomass and its N content in a drought year with poor maize growth, intercrop species as well as sowing and harvest dates (relative to the main crop) emerge as viable management factors for controlling the accumulation of legume biomass between the maize rows and hence the risk for increased N_2O emission. Legume species and cultivar in intercropping systems are known to be critical for N loss, both during the intercropping and the subsequent seasons (Pappa et al., 2011, Weiler et al., 2018). The stimulating effect of crop residues on N_2O emission has been reported to depend on residue quality and soil moisture, with denitrification being the likely process (Li et al., 2016). Our study provides evidence that vigorous growth of high yielding legume intercrops can enhance N_2O emissions in years unfavorable for maize growth, whereas in years with sufficient water availability early in the growing season, maize growth is favored preventing excessive growth of the intercrop. Our study therefore points to optimizing the sowing date in response to expected emergence and growth of maize as a promising option to control growth of the intercrop and hence to deal with the risk of increased N_2O emissions.

4.2 Seasonal N_2O and CH_4 emission, EF_{N2O} and total GHG emission

Growing season N_2O emissions in fertilized treatments varied from 0.17 to 0.33 and 0.23 to 0.3 kg N_2O-N ha^{-1} in 2015 and 2016 covering 107 and 123 days, respectively (Fig. 2), and a range of estimated total N inputs from 36.4 to 97.8 kg N ha^{-1} (Table 1). There are no N_2O emissions studies for maize-legume intercropping in the Ethiopian Rift valley so far. Hickman et al. (2014a) reported N_2O emissions of 0.62 and 0.81 kg N ha^{-1} over 99 days for 100 and 200 kg N input ha^{-1}, respectively, for a maize field without intercropping in humid western Kenya, which seems to be higher than seasonal emissions we found. Baggs et al. (2006), working in the same region with maize intercropped with legumes in an agroforestry system reported N_2O emissions ranging from 0.2 to 0.6 kg N ha^{-1} with higher emissions in tilled intercropping treatments; our values are in the lower end of the range they reported. The largest seasonal N_2O emission for intercropping reported so far from SSA is 4.1 kg N ha^{-1} (84 days) after incorporating 7.4 t ha^{-1} of a *Sesbania-Macroptilium* mixture in humid western Kenya (Millar et al., 2004). Compared to the N_2O emissions reported for humid tropical maize production systems, our data suggest that maize-legume intercropping based on mulching in the sub-humid to semi-arid rift valley appears to be a minor N_2O source, mainly because of the relatively small amount of legume biomass mulched (Table 1). Growing season N_2O emission factors (EF) in our study ranged from 0.02 to 0.25 and 0.11 to 0.20% of the estimated total N input in 2015 and 2016, respectively, including assumed N inputs from legume mulch as well as belowground additions and carryover between the years (Table 1). Even if the estimated EF is doubled to account for off-season emissions, it is still lower than the annual IPCC default value of 1% N_2O-N per unit added N (IPCC, 2014). Our estimated EFs thus seem to be at the lower end of those reported by Kim et al. (2016) for SSA smallholder agriculture estimated from literature data (0.01 to 4.1%). The reasons for the low EFs in our study are probably the high background emissions in the fertile soil of the Hawassa University research farm which supports high maize yields even in the unfertilized control (Table 1) and the low levels of N input. The soil has been used over decades for agronomic trials with various fertilization rates with and without crop residue retention and legume intercropping (e.g. Raji et al., 2019). Thus, our field trial has to be considered representative for intensive management as opposed to smallholder systems with minimal or no fertilization history.

Methane uptake by the soil in both seasons varied between 1.0 to 1.5 kg CH_4-C ha^{-1} without showing any significant treatment effect, even though maize-legume intercrops tended to take up less CH_4 than maize monocrops (Fig. S1). The observed trend might relate to competitive inhibition of CH_4 oxidation by higher NH_4^+ availability (Le Mer and Roger, 2001, Dunfield and Knowles, 1995) in the presence of legume intercrops, even though estimated total N inputs remained below 100 kg N ha^{-1}, which is considered a threshold for NH_4^+ inhibition (Aronson and Helliker, 2010). Alternatively, densely growing legumes may have lowered CH_4 uptake through impeding CH_4 and/or O_2 diffusion into the soil (Ball et al., 1997). We did not observe stimulation of CH_4 uptake by legume intercropping, which we attribute to the absence of N and P deficiency in this fertile soil. Methane uptake rates varied from 20 to 140 µg CH_4-C m^{-2} h^{-1} which is in the range of rates reported previously for SSA upland soils (Pelster et al., 2017). Seasonal CH_4 uptake in our experiment offsets between 22 and 69% of the CO_2 equivalents associated with N_2O emissions without revealing any significant treatment effect (Fig. S1a, b), but the offset was relatively largest in the unfertilized maize monocrop and smallest in lablab intercropping. Hence, CH_4 uptake is an important component of the non-CO_2 climate footprint of SSA crop production.

4.3 Legume intercropping and climate smart agriculture

Legumes are an important N source in smallholder farming systems, where mineral fertilizers are unaffordable or unavailable. Legume intercrops maximize resource use efficiency as total productivity is often higher than in mono-cropping systems (Banik et al., 2006). Moreover, N fixed biologically by legume intercrops can partly replace synthetic N fertilizers, if the release is synchronized with the nutrient demand of the cereal crop. On the other hand, surplus N from legumes may result in N losses as NO_3^-, NH_3 and NO, N_2O or N_2. Mulching and incorporation of legume biomass has been found to increase N_2O emissions under temperate conditions (Baggs et al., 2000, Baggs et al., 2003) and under humid tropical conditions (Millar et al., 2004). Also under semi-arid, Mediterranean conditions, vetch (*V. villosa*) used as a winter catch crop and mulched in spring significantly increased N_2O emissions during the fallow period while rape did not (Sanz-Cobena et al., 2014). This was later confirmed by a ^{15}N study, highlighting the role of N mineralization from legumes as a source of N_2O (Guardia et al., 2016). None of the studies found an overall N_2O saving effect of catch crops when scaling up to the entire crop cycle, even though the latter study used reduced mineral N fertilization rates in treatments with catch crops. By contrast, reduced NO_3^- leaching and N_2O emission has been reported from maize intercropped with legumes in the semi-arid North China plain, which the authors attributed to enhanced N uptake by both the inter and main crop and reduced soil moisture in treatments with intercrops during the rainy season (Huang et al., 2017). This shows that legume intercrops have a potential to both increase or reduce N_2O emissions with consequences for the non-CO_2 footprint of cereal production and hence for the viability of intercropping as a central component of CSA (Thierfelder et al., 2017).

The legume intercrops used in our study had low C:N ratios (Table S1) and can be expected to release a significant part of their N through decomposition of roots and nodules or root exudation as well as during decomposition of mulches (Fustec et al., 2010). The effect of mulching on N_2O emissions depends on the C:N ratio with increased emissions for low C:N ratio residues (Baggs et al., 2000, Shan and Yan, 2013). In line with this, N_2O emissions in intercrop treatments of our study exceeded those in fertilized maize monocrop on several sampling dates, both during active growth of legumes and after mulching. Another important aspect is the amount of legume N carried over between years which depends, among others, on amount and quality of the legume and the weather between the growing seasons. Abera et al. (2014) showed that surface-placed residues of haricot bean and pigeon pea decompose quickly despite relatively dry conditions during offseason. Vigorous rainfalls in the beginning of the growing season like in 2016 (Fig. 1) could lead to dissolved N losses, which could lead to indirect N_2O emissions elsewhere, which should be taken into account when evaluating intercropping as a CSA strategy.

5. Conclusion

While legume intercrops have the potential to improve cereal yields and diversify produces for smallholders in central Ethiopian rift valley, a risk of enhanced N_2O emissions remains, which became apparent as increased "N_2O intensity" of the main crop in a drought year (2015). At the same time, our study points at possibilities to counteract this trend by actively controlling legume biomass development and hence potential N input through "climate-smart" choices of legume species, sowing date and mulch amounts in response to prevailing environmental conditions. This approach, however, is complicated by the annual variability in growth conditions and requires active planning of sowing and mulching time by the farmer. Our study was conducted on a relatively nutrient-rich soil (as compared to typical smallholder farms) which supports high yields of both maize and leguminous intercrops. Under these conditions, intercropped legumes can potentially replace a considerable part of synthetic fertilizer, thus supporting common CSA goals. However, more studies are needed to fully explore intercropping options in the framework of CSA in the rift valley, particularly in nutrient-poor smallholder fields. Future studies on CSA approaches in the rift valley should address, in addition to greenhouse gas emissions, N-runoff and soil organic matter build up, ideally in long-term field trials with and without legume intercropping. Future studies should also attempt to combine flux measurements with inorganic N dynamics and BNF measurements. Given that seasonal N_2O emission factors and intensities in our study were in the lower range of published values for SSA, intercropping appears as a promising approach to sustainable intensification in the Ethiopian Rift Valley.

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Table 1: N inputs from forage legumes and fertilization per treatment. Shown are mean values (n=4 ± standard error)

Legume	DMY	Aboveground N yield[a]	Belowground N yield[b]	N from mulch[c]	Mineral N	Carryover[d]	Total N input
	kg N ha^{-1}						
	2015						
Crotalaria							
3w	1516±183	53.3±6.4	17.7±2.1	26.6±3.2	64		75.8
6w	345±65	12.1±2.3	4.0±0.8	6.1±1.1	64		66.4
Lablab							
3w	2221±340	96.8±14.8	32.3±4.9	48.4±7.4	64		82.9
6w	467±137	20.3±6.0	6.8±2.0	10.2±3.0	64		67.7
2016							
Crotalaria							
3w	468±85	16.4±3.0	5.47±1.0	8.21±1.5	32	11.1±1.3	56.8
6w	65±44	2.3±1.5	0.75±0.5	1.13±0.8	32	2.5±0.5	36.4
Lablab							
3w	1256±221	54.7±9.6	18.25±3.2	27.4±4.8	32	20.2±3.1	97.8
6w	186±60	8.1±2.6	2.70±0.9	4.06±1.3	32	4.2±1.2	43.0

[a] N content of crotalaria and lablab was 3.51 and 4.36%, respectively, measured in 2 representative samples, DMY=Dry matter yield

[b] assuming a shoot-to-root ratio of 2 and an average belowground N input from the standing legumes of 50% during the growing season

[c] returning half of the aboveground yield as mulch; assuming an average N release of 50% and 30% for 3-week and 6-week treatments, respectively, during the growing season

[d] assuming that 50% of the remaining N becomes available in the following cropping season

Table 2: Grain yields, growing-season N_2O emission factors and non-CO_2 GHG emission associated with N_2O and CH_4 and N_2O emission intensities for fertilized treatments with and without legume intercropping during 107 and 123 days in 2015 and 2016, respectively. N input was estimated as outlined in Table 1. Shown are mean values (n=4 ± standard error). Different letters indicate statistical difference at $p < 0.05$.

	2015				2016			
Treatment	Maize Grain yield (kg ha^{-1})	N_2O emission factor (%)	Non-CO_2 GHG emission (kg CO_2 eq. ha^{-1})*	N_2O emission intensity (g N_2O-N ton^{-1} grain)	Maize Grain yield (kg ha^{-1})	N_2O Emission factor (%)	Non-CO_2 GHG emission (kg CO_2 eq. ha^{-1})*	N_2O emission intensity (g N_2O -N ton^{-1} grain)
M-F	4313±235^{a}		17.4±12^{a}	29.7±4.2^{ab}	6558±217^{a}		29.7±18^{a}	26.3±4.0^{a}
M+F	5022±133^{ab}	0.07±0.07^{ab}	38.4±25^{a}	34.4±8.8^{ab}	8403±342^{b}	0.20±0.03^{a}	91.4±16^{ab}	37.0±4.0^{a}
M+Cr3w	5882±249^{ab}	0.17±0.05^{ab}	78.0±12^{ab}	42.2±5.5^{b}	8276±236^{b}	0.16±0.08^{a}	78.3±19^{ab}	33.6±4.7^{a}
M+Cr6w	5316±316^{ab}	0.07±0.06^{ab}	47.0±15^{ab}	34.8±5.4^{ab}	8283±148^{b}	0.16±0.05^{a}	69.0±12^{ab}	27.8±2.0^{a}
M+Lb3w	5989±528^{b}	0.25±0.06^{b}	120.5±27^{b}	54.3±6.1^{ab}	8557±262^{b}	0.15±0.03^{a}	111.7±9^{b}	36.8±2.1^{a}
M+Lb6w	5541±492^{ab}	0.02±0.01^{a}	21.2±7^{a}	24.6±1.5^{a}	8306±501^{b}	0.11±0.07^{a}	62.3±25^{ab}	26.8±3.9^{a}

* N_2O: 300 CO_2 eq; CH_4: 25 CO_2 eq

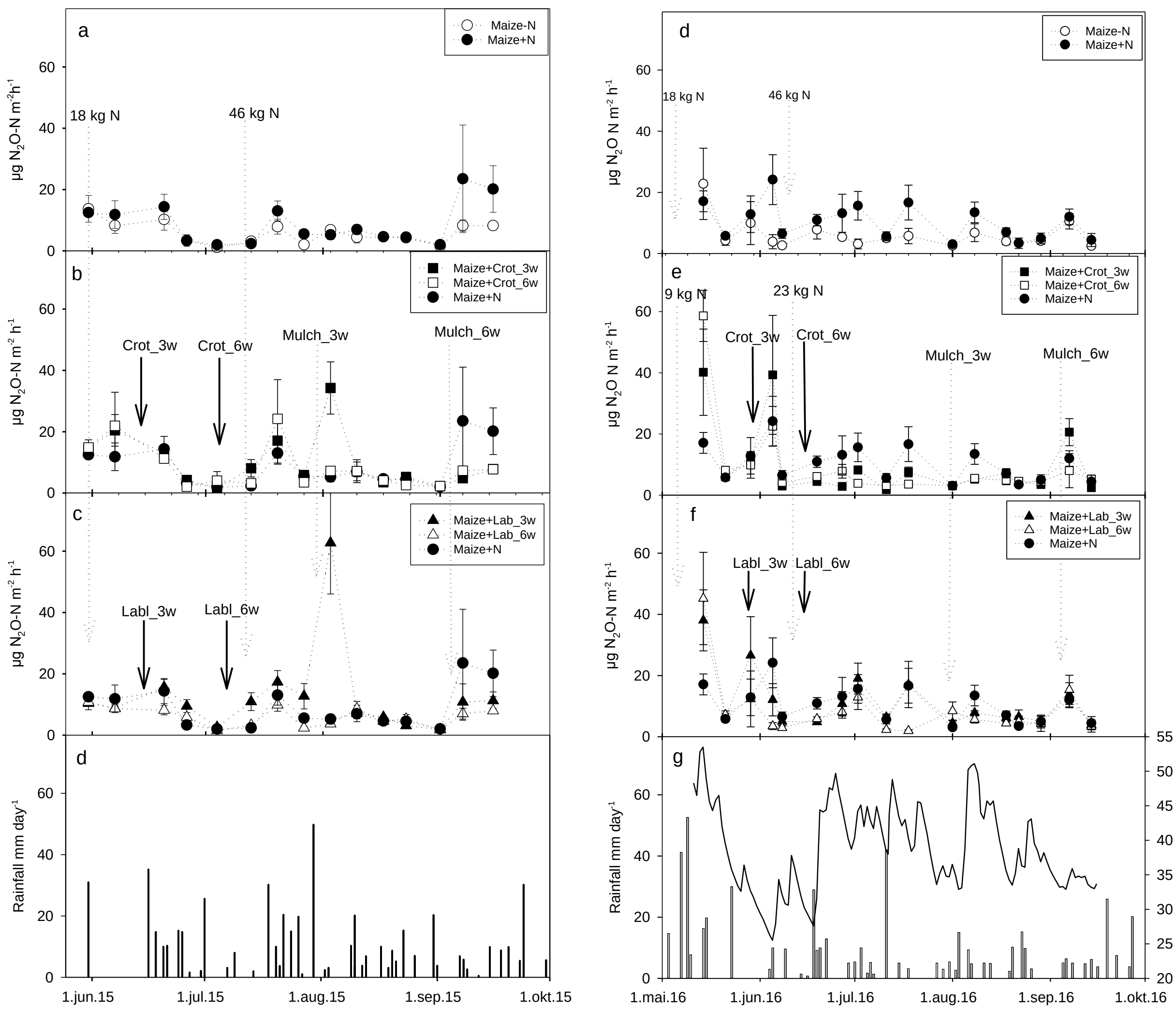


Figure 1: Mean N_2O emission rates (n=4; error bars = SEM) in 2015 (left panel) and 2016 (right panel) and daily rain fall and water-filled pore space (in 2016 only). Figures a and d show emission rates in the absence of intercrops, b and e with crotalaria and c and f with lablab intercrops.

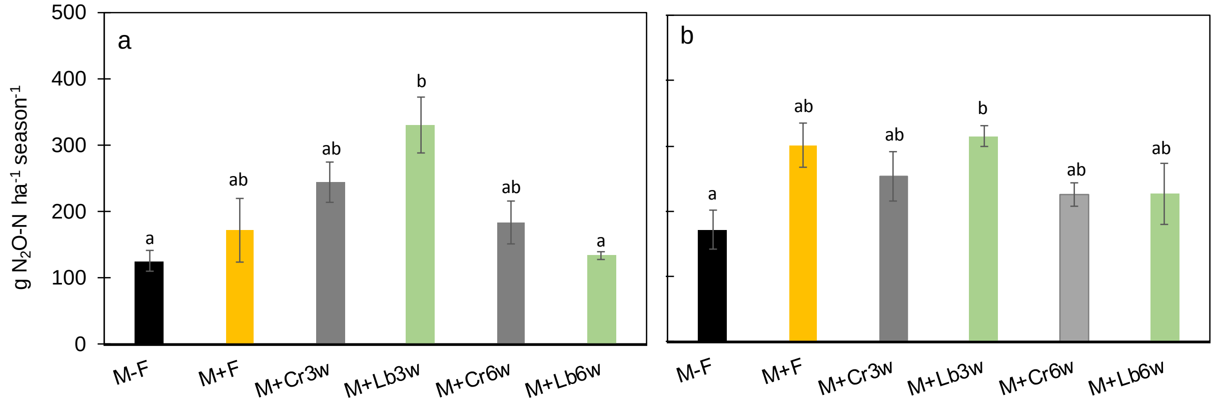


Figure 2: Cumulative seasonal N_2O-N (g N ha^{-1} $season^{-1}$) in 2015 (a) and 2016 (b) throughout 107 and 123 days, respectively, in treatments with and without legume intercropping. Error bars denote SEM (n=4). Different letters indicate significant differences at $p < 0.05$. M+F: fertilized maize; M+Cr3w: fertilized maize with crotalaria sown 3 weeks after maize; M+Cr6w: fertilized maize with crotalaria sown 6 weeks after maize; M+Lb3w: fertilized maize with lablab sown 3 weeks after maize; M+Lb6w: fertilized maize with lablab sown 6 weeks after maize

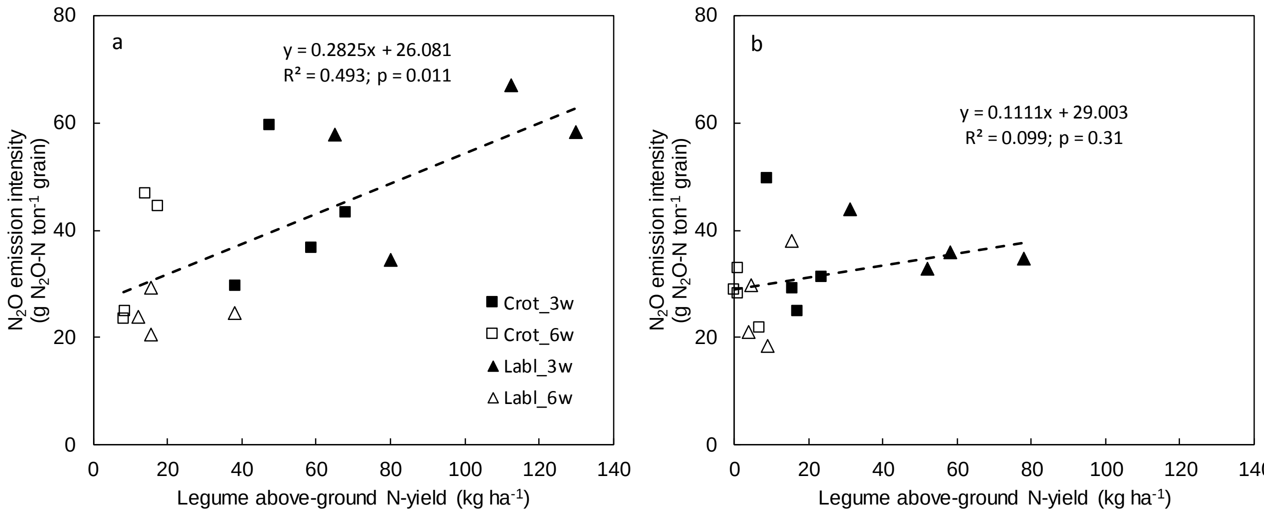


Figure 3: Relationship between N_2O emission intensity and aboveground intercrop legume N yield in intercrop treatments in 2015 (a) and 2016 (b). Shown are single-plot values for each treatment (n=4).

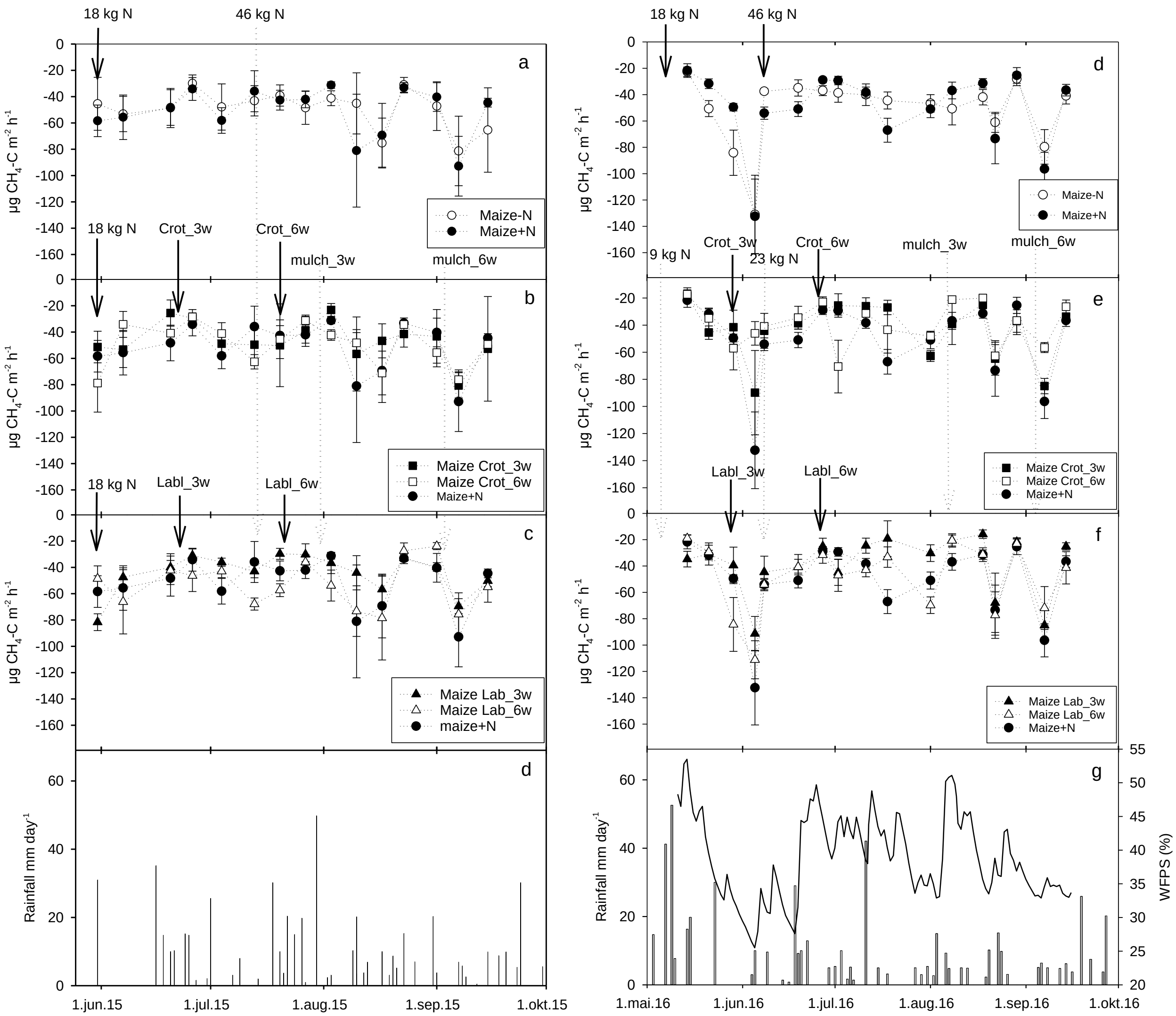
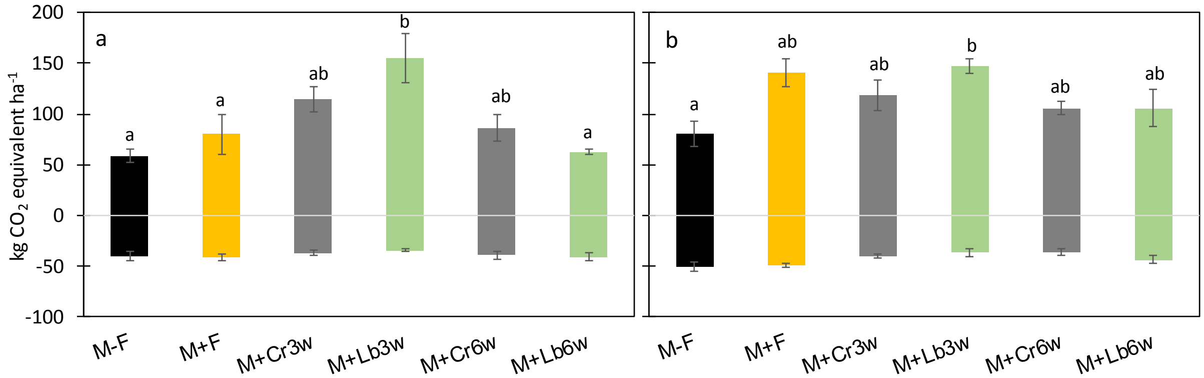


Figure 4: Mean CH_4 flux in 2015 (left panel) and 2016 (right panel) and daily rainfall and water-filled pore space (in 2016 only). Error bars show standard error of the mean (n=4). Figures a and d show emission rates in the absence of intercrops, b and e with crotalaria and c and f with lablab intercropping.



785 Figure 5: Relative contribution of CH_4 uptake and N_2O emission to seasonal total non-CO_2 GHG emissions in mono- and intercropping treatments in 2015 (a) and 2016 (b). Error bars indicate standard deviation (n=4).