INFLUENCE OF COVER CROPS AND THEIR MANAGEMENT ON POST-INCOPORATION N_2O EMISSIONS AND NITROGEN TRANSFER TO RECIPIENT CROP

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Abstract:

Including cover crops in production systems for improving soil health and nutrient cycling has gained interest in recent years. Although cover crops may provide many agronomic and environmental benefits, they may also increase nitrous oxide (N_2O) emissions, a potent greenhouse gas, during residue decomposition. Increased N_2O emissions from decomposing cover crop residues may offset potential benefits associated with increased carbon uptake due to greater radiative forcing of N_2O. Emissions from decomposing cover crops depend on various factors such as type of cover crop, management of cover crop residue, physiochemical properties of cover crops, soil temperature, and soil moisture. Therefore, different field and greenhouse studies were conducted to evaluate the impacts of types of cover crops (leguminous (grass pea and hairy vetch) and non-leguminous (oat)), different forms of management of cover crop residues (removal and retention of aboveground biomass), incorporation at different maturity levels (vegetative or reproductive stages), and different moisture levels (rainfall immediately after cover crop incorporation and rainfall a week after cover crop incorporation) at time of soil incorporation of cover crops on N_2O emissions. A treatment with no cover crop was included as the control in each experiment. Results showed that effect of maturity level at termination on cumulative N_2O emissions was significant ($P < 0.05$) with 30–35% greater emissions recorded from both leguminous and non-leguminous cover crops terminated at the reproductive stage than the vegetative stage. It was also observed that greater biomass yields by a non-leguminous cover crop (oat) could lead to greater N_2O emissions after soil incorporation as compared to incorporation of a less-productive leguminous (grass pea) cover crop. Additionally, the removal of aboveground biomass of leguminous cover crop (grass pea) was an effective management strategy to mitigate N_2O emissions. Soil incorporation of legumes based on a short-term rainfall forecast may not be an effective tool to avoid large N_2O emissions as emissions were not significantly different between early and late simulated rainfall treatments. Therefore, it can be concluded that post-incorporation N_2O emissions from cover crop residue can be mitigated by incorporating at proper stages of maturity and removing aboveground biomass of the cover crop for forage. Future research should consider interaction of cover crop incorporation with various environment variables such as timing and frequency of rainfall events, soil temperature, and abundance of denitrifying communities.

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CHAPTER I

LITERATURE REVIEW

This chapter is a part of a review article published in 'Mitigation and Adaptation Strategies for Global Change' under title of 'Greenhouse gas mitigation strategies for agronomic and grazing lands of the US Southern Great Plains'

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Background

Emission of greenhouse gases (GHGs) from human activities is a global phenomenon related to a wide range of activities. Included are activities related to industrial production, transportation and movement of goods and people, and the production of foods for humans and animals (Fissore et al., 2010; Conant et al., 2011). Fluxes in GHGs as part of the soil-plant-animal-human interface are not uniform across the planet, and emissions from any region into the atmosphere have a global interface. The concentration of GHGs in the atmosphere has increased over the past centuries, has been correlated to an array of human activities, and has also been correlated to increases in global temperatures (Signor and Cerri, 2013).

While developing techniques and systems at a global scale would be a direct, more strategic, method of addressing GHG emissions, such an approach will not likely occur due to the effects of regional geopolitics, and demands for services from landscapes by human populations. However, there are increasing regional-scale concerns related to effects of GHG emissions on climate, and some desire as to how they can be addressed. One specific issue in

regions with agricultural economies is an increased concern of how different types of land use and landforms may affect concentrations of the three primary GHGs (carbon dioxide (CO_2), nitrous oxide (N_2O), and methane (CH_4)) at the landscape-atmosphere interface of grazing lands and croplands (Fissore et al., 2010; Conant et al., 2011). According to an IPCC, (2014) report, agriculture accounts for roughly 6% and 24% of the United States (US) and globally produced GHG emissions, respectively. Although these proportions are relatively small compared to GHGs added to the atmosphere through other human activities, releases from agriculture are still significant (Cole et al., 1997; Paustian et al., 1998). The proportions of total GHG emissions contributed by different agricultural sources are presented in Fig. 1 (U.S. EPA, 2008). In agriculture, CO_2 is produced by burning of plant materials or the decomposition of plant litter and soil organic matter by microbial communities through a number of production activities (Janzen, 2004). In contrast, the production of N_2O from agriculture is mostly contributed by biological processes (nitrification or denitrification), with small amounts produced by non-biological processes such as chemo-denitrification (Hénault et al., 2012). Biological processes are sources of N_2O production when available soil N exceeds the amount of N required for plant growth, and the water-filled pore space of soils are greater than 60% (Smith and Conen, 2004). Denitrification is a microbial process that contributes to N_2O emissions from biomass incorporated into the soil (Li et al., 2016). Nitrate (NO_3^-) or nitrite (NO_2^-) are reduced to N_2 through intermediate products of nitric oxide (NO) and N_2O in denitrification.

Methane is produced during microbial decay of organic material under anaerobic conditions, particularly from stored manures and flooded conditions in rice production (Smith et al., 2007). Fermentation of consumed forages in the rumen of ruminant animals, such as cattle, sheep, and goats, is also a form of microbial consumption of plant materials within an anaerobic

environment (Kebreab et al., 2006; Liu and Liu, 2018). Certain landforms in agricultural areas, such as transient wetlands, or wet puddled soils, are also short-term sources of CH_4 (Conant et al., 2011).

This review presents and discusses literature related to GHG emissions from croplands and grazing lands in the US SGP, and different potential mitigation strategies. The paper focus is on factors related to the production of CO_2, CH_4, and N_2O in Southern Great Plains (SGP). Carbon dioxide is the most significant contributor to climate change and variability due to its high concentration, while N_2O is the most potent GHG affecting global warming. Nitrous oxide is 265–290 times as potent as CO_2 in its effects and can remain in the atmosphere for over 114 years (Follett et al., 2005; Signor and Cerri, 2013). Methane has 34 times greater potential effects than CO_2 and can persist in the atmosphere for a period of over 100 years (Smith et al., 2007). The contribution of GHG emissions from other landforms of the broad agricultural landscape that exists in the SGP, such as agroforestry or buffer strips, has not been considered in this review. However, the issues related to agricultural emissions in SGP also translates to other agricultural regions and systems in semiarid and sub humid environments.

SOUTHERN GREAT PLAINS

Climatic conditions

The SGP is one of the six major regions in the US, and mainly consists of Kansas, Oklahoma, Texas, and parts of Colorado and New Mexico. About a third of the total area of these five states is represented as SGP. The boundary of this region was defined originally by Savage and Castello (1948), who wrote about the SGP and its needs (Fig. 2). The area is bordered by the high-elevation mountainous states of Colorado and New Mexico to the west and humid states (Missouri, Arkansas, and Louisiana) to the east (Mullens et al., 2018). The boundary extends up

to the southern border of New Mexico including adjacent areas of western Texas and eastern New Mexico (Savage and Costello, 1948). Due to this variation along the borders, SGP experiences varied elevation levels and annual rainfall amounts across the region. The elevation on the western edge of the region extends to 1500 to 1800 m while elevation on the eastern and southern edges are < 600 m. Precipitation in the SGP is also variable, ranging from 395 to 449 mm in western areas, to 755 to 890 mm in eastern areas (Baath et al., 2018). About two-thirds of total annual precipitation is received during late-spring through summer (May-September) which is the active growing season of summer crops and forages (Savage and Costello, 1948; Northup and Rao, 2015). Variability in amounts of precipitation received within this time period is high as compared to the rest of the year (Fig. 3). However, prolonged drought periods are frequent in the SGP, resulting in erratic amounts and occurrence of rainfall on a monthly basis (Rao and Northup, 2011; Patrignani et al., 2014). The average temperature also varies dramatically across the region with a range of 50°F in the northern part and 65°F in the southern region, with a summer mean usually above 70°F (Savage and Costello, 1948) (Fig. 4).

Soil and land use

The soil of this region also vary dramatically, from heavy clays to dune sands in some regions. Soils of the region include mollisols, alfisols, inceptisols, aridisols, and vertisols (Singh et al., 2019). Most of the soils of the region have an ustic moisture regime and lie within thermic and mesic temperature regimes.

SGP covers an area of approximately 412,000 square miles, which comprises 12% of total US land area with most of the area covered by grazing lands. According to NASS 2014, approximately 156.6 million acres of land is covered by grazing lands (i.e., rangeland and pastureland) in SGP states (Kansas, Oklahoma, and Texas) which comprises roughly 30% of

total grazing land in the US. About one-third of the available US feeder cattle supplies are found in the SGP on January 1 of each year (Peel, 2003). One reason explaining this contribution is that a large number of weaned calves from cow-calf operations across the US spend time as stocker cattle grazing in SGP, therefore SGP contributes greatly to the beef production industry (Peel, 2003; Baath et al., 2018). Kansas and Texas are ranked in the top five states for number of total cattle on feed while Oklahoma, Kansas, and Texas are ranked in top ten states for total cattle inventory and cattle sales.

Among the area covered by cropland, the major crop is winter wheat, which is planted on approximately 20.6 million acres of SGP states (Kansas, Oklahoma, and Texas) annually (NASS, 2014). The winter wheat acreage in the SGP represents approximately 30% of the total acreage, and roughly 43% of total wheat production in the US. Roughly 60 to 65% of the area under winter wheat in this region is utilized in a production system known as graze-grain, that provides fall and winter forage for beef cattle and is harvested for grain in the spring; the remaining area is largely managed as either grain only or graze out systems Baath, et al., 2018). Other major crops grown in the cropland area of SGP are cotton (7.2 million acres in a semi-arid area of Texas), corn (5.86 million acres), sorghum (4.2 million acres), and soybean (4.17 million acres in total and primarily grown in eastern Kansas). The area under sorghum cultivation is increasing due to increased demand as a bioenergy crop.

The remaining area of SGP is occupied with commercially or naturally managed forests, comprising a small but important land area. Among the three major states of SGP, Texas has approximately 12 million acres of commercial forest cover. Oklahoma has roughly 10 million acres of forest cover, mainly in central and eastern parts, and Kansas has nearly 5.2 million acres (10% of state area) of forest cover (Atchison et al., 2010, Johnson et al., 2010, Simpson et al.,

2013). The forest area provides beneficial effects to the SGP by regulating climate change through carbon (C) sequestration, biological diversity, and watershed regulating services (Steiner et al., 2015).

GREENHOUSE GAS EMISSIONS

The potential for GHG emissions to affect the global climate has led to increased concern of how different land uses may either increase or mitigate the production of the main GHGs, CO_2, N_2O, and CH_4. The concentration of these GHGs in the atmosphere has changed in the past years and been correlated with increases in global temperature (Signor and Cerri, 2013). According to an IPCC, (2014) report, approximately 6% and 24% of the US and globally produced GHG emissionss are contributed by agriculture, respectively. Although these proportions are relatively small compared to GHG added to the atmosphere through other human activities, releases from agriculture are still significant (Cole et al., 1997; Paustian et al., 1998).

In agriculture, CO_2 is produced by burning of plant materials or the decomposition of the plant litter and soil organic matter by microbial communities (Janzen, 2004). The production of N_2O from agriculture is mostly contributed by biological processes (nitrification or denitrification), with small amounts produced by non-biological processes such as chemo-denitrification (Hénault et al., 2012). Biological processes are sources of N_2O production when available soil nitrogen (N) exceeds the amount of N required by plants, and the water-filled pore space is greater than 60% (Smith and Conen, 2004). Methane is produced during microbial decay of organic material under anaerobic conditions, particularly from stored manures and flooded conditions in rice production (Smith et al., 2007).

Agricultural soils are major contributors of N_2O, which is 265-298 times as potent as CO_2 as a GHG (Myhre et al., 2013; Parton et al., 2015). Application of synthetic N fertilizers,

livestock manures, green manures and cover crops all have potential to produce N_2O and CO_2 emissions (Ciais et al., 2013; Cai et al., 2017; Han et al., 2017), depending on N and water inputs to soils, aerobic conditions within soil profiles, and soil temperatures. As discussed earlier, increasing N_2O emissions from agricultural land is a result of decreased nitrogen use efficiency. Therefore, reducing N_2O emissions from agricultural land have potential to increase nitrogen use efficiency of crops.

The major sources of GHG emissions in SGP are croplands and grazing lands while forest areas are a source of C storage at the rate of -26 teragrams carbon dioxide equivalent (Tg CO_2 eq.) (Fig. 5). Crop-related N_2O emissions are the largest contributor to SGP GHG emissions at a rate of 33 Tg CO_2 eq. which mostly comes from inappropriate methods, timing, and quantity of N based fertilizer applied to corn, wheat, and cotton (Ribaudo et al. 2011) (Fig. 5). Best management practices for N application is to inject or incorporate N fertilizer instead of surface application (Tenuta and Beauchamp, 2000). In terms of timing of N application, there should be synchronization between the supply of N and uptake by the growing crop, i.e. limiting application of N fertilizer at planting and supplying enough N later in the growing season to meet needs of the crop as it matures (Hodge et al., 2000). As defined by Ribaudo et al., (2011) the best quantity that can be applied to mitigate N_2O emissions is no more than 40% extra N above what is being removed at crop harvest, which includes both commercial and manure sources, and carryover from the previous crop, irrigation, or atmospheric deposits.

COVER CROPS

Growing cover crops during fallow periods between cash crops could serve as a strategy to reduce GHG emissions, and provide other ecosystem services that benefit the environment. Included are reducing wind and water erosion, reducing NO_3^- leaching, fixing atmosphere N, and

improving sequestration of C (Blanco-Canqui et al., 2015; Tonitto et al., 2006). Some studies have cited the value of cover crops in mitigating climate change. Cover crops are capable of reducing GHG emissions, especially CO_2 and N_2O, by affecting C and N cycling (Kaye and Quemada, 2017). The C cycle is impacted as root and shoot biomass produced by cover crops store C in organic matter after the incorporation of crop residues into the soil. The reduced soil erosion by cover crops also reduces decomposition of soil C caused by water transport (Berhe et al., 2007). A meta-analysis using data from 37 different sites reported sequestration rates of 32 ± 8 g C m^{-2} $year^{-1}$ with cover crops compared to a control, which is equivalent to mitigating 117 ± 29 g CO_2 m^{-2} $year^{-1}$ (Poeplau and Don, 2015).

The effect of cover crops on mitigating N_2O, the most-potent GHG, is still debatable. Emissions of N_2O are dependent on available soil mineral N, soil water content, available electron donors (C), and the physical properties of the soil (Basche et al., 2014). Fluxes in agricultural N_2O generally result from denitrification of NO_3^-, which occurs under saturated soil conditions. It is assumed the conditions for N_2O production would be less conducive as cover crops take up NO_3^- and soil water when growing (Tribouillois et al., 2016). However, incorporation of legume-based cover crops at maturity would lead to higher C (electron donor) inputs, and mulching effects of cover crops may stimulate saturated conditions, thus enhancing denitrification and N_2O production (Mitchell et al., 2013).

A meta-analysis investigating the impact of cover crops on N_2O emissions reported that environmental and management factors, involving fertilizer N rate, soil incorporation, rainfall, and type of cover, (legume or non-legume) altered the impact of cover crops on N_2O emissions (Basche et al., 2014). The meta-analysis reported that the use of non-legumes with high C/N ratios as cover crops, would have the greatest potential to mitigate N_2O emissions. This approach

might have some potential in the SGP if the cover crop is used for other services than strictly as a cover. The aboveground biomass produced by a cover crop could be used as forage for beef production. Haying would reduce the amount of electron donors (C) input to the soil at the termination of the cover crop, and reduce N_2O emissions after incorporation. Although other studies reported slight increases in N_2O emissions after incorporation of cover crops, this increase could be compensated through increased C sequestration. An improvement in GHG balance of 315 kg CO_2 ha^{-1} $year^{-1}$ was reported with cover crops compared to bare soil (Basche et al., 2014). Therefore, there is a need to investigate the impact of different types of cover crops (leguminous and non-leguminous) grown in SGP region on N_2O and CO_2 emissions after their incorporation.

The results of meta-analysis done by Basche et al., (2014) also reported that removing the cover crop biomass would be helpful in reducing N_2O emissions reported after incorporation of cover crop biomass. Brozyna et al., (2013) also evidenced that management of cover crop biomass (incorporation vs removal of biomass) had effects on N_2O emissions. Therefore, the effect of different forms of management of cover crops, such as removing above ground biomass for forage, need to be tested for cover crops grown in the SGP region. Since biochemical composition of cover crop biomass is the primary driver of C and N mineralization after termination, termination of green manures at optimal stages of maturity would be an important tool for increasing synchronization between nutrient mineralization of cover crop biomass and uptake by the succeeding crop (Trinsoutrot et al., 2000). Therefore, there is need to analyze the optimal maturity levels for termination of cover crops grown in SGP. Soil moisture greatly impacts N_2O emissions, as it is a key factor governing the activity of soil microbial communities which plays an important role in nutrient transformation and chemical cycling (Schulthess and

Gujer, 1996). One possible management option to reduce large emissions of N_2O would be to incorporate biomass into the soil during dry periods, based on short term-rainfall forecasts. Effects of soil moisture on N_2O emissions has been extensively studied. However, there is limited information in the US SGP on effects of amount and timing of soil moisture on N_2O emissions from fall- and spring-planted legumes. Therefore, impact of timing of rainfall after incorporation of a cover crop is needed to test for N_2O and CO_2 emissions and their capacity to transfer N to succeeding cash crop.

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FIGURES

Figure 1. Amount of greenhouse gas emissions from different agricultural sources in the United States in 2008. (Tg CO_2 eq. is teragrams carbon dioxide equivalent). Source: (U.S. EPA, 2008)

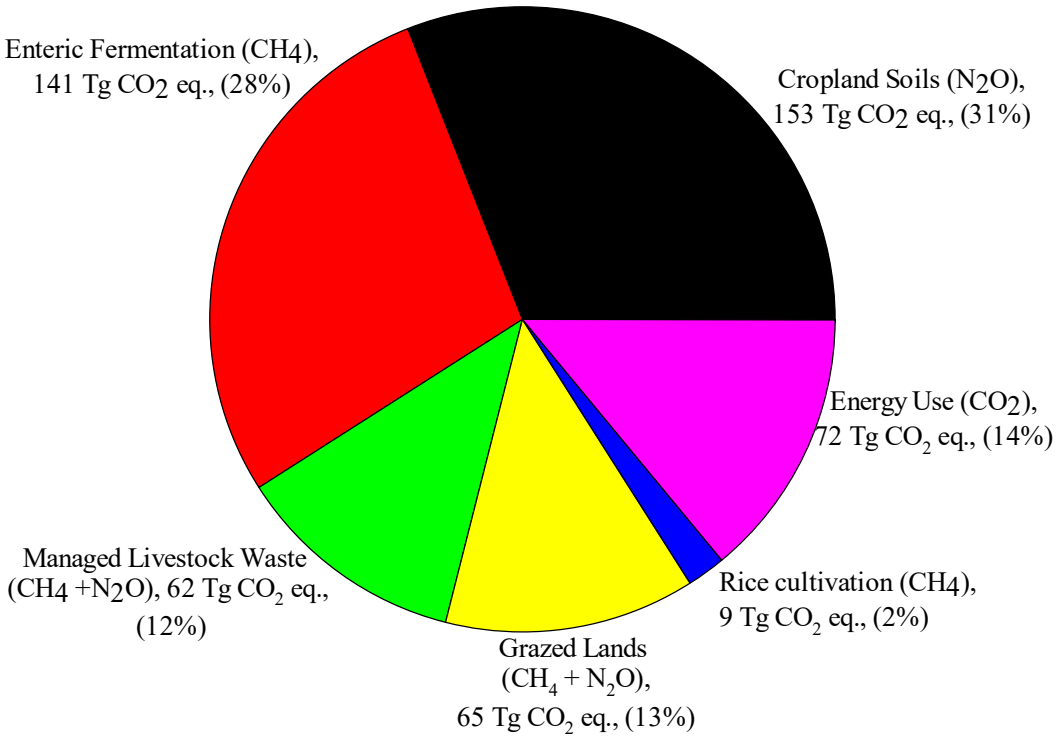


Figure 2. Location of Southern Great Plains and the six locations used for representing climate conditions.

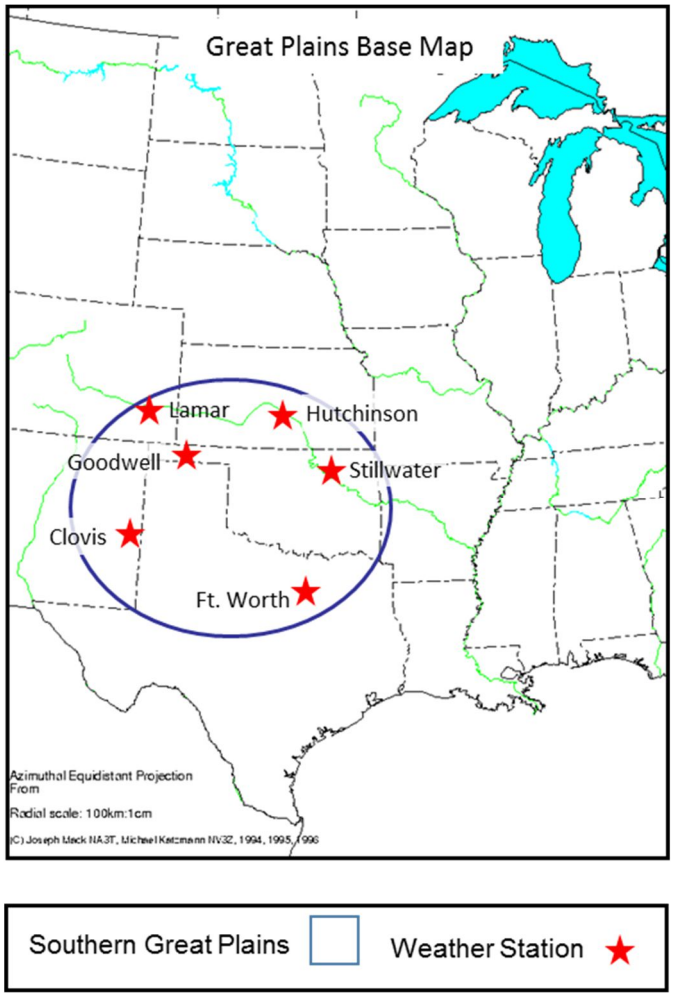


Figure 3. Average monthly precipitation for six stations within the Southern Great Plains of United States from 1966 to 2016. Error bars indicate the standard deviation for each month.

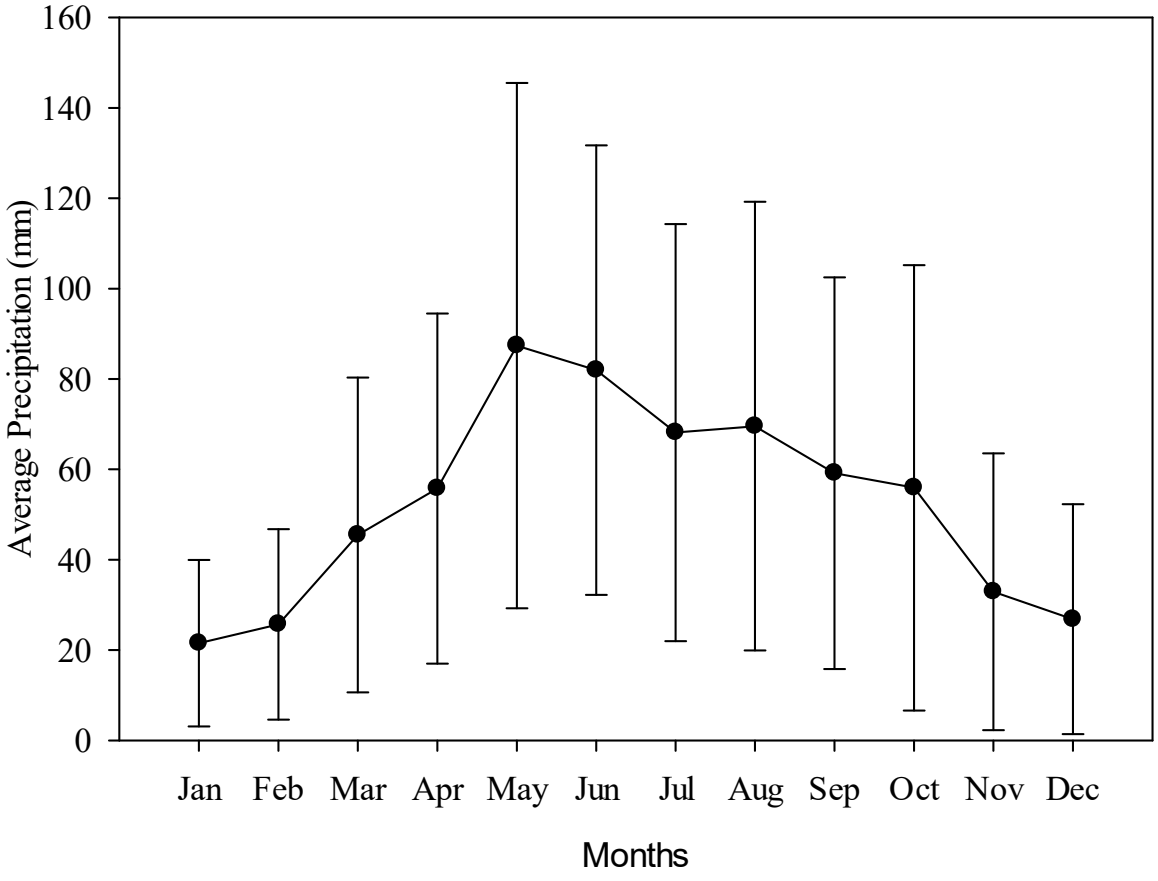


Figure 4. Average monthly maximum and minimum air temperatures for six stations within the Southern Great Plains of United States from 1966 to 2016.

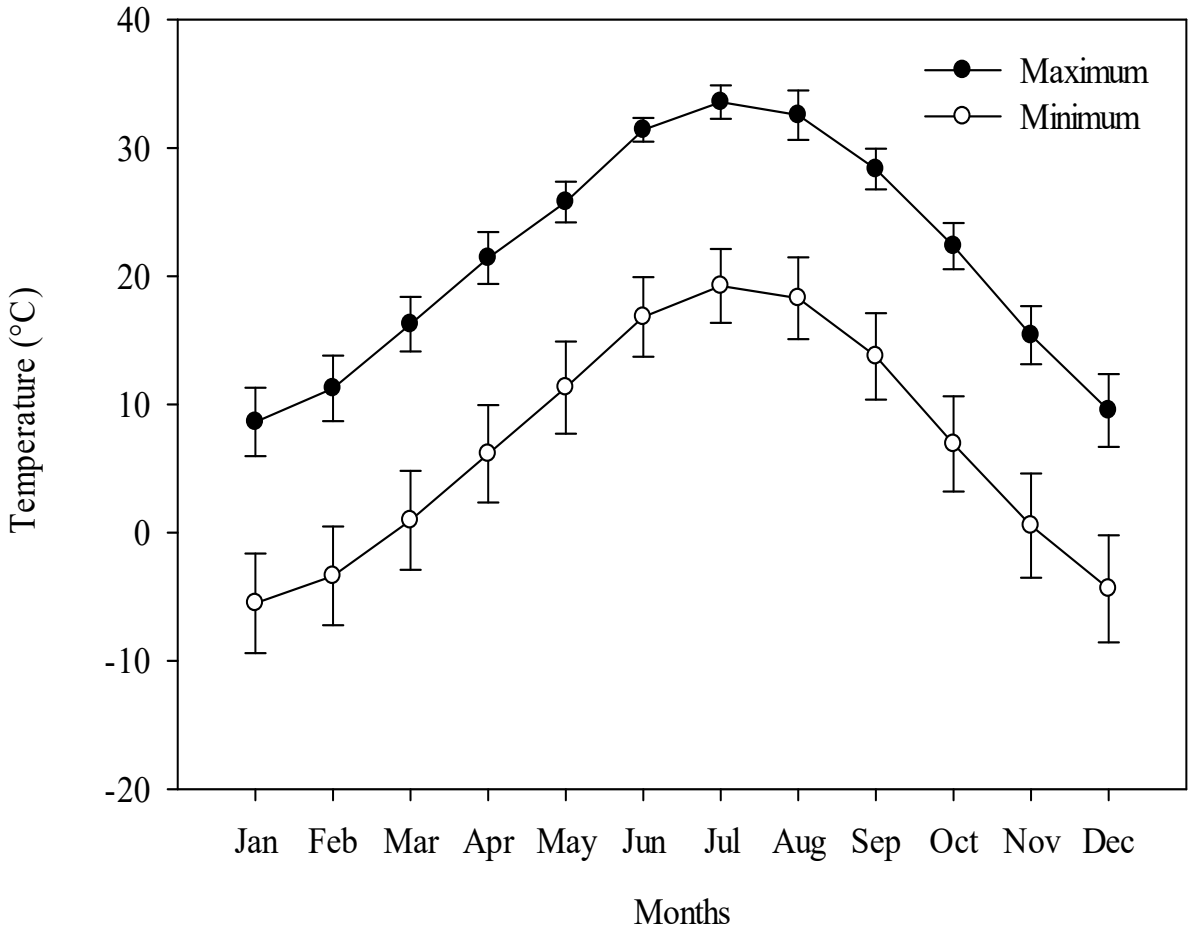
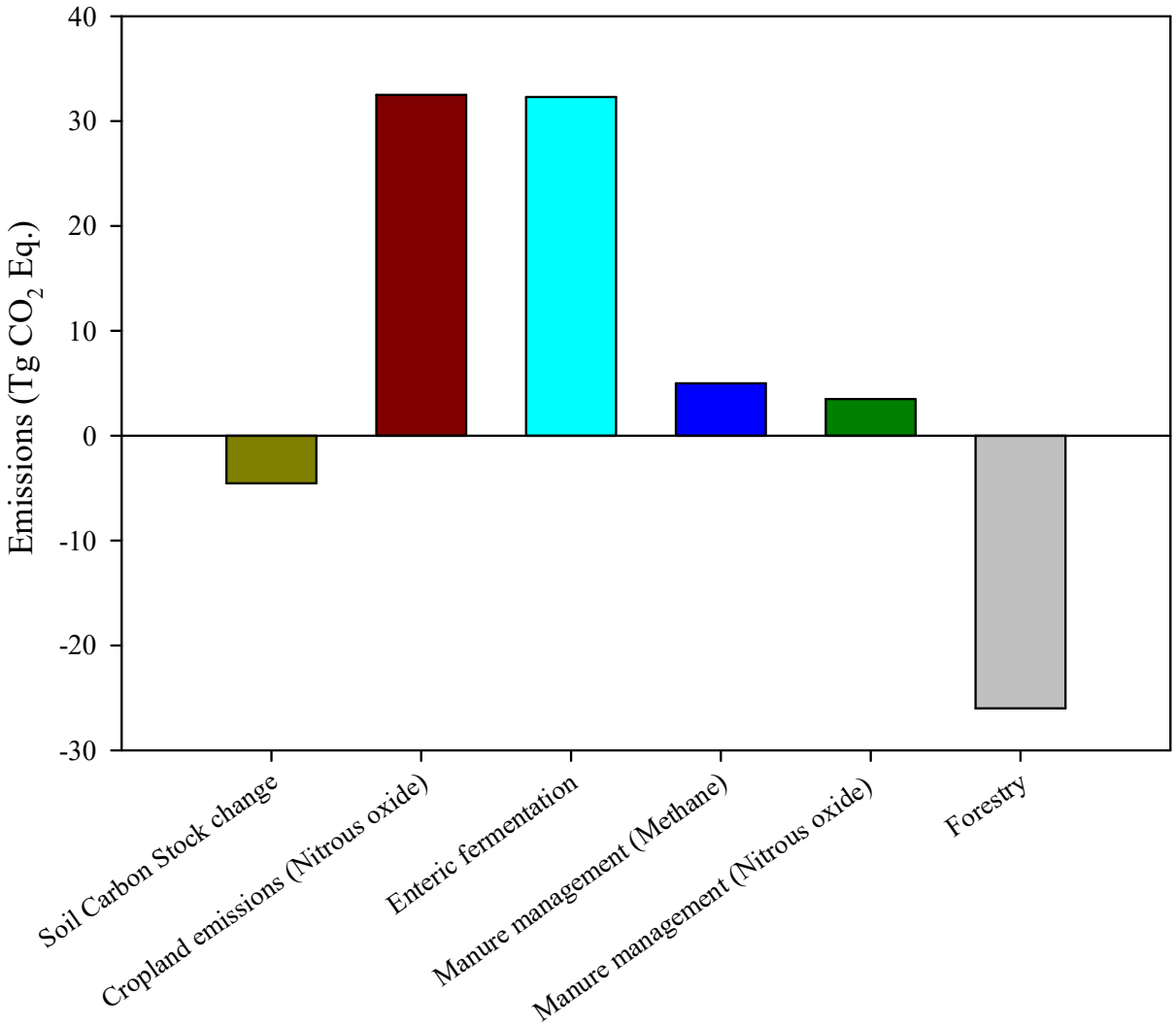


Figure 5. Amount of greenhouse gas emissions from different sources in Southern Great Plains.

Source: Steiner et al. (2015)



CHAPTER II

SOIL N_2O EMISSIONS FOLLOWING TERMINATION OF GRASS PEA AND OAT COVER CROP RESIDUES WITH DIFFERENT MATURITY LEVELS

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ABSTRACT

Although cover crops provide many agronomic and environmental benefits, they may also increase N_2O emissions after termination. The N_2O emissions from decomposing biomass of cover crops largely depends on the type of cover and maturity level at termination. The objective of this study was to quantify N_2O emissions following soil incorporation of residues of a non-legume (oat; *Avena sativa* L.) and legume (grass pea; *Lathyrus sphaericus* Retz.) cover crop at two different maturity levels. Oat and grass pea were terminated at vegetative (early-termination) and reproductive (late-termination) stages, and stored fresh before soil incorporation (2.5 Mg dry matter ha^{-1}) in late-May. A treatment with no cover crop was included as the control. The experiment was laid out as completely randomized block design with three replicated plots (2 m × 2 m) in each treatment combination. Finger millet (*Eleusine coracana* Gaertn L.) seedlings were transplanted as a summer crop. The N_2O fluxes were measured with a closed chamber with a portable gas analyzer on 25 dates over an experimental period of 98 days. In general, fluxes

of N_2O increased after rainfall or irrigation events and approximated zero during dry periods. Cumulative N_2O emissions during the 98 days study period were higher ($P < 0.01$) from all cover crops treatments than the control. Effects of maturity level at termination on cumulative N_2O emissions were significant ($P < 0.05$), with 30–35% higher emissions recorded from both cover crops terminated at the reproductive stage. Biomass yields of finger millet from oat incorporated plots were 15–19% greater ($P < 0.05$) than grass pea incorporated plots, while the yields from control plots were not significantly different from plots receiving cover crop residues. Therefore, it can be concluded that stage of maturity at termination alone was not a strong predictor of total cumulative N_2O emissions from decomposing cover crop residues, as maturity level interacted with environment variables (e.g., timing of rainfall events).

INTRODUCTION

Cover crops are being promoted throughout the US as a tool to improve soil health, reduce soil erosion, and increase nutrient cycling (Foster et al., 2017; Snapp et al., 2005). In the US SGP, cool-season cover crops can be cultivated outside the growing season of summer crops, which are generally grown between May and October (Biederbeck et al., 1993; Kandel et al., 2019a; Singh et al., 2019a). Cereals and legumes cultivated as spring-planted cover crops in the U.S. SGP have short growing periods (generally spanning between March and May) and decompose rapidly after termination, which is a useful trait for effective transfer of nutrients from the decomposing biomass to the following summer crops (Sievers and Cook, 2018).

Although legume-based cover crops can biologically fix atmospheric N, non-legume cover crops can also increase the soil N pool by scavenging N and decreasing N loss through leaching and gaseous emissions (White et al., 2017). Regardless of type of cover crops, their successful use as source of N for the following crops depends on synchronization of N

mineralization from decomposing plant tissues and demand of the following recipient crops (Kandel et al., 2019a; 2019b; Myers et al., 1994). While rapid decomposition and mineralization of biomass immediately after termination may risk N lose prior to establishment of following crop, slow mineralization rates may hinder N transfer to following cash crops (Kandel et al., 2018; 2019b; Kumar and Goh, 1999). Additionally, if N in residues of cover crop mineralizes outside the growing season of the recipient crops, it is prone to loss from the soil (Kandel et al., 2019b).

Although cover crops provide many potential agronomic and environmental services, they may also increase emissions of N_2O after soil incorporation (Baggs et al., 2003; Kandel et al., 2018; Millar et al., 2004). Nitrous oxide is a highly potent GHG, produced mostly as a by-product of autotrophic nitrification, and an intermediate product of the heterotrophic denitrification process (Bremner, 1997; Hu et al., 2015). The denitrification pathway, which dominate N_2O emissions from decomposing biomass, is favored by high soil moisture (Cardenas et al., 2017; Li et al., 2016; Tiedje et al., 1984). High and frequent rainfall during late spring to early summer (April – June) is a normal precipitation pattern in the US SGP, which is also the termination period of spring-planted cover crops. Thus, rapid increases in soil NO_3^- concentrations after termination of spring-planted cover crops may provide substrate for denitrifying microbiota during rainfall events, potentially contributing to large N_2O emissions (Kandel et al., 2018). Therefore, systems of crop management that avoid rapid N mineralization from decomposing cover crop biomass prior to establishment of following summer crops could be crucial for mitigating N_2O emissions (Hoorman et al., 2009).

Nitrogen mineralization from decomposing biomass and resulting soil N_2O emissions largely depend on biomass C/N ratios and lignin concentrations (Garcia-Ruiz et al., 2007;

Kushwah et al., 2014). High C/N ratio and lignin in plant biomass delays N mineralization by producing polyphenols, which can increase amounts of recalcitrant N, by formation of humic polymers (Fox et al., 1990; Haynes, 2012). Likewise, inverse correlations between biomass C/N ratios and N_2O emissions from biomass residues are frequently reported (Han et al., 2017; Huang et al., 2004; Nicolardot et al., 2001; Trinsoutrot et al., 2000). Ratios of C/N and lignin concentrations in plant biomass generally increase with crop maturity (Kandel et al., 2013). Therefore, identifying the optimal maturity level of cover crops for termination can be useful for mitigation of N_2O emissions from decomposing residue of cover crops planted during spring in the SGP.

After termination and soil incorporation, legume cover crops generally increase N_2O emissions compared to non-legumes due to increased soil pools of soil N through biological N fixation, and incorporated biomass with relatively low C/N ratios and lignin concentrations (Baggs et al., 2000). Additionally, the rapid decomposition of legume residues in the soil can quickly deplete O_2 concentrations and provide soil conditions conducive for denitrification (Højberg et al., 1994). A meta-analysis by Basche et al., (2014) reported significantly greater N_2O emissions from legume cover crops than non-legumes during the growth phase of following cash crops. In general, cereals have higher C/N ratio and lignin concentrations compared to legume species at similar level of maturity. However, cereals grown as cover crops during spring in the SGP, are generally terminated within 3 months after planting, and can have C/N ratios and lignin concentrations that are similar to legumes at termination. Thus, although lower N_2O emissions from cereal cover crops are generally expected, this may not be achieved from spring-planted cereals in the region.

A recent study in the US SGP reported large N_2O emissions after soil incorporation of hairy vetch (*Vicia villosa*, Roth; a fall-planted legume) terminated in early-May (Kandel et al., 2018). Further study by Kandel et al., (2019a) suggested that removal of hairy vetch biomass for forage rather than soil incorporation could mitigate N_2O emissions, but quantity and N concentration of biomass produced by the following summer crop was constrained. Thus, further studies are required to identify management options for termination of cool-season species used as cover crops to mitigate N_2O emissions while maintaining crop yield and quality of following crops.

The objective of this study was to examine the impact of maturity at termination of spring-planted grass pea and oat grown as cover crops on N_2O emissions. Grass pea and oat are potential cover crops in the SGP that can be planted in spring and terminated prior to planting of summer crops (Clark, 2008). Treatments included grass pea and oat terminated at vegetative and reproductive stages, and a control without a cover crop residue incorporation. Emissions of N_2O were measured for a period of during 98-days following soil incorporation of the cover crop biomass. Finger millet, a potential forage crop for the region, was cultivated as a summer crop during this period. We hypothesized that (i) emissions of N_2O would be lower from oat-based cover crops than from grass pea, and (ii) emissions of N_2O from late-terminated cover crops would be lower than from early-terminated crops.

MATERIALS AND METHODS

Experimental site and soil properties

This study was conducted on an agronomic field site at the USDA-ARS Grazinglands Research Laboratory near El Reno, OK, USA (35°34′23″ N, 98°02′13″ W; 411 m elevation). The site was within the bottomland area of the North Canadian River drainage basin, and considered as a

highly fertile soil for the region (Goodman and Morris, 1977). The soil was a Brewer silty clay loam (fine, mixed, super active, thermic Udertic Argiustolls). The site was moderately well drained with 0 to 1% slope. The soil had permeability of 0.2−1.5 cm $hour^{-1}$, average water-holding capacities of 4 mm mm^{-1} soil, cation exchange capacity of 17.5 cmol kg^{-1} soil, and a pH of 6.9. Average soil organic C and N content were 1.31% and 0.10%, respectively (USDA-NRCS, 1999). The topsoil (0–0.15 m) had particle fractions of 36% sand, 42% silt, and 22% clay.

Collection and characterization of cover crop biomass

Biomass of the cover crops were collected from adjacent plots of grass pea and oat. Biomass including roots for the early- and late termination treatments were sampled on 9 and 30 May, 2018, respectively. Thereafter, plant materials were thoroughly cleaned and cut into 1.0-cm pieces and stored frozen until soil incorporation.

A portion of biomass samples from both cover crops was oven-dried at 60°C to constant weight and analyzed for total C, N and cell wall components (cellulose, hemicellulose, and lignin). Analyses were done on triplicate samples from each species/maturity level combination. Concentrations of C and N were assayed by flash combustion (900°C for 10 min) method (Model VarioMacro, Elementar Americas, Inc., Mt. Laurel, NJ, USA). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined by the van Soest and Wine (1967) method. Concentration of cellulose were calculated as the difference between ADF and ADL, and hemicellulose as the difference between NDF and ADF. The ADL fraction was presented as lignin concentration.

Experimental design and crop management

This field study was conducted in the summer of 2018, with the experimental plots managed by regular tillage operations (roto-till) during the spring prior to the study. To evaluate the isolated effects of crop maturity on C and N fluxes, plant materials were collected from adjacent plots planted to pure stands of grass pea and oat. The experimental design of this study was a completely randomized block design with three blocks, within which a factorial treatment structure of two cover crops (grass pea and oat) with two maturity levels (vegetative and reproductive) at incorporation were included. The maturity treatments are described as early- and late-termination, based on stage of plant development. Additionally, a control treatment without cover crop residue was included. Thus, the experiment consisted of 15 plots (2-m^2) in total.

A set of two PVC collars (0.65 m × 0.65 m) were inserted to 0.10 m depth in each plot immediately after tillage operations on 2 June. One of the two collars in each plot was used for gas flux measurements, while the second collar was used for simultaneous soil sampling at times of flux measurements. Biomass of the cover crops wasincorporated in the soil by hand within the upper 15 cm of freshly tilled soil within the collars. Biomass was incorporated at a rate equivalent to 2.5 Mg dry matter ha^{-1} based on average yield of these crops at the first harvest. One-month old finger millet (PI302662) plants obtained from a nearby field were transplanted in all study area on 12 June, 2018, 10 days after soil incorporation of cover crop biomass. A long dry spell followed transplanting of finger millet, so irrigation equivalent to a 30-mm rainfall was provided on 10 days after biomass incorporation (i.e., day finger millet was transplanted). Thereafter, further irrigations were applied on 38 and 45 days after biomass incorporation (30 mm on both events).

Biomass decomposition measurements

Portions of the fresh biomass cut to 1-cm pieces was used for litter decomposition study in litter-bags (0.10 m × 0.20 m) with a mesh size of 50 μm. Amounts of biomass inside the litter-bags was incorporated at the same rate (2.5 Mg dry matter ha^{-1}) used for gas flux measurements and soil sampling. The bags were closed at the top by folding the top 0.05 m of the bag over and stapled twice. Three litter-bags from each cover crop treatment combination were buried horizontally inside four additional collars not used for gas flux or soil sampling to a depth of 0.15 m. All 12 bags placed inside a collar were extracted at intervals of 2–4 weeks. Extracted biomass was cleaned thoroughly and oven-dried at 60°C to constant weight. Thereafter, the biomass was milled (1-mm sieve) to determine concentrations of N remaining in biomass using the dry combustion method (900°C for 10 min).

Gas flux measurements

Fluxes of CO_2 and N_2O were measured using a closed chamber system from 2 June to 7 September, 2018. Fluxes were measured by placing a white-colored chamber (0.70 × 0.70 × 0.21 m^3) on the preinstalled support collars. An extension with similar dimension as the top chamber was used when the plant height of finger millet exceeded the chamber height. The collars had a 0.04 m wide outer flange that remained parallel to the soil surface to support the chamber used for flux measurements. Flux measurements were taken more frequently (often daily) after rainfall and irrigation events to capture rainfall/irrigation induced pulses of N_2O emissions. Less frequent measurements (longest interval 10 days) were taken during dry spells when N_2O emissions remained close to zero. During chamber enclosure, headspace air was mixed by two small battery-driven fans. Air in the chamber headspace was circulated through 3.0 mm inlet and outlet tubing to a portable Fourier transform infrared-based gas analyzer (DX4040; Gasmet

Technology Oy, Helsinki, Finland). The concentrations of CO_2 and N_2O were recorded at 20-s intervals during 6–8 min enclosures for each measurement. Flux measurements were taken between 11:00 and 13:00 on the dates of sampling.

Though emissions of N_2O from biomass of the incorporated cover crops was the primary interest of the study, concurrently measured CO_2 emissions were also reported as CO_2 emissions during first 10 days of the study (prior to planting of finger millet), to reflect the decomposition rates of cover crop biomass.

Fluxes were calculated by linear regression using the routine developed by Kutzbach et al., (2007). The first few records after chamber enclosure were discarded as dead-band based on visual inspection of the CO_2 flux curve. Total cumulative emissions of N_2O during the measurement period were calculated using linear interpolation of measured fluxes between the measurement dates.

Measurements of environmental variables

Soil temperature was recorded hourly using soil sensors (TMC-6, Onset Computer Corporation, Bourne, USA) inside an additional collar. Three soil sensors were placed at 0.05, 0.10 and 0.15 m soil depths, and the average was presented. Air temperature and precipitation measurements for the study period were obtained from a weather station (Oklahoma Mesonet, Oklahoma Climatological Survey) located approximately 1.0 km from the study site.

Volumetric water content (VWC) at the 0–0.15 m cm depth was continuously recorded at hourly interval using soil moisture sensors (model EC-10; Meter Environment, Pullman, WA). Three sensors were inserted at a soil depth of 0–0.05, 0.05–0.10 and 0.10–0.15 m in the same collar where the soil temperature sensors were installed, and an average measurement by the

three sensors is presented. The VWC was presented as water filled pore space (WFPS) calculated as relative-VWC at saturation.

Measurements of plant growth, yield, and quality of finger millet

Growth of finger millet was monitored non-destructively by taking canopy reflectance measurements inside the collars using a portable spectro-radiometer (PSR-3500; Spectral Evolution, Lawrence, USA). Ratio vegetation index (RVI) was calculated as a ratio of canopy reflectance at red (656 nm) and near-infrared (779 nm) wavelengths. Total aboveground biomass of finger millet was determined by harvesting all biomass inside all collars on 6 September, one day prior to the last flux measurement. The biomass was oven-dried at 60°C to constant weight and N concentration was determined by flash combustion method. Total N uptake per hectare was calculated as the product of biomass yield and N concentration.

Analyses of soil samples

Soil samples (0–0.15 m) were collected from all collars assigned for soil sampling on all dates of flux measurements to determine concentrations of the two main fractions of mineral N as NO_3^- and ammonium (NH_4^+), soil pH and electrical conductivity (EC). Additionally, one sample from each block was taken prior to cover crop residue incorporation on 2 June (day 0) to characterize soil prior to the residue incorporation. On each sampling date, three soil cores (diameter, 0.02 m) were taken from all 15 collars assigned for soil sampling and pooled to form a composite sample for analyses. Aliquots of samples were extracted in 1.0 M KCl and concentrations of NO_3^- and NH_4^+ was determined by flow injection method (Timberline Instruments, Boulder, CO, USA). Soil pH and EC were determined in 1:2 soil: water solution by a benchtop pH/conductivity meter (Orion Star A215; Thermo Scientific, Waltham, MA, USA).

Statistical analysis

Averages of three plots in a treatment is presented and standard errors denote spatial variations in responses unless stated otherwise. The difference of measured fluxes among the treatments were determined using a mixed model in SAS 9.4 (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. Contrasts were used for pairwise comparisons at 5% level.

Pearson correlations (*R*) showed the relationship between 10 days (prior to planting of finger millet) accumulated CO_2 and N_2O emissions from individual collars after cover crop incorporation. Likewise, correlations among soil variables (soil moisture, concentrations of NO_3^- and NH_4^+, pH, and EC) and emissions of N_2O were presented in a correlation matrix. Averages of the soil variables and N_2O emissions across treatments at 25 measurement dates were used for the test. Additionally, relative importance of the soil variables on dynamics of N_2O emissions was tested using PROC HPFOREST in SAS.

RESULTS

Properties of cover crop biomass

As expected, moisture content of the early-terminated biomass of both species was greater than late-terminated biomass (Table 1). Carbon concentrations in the late-terminated biomass of both species were greater than early-terminated biomass but the concentrations were similar between the crop species within a termination date. Nitrogen concentration in the early- terminated biomass was significantly greater than the late-terminated biomass. Biomass N concentration was significantly greater in grass pea than that of oat on both sampling dates. As expected, early-terminated biomass had less C/N ratios than the late terminated biomass. Between the two

species, the C/N ratio of grass pea biomass was significantly less than that of oat biomass. As similar amounts of dry matter were incorporated in all cover crop treatments and N concentration was greater in grass pea biomass, N application rate by grass pea was greater than oat. Concentrations of cell wall components (cellulose, hemicellulose, and lignin) were greater in oat biomass on both sampling dates. Likewise, concentrations of the cell wall components were greater on late-terminated biomass than in early-terminated biomass for both species.

Environmental conditions

During the 98 days period of flux measurement between June and August, the average daily soil temperatures ranged between 18 and 35°C, and average daily air temperature ranged between 15 and 28°C (Fig. 1a). The average air temperature at study site in June (26.2°C) was 1.5°C greater than long-term (1981–2010) average. However, average air temperature in July (27.4°C) and August (25.9°C) were 0.4 and 1.4°C less than the long-term averages for those months.

Total precipitation in June (93 mm) and July (33 mm) were 27 and 29 mm less than the long-term averages (Fig. 1b). However, total precipitation in August (109 mm) was 21 mm more than the long-term average precipitation. Soil moisture was low at incorporation of residues but increased after the irrigation event. Soil moisture declined gradually but increased after rainfall and additional irrigation events. Soil moisture had reached high (>70% WFPS) after heavy rainfall events (>25 mm).

Dynamics of CO_2 flux

The fluxes were low on day 1 after biomass incorporation but increased after the first irrigation event (Fig. 1c). Thereafter, the CO_2 flux rates decreased with declining soil moisture, but frequent peaks occurred after rainfall and irrigation events. Prior to planting of finger millet on day 10 of cover crop incorporation, CO_2 emissions from grass pea incorporated plots were

greater than the emissions from oat-incorporated plots within both maturity groups. The difference in CO_2 fluxes among the cover crop and control treatments was not prominent during effective growth of finger millet (i.e., 2 weeks of transplanting).

Dynamics of N_2O flux

In general, cover crop treatments generated larger N_2O fluxes than the control at the early period of residue incorporation (Fig. 1d). Fluxes of N_2O remained close to zero from all treatments on the first two measurements, but peak emissions from cover crop treatments observed following the irrigation event applied on 10 days after biomass incorporation. The flux rates decreased with declining soil moisture but increased after subsequent rainfall and irrigation events. Although N_2O fluxes were close to zero from control treatment until day 38, emission peaks were observed during the rainfall events thereafter. During the last week of the measurements, emission peaks were not recorded despite heavy rainfall events. Average N_2O fluxes from the late-terminated grass pea (31.1 g N_2O-N ha^{-1} d^{-1}) across measurement dates were significantly greater (12.6 %) than flux from the early-terminated grass pea (27.6 g N_2O-N ha^{-1} d^{-1}). Likewise, N_2O fluxes from late-terminated oat (32.3 g N_2O-N ha^{-1} d^{-1}) across the measurement dates were significantly greater (26.6 %) than from early-terminated oat (25.5 g N_2O-N ha^{-1} d^{-1}).

Cumulative N_2O emissions

The cumulative N_2O emissions from plots receiving all cover crop treatments were significantly greater ($P < 0.05$) than that from control plots (Fig. 2). Effects of maturity level at termination of the cover crops was significant ($P < 0.05$), with greater emissions recorded from late-terminated cover crops. During the first 10 days of study, when CO_2 emissions primarily represented heterotrophic respiration due to the absence of green plants, cumulative emissions of CO_2 and N_2O from individual collars correlated ($R = 0.93$) strongly (Fig. 3).

Soil pH and EC

Soil pH across sampling dates fell within a range of 6.3−7.5 (Fig. 4a). There was no significant difference among the applied treatments on soil pH, but effect of sampling date was significant. In general, soil pH declined after rainfall events but increased during dry periods.

Similar to soil pH, there was no significant difference among the applied treatments on soil EC, but influence of sampling dates was significant (Fig. 4b). Soil EC generally increased after the rainfall events and decreased during dry periods. However, when rapid growth of finger millet occurred during the last week of measurements, soil EC did not fluctuate prominently during the rainfall events.

Dynamics of soil mineral nitrogen

Initial soil NH_4^+ concentration was low (average, 3.42 mg kg^{-1} soil) but increased after the first irrigation event on day 10 (Fig. 4c). Average soil NH_4^+ concentrations across the sampling dates in response to grass pea treatment (6.87 and 6.73 mg kg^{-1} soil for early- and late-terminated, respectively) were significantly greater than oat (5.80 and 5.99 mg kg^{-1} soil for early- and late-terminated, respectively) and the control (5.90 mg kg^{-1}) treatments. Likewise, the influence of date of soil sampling was significant as soil NH_4^+ concentrations generally increased after rainfall or irrigation events, and declined during dry periods. However, when finger millet was growing rapidly in the last week of the study, soil NH_4^+ concentrations remained low and did not fluctuate even during rainfall events.

Soil NO_3^- concentrations remained more stable than soil NH_4^+ concentrations and no large fluctuations occurred within short periods after rainfall or irrigation events (Fig. 4d). Soil NO_3^- concentrations declined slightly until day 55, but more rapidly thereafter. Soil NO_3^- concentration in the control treatment was lesser than the cover crop treatments during most of

the sampling events. Average soil NO_3^- concentrations across measurement dates were significantly greater for early-terminated grass pea and oat (10.86 and 11.32 mg kg^{-1} soil, respectively) than under the control treatment (7.70 mg kg^{-1} soil), while late-terminated grass pea and oat resulted in intermediate amounts (8.82 and 8.90 mg kg^{-1} soil, respectively).

Decomposition rates of cover crops

A rapid decrease in relative mass of decomposing biomass occurred during the first 14 days after biomass incorporation across both cover crops and maturity levels (Fig. 5a). Thereafter, the rate of decrease in biomass weight slowed and total remaining mass remained mostly constant. The effects of sampling date and maturity level were significant for relative remaining mass of incorporated biomass, but the interaction was not significant. The undecomposed biomass of late-terminated cover crops was significantly greater than early-terminated cover crops throughout the study period.

Effects of cover crops and sampling date on N concentration of decomposing biomass were significant, while the treatment by date interaction was not (Fig. 5b). The N concentration of decomposing grass pea remained significantly greater than oat within a maturity group throughout the study. Nitrogen concentration of early-terminated biomass declined at slightly higher rate than the late-terminated biomass during the first 2 weeks after soil incorporation. After 42 days of incorporation, N concentrations of decomposing biomass remained mostly constant.

As loss of biomass in decomposition was greater than change in biomass N concentration within the first 2 weeks of incorporation, relative loss of biomass N was greater within the first 2 weeks (Fig. 5c). Overall, early-terminated cover crops lost more N during decomposition than the late-terminated cover crops. Total amount of N released from decomposing biomass

calculated as a product of N incorporation rate (Table 1) and relative N release was 80, 51, 53, and 39 kg N ha^{-1} from early-terminated grass pea, late-terminated grass pea, early-terminated oat, and late-terminated oat, respectively.

Importance of soil variables for N_2O emissions

The soil NH_4^+ level was the most important variable for predicting temporal dynamics of N_2O emissions (Fig. 6a). The importance of soil variables was revealed in the order as NH_4^+ > EC > Moisture > NO_3^- > pH by the regression model with random forest. The stronger correlation between soil NH_4^+ level and N_2O emissions was also supported the importance of NH_4^+ to N_2O emissions, relative to the other soil variables (Fig. 6b).

Biomass growth, yield, N concentrations of finger millet

Dynamics of RVI measured as a proxy of finger millet biomass growth was mostly similar among the treatments (Fig. 7). During the first 30 days, finger millet had slow growth due to a long drought spell. However, a rapid increase in RVI occurred after 30 days with the occurrence of frequent rainfall events.

Finger millet cultivated on oat-incorporated plots produced significantly more (15-19%) biomass than grass pea incorporated plots (Fig. 8a). However, finger millet biomass yield in control was not significantly different from plots receiving cover crop residue. Nitrogen concentrations in biomass of finger millet was greater in response to all cover crop treatments than in response to the control (Fig. 8b). Finger millet biomass cultivated on early-terminated cover crops had significantly greater ($P < 0.05$) concentrations of N than in response to late-terminated cover crops. Finger millet cultivated on plots receiving cover crop treatments had significantly higher amounts of total accumulated N in biomass than in response to the control (Fig. 8c). Total uptake of N in biomass of finger millet was higher from early- than late-

terminated oat treatment while there was no significant different between early- and late terminated grass pea.

DISCUSSION

Soil moisture is the major environmental factor that controls decomposition of cover crop biomass and N_2O emissions (Singh et al., 2019b). As expected, N_2O emissions increased from plots with incorporated cover crops after rainfall and irrigation events, as reported by previous studies in the region (Kandel et al., 2018; 2019a). Increased soil moisture after rainfall and irrigation events increased decomposition of residues of cover crops, and soil organic matter as indicated by increased CO_2 emissions rate, to contribute for increased soil concentrations of inorganic N. Therefore, this increased soil concentrations of inorganic N coupled with anaerobic conditions under high soil moisture is conducive for denitrification, which dominates N_2O emissions from agricultural soils (Aulakh and Singh, 2001). The similar dynamics of WFPS and N_2O emissions indicated that soil moisture was a key factor to controlling N_2O emissions from managed cover crops.

Type of crop, chemical composition, and applied management practices all affect fluxes of N_2O from decomposing green manures (Basche et al., 2014; Huang et al., 2004; Kaiser et al., 1998; Kaspar and Singer, 2011; Omonode et al., 2011; Soon and Arshad, 2002). Greater N_2O emissions generally occur from soil incorporated residues with low C/N ratios and lignin concentrations as C and N in biomass residues with low C/N ratio mineralize rapidly after soil incorporation and increases soil availability of inorganic N for N_2O production (Basche et al., 2014; Gomes et al., 2009; Huang et al., 2004). However, in this study, similar cumulative emissions were observed in response to grass pea and oat cover crops within a maturity group. Yet, the fluxes of N_2O were greater from early-terminated crops were greater than late-

terminated crops. Likewise, soil NO_3^- concentrations in the early days of soil incorporation were often greater in early-termination treatments than in late-termination treatments. Thus, finger millet could have taken more N mineralized from biomass of early-terminated cover crops during the early part of the summer growing season. This assumption of higher use of soil N by finger millet was supported by the greater amounts of N in finger millet biomass cultivated under early-terminated cover crops. Although more N could have been mineralized from early-terminated biomass immediately after soil incorporation, low availability of soil moisture during this period might have constrained N_2O emissions. While C/N ratios were greater in late terminated cover crops, N_2O emissions remained greater after 30 days of biomass incorporation, when relatively greater amount of soil moisture was available. These results indicated that chemical composition of cover crops alone was not a strong predictor of total cumulative N emissions after soil incorporation, as the maturity level interacted with environment variables (e.g., timing of rainfall events) and crop N uptake to influence cumulative emissions of N_2O.

In general, higher N_2O emissions are expected from decomposing legume biomass than grass residues, since legumes have lower C/N ratios and higher N concentrations than grasses at similar stages of maturity (Basche et al., 2014). However, in this study, N_2O emissions from grass pea and oat residues were similar, even though grass pea N application rate by grass pea residue was about 1.5 times more N than that of oat within both maturity groups (Table 1). This indicated that oat biomass terminated within 2–3 months of planting, and had a C/N ratio in the range of 14–23 at termination, could decompose and mineralize as rapidly as grass pea. This was also seen as similar level of CO_2 emissions from both oat and grass pea incorporated plots and similar level of biomass decomposition rates in the litter-bag assay.

Decomposition rate of residues with low C/N ratios is generally greater than residues with high C/N ratios (Basche et al., 2014; Gomes et al., 2009; Seneviratne, 2000). This response occurred in the current study, since the relative mass loss of early-terminated biomass in the litter-bag experiment was greater than for late-terminated biomass of both grass pea and oat during the first two weeks after soil incorporation. As reported by Shi (2013), biomass from early growth stage biomass decomposed more rapidly than of later growth stages. This is possibly due to rapid decomposition of non-structural cell soluble components of biomass after soil incorporation (Berg and McClaugherty, 2007). The slower rate of biomass decomposition after two weeks of soil incorporation was due to slow degradation rates of structural cell wall components. The difference in total accumulated N in aboveground finger millet biomass in cover crop and control treatment ranged from 47 to 133 kg N ha^{-1}. The greater amounts of N uptake from cover crop treatments than N released from decomposing cover crops could be due to priming effect of soil organic matter (SOM) by N and other nutrients available in the cover crop residues and increased N mineralization from SOM (Kuzyakov, 2002). Thus, a large proportion of N_2O emissions from cover crops could have been originated from the N mineralized from priming of SOM rather than N released from decomposing cover crop residues.

Previous studies have documented decreased soil pH for a brief period following termination and soil incorporation of cover crops due to production of organic acids by sugars in the glycolytic pathway (Adeleke et al., 2017; Kiiya et al., 2010). The newly formed organic acids subsequently decompose to H_2O and CO_2 and soil pH increases (Yan et al., 1996). Additionally, use of H^+ ions in decarboxylation and protonation of NH_3 contributes to increased soil pH. In this study, however, we did not observe a strong influence of incorporated biomass on soil pH, since the dynamics and magnitude of soil pH was mostly similar among the cover crop and

control treatments. Previous studies have reported strong correlations between soil pH and soil N_2O emissions, as low pH inhibits the reduction of N_2O to N_2 in the denitrification process (Simek and Cooper, 2002). In the current study, although soil pH fluctuated rapidly within short periods during the rainfall events, soil pH did not drop below 6.3, and thus, such inhibition might not have occurred.

A rapid increase in soil EC in all cover crops incorporated plots after every rainfall or irrigation events can be attributed to release of minerals from biomass during decomposition (Moreno-Barriga et al., 2017; Singh et al., 2019b). Therefore, soil EC showed a similar trend as soil NH_4^+ concentration which was also supported by significant correlation (R = 0.81) between both (Fig. 6b). A possible explanation to this correlation is can be that there can be inhibition of nitrification of NH_4^+ to NO_3^- in soils with high EC (Adviento-Brobe et al., 2006). Additionally, results from the variable importance matrix suggested soil EC to be the second most important soil variable for predicting N_2O emissions after soil NH_4^+, and correlations between N_2O emissions and soil EC were significant (R = 0.70). Stable soil EC after rainfall events noted at the end of this study might be due to uptake of minerals in decomposing biomass and NH_4^+ by finger millet (Kandel et al., 2019).

CONCLUSIONS

The current study showed that incorporation of grass pea and oat-based cover crops at different stages of maturity (21-day age difference) had only minor influences on cumulative N_2O emissions within the first 3 months after soil incorporation. Emissions of N_2O from early-terminated cover crops were either similar or slightly greater than late-terminated crops during the first 30 days of soil incorporation. Thereafter, greater emissions were observed from late terminated crops when more and frequent rainfall was received. Nitrogen concentrations and

total N uptake by finger millet grown as a recipient crop was greater than in response to the unfertilized control, which indicated effective transfer of biomass N from cover crops to the recipient crop. Results in this study indicated the properties of biomass of incorporated cover crops alone are not a strong predictor of total cumulative N_2O emissions, as biomass properties had complex interaction with environment variables (e.g., timing of rainfall events) to influence cumulative emissions of N_2O. Overall, this study indicated terminating oat and grass pea based cover crops at vegetative or reproductive states could have only minimal influence on emissions of N_2O if environmental conditions are similar at termination.

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TABLES AND FIGURES

Table 1. Chemical composition of early- and late-terminated cover crops. Measurements are averages of three replications of each species and each maturity level. DM: Dry matter

Properties	Grass pea		Oat	
	Early-terminated	Late-terminated	Early-terminated	Late-terminated
Moisture (%)	86.6	80.4	83.7	77.6
Biomass incorporation rate (Mg ha^{-1})	2.5	2.5	2.5	2.5
N concentrations (% of DM)	4.6	3.4	3.06	2.4
C concentrations (% of DM)	41.1	58.5	42.6	57.0
C/N	8.7	17.1	13.9	23.1
Total N application (kg ha^{-1})	112.5	84.4	74.3	61.2
Cellulose (% of DM)	21.0	22.6	25.8	30.0
Hemicellulose (% of DM)	6.8	14.0	16.1	26.9
Lignin (% of DM)	7.4	8.0	6.7	8.4

Figure 1. (a) Average air and soil temperatures during flux measurements, (b) dynamics of daily precipitation (black bars), irrigation amount (gray bars with arrows) and water-filled pore space (WFPS) measured at 0–0.15 m soil depth (black line) during flux measurement. Dynamics of (c) CO_2 and (d) N_2O emissions. Unidirectional error bars are shown for clarity. Error bars (c–d) represent the spatial variations at the plot scale (S.E., n = 3).

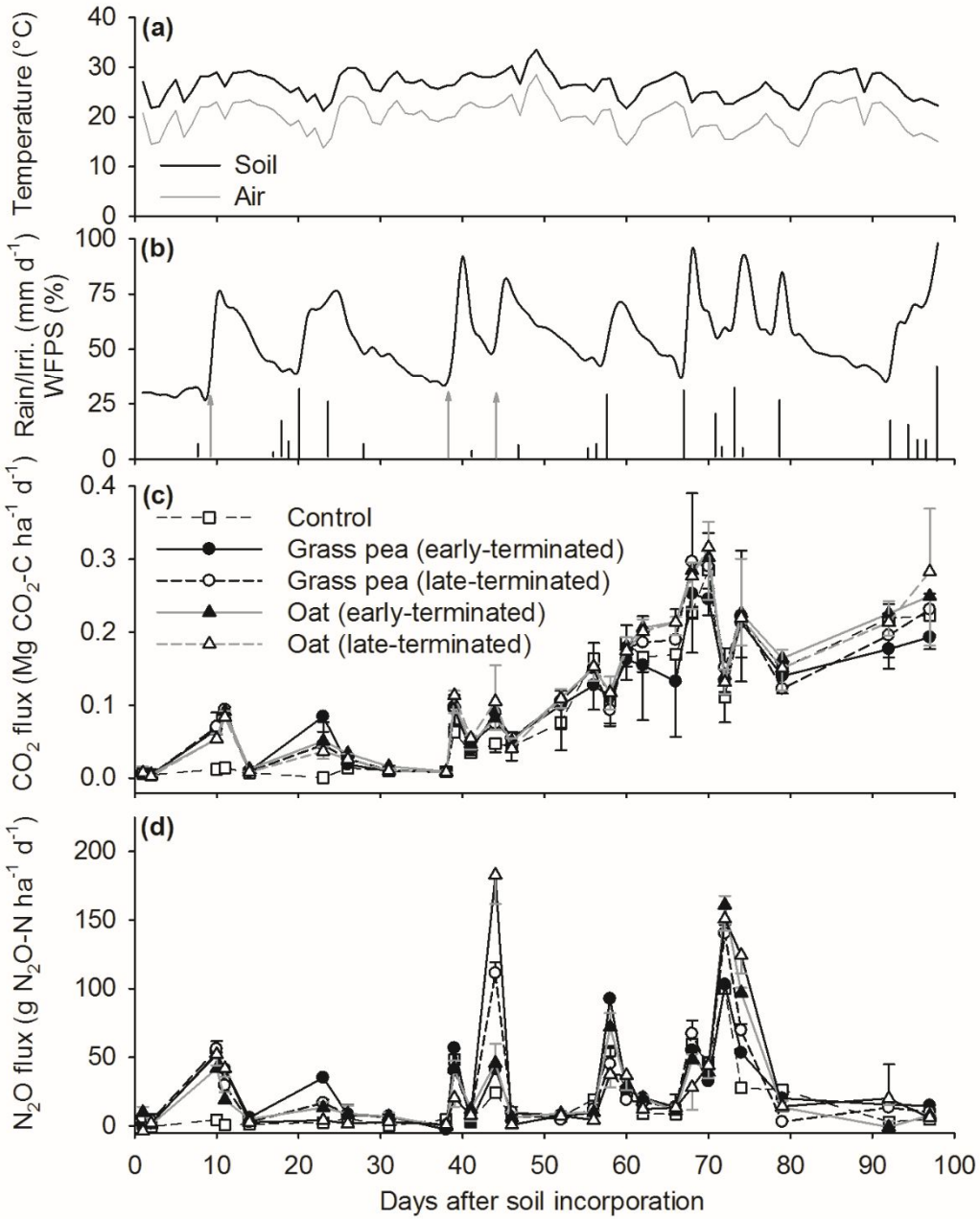


Figure 2. Cumulative estimates of N_2O emissions during the 3-month study period. Error bars represent the spatial variations at the plot scale (S.E., $n = 3$).

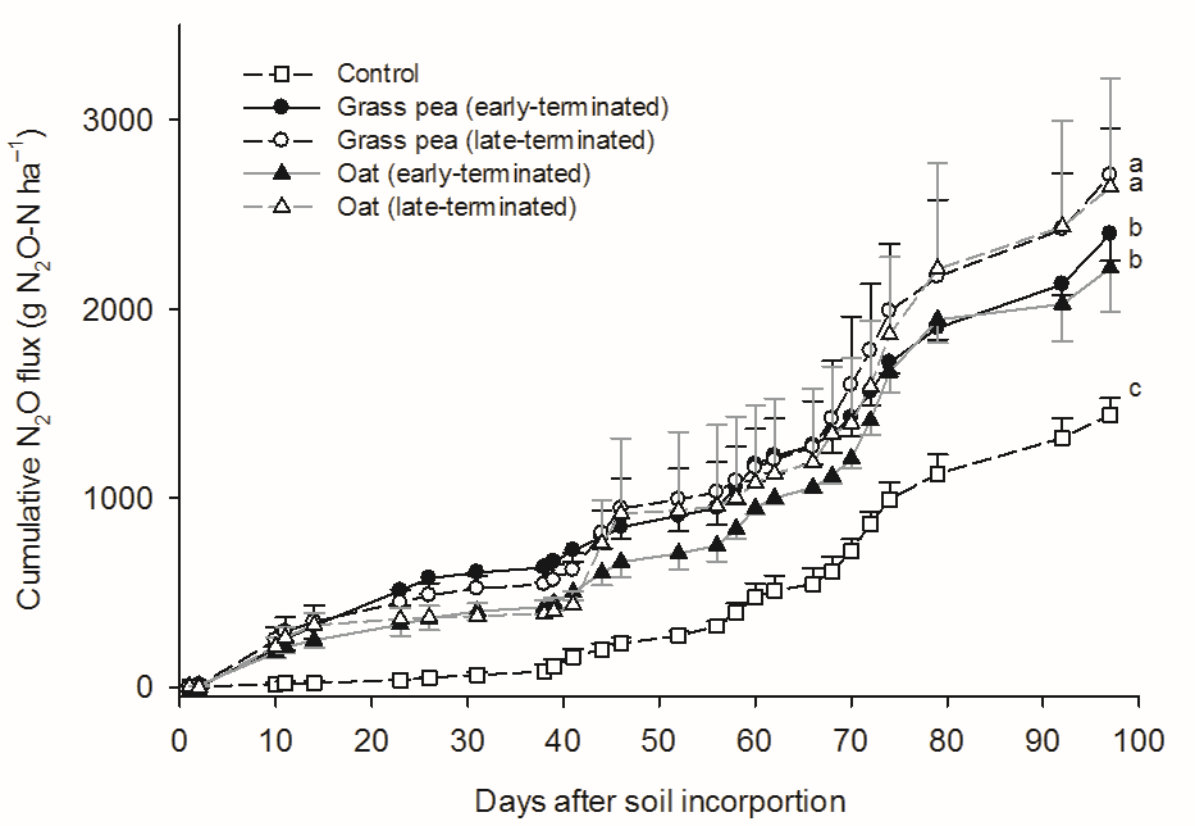


Figure 3. Correlation of cumulative CO_2 and N_2O emissions during the first 10 days of incorporation of cover crop biomass. Pearson's correlation coefficient (*R*) between the cumulative emissions is shown.

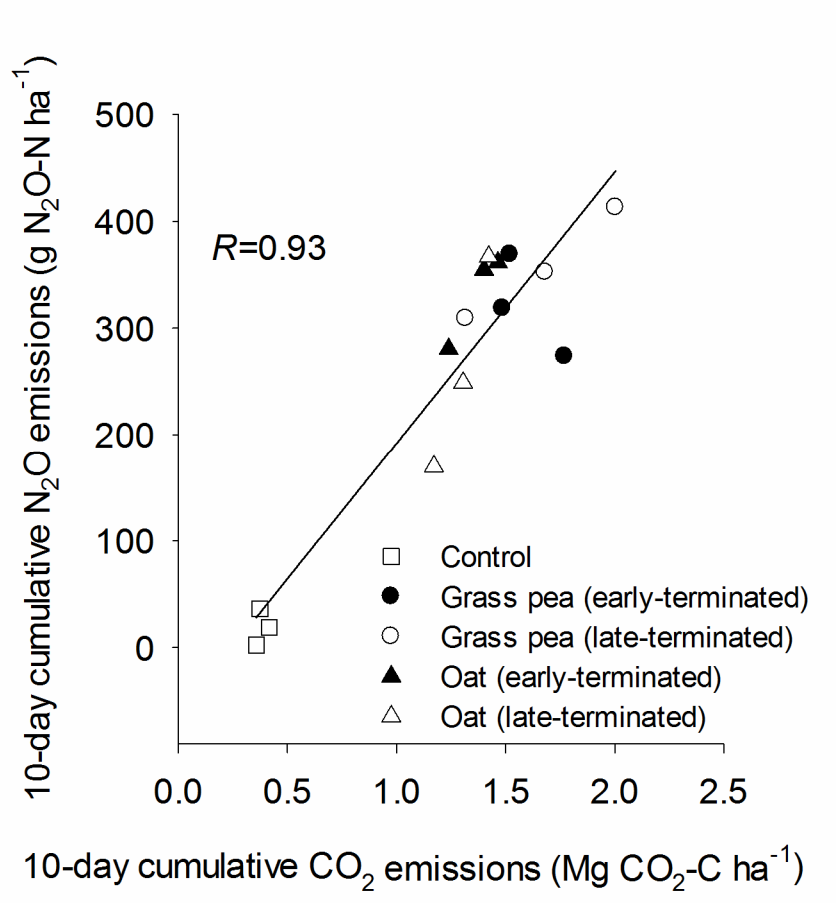


Figure 4. Dynamics of (a) soil pH, (b) electrical conductivity (EC), (c) ammonium (NH_4^+) and (d) nitrate (NO_3^-) N in the 0-0.15 m soil depth. Average measurements from three plots (n =3) are presented. Error bars are not shown for clarity.

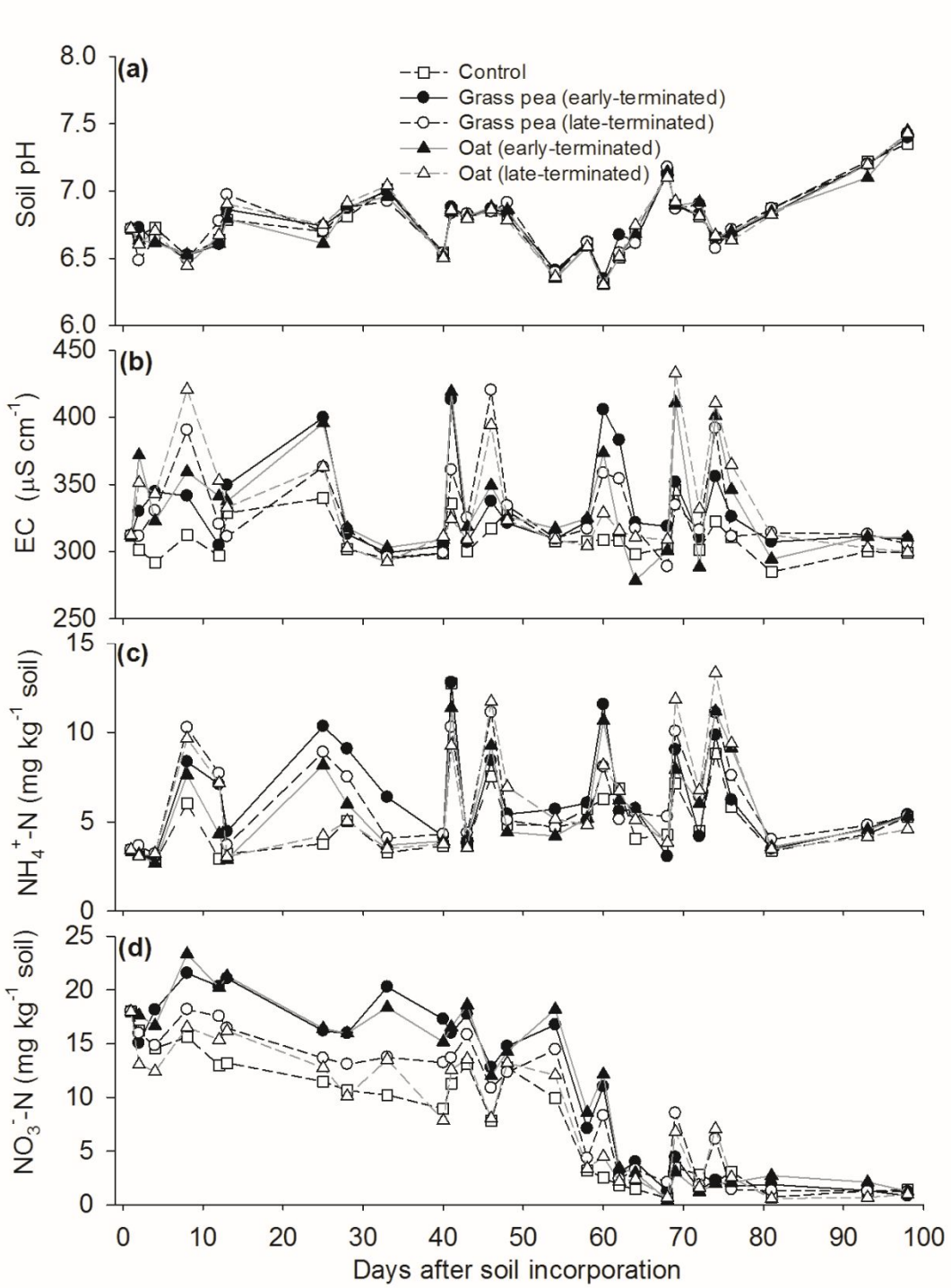


Figure 5. Dynamics of (a) relative remaining mass, (b) nitrogen concentration, and (c) relative remaining N of decomposing cover crops residues. (Error bars represent the spatial variations at the plot scale (S.E., n = 3). Unidirectional error bars are shown for clarity.

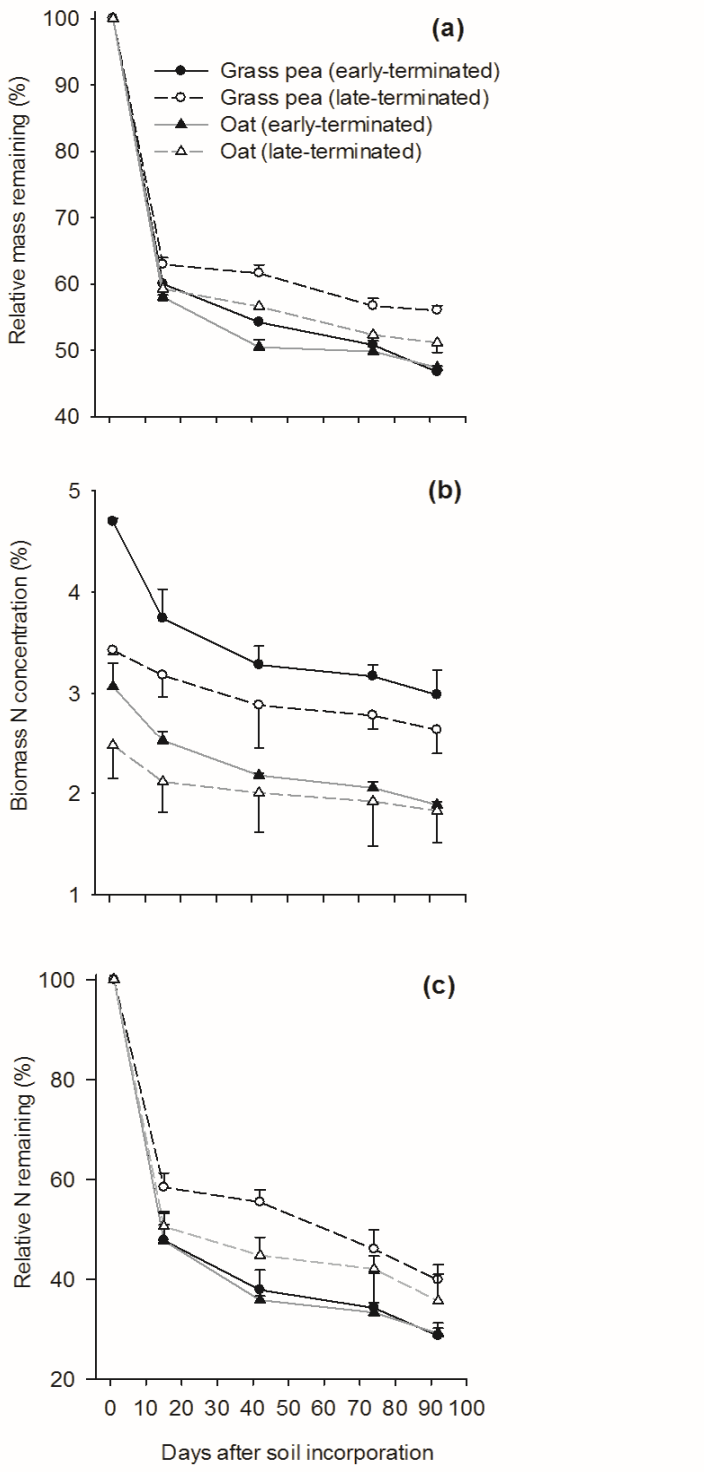
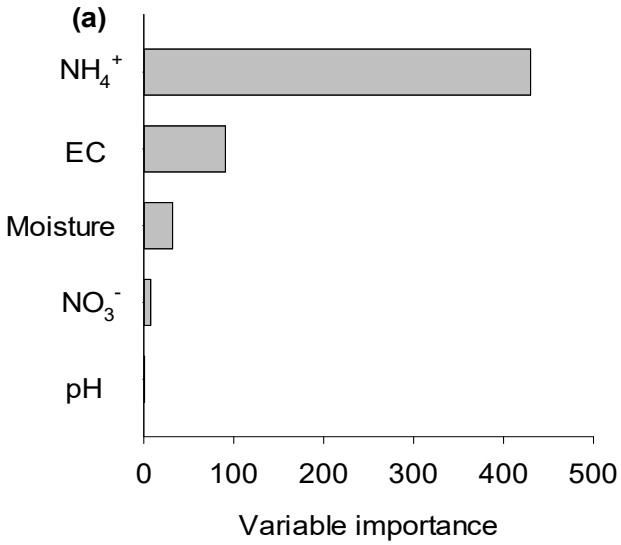


Figure 6. (a) Variable importance (VIMP) for predicting temporal trends in N_2O emissions. (b) Correlation matrices with Pearson's correlation coefficients (*R*) of N_2O emissions and soil variables on 25 measurement dates during the study period. EC, electrical conductivity.



(b)

	pH	EC	NH_4^+	NO_3^-	Moisture
EC	-0.30				
NH_4^+	-0.18	**0.81**			
NO_3^-	-0.34	0.19	-0.08		
Moisture	0.30	0.39	0.50	-0.33	
N_2O	-0.17	**0.70**	**0.83**	-0.29	**0.57**

Figure 7. Dynamics of ratio vegetation index (RVI) measured as a proxy for green biomass of finger millet. Error bars represent the spatial variations at the plot scale (S.E., $n = 3$). Unidirectional error bars are shown for clarity.

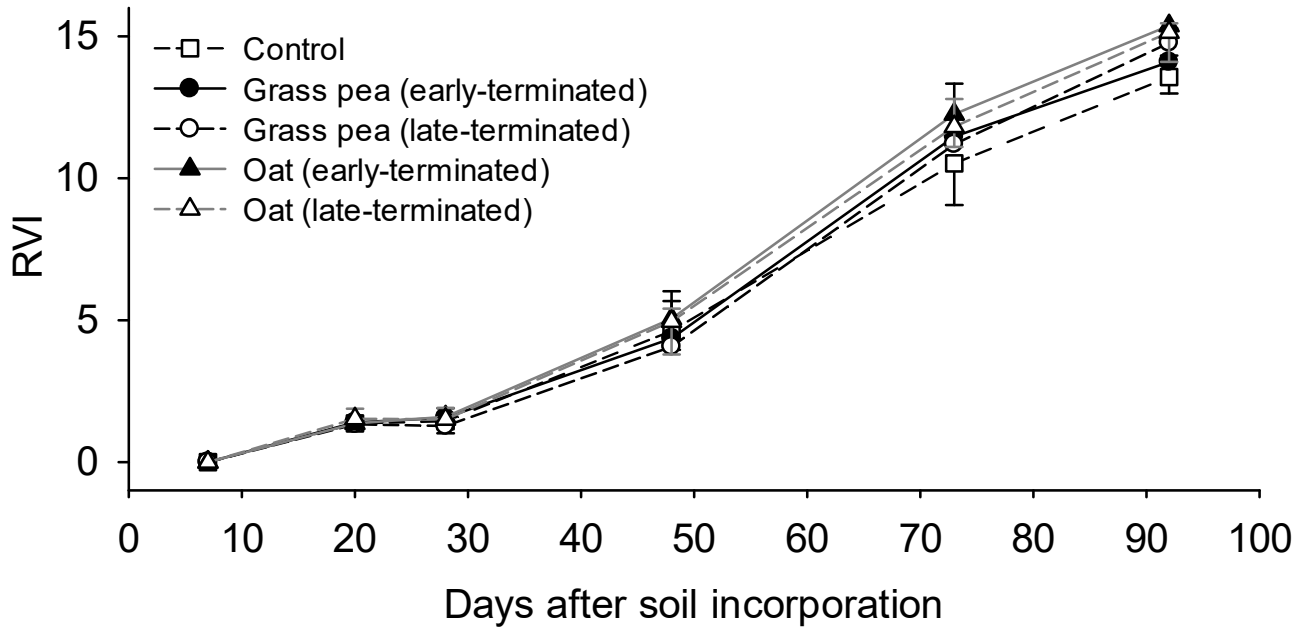
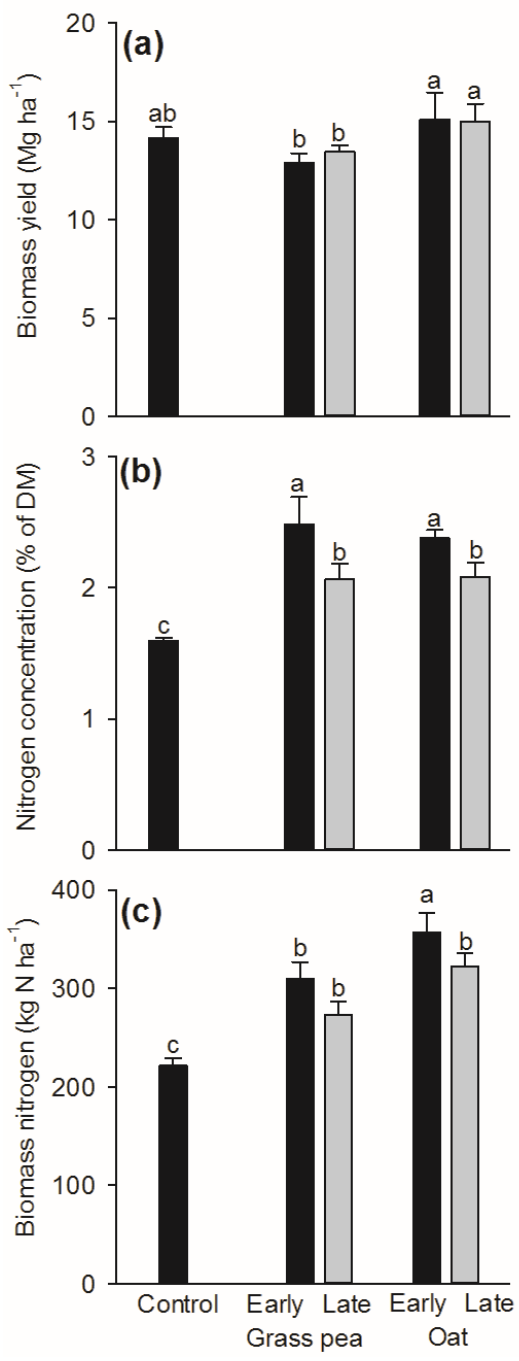


Figure 8. (a) Mean aboveground biomass produced by finger millet in early-September. (b) Nitrogen concentrations of the harvested biomass of finger millet. (c) Total amount of nitrogen in the harvested biomss of finger millet. Standard error (S.E., $n = 3$) bars represent spatial variations at the plot scale. The statistical differences ($P < 0.05$) in biomass yield are indicated by different letters on the top of bars.



CHAPTER III

N_2O EMISSIONS FROM RESIDUES OF OAT AND GRASS PEA COVER CROPS CULTIVATED IN US SOUTHERN GREAT PLAINS

Manuscript is submitted to Frontiers in Sustainable Food Systems

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ABSTRACT

Grass pea and oat are potential cover crops in lieu of spring fallow periods within summer crop systems in the US SGP. The main objective of this study was to compare N_2O emissions from residues of a legume (grass pea) and a cereal (oat) cover crops. The comparisons included oat and grass pea cultivated solely as cover crops where all biomass was terminated by tillage at flowering (18 May), removal of grass pea biomass for forage use, and a control with plots fallowed during the spring (March to May). Crabgrass (*Digitaria sanguinalis*) was cultivated as a main summer crop immediately after termination of the cover crops. Fluxes of N_2O were measured with a closed chamber connected to a portable gas analyzer on 23 dates during a 3-month growth period of crabgrass. At termination, oat produced more aboveground biomass than grass pea (2.17 vs. 3.56 Mg ha^{-1}) but total N in biomass was similar (102-104 kg ha^{-1}) as N concentrations in grass pea was greater biomass than oat (4.80 vs. 2.86% of dry mass). Three-month cumulative emissions of N_2O from grass pea incorporated plots (0.76 ± 0.11 kg N_2O-N ha^{-1}; mean ± standard error, $n = 3$) were significantly lower ($P < 0.05$) than from oat

incorporated plots (1.26 ± 0.14 kg N_2O-N ha^{-1}). Emissions from grass pea harvested plots (0.48 ± 0.04 kg N_2O-N ha^{-1}) were significantly lower ($P < 0.05$) than grass pea incorporated plots. Yields produced by crabgrass were similar ($P > 0.05$) for oat incorporated, grass pea incorporated, and grass pea harvested plots (8.65−10.46 Mg ha^{-1}), but yield responses to the control (18.53 Mg ha^{-1}) was significantly greater ($P < 0.05$). Nitrogen concentration in crabgrass biomass was greater from oat and grass pea incorporated plots (2.86−2.87 %) than grass pea harvested (1.93 %) and control (1.44 %) plots. In conclusion, results indicated that greater biomass yields by an oat-based cover crop could lead to greater N_2O emissions after soil incorporation than legume-base cover crop, and removal of aboveground biomass of grass pea could mitigate N_2O emissions.

INTRODUCTION

Interest in including cover crops in production systems used in the US SGP has been increasing. Cover crops are seen to provide a number of environmental services, including; reducing soil erosion, improving soil aggregation and infiltration, suppressing weeds, increasing the pool of N in soils, reducing leaching and runoff, and increasing soil organic matter, (Foster et al. 2017; Snapp et al., 2005). The predominant cropping systems used by producers in the SGP are different forms of winter wheat – summer fallow rotations, and are generally applied continuously each year (Decker et al., 2009). However, warm-season crop – winter fallow rotations are also utilized in some years to diversity production and increase income (Decker et al., 2009; Aiken et al., 2013). Among the potential warm-season–winter fallow systems, for the SGP region cool-season legumes or grasses with short growing seasons (generally spanning March to May) can be grown as cover crops to support a summer cash crop (Biederbeck et al., 1993; Singh et al., 2019a).

One issue with growing cover crops to support a following grain or forage crop in place of fallowing is the availability of water to support the entire crop rotation (Holman at al., 2018; Decker et al., 2009). Precipitation in the SGP is extremely erratic within the region in terms of timing and amount, resulting in a degree of uncertainty for many cropping systems (Garbrecht and Schneider, 2003; Decker et al., 2009; Aiken et al. 2013). Further, irrigation within the SGP is limited to a small part of the total land area. Therefore, plant species selected as cover crops should not overly deplete soil moisture during growth, as this water use could reduce establishment and yield of following crops, such as; corn (*Zea mays L.*), sorghum (*Sorghum bicolor* L.) or annual forage grasses (Singh et al., 2019b).

Given the limitations on available water, spring-planted crops with short growing seasons have potential as cover crops within summer cropping systems of the SGP. Among the candidates are grass pea, a grain legume with a short-growing season and oat, a short-duration cereal. Rao et al., (2005, 2007) reported grass pea as an adaptable crop suited to the dry growing conditions of the SGP. Legume-based cover crops can fix atmospheric N and serves as an N source for following summer crops. However, both legume and non-legume species can contribute to increased N in soil pools by reducing losses through leaching, runoff and gaseous emissions (White et al., 2017).

One key aspect for the success of cover crops grown as nutrient sources for following crops is the synchronization between nutrient mineralization from decomposing residues, and the demand for nutrients by the recipient crop (Kandel et al., 2019a, b; Myers et al., 1994). Due to short growing seasons, spring-planted, cool-season crops have low C/N ratios at termination, which may result in rapid biomass decomposition and N mineralization after termination, to transfer nutrients to following summer crops (Kandel et al., 2019b). However, rapid

decomposition and N mineralization from residues of cover crops prior to establishment of the following crop also may contribute to large emissions of N_2O, a highly potent GHG (Huang et al., 2004; Basche et al., 2014; Kandel et al., 2018). Heavy or frequent rainfall events are common in the US SGP during the late spring, and occurs during the termination period of spring-planted cover crops. Such simultaneous increase in soil concentrations of mineral N and moisture after termination of cover crops could be conducive for denitrification, a major microbial pathway for N_2O production (Kandel et al., 2018). Therefore, a better understanding of how type of cover crop and their management impact N_2O emissions from decomposing biomass are crucial for mitigation (Hoorman et al., 2009).

Nitrogen mineralization from decomposing biomass and resulting soil N_2O emissions can be influenced by type of the cover crop (Basche et al., 2014). Legume cover crops in general contribute greater N_2O emissions compared to non-legumes due to increased pools of N from biological fixation (Baggs et al., 2000). Additionally, legume biomass generally has lower C/N ratios than grasses, decompose rapidly and deplete soil O_2 concentrations, a condition conducive to denitrification (Aulakh et al., 1991, Thilakarathna et al., 2016). However, since spring-planted cereals used as cover crops are generally terminated before flowering, the C/N ratio and lignin concentrations could be low enough for rapid decomposition, and may also result high N_2O emissions.

One potential strategy recommended for limiting N_2O emissions from residues of cover crops is removal of aboveground biomass as forage or other uses, instead of soil incorporation (Li et al., 2015; Kandel et al., 2019a). Beef cattle are a major agricultural commodity in the US SGP, so harvesting biomass for forage could be more profitable than soil incorporation, by lowering risks of forage shortages in dry years, which are common in the region (Holman et al.,

2018). However, biomass removal also results in significant removal of legume N from the ecosystem, as root systems of legumes retained in the soil contain <20% of total plant biomass (Biederbeck et al., 1993), and generally have tissues with high C/N ratios (Kandel et al., 2019b). Therefore, biomass removal of cover crops may impact the growth of following crops due to reduced N supply, if supplemental N is not supplied from external sources (Kandel et al., 2019a). Although interest in cover crops is increasing in the US SGP, there is limited information on the influences of type of cover crop on fluxes of N_2O after termination. Additionally, although large fluxes of N_2O after termination of legume-based cover crops have been reported (Kandel et al., 2018), there is limited information on how to mitigate N_2O emissions from residues of cover crops through management strategies. Therefore, we tested how type of cover crop (legume vs. cereal) and management strategy (incorporation vs. harvest of a legume residues) influenced fluxes of N_2O. The objective of this study was to compare N_2O emissions from legume (grass pea) and cereal (oat) residues of spring-cultivated cover crops, within a summer crop system in the US SGP. We hypothesized that (i) emissions of N_2O would be lower from decomposing oat residue than grass pea residue, and (ii) emissions of N_2O could be mitigated by removing biomass for use as forage compared to incorporation of biomass to supply N to a following forage grass.

MATERIALS AND METHODS

Experimental 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 study was conducted during the March to August time period of 2018, and included periods of growth by both; 1) sources of spring-grown (March to May) green N, and 2) a recipient hay crop grown during summer (May

to August). The study site was situated on an upper terrace of a bottomland area along the North Canadian River (Goodman and Morris, 1977). The soils for the site were classified as Brewer silty clay loams (fine, mixed, superactive, thermic Udertic Argiustolls). These soils average (±1 standard deviation) near-neutral pH [6.9 (±0.7)], high moist bulk densities [1.3 ± 0.1) Mg m^{-3}], low permeability [12 (± 6) mm h^{-1}], available water capacity of 0.47 (± 0.12) cm cm^{-1}, and cation exchange capacity of 17.5 cmol kg^{-1} within the 0-15cm soil depth (USDA-NRCS, 1999). Brewer series are among the more fertile soils in the Canadian River basin. Measured average soil organic C and N contents of these soils prior to the study were 1.43% and 0.11%, respectively. The topsoil (0–0.15 m) had particle fractions of 18% sand, 52% silt, and 30% clay.

Long-term annual (1977–2019) precipitation (±1 standard deviation) received during the calendar year (January–December) at the study site was 920 (± 186) mm. Mean quarterly precipitation for the March to May (major growth period of spring-planted cover crops) and June to August (major growth period of following summer crops) periods were, respectively, 299 (± 110) and 266 (± 114) mm. Mean quarterly daily temperatures for the March to May and June to August periods were, respectively, 15.6 (± 1.1) and 26.9 (± 1.4)°C.

Experimental design and crop management

The experiment consisted of 12 plots (4 m × 3 m) arranged in completely randomized design. Grass pea was planted on six plots, and oat was planted on three plots on 10 March, while three plots were left fallow during spring. Three oat and three grass pea plots were terminated by tillage (disked once to ∼10 cm depth and roto-tilled once) on 18-May. Grass pea biomass from the remaining 3 plots were harvested manually on the same day. Crabgrass was then planted on all plots at a rate of 5 kg ha^{-1} at 0.03 m spaced rows on 19-May.

A long dry spell (>1 month) started 7 days after crabgrass was sown. This dry period restricted growth of crabgrass, and plants showed symptoms of water shortage. Therefore, the plots were irrigated with 30-mm water on 23 days after terminating cover crops. The plots were again irrigated with 30-mm water 64 days after termination of cover crops. The entire area of the plots, except that covered by collars placed to measure N_2O fluxes (described in the following section), was irrigated with a sprinkler system. Irrigation inside collars was subsequently applied using a watering can to apply precise amounts of water.

Measurements of yield and quality of cover crop biomass

Total aboveground biomass of grass pea was determined by oven drying all biomass harvested from the 12-m^2 areas of plots (n = 3) assigned to harvest treatments. In contrast, biomass yield of oat was determined by drying biomass harvested from 1-m^2 areas from each plot (n = 3). The root biomass of oats and grass pea was collected by collecting soil from the upper 15 cm of the profile from the 1 m^2 area by shovel, and cleaning the soil form roots manually by washing roots through a 2.0 mm sieve under running water. Biomass samples (roots and shoots) from both green manures were oven-dried at 60°C to constant weight to determine amount of dry matter. Portions of samples were then ground through a 2.0 mm screen by Wiley mill for analyses.

Samples were analyzed for total C, N and cell wall components (cellulose, hemicellulose, and lignin). Concentrations of C and N were assayed by flash combustion (900°C for 10 min) method (Model VarioMacro, Elementar Americas, Inc., Mt. Laurel, NJ, USA). Neutral detergent fiber, ADF, and ADL were determined by the van Soest and Wine (1967) method. Concentrations of cellulose were calculated as the difference between ADF, ADL, and hemicellulose as the difference between NDF and ADF. The ADL fraction was presented as an estimate of lignin concentration in samples.

Gas flux measurements

Nitrous oxide and CO_2 fluxes were measured on 23 dates at irregular intervals using a closed chamber system during 19 May to 16 August 2018. In each plot (total $n = 12$), a white painted steel collar (0.65 m × 0.65 m) was inserted to a 0.10 m depth immediately after tillage operations and crabgrass was sown. These collars had a 0.04 m wide outer flange to support the top chamber used for flux measurements. During the flux measurement, a white-colored PVC chamber (0.70 m × 0.70 m × 0.21 m) was placed on the permanently installed collars. Fluxes were measured frequently (often daily) between 10:00 and 12:00 after rainfall and irrigation events, to capture N_2O emission peaks observed after the events, but less frequently during dry periods when N_2O emissions remained close to zero.

During chamber enclosure, air in the chamber headspace was mixed using two small battery-driven fans. Air in the chamber headspace was circulated through 3.0 mm inlet and outlet tubing to a portable Fourier transform infrared-based gas analyzer (DX4040; Gasmet Technology Oy, Helsinki, Finland). The concentrations of N_2O and CO_2 were recorded at 20-s intervals, resulting in 18-24 data points during 6–8 min enclosures for each measurement period.

Fluxes were calculated by linear regression using the routine developed by Kutzbach et al. (2007). Based on visual inspection of the CO_2 flux curve, the first few records after chamber enclosure were discarded as dead-band. Total cumulative emissions of N_2O during the measurement period were calculated using linear interpolation of measured fluxes between the measurement dates.

Measurements of environmental variables

Soil temperatures were recorded continuously at 1-hour intervals in one of the control plots using TMC-6 soil sensors (Onset Computer Corporation, Bourne, USA). Three soil sensors were

placed at 0.05, 0.10, and 0.15 m soil depths, and the average temperature of the three depths is presented. Air temperature and precipitation data for the study period were obtained from a weather station (Oklahoma Mesonet, Oklahoma Climatological Survey) located roughly 1.0 km from the study site.

Volumetric water content was continuously recorded at hourly intervals in the same control plot where soil temperature was recorded using soil moisture sensors (model EC-10; Meter Environment, Pullman, WA). Three sensors were inserted at soil depths of 0–0.05, 0.05–0.10, and 0.10–0.15 m and an average of three sensors is presented. The VWC was presented as WFPS calculated as relative-VWC at saturation.

Analyses of soil samples

To determine the concentrations of NO_3^- and NH_4^+ in soils, samples were collected from all plots at the 0−0.15 m depth on all 23 dates of flux measurements. Two soil cores (diameter, 0.02 m) were taken 0.10 m distance from opposite sides of the collars, and the cores were pooled to form a composite sample for each date for analyses. Aliquots of samples were extracted in 1.0 M KCl, and the flow injection method (Timberline Instruments, Boulder, CO, USA) was used to determine the concentrations of NO_3^- and NH_4^+.

Measurements of plant growth, yield, and quality of crabgrass

Canopy reflectance was measured periodically inside the collars using a portable spectro-radiometer (PSR-3500; Spectral Evolution, Lawrence, USA) to monitor the growth of crabgrass non-destructively. Ratio vegetation index was calculated as a ratio of canopy reflectance at red and near-infrared (656 and 779 nm, respectively) wavelengths.

All biomass of crabgrass inside the 0.42-m^2 collars was harvested manually on 16 August, 2018. The biomass was oven-dried at 60°C to a constant weight, and the dried biomass

was milled to pass through a 1 mm sieve. Concentration of N in biomass of crabgrass was determined by flash combustion (900°C for 10 min) method. The amount of N uptake per hectare in crabgrass biomass was calculated as a product of biomass yield and N concentration.

Statistical analysis

The normality of data was tested using Shapiro-Wilk test and homogeneity of variances was tested using Levene's test. The data is presented as averages and standard errors of three plots from a treatment unless stated otherwise. The difference of measured fluxes among the treatments were determined using a mixed model in SAS 9.4 (SAS Inc., Cary, NC, USA). The effect of sampling dates was included in the model and treated as repeated measurements for the measured dynamic variables. Contrasts were used for pairwise comparisons at the 5% level. Pearson correlation coefficients (R) were applied to test for relationships between accumulated CO_2 and N_2O emissions 7 days (prior to emergence of crabgrass) after cover crop incorporation, and to test for correlations of N_2O emissions and soil variables (soil moisture, concentrations of NO_3^- and NH_4^+). Averages of the soil variables and N_2O emissions across treatments at 23 measurement dates were used for the test.

RESULTS

Cover crop yield and biomass properties

Grass pea produced 2.17 Mg ha^{-1} aboveground biomass with N concentrations of 4.81%, resulting to 104.37 kg N ha^{-1} (Table 1). Additionally, grass pea produced 0.30 Mg ha^{-1} root biomass containing 7.86 kg N ha^{-1}. Oat produced greater ($P < 0.05$) amounts of aboveground biomass (3.56 Mg ha^{-1}) than grass pea but had significantly lower N concentration (2.86%) which resulted in similar ($P > 0.05$) amounts of N in aboveground biomass (101.81 kg ha^{-1}). Yield of root biomass (0.40 Mg ha^{-1}) and their N content (1.81%) in oat was similar to amounts

noted for grass pea. The amount of N in root biomass represented <7% of N in total biomass for both species. Cellulose concentrations were similar in both crops, but hemicellulose concentrations were greater in oat biomass and lignin concentrations were greater in grass pea.

Environmental conditions

Average daily air temperatures during the 90-day period of flux measurements ranged between 18 and 33°C, while average daily soil temperatures ranged between 21 and 35°C (Fig. 1a). The average air temperatures for the months of May and June at the study site were 23.2 and 26.2°C, respectively which was 2.8 and 1.1°C higher than long-term (1977-2019) average air temperature of both months. In contrast, the average air temperature of July (27.4°C) and August (25.9°C) was 0.7 and 1.7°C lower than the long-term averages for those months.

A 15 mm rainfall was recorded within the first two days after soil incorporation of cover crops, followed by a prolonged period without precipitation (Fig. 1b). In comparison, approximately 87 mm of rainfall was recorded in mid-June, while a long dry period occurred during the month of July, with approximately 33 mm of rainfall received towards the end of the month. The remaining precipitation events were recorded towards the end of study period in mid-August. A heavy rainfall event at the beginning of the study resulted in high WFPS during the first few days, but declined thereafter due to a long drought period. However, amounts of soil moisture increased significantly after all major rainfall or irrigation events.

Total precipitation during the months of May, June, and July were 50 mm, 93 mm, and 33, respectively, which was 93 mm, 27 mm, and 34 mm lower than the long-term (1977-2019) average for those months [143 (± 88), 120 (± 63), and 67 (± 45) mm for May, June and July, respectively]. However, total precipitation in August (109 mm) was 30 mm greater than long-

term average [79 (± 149) mm]. In total, approximately 230 mm of rainfall was recorded at study site during the May to August period when flux measurements were recorded, compared to 409(± 63) mm for the long-term average. Therefore, this study was undertaken during a drought-affected summer period (56% of long-term precipitation).

N_2O and CO_2 emissions

CO_2 emissions were greater from the oat and grass pea incorporated plots than the control or grass pea harvested plots during the first week after incorporation of green manures (Fig. 1c). The emission rates declined subsequently with declining WFPS, but increased slightly after the first irrigation event. Crabgrass in the control plots had better growth after rainfall events that occurred during the second drought period (day 35-72 after biomass cover crop incorporation), and greater CO_2 fluxes were recorded from control plots during this period.

Immediately after soil incorporation of residues of oat and grass pea, N_2O emissions increased compared to emissions from the control and grass pea harvested plots, indicating the contribution of N from decomposing residue to N_2O emissions (Fig. 1d). The N_2O emissions from control and grass pea harvested plots approximated zero until 22 days after soil incorporation, but few rainfall-induced peaks were recorded thereafter. Emissions of N_2O were observed after rainfall or irrigation events until 85 days after soil incorporation, but emissions did not increase on the last measurement, in response to a rainfall that followed a previous event of >25 mm. Average emissions from oat incorporated plots (14.02 g N_2O-N ha^{-1} d^{-1}) were significantly greater ($P < 0.05$) than average emissions from the grass pea incorporated plots (8.52 g N_2O-N ha^{-1} d^{-1}). Likewise, average N_2O emissions across measurement dates from grass pea incorporated plots were significantly greater than the average emissions from grass pea harvested plots (5.36 g N_2O-N ha^{-1} d^{-1}).

Dynamics of Soil Mineral N

Soil NH_4^+ concentrations remained low and stable in response to all treatments during the first 20 days of the study, but increased after the first irrigation event on day 23 (Fig. 1e). Effects of sampling dates on soil NH_4^+ concentrations were significant ($P < 0.05$) while there were no significant differences between applied treatments except three sampling dates. Soil NH_4^+ concentrations were significantly greater ($P < 0.05$) in oat and grass pea incorporated plots compared to concentrations in control and grass pea harvested plots on day 12 and 19 days after soil incorporation of cover crops. On day 27 after soil incorporation, soil NH_4^+ concentrations were significantly greater ($P < 0.05$) in oat incorporated plots than NH_4^+ responses to the other treatments.

There were decreases in soil NO_3^- concentrations on day 66 and 84 after soil incorporation of cover crops (Fig. 1f). Similar to NH_4^+ concentrations, effects of sampling dates on soil NO_3^- concentrations were significant ($P < 0.05$) while there was no significant difference ($P < 0.05$) among applied treatments. Therefore, the average soil NO_3^- concentrations across sampling dates from grass pea and oat incorporated plots (13.38 and 11.67 mg kg^{-1} soil, respectively) were not different from NO_3^- concentrations in control and grass pea harvested plots (13.74 and 10.84 mg kg^{-1} soil, respectively). Additionally, average of weekly NH_4^+ and NO_3^- concentrations, and soil moisture content were significantly ($P < 0.05$), correlated with average weekly N_2O emissions, though Pearson's correlation coefficients (*R)* were weak (0.28, 0.24, and 0.33, respectively).

Cumulative N_2O emissions

The cumulative N_2O emissions from the oat incorporated plots were significantly greater ($P < 0.05$) than emissions from other treatments (Fig. 2). Additionally, cumulative N_2O emissions

from grass pea incorporated and control plots were significantly greater ($P < 0.05$) than cumulative emissions from grass pea harvested plots (Fig. 2). There was no significant difference ($P < 0.05$) between cumulative N_2O emissions from grass pea incorporated and control plots. During the first 7 days of the study, when CO_2 emissions primarily represented heterotrophic respiration due to the absence of green plants, cumulative emissions of CO_2 and N_2O from individual collars were strongly correlated ($R = 0.97$; $P < 0.01$), indicating rapid contribution of decomposing biomass of cover crops to N_2O fluxes (Fig. 3).

Biomass growth, yield and N concentrations and uptake of crabgrass

Biomass growth of crabgrass measured as RVI was significantly greater ($P < 0.05$) on the control plots on 41 and 46 days after incorporation of cover crops than the other treatments, and remained nominally greater thereafter (Fig. 4). Crabgrass germination occurred in all plots 7 days after planting, but growth by crabgrass on plots receiving cover crops were affected by drought. Crabgrass expressed symptoms of dark bluish-green rolled leaves, and small plant size. A rapid increase in RVI was observed on control plots after irrigation event on day 23, and such increases were observed in response to the remaining treatment after day 45.

The biomass yield of the crabgrass in response to the control was roughly twice ($P < 0.05$) the yields generated by cover crop treatments (Fig. 5a). The N concentrations of crabgrass biomass produced on grass pea and oat incorporated plots were significantly greater than in crabgrass biomass produced by the control and grass pea harvest treatments (Fig. 5b). However, there were no significant differences among treatments for total N accumulated in crabgrass biomass (Fig. 5c).

DISCUSSION

Our hypothesis of lower N_2O emissions from decomposing oat residue than from grass pea residue was rejected since the cumulative emissions from the oat incorporated treatment were significantly greater. In particular, emissions from oat cultivated plots were greater than the plots cultivated with grass pea during the first two weeks after soil incorporation. Generally, greater amounts of N_2O emissions are expected from decomposing legume residues, due to rapid N mineralization of the low C/N ratio residues of legume biomass, and increased NO_3^- in soil pools, which serves as substrate for denitrification (Basche et al., 2014; Baggs et al., 2006; Gomes et al., 2009; Huang et al., 2004). However, this response was not observed during the study.

A possible reason for the greater level of N_2O emissions from the oat treatment could be the greater amounts of C provided by oat biomass, and relatively low C/N ratios in shoots, which increased the amounts of mineralizable C available for denitrification by soil microbes, as indicated by greater CO_2 flux rates (Cameron et al., 2013). Additionally, since the amount of N in oat biomass was comparable to that in biomass of grass pea, oats may have efficiently scavenged soil N, which would be available for denitrification after termination. Therefore, the greater denitrification rates due to greater availability of mineralizable C, combined with amounts of N in oat biomass likely contributed to the greater N_2O emissions from oat incorporated plots.

The cumulative N_2O emissions from control plots were not significantly different from the N_2O emissions generated by the grass pea incorporated plots. The possible explanation for this response might be greater accumulation of N by grass pea when growing than was released during decomposition. This effect was also supported by generally greater NO_3^- in control plots

during the initial stages of the study. Further, growth rate of crabgrass in the control plots was greater than in plots assigned to cover crops. Similar results on growth and yields were reported in double-cropped systems of production in the SGP related to water use by summer crops within a wheat-summer legume rotation (Nielsen et al., 2002; Rao and Northup, 2009; Aiken et al., 2013), and effects of double cropping summer cover crops on wheat production (Northup and Rao, 2016).

Previous research reported that incorporation of cover crop residues is an important factor for N_2O emissions from agricultural soils, though mitigation is possible by management of the residues of cover crops (Sanz-Cobena et al., 2014; Kim et al., 2017; Kandel et al., 2019a; Singh et al., 2020). Residues of cover crops that are incorporated into soil generally increase amounts of mineralizable C and NO_3^- in soils, which are conducive for N_2O emissions (Mitchell et al., 2013). In the current study, the increase in mineralizable C in residues within the incorporation treatments was evidenced by greater CO_2 emissions compared to control and grass pea harvested treatments during first 7 days of the study (before germination of crabgrass). However, similar average concentrations of soil NO_3^- among treatments across sampling dates did not support this premise (Mitchell et al., 2013). Thereby, it can be deduced that increased soil-mineralizable C provided by residues of incorporated cover crops in the current study had a stronger influence than mineralized N on N_2O emissions from incorporated residues. Poor growth and yield of crabgrass in response to removal of biomass of grass pea mat relate to lower fertilizer values of residues of remaining biomass, since soil in this study is considered highly fertile in the region. In a nearby site with less fertile soil, biomass removal of a cover crop of hairy vetch resulted in poor growth and yields of crabgrass in the same season as in this study (Kandel et al., 2019a). The similar results generated by biomass removal from two nearby sites with different soils

during the same year indicate management techniques, like biomass removal, should be based on soil type and their fertility status.

Yields generated by crabgrass in response to the control treatment was significantly greater than yields generated by treatments that included cover crops. This response was likely due to depletion of available soil moisture by the cover crops that would normally be available for establishment and growth by the recipient crop. The total precipitation received during growth of cover crops, and the following summer crop, was lower than was recorded in long term averages. This indicated that replacing spring fallow with a spring-planted cover crop can affect yields by following summer crop in dry years. Such depletion of moisture is a common phenomenon in double-cropped systems in the drought-prone SGP (Nielsen et al., 2002; Rao and Northup, 2009; Aiken et al., 2013). Crabgrass biomass produced on grass pea incorporated plots contained approximately 40 kg ha^{-1} more N than crabgrass produced on grass pea harvested plots, but these responses were not significantly different. This response indicated approximately 39% of N in aboveground biomass of grass pea was transferred to crabgrass. This transfer indicates some degree of function of grass pea as an N fertilizer when residues as soil-incorporated.

The effectiveness of the cover crops as sources of N to following crops mainly depends amount of N in their biomass (Kaye et al., 2019, Singh et al., 2020). Additionally, the chemical properties of biomass, particularly cell wall fractions that govern decomposition and mineralization are also important for the transfer of nutrients from residues of cover crops to recipient crops (White et al., 2014, Singh et al. 2020). Although we expected better N fertilizer values from grass pea due to biological fixation of N, and higher N content in biomass, crabgrass performed at similar levels under both grass pea and oat cover crops. This response was likely

related to greater uptake and recycling of soil N by oat, since the total amount of N in biomass of both oat and grass pea were similar. Further, oat biomass was terminated at an earlier growth stage (~60-days after emergence), so biomass was less mature, and would decompose rapidly, as noted in the larger CO_2 fluxes compared to responses to grass pea.

CONCLUSIONS

It was observed that post-incorporation N_2O emissions were significantly greater from an incorporated oat cover crop than an incorporated legume (grass pea) cover crop. Although greater amounts of N_2O emissions were expected from grass pea, oat produced significantly greater amounts of biomass, and hence had greater amounts of mineralizable C, and similar amounts of N in biomass, which contributed to greater levels of N_2O production. The 90-days cumulative N_2O emissions from the grass pea plots that were harvested were two times lower than from grass pea plots where biomass was incorporated, showing incorporated biomass was a major source of N_2O emissions and the potential to mitigate emissions by harvesting biomass for forage. The crabgrass biomass yield from all treatments with cover crops was roughly half of the control with fallow plots during spring. This response indicated that replacing the spring period of fallow with a spring cover crop can severely affect yield of following summer forages in the region during dry years.

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TABLES AND FIGURES

Table 1. Average (n = 3) yield and chemical composition of grass pea and oat cover crops. DM: Dry matter

Properties	Grass pea			Oat		
	Shoot	Root	Total	Shoot	Root	Total
Biomass yield (Mg ha^{-1})	2.17	0.30	2.20	3.56	0.40	3.60
N concentrations (% of DM)	4.81	2.62	3.71	2.86	1.81	2.33
C concentrations (% of DM)	46.32	49.44	47.88	53.67	57.06	55.36
C/N	9.62	18.87	12.90	18.76	31.52	23.75
Total N in biomass (kg ha^{-1})	104.37	7.86	112.23	101.81	7.24	109.05
Cellulose (% of DM)	22.63	20.81	21.72	23.01	21.15	22.08
Hemicellulose (% of DM)	8.11	7.82	7.96	19.46	16.90	18.18
Lignin (% of DM)	8.71	14.01	11.36	5.73	10.41	8.07

Figure 1. (a) Average soil and air temperatures during flux measurements, (b) dynamics of daily precipitation (black bars), irrigation amount (gray bars with arrows) and water-filled pore space (WFPS) measured at 0–0.15 m soil depth (black line) during flux measurement. Dynamics of (c) CO_2, (d) N_2O emissions, (d) ammonium (NH_4^+), and (e) nitrate (NO_3^-) N in the 0–0.15 m soil depth. Unidirectional error bars are shown for clarity. Error bars (c–f) represent standard error (n = 3).

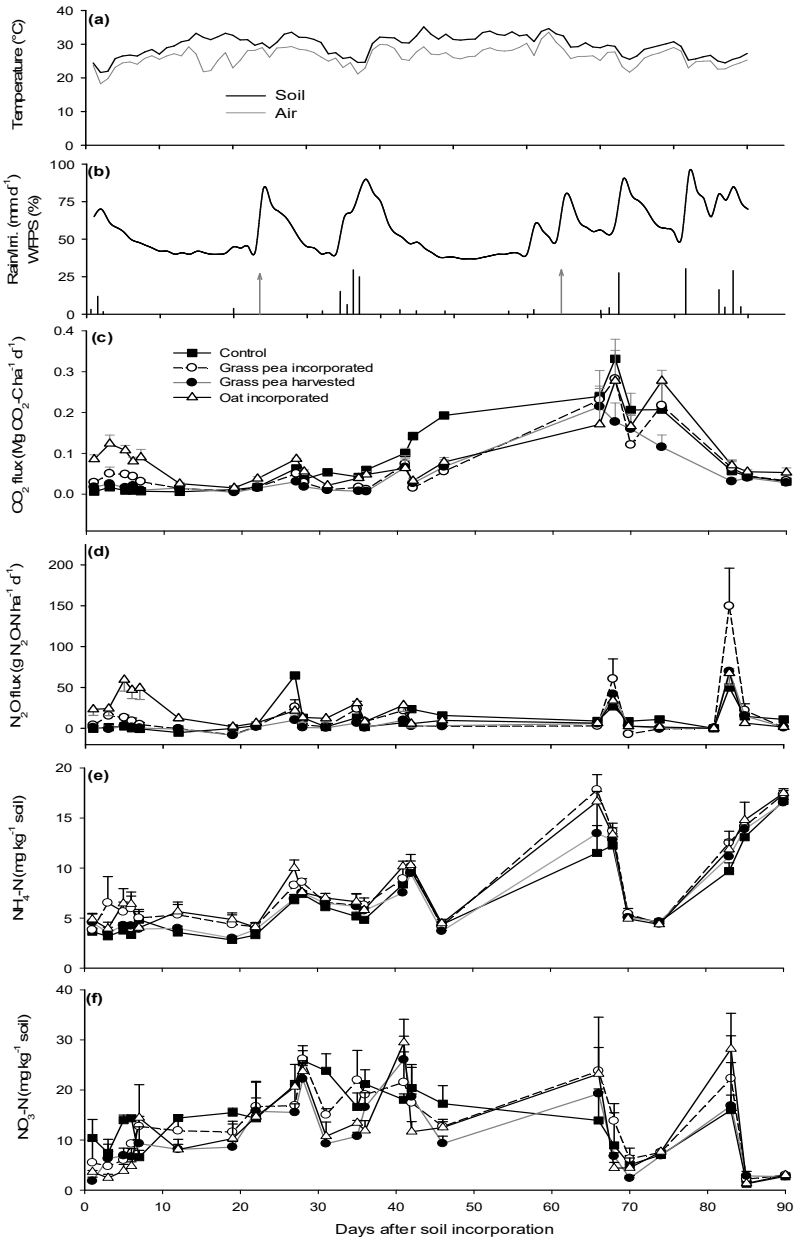


Figure 2. Cumulative estimates of N_2O emissions during the 90-day study period. Error bars represent standard error (n = 3).

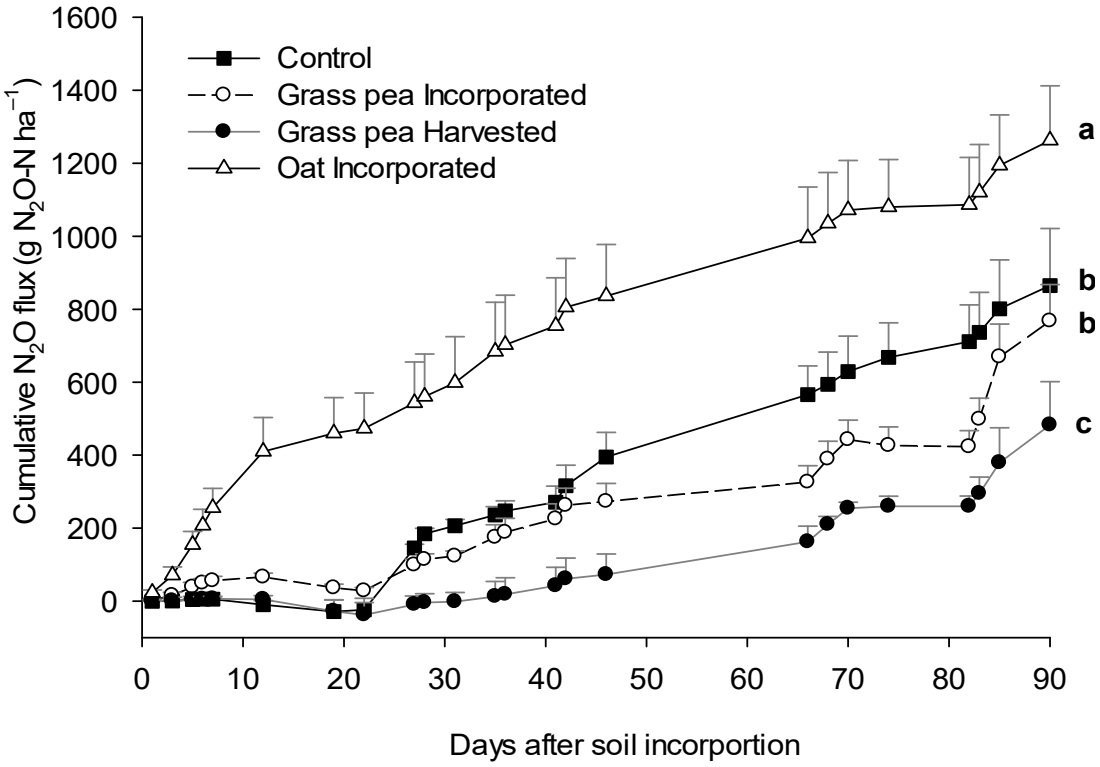


Figure 3. Correlation of cumulative CO_2 and N_2O emissions from individual collars across the treatments during the first 7 days of soil incorporation of cover crop biomass. Pearson's correlation coefficient (*R*) between the cumulative emissions is shown.

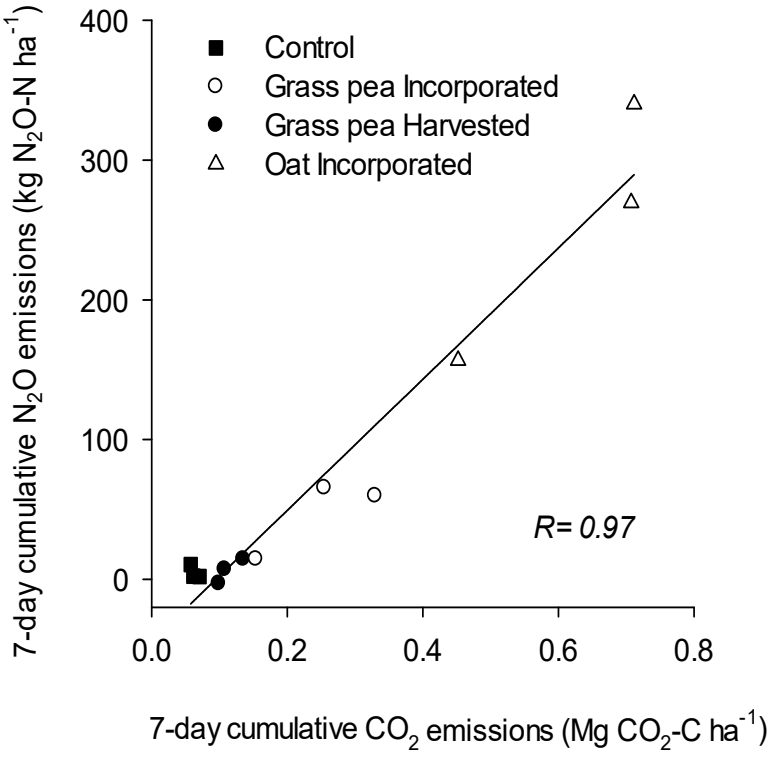


Figure 4. Dynamics of ratio vegetation index (RVI) measured as a proxy for green biomass of crabgrass. Error bars represent standard error (n = 3). Unidirectional error bars are shown for clarity.

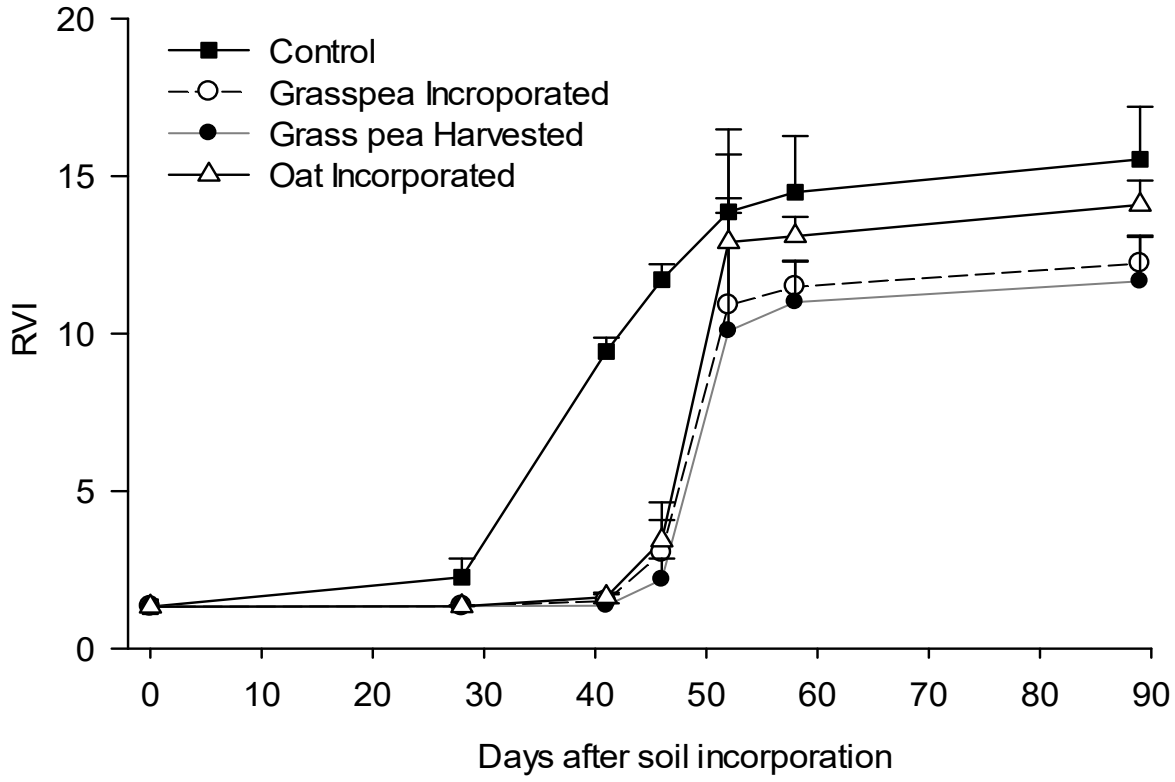
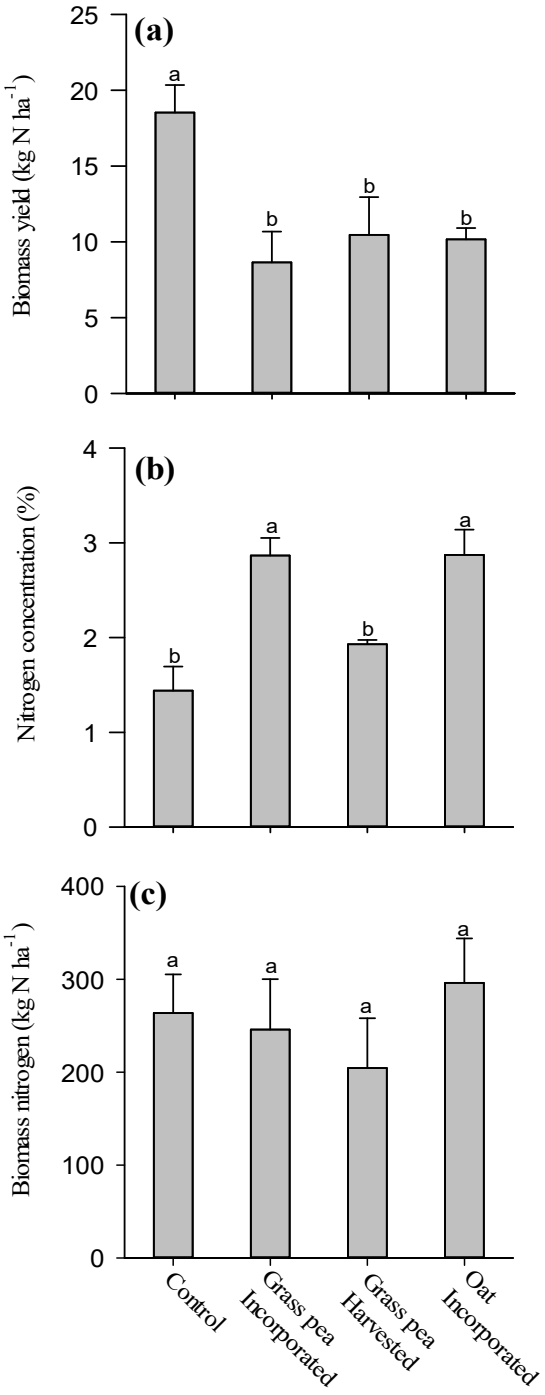


Figure 5. (a) Mean aboveground biomass produced by crabgrass after 90 days of growth. (b) Nitrogen concentrations of the harvested biomass of crabgrass. (c) Total amount of nitrogen in the harvested crabgrass biomass. Error bars represent standard error (n = 3). Different letters on the top of bars indicate statistical difference ($P < 0.05$).



CHAPTER IV

INFLUENCE OF CONTRASTING SOIL MOISTURE CONDITIONS ON CARBON DIOXIDE AND NITROUS OXIDE EMISSIONS FROM TERMINATED GREEN MANURES

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ABSTRACT

Carbon dioxide (CO_2) and N_2O emissions from decomposing legume green manures largely depend on soil moisture. A potential management to mitigate N_2O emissions could be to incorporate legumes during dry periods based on the short-term rainfall forecast. The present mesocosm study was designed to examine the impact of soil moisture due to different timing of rainfall after incorporation of legume cover crops on CO_2 and N_2O emissions. Two timings of rainfall were simulated as early and late rainfall that received 80 mm deionized water at or 1 week after incorporation of the legumes. An additional 20 mm water was added after 2 wk of the first simulated rainfalls. Gas fluxes of CO_2 and N_2O were measured using closed chamber method for 28 d incubation assay. Soil concentrations of NH_4^+ and NO_3^-, concentrations of N in undecomposed biomass, and abundances of denitrifier bacterial genes (nirK, nirS, and nosZ) and arbuscular mycorrhiza fungi (AMF) were determined at weekly intervals. Carbon dioxide emissions increased immediately after the first simulated rainfall events and peaked around Day 2 to 3, whereas N_2O emissions reached peak level around Day 8 to 10 from both legume treatments. After the first rainfall simulations, soil NH_4^+ and NO_3^- concentrations increased,

whereas biomass N concentrations decreased rapidly. Abundance of nirK, nosZ, and AMF was positively correlated ($P < 0.05$) to N_2O emissions. Dynamics and magnitude of emissions after first rainfall events remained similar irrespective of the timing of simulated rainfall. In conclusion, our results indicated that soil incorporation of legumes based on a short-term rainfall forecast may not be an effective tool to avoid large N_2O emissions.

INTRODUCTION

The interest in cultivating legumes as green manures is increasing in the US SGP (Bergtold et al., 2017; Foster et al., 2017; Kandel et al., 2018). The growing periods of summer crops such as corn, sorghum, and annual grasses for the region normally spans May to October, followed by long fallow periods. Croplands in the SGP are largely left bare or with minimal ground cover during these fallow periods (Unger, 1994). During the fallow periods, fall-planted legumes such as hairy vetch or various annual clovers (*Trifolium* spp.) or short growing-season spring legumes such as grass pea, singletary pea (*Lathyrus hirsutus* L.), or field pea (*Pisum sativum* L.) can be cultivated as a green source of N for the summer crops that follow (Rao and Northup, 2008). In comparison, fall-planted legumes are winter hardy, have long growing seasons, and can produce relatively higher amounts of biomass than spring-planted legumes, though with greater levels of maturity at termination (Moncada and Sheaffer, 2010).

Although legumes provide an organic source of N, they can also be significant sources of N_2O, particularly after termination and incorporation into the soil (Kandel et al., 2018). Rates of N_2O emissions from soil-incorporated legumes largely depend on the quantity and quality of incorporated biomass. Fall- and spring-planted legumes grown as green manures within the SGP normally have low C/N ratios compared with summer legumes (Kandel et al., 2018). A low C/N ratio is a biomass trait conducive to rapid mineralization of C and N after soil incorporation. In

addition to biomass factors, soil environmental factors at incorporation, such as temperature and moisture, control mineralization of both C and N and thereby emissions of CO_2 and N_2O (Blanco-Canqui et al., 2012; Sims, 1986; Whalen et al., 2001). Elevated concentrations of mineral N in soil after incorporation of legumes may lead to large emissions of N_2O during fallow periods.

Soil moisture greatly affects N_2O emissions because it is a key factor governing the activity of soil microbial communities and plays an important role in nutrient transformation and chemical cycling (Breezee et al., 2004). Nitrous oxide in soils is primarily produced as an intermediate product of denitrification, which is favored at high moisture levels (Schulthess and Gujer, 1996). Fall- and spring planted legume green manures in the SGP are generally terminated in May, which is among the wetter months of the year. A recent study in the US SGP reported extremely large emissions of N_2O during high rainfall events after soil incorporation of hairy vetch in May (Kandel et al., 2018). The emissions, however, remained close to zero during an active growth phase of a recipient summer crop. Therefore, reducing emissions of N_2O during the fallow period between termination of green manures and active growth of recipient crops is crucial for mitigating N_2O emissions.

Intense, but infrequent, precipitation events are common in the SGP during summer, as are prolonged periods of drought (Baath et al., 2018). One possible management option to reduce large emissions of N_2O would be to incorporate legume biomass during dry periods based on short-term rainfall forecasts. The effects of soil moisture on N_2O emissions have been extensively studied. However, there is limited information in the US SGP on the effects of available soil moisture on N_2O emissions after soil incorporation of fall- and spring-planted legumes. Therefore, we undertook a mesocosm study to examine the impact of simulated rainfall

at different times after soil incorporation on the emissions of CO_2 and N_2O from fall-planted hairy vetch and spring-planted grass pea, and the responses of soil microorganisms that drive denitrification. The hypotheses of the study were (i) emissions of CO_2 and N_2O would not differ among timing of simulated rainfall events during 28-d incubation periods after soil incorporation of the legumes and (ii) responses of soil microorganisms that drive denitrification would not differ among applied green N and rainfall treatments.

MATERIALS AND METHODS

Soil collection

Samples of Norge silt loam soils (fine, mixed, thermic, Udic Ustochrepts) for this mesocosm study were collected on 21 Feb. 2018 from a 1 m × 1 m area at the 0- to 20-cm depth at the USDA– ARS Grazinglands Research Laboratory (35°40′ N, 98°00′ W) near El Reno, OK. Norge silt loams contain high proportions of finer particles (42% silt; 22% clay) (USDA–NRCS, 1999). The soil in the field was repeatedly wetted for 2 wk prior to collection to minimize CO_2 and N_2O emissions from control treatment without legumes (described below) during the incubation period. The collected soil was then air-dried at 25°C in a greenhouse for 7 d to reach about 15% WFPS. The dried soil was homogenized by grinding prior to using it for the incubation experiment.

Plant materials used in the study

On 20 May 2017, the aboveground biomass of hairy vetch and grass pea was collected from a field near the soil sampling site. Hairy vetch was sown on 15 Sept. 2016, and grass pea was sown

on 9 Mar. 2017. Hairy vetch had completed flowering at biomass collection while grass pea was actively flowering.

The biomass was stored frozen at –20°C prior to use in the incubation experiment. A portion of this biomass was oven dried at 60°C to constant weight and analyzed for total C, N, and cell wall components (cellulose, hemi-cellulose, and lignin). Chemical composition of biomass was analyzed on three samples of each species. Concentrations of C and N were assessed by flash combustion (900°C for 10 min) method (VarioMacro, Elementar Americas, Inc.). Neutral detergent fiber, ADF, and ADL were determined by the van Soest and Wine (1967) method. Cellulose concentration was estimated as the difference between ADF and ADL, and hemicellulose concentration was estimated as the difference between neutral detergent fiber and ADF. The ADL was presented as lignin concentration.

Experimental setup

This mesocosm experiment was conducted inside a greenhouse as a factorial design with legume species and moisture levels as two treatments. Legume treatments consisted of three factors: a control without legumes, grass pea, and hairy vetch. Each legume treatment received two contrasting levels of soil moisture at incorporation. The moisture treatment included soil at 15% WFPS and 80 mm simulated rainfall at soil incorporation of legume. Each water treatment was replicated three times per legume treatment, resulting a total of 18 experimental units. Experimental units of the study were PVC cylinders (diameter, 10 cm; height, 25 cm) packed with soil for gas flux measurements. Additional cylinders were included for sampling soils at weekly intervals for mineral N analysis.

The incubation experiment was initiated on 26 Feb. 2018. Soil was packed (bulk density, 1.2 g cm^{-3}) to 25 cm depth in bottom capped cylinders (inner diameter, 10 cm; height, 30 cm) using a custom-made piston. The bottom 15 cm of all the cylinders was filled only with soil. For the untreated control, the upper 10 cm of the cylinder was also filled only with soil. For legume treatments, legume biomass was cut to 1-cm pieces and thoroughly mixed with the soil and packed in the upper 10 cm of the cylinders. Biomass was added at a rate equivalent to 8 Mg dry matter ha^{-1}. The simulated early rainfall treatment received 80 mm deionized water immediately after biomass incorporation. The simulated late rainfall treatment received 80 mm deionized water 7 d after biomass incorporation. An additional 20 mm of water was added to both treatments 14 d after the first simulated rainfalls. When the soil started to lose moisture after the first simulated rainfall, it formed a gap between the soil and inner wall of the pots. Therefore, liquid petroleum jelly was used to fill that gap prior the second simulated rainfall event. The temperature inside the greenhouse was kept at 22°C, and the tops of the cylinders were left open. The cylinders were kept inside a plastic box, and the gaps between the cylinders were filled with sand for heat insulation.

Gas flux measurements

Fluxes of CO_2 and N_2O were measured using a closed chamber (diameter, 10 cm; height, 15 cm) on 14 different dates during the 28-d incubation period. Fluxes were measured by placing the chamber on the top of the cylinders. The chamber was connected to a portable Fourier transform infrared–based analyzer (DX4040, Gasmet Technologies Oy). During flux measurement, headspace air in the chambers was circulated through 3-mm inlets and outlet tubing to the gas analyzer. The chamber was enclosed for 8 min during each measurement, and concentrations of CO_2 and N_2O were measured at 40-s intervals. Fluxes were calculated by linear regression using

the MATLAB (MathWorks, Inc.) routine developed by Kutzbach et al. (2007). The first few records after the chambers were enclosed were discarded as dead band based on visual inspection of the CO_2 flux curve. Cumulative emissions during the incubation period were estimated using a linear interpolation method.

Soil and biomass analysis

Chemical properties of soil on inception of incubations were determined on three replicate samples. Thereafter, soil samples were collected from one cylinder (not used for flux measurement) receiving each treatment at weekly intervals. After the final flux measurements (Day 28), soil samples were collected from all 18 cylinders used for flux measurements. To collect soil samples from the cylinders, the top 10 cm of soil was removed from the cylinders, and biomass and soils were separated by sieving and thoroughly mixed to obtain representative samples for analysis. Soil samples were subsequently split for microbial and biochemical assays. A fraction of soil samples was also dried at 60°C to constant weight for analyses of concentrations of NO_3^-, NH_4^+, pH, and EC. Additional soil samples were stored at –80°C for microbial analysis.

Aliquots of samples were extracted in 1.0 M KCl and analyzed by flow injection (FIAstar 5010 Analyzer, Foss North America, Inc.) to determine concentrations of NO_3^- and NH_4^+ N. The pH and EC of soils were assessed using a 1:2 soil:water solution with a benchtop pH/conductivity meter (Orion Star A215, Thermo Scientific). For each soil sampling, the undecomposed biomass was separated, cleaned thoroughly, and milled, and concentrations of N were assessed by flash combustion as described previously.

Measurements of environmental variables

Soil temperature was recorded at 1-h intervals from two additional cylinders (one for each rainfall treatment) that were not used for flux measurements. Soil sensors (TMC-6, Onset Computer Corp.) were placed at the center of the cylinder at a soil depth of 10 cm. Similarly, air temperatures during chamber enclosure were recorded on each date of flux measurements. Volumetric water content at the 0- to 10-cm depth was recorded hourly using EC-10 soil moisture sensors (Meter Environment) in two spare cylinders not used for gas flux measurement. Volumetric water content was presented as WFPS calculated as relative volumetric water content at saturation.

Estimation of denitrification gene copy numbers

The soil samples stored at –80°C were subsequently vacuum dried and stored in air-tight tubes for microbial analysis. Approximately 0.4 g of soil sample was used for microbial DNA extractions. Microbial community DNA was extracted using MoBio soil DNA extraction kits (Qiagen Inc.) according to the manufacturer's protocols. The concentration and quality of DNA were determined by spectrophotometry (SimpliNano, GE Healthcare LifeSciences, Inc.). The abundance of arbuscular mycorrhizae fungi (AMF) and the bacterial denitrification functional groups (nirK, nirS, and nosZ) were identified by targeting phylogenetic and functional marker genes.

Gene marker abundance was estimated using SsoAdvanced Universal SYBR Green Supermix (Bio-Rad Laboratories); genespecific primers and PCR conditions are provided in Table 2. Each sample was quantified in duplicate using the Rotor- Gene 6000 Real-Time PCR Detection System and Rotor-Gene Q Software 2.3 (Qiagen Inc.). Each quantitative polymerase chain reaction (qPCR) was organized to include appropriate standard curves, quality controls

(positive and negative controls, standard checks, spikes, no template controls), and evaluation of qPCR runs following MIQE guidelines (Bustin et al., 2009). After each qPCR run, the amplicon products were verified using both melting curve analysis and agarose gel electrophoresis of the products. The template gene copy numbers per qPCR reaction volume were calculated by comparing with standard curves plotted to known concentrations of individual gene marker templates in a synthetic DNA gBlocks Gene Fragments (Integrated DNA Technologies Inc.). Gene-copy numbers in the reaction volume were converted to per gram dry soil to quantify the abundance of particular genes.

Statistical analysis

Measurements of fluxes in each treatment are presented as the averages of three cylinders per treatment and standard errors. The differences in measured CO_2 and N_2O fluxes among the treatments were analyzed using analysis of variance. The sampling date effect was included in the model as repeated measurements. The difference in cumulative fluxes were analyzed using single factor ANOVA. Fisher's LSD method was used for pairwise comparisons of treatments at the 5% level. Spearman rank order correlations between N_2O emissions and data pertaining to gene abundance through all dates were estimated using the PAST 3.1 software (Hammer et al., 2001; Harter et al., 2014).

RESULTS

Properties of legume biomass

Moisture content of the biomass of grass pea was higher than that in the biomass of hairy vetch (Table 1). Carbon concentration of biomass was similar in both species. In contrast, N concentration in the biomass of grass pea (4.8%) was greater than in hairy vetch (3.2%).

Therefore, the lack of difference in C concentration and the difference in N concentration between the two species resulted in a significantly higher C/N ratio for hairy vetch (14.4) than for grass pea (9.8). Likewise, although the similar amount of dry matter was incorporated into cylinders for both species, N supplied by the biomass of grass pea (382 kg N ha^{-1}) was greater than N supplied by hairy vetch (256 kg N ha^{-1}). Concentrations of cellulose, hemicellulose, and lignin in the biomass of hairy vetch biomass were higher than in grass pea, indicating plants of hairy vetch were at higher levels of maturity.

Environmental conditions

Soil temperature during flux measurements ranged from 19 to 24°C (Fig. 1a). For early rainfall simulation, soil moisture was 88% WFPS on Day 1, decreased to 70% on Day 15, and then increased to 80% WFPS after application of the second simulated rainfall of 20 mm (Fig. 1b). For the late rainfall simulation, WFPS remained at 15% prior to the simulated rainfall of 80 mm on Day 7 and then reached 95%. Thereafter, WFPS decreased gradually until the second simulated rainfall of 20 mm on Day 21 of the incubation.

Biomass nitrogen concentrations

For early rainfall simulation, the N concentration of biomass decreased rapidly, reached ~1% for both species within the first week of incubation, and remained constant thereafter (Fig. 2). For late rainfall simulation, N concentrations in biomass decreased slightly prior to the first simulated rainfall. However, the N concentrations in biomass decreased rapidly over a week after the first simulated rainfall and then remained at ~1% thereafter.

Soil pH and EC

Initial soil pH was ~7.5 and remained mostly constant in the control treatment throughout the incubation assay (Fig. 3a). For legume-incorporated treatments, soil pH decreased slightly 1 week after the first simulated rainfall events.

Initial soil EC was ~400 μs cm^{-1} and decreased slightly over the first week after the first simulated rainfall in control treatments and remained mostly constant thereafter (Fig. 3b). In early rainfall simulation, EC of legume treatments increased over 1 week after the first simulated rainfall but decreased slightly in the last measurement. Overall, soil EC in grass pea treatment remained higher than hairy vetch treatment. For late rainfall simulation, soil EC decreased for hairy vetch treatment in the last measurement.

Soil mineral nitrogen concentrations

Concentrations of soil NH_4^+ at initial sampling were close to zero and did not increase in control treatments throughout the incubation assay (Fig. 4a). For early rainfall simulation, soil NH_4^+ concentrations increased within 1 week of the first simulated rainfall in both legume treatments. Overall, NH_4^+ concentrations remained higher with grass pea treatment than with hairy vetch treatment. For the late rainfall simulation, concentrations of soil NH_4^+ also increased in both legume treatments after the first simulated rainfall and peaked within 1 week. Soil NH_4^+ concentration declined thereafter and reached close zero at the last measurement.

Soil NO_3^- concentration at initial sampling was close to zero and remained mostly constant in the control treatment throughout the incubation assay (Fig. 4b). For early rainfall simulation, concentrations of NO_3^- in the grass pea treatment increased in the first week, whereas concentrations in hairy vetch did not increase until Week 2. Concentrations of soil NO_3^- peaked in Week 3 and remained unchanged through Week 4. For late simulated rainfall, concentrations

of soil NO_3^- increased after the first simulated rainfall for both legume treatments. After the second simulated rainfall, NO_3^- concentrations increased slightly in grass pea but decreased in the hairy vetch treatment.

Carbon dioxide emissions

Emissions of CO_2 from the control treatments remained low throughout the 28-d incubation period (Fig. 5a). For early rainfall simulation, CO_2 emissions reached peak levels between Days 2 and 4, respectively, in the grass pea and hairy vetch treatments. Thereafter, rates of emission declined significantly ($P < 0.05$) and did not increase considerably after the second simulated rainfall. For late rainfall simulation, CO_2 emissions were slightly higher in the grass pea treatment than in the control and hairy vetch treatments prior to the first simulated rainfall. The emission rates from legume treatments were significantly higher ($P < 0.05$) after the first simulated rainfall and followed similar trends as those noted in the early simulated rainfalls.

Nitrous oxide emissions

Emissions of N_2O from the untreated control remained close to zero throughout the 28-d incubation period (Fig. 5b). For the early rainfall simulation, N_2O emissions were detected from legume treatments beginning on Day 1 of an incubation and reached peak levels within 7 d. The emission rates from grass pea treatment were significantly higher ($P < 0.05$) than that from hairy vetch treatment. The second simulated rainfall slightly increased N_2O emissions from the grass pea treatment. For the late rainfall simulation, N_2O emissions were approximately zero from both legume treatments prior to the first simulated rainfall. The emission rates started to increase after rainfall events and followed similar trends as observed for the early simulated rainfall prior to the second simulated rainfall. However, significantly large rates of N_2O emissions ($P < 0.05$), some approaching 5.4 kg N_2O–N ha^{-1} d^{-1}, were observed from both legume treatments after the second

simulated rainfall. The large emissions were observed from only one of the three replicated cylinders of both legume treatments, which contributed to large within-treatment variations.

Cumulative carbon dioxide and nitrous oxide emissions

Cumulative emissions of CO_2 from cylinders receiving the control treatment were low in both the early and late simulated rainfalls. In contrast, the cumulative emissions for the 28-d incubation period for the early rainfall simulation from the grass pea treatment (3.3 Mg CO_2–C ha^{-1}) were significantly higher ($P < 0.05$) than emissions from the hairy vetch treatment (1.8 Mg CO_2–C ha^{-1}). For the late rainfall simulation, there was no significant difference between CO_2 emissions for the grass pea (2.9 Mg CO_2–C ha^{-1}) and hairy vetch (2.2 Mg CO_2–C ha^{-1}) treatments (Fig. 6a).

Because no N_2O emissions were observed from the control treatments throughout the incubation assay, cumulative emissions remained close zero under both rainfall simulations. In contrast, the 28-d cumulative emissions of N_2O were higher from the late rainfall simulation for both the grass pea (16.9 kg N_2O–N ha^{-1}) and hairy vetch (20.6 kg N_2O–N ha^{-1}) treatments but were not significantly different than early rainfall simulation treatment (Fig. 6b). Emissions from early rainfall simulation treatment were largely contributed by peak emissions observed after the second rainfall event (20 mm), which was scheduled 15 days after first rainfall event.

Abundance of denitrifier genes

The dynamics of abundance of nirK in the grass pea and control treatments were similar for the early rainfall simulation, although the magnitude of response was higher in the grass pea treatment. Abundance of nirK in hairy vetch treatment was slightly lower than the control on Day 7 but was higher than other treatments on Days 14 and 21 (Fig. 7a). The abundance of nirK in the legume treatments in the late rainfall simulation was higher than the control treatment,

except on Day 7. The magnitude of nirK abundance was similar in both legume treatments except on Day 21, when it was higher in hairy vetch.

The abundance of nirS mostly showed trends in abundance that were similar to nirK responses. For both early and late rainfall simulations, the abundance of nirS in the legume treatments was higher than in the control treatments except on Day 7. Among legume treatments, nirS abundance in the hairy vetch treatment was higher than grass pea treatment on Days 14 and 21 in the early rainfall simulation (Fig. 7b). In comparison, abundances among legume treatments in the late rainfall simulation were similar except for Day 28.

For early rainfall simulation, the abundance of nosZ remained higher in the grass pea treatment than in the control throughout the incubation period. The abundance of nosZ was lowest in the hairy vetch treatment on Day 7 of incubation but was higher than grass pea thereafter before declining on Day 28 (Fig. 7c). In comparison, nosZ abundance in the late rainfall simulation was higher in the control than in legume treatments on Day 7 but decreased thereafter, whereas responses to legume treatments were stable.

For early rainfall simulation, AMF abundance in the grass pea treatment was highest on Day 7 and then decreased through Day 21 before increasing through Day 28. Arbuscular mycorrhiza fungi abundance in the hairy vetch treatment was lower than responses recorded for the control treatment on Day 7 but was higher than after grass pea treatment on Days 14 and 21 (Fig. 7d). Abundance of AMF on Day 7 of the late-rainfall simulation in the legume treatments were lower than amounts under the control treatment. After Day 7, AMF abundance increased in both legume treatments. Among legumes, the response of AMF abundance was higher for hairy vetch than grass pea, except on Day 28.

There were moderate correlations between N_2O emission and nirK ($R_2 = 0.60$; $P < 0.05$) and nosZ ($R_2 = 0.57$; $P < 0.05$) abundances, whereas the correlation between nirS abundance and N_2O emissions was not significant. However, correlation between N_2O emissions and abundance of AMF ($R_2 = 0.81$; $P < 0.05$) was stronger as compared with abundance of bacterial denitrifier genes.

DISCUSSION

Rapid mineralization of C and N from both legume species after soil incorporation and simulated rainfall are in accord with findings of previous studies that reported increased CO_2 and N_2O emissions within a few days of soil incorporation of legumes with low C/N ratios (Kandel et al., 2018; Shaaban et al., 2016). As seen in the current study, soil moisture was a key controlling factor for biomass decomposition and N_2O emissions. Higher CO_2 emissions from grass pea treatments were possibly related to more rapid decomposition of biomass that was (relatively) less mature than biomass of hairy vetch. Such less mature biomass had higher concentrations of N and lower C/N ratios (Table 1). As seen in the current study and in previous studies (Nicolardot et al., 1994; Trinsoutrot et al., 2000), decomposition of biomass is strongly influenced by biomass C/N ratios and lignin concentrations of legume green manures.

The increased concentrations of mineral N in soils in the legume treatments after the simulated rainfalls corresponded to the decreased concentrations of N in legume biomass. However, the changes in biomass N and soil mineral N were observed only after simulated rainfalls, which indicated the crucial role of soil moisture for mineralization of N from biomass residues (Quemada and Cabrera, 1997; Wang et al., 2006). The rapidly decreased N concentrations in legume biomass and concurrent higher increases in mineral N in soil in response to grass pea compared with hairy vetch were due to lower C/N and lignin/N ratios of

grass pea (Nicolardot et al., 2001; Trinsoutrot et al., 2000). Such low ratios in legume green manures are key to rapid turnover of N from pools in plant materials to soil pools.

The higher rates of N_2O emissions from legume treatments after application of the first simulated rainfall were expected because the amount of soil moisture was favorable for N mineralization and N_2O emissions (Kandel et al., 2018). Higher N_2O emissions from the grass pea compared with the hairy vetch treatments can be explained by the higher rates of N mineralization that were supported by low C/N ratios and the higher amounts of N input by grass pea residues (Table 1). The higher rate of N mineralization of grass pea was evidenced by higher concentrations of mineral N in the grass pea treatment after simulated rainfall as compared with hairy vetch (Fig. 3). In addition to higher concentrations of N and the lower C/N ratio of grass pea biomass, lower concentrations of lignin may have contributed to the greater mineralization rate recorded for grass pea. According to Haynes (2005), lignin from decomposing biomass produces polyphenols, which form recalcitrant N-containing humic polymers that inhibit N mineralization rates.

The higher cumulative N_2O emissions in response to late simulated rainfalls were mostly affected by large emission rates from both legume treatments after the second rainfall event. This indicates that if the soil concentration of mineral N is increased after decomposition of legume and if the mineralized N is not utilized by a recipient crop, large rates of N_2O emissions are possible during rainfall events. Such large peaks of N_2O emissions were also reported after rainfall events in a recent field study in the US SGP after incorporation of hairy vetch in early May (Kandel et al., 2018). In the current study, we measured N_2O emissions on alternate days to capture possible ephemeral peaks as observed after the second simulated rainfall event of the late simulated rainfall. However, the exact duration of peak emissions within the 48-hour periods was

uncertain, and therefore cumulative emissions of N_2O may have been over- or underestimated. The possibility of missing ephemeral peaks after the second rainfall event in early rainfall simulations also exists. Nevertheless, N_2O emissions from the legume treatments were consistently higher than in response to the control. Such peak emissions showed that these legumes can be a significant source of N_2O immediately after soil incorporation in the absence of plants to compete for increased soil mineral N.

This lower soil pH in legume treatments after simulated rainfall might be due to accumulation of organic acids from decomposing biomass (Kiiya et al., 2010; Šimek and Cooper, 2002). Previous studies have shown strong effects of soil acidity in N_2O production because reduction of N_2O to N_2 is inhibited in acidic soils (Šimek and Cooper, 2002). In this study, soil pH mostly remained >7.0, despite reduction in the legume treatments. Therefore, the strong influence of the small change in soil pH on rates of N_2O emission was not expected. Increased EC in legume treatments after simulated rainfall might be due to increased concentrations of nutrients released from decomposing biomass (Kabirinejad et al., 2014). Although N_2O emissions and EC increased from legume treatments after simulated rainfall, their dynamics did not follow similar trends. This might be related to the stronger response of soil moisture than soil EC on N_2O emissions (Kandel et al., 2019).

The strong correlations between abundance of AMF and N_2O emissions were also reported previously, indicating different fungal taxa are also responsible for N_2O production, in addition to bacterial nitrifiers and denitrifiers (Jirout et al., 2013; Shoun and Takaya, 2002). A study analyzing fungal diversity affected by soil characteristics reported that most of the fungal species were positively correlated with the fine texture of soil (Tančić Živanov et al., 2017). Because the soil used for the current experiment was a finer-textured soil, we can surmise the

presence of a rich fungal population in soil, which can act as an additional source of N_2O emissions. Therefore, it can be suggested that N_2O production from soil fungi should not be neglected.

CONCLUSION

In this incubation study, we studied the impacts of moisture at soil incorporation of two legumes (fall-planted hairy vetch and spring-planted grass pea) on the CO_2 and N_2O emissions that mimicked conditions during the early period of soil incorporation. The results indicated that both legumes with low C/N ratio and low lignin concentrations decompose rapidly and generate higher concentrations of mineral N in soil if soil moisture is not limiting. Emissions of CO_2 and N_2O from both legumes within 28 d of the incubation study were not significantly different even though grass pea provided a greater amount of N (382 kg ha^{-1}) as compared with N provided by hairy vetch (256 kg ha^{-1}). Emissions of N_2O were increased after simulated rainfalls at the time of legume incorporation and 1 week after incorporation. The results indicated that avoiding rainfall events at incorporation of legume biomass may not be a useful tool for avoiding large emissions of N_2O after incorporation of green manures. Therefore, future research is required to evaluate other management techniques to lower the loss of N as N_2O, like maturity level of the crops at time of incorporation or different types of the cover crops (legumes or non-legumes etc.).

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Tables and Figures

Table 1. Chemical composition of grass pea and hairy vetch biomass. Measurements are presented as average of three replications of each species. DM: dry matter.

Parameter	Hairy vetch	Grass pea
Moisture (%)	76.3	87.7
C concentration (% of DM)	46.1	46.6
N concentration (% of DM)	3.2	4.8
C/N	14.4	9.8
Total N in biomass (kg ha^{-1})	256	382
Cellulose (% of DM)	25.1	20.2
Hemicellulose (% of DM)	14.8	8.3
Lignin (% of DM)	7.0	4.1

Table 2. Primers used and qPCR parameters for evaluation of microbial community abundance.

Gene	Primer	Sequence	Amplicon size [bp]	PCR conditions	Strain*	Reference	Efficiency (%)	R^2
16S rRNA	341f 797r	CCTACGGGAGGCAGCAG GGACTACCAGGGTATCTAATCC TGTT	466	98°C 10s, 61.5°C 45s, 40 cycles	*Pseudomonas denitrificans*	Muyzer et al. (1993) Harter et al. (2014)	1.07	0.99
nirK	nirK876C nirK1040	ATYGGCGGVCAYGGCGA[a] GCCTCGATCAGRTTRTGG	164	98°C 15s, 63-58°C 30s, 72°C 30s, 6cycles 98°C 15s, 58°C 30s, 72°C 30s, 40 cycles	*Pseudomonas denitrificans* (ATCC 13867)	Harter et al.(2014)	92.3	0.99
nirS	cd3af R3cd	GTNAAYGTNAARGARACNGG GASTTCGGRTGSGTCTTGA	413	98°C 60s, 57°C 60s, 72°C 60s, 40 cycles	*Ralstonia eutropha* H16	Michotey et al. (2000) Throback et al. (2004)	91.2	0.99
nosZ	nosZ2F nosZ2R	CGCRACGGCAASAAGGTSMSSG T CAKRTGCAKSGCRTGGCAGAA	267	98°C 30s, 65-60°C 30s, 72°C 30s, 6cycles 98°C 15s, 60°C 15s, 72°C 30s, 40 cycles	*Sinorhizobium melliloti 1021A*	Henry et al. (2006)	86.9	0.98
ITS		ITS1f-(5'-TCC GTA GGT GAA CCT GCG G-3')/5.8s-(5'-CGC TGC GTT CTT CAT CG-3')		10 min at 98°C for initial denaturation; 35 cycles of 60 s at 98°C, 30 s at 53°C, extension for 45 s at 72°C, and acquisition for 10 s at 82°C. Melt curve produced at 48-98°C (1° and 5 s/cycle melt).	Rhizopus microsporus	(Fierer et al., 2005)		
AMF		GC-AMV4.5NF- (5'-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG G [GC clamp] AAG CTC GTA GTT GAA TTT CG-3′)/ AMDGR-(5′-CCC AAC TAT CCC TAT TAA TCA T-3′)		10 min at 98°C for initial denaturation; 35 cycles of 30 s at 98°C, 30 s at 55°C, extension for 45 s at 72°C, and acquisition for 10 s at 82°C. Melt curve produced at 50-98°C (1° and 5 s/cycle melt).	Glomus intraradices	(Sato et al., 2005)		

Figure 1. (a) Average soil temperatures at 0–10 cm depth during flux measurement and **(b)** dynamics of water filled pore space (WFPS) measured at 0–10 cm soil depth during flux measurement. Left panels are measurements from early rainfall simulation and right panels are measurements from late rainfall simulation treatments. Long arrows indicate the times of the first simulated rainfall (80-mm) and short arrows indicate the times of the second simulated rainfall (20-mm).

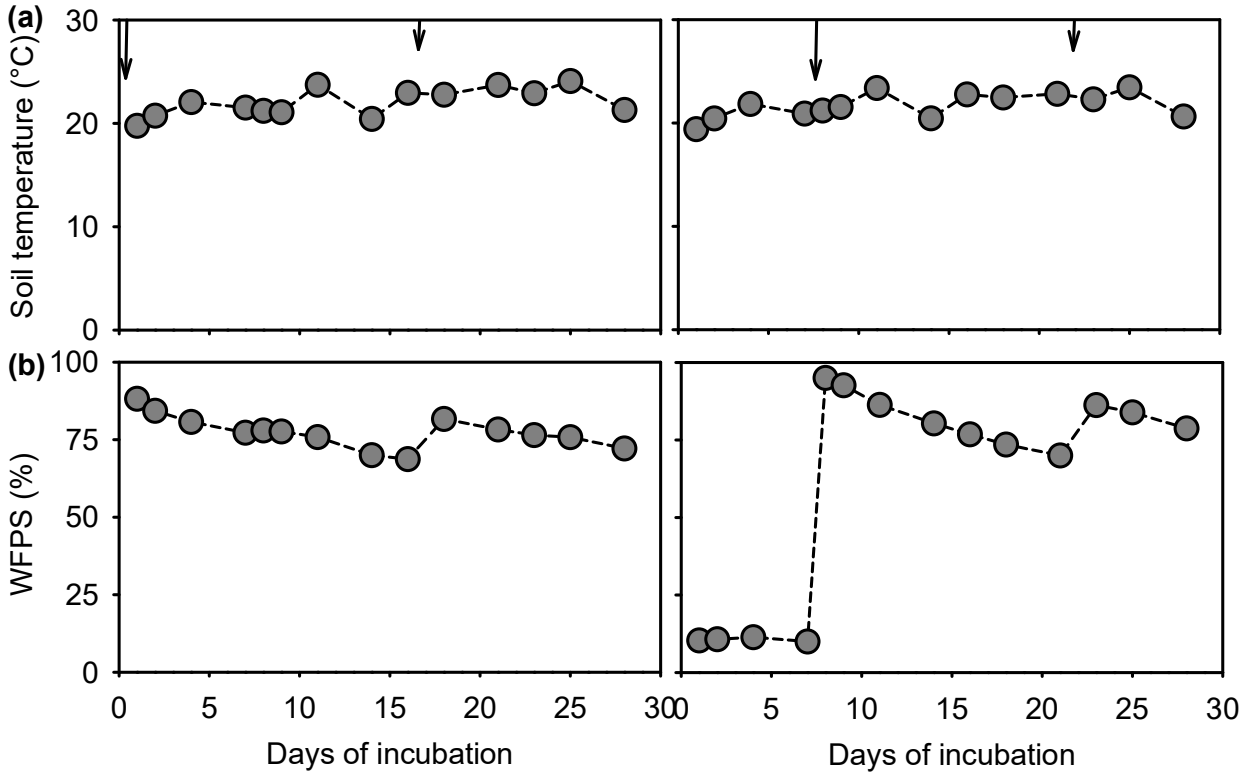


Figure 2. Dynamics of biomass N concentrations during the batch assay. Left panels are measurements from early rainfall simulation and right panels are measurements from late rainfall simulation treatments. Long arrows indicate the times of the first simulated rainfall (80-mm) and arrows short arrows indicate the times of the second simulated rainfall (20-mm).

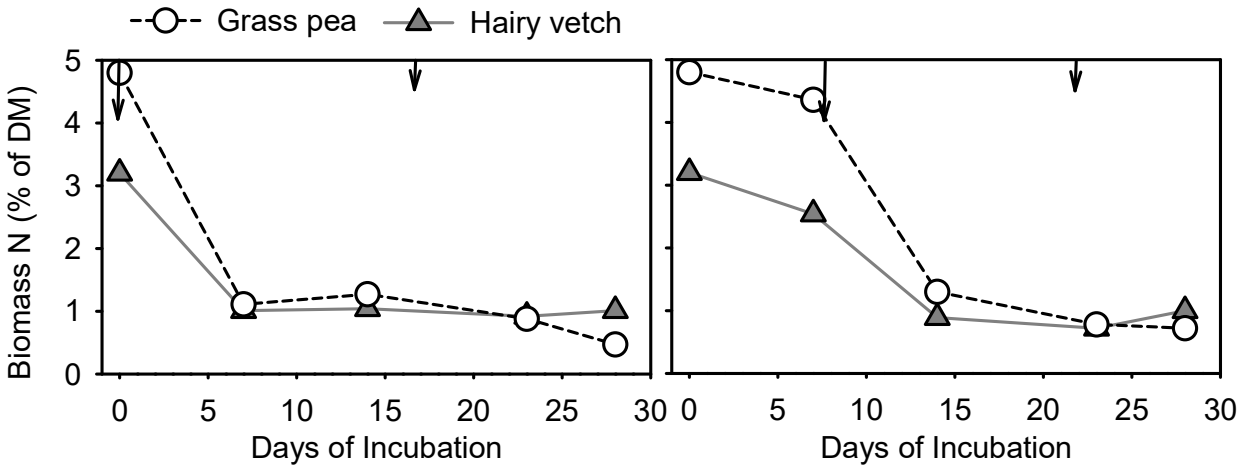


Figure 3. Dynamics of soil (**a**) ammonium (NH_4^+) and (**b**) nitrate (NO_3^-) concentrations in the 0–10 cm soil depth. The first and last measurements are shown as mean and standard error of individual cylinders (n = 3). Other points represent measurements from a cylinder from each treatment combinations. Left panels are measurements from early rainfall simulation and right panels are measurements from late rainfall simulation treatments. Long arrows indicate the times of the first simulated rainfall (80-mm) and arrows short arrows indicate the times of the second simulated rainfall (20-mm).

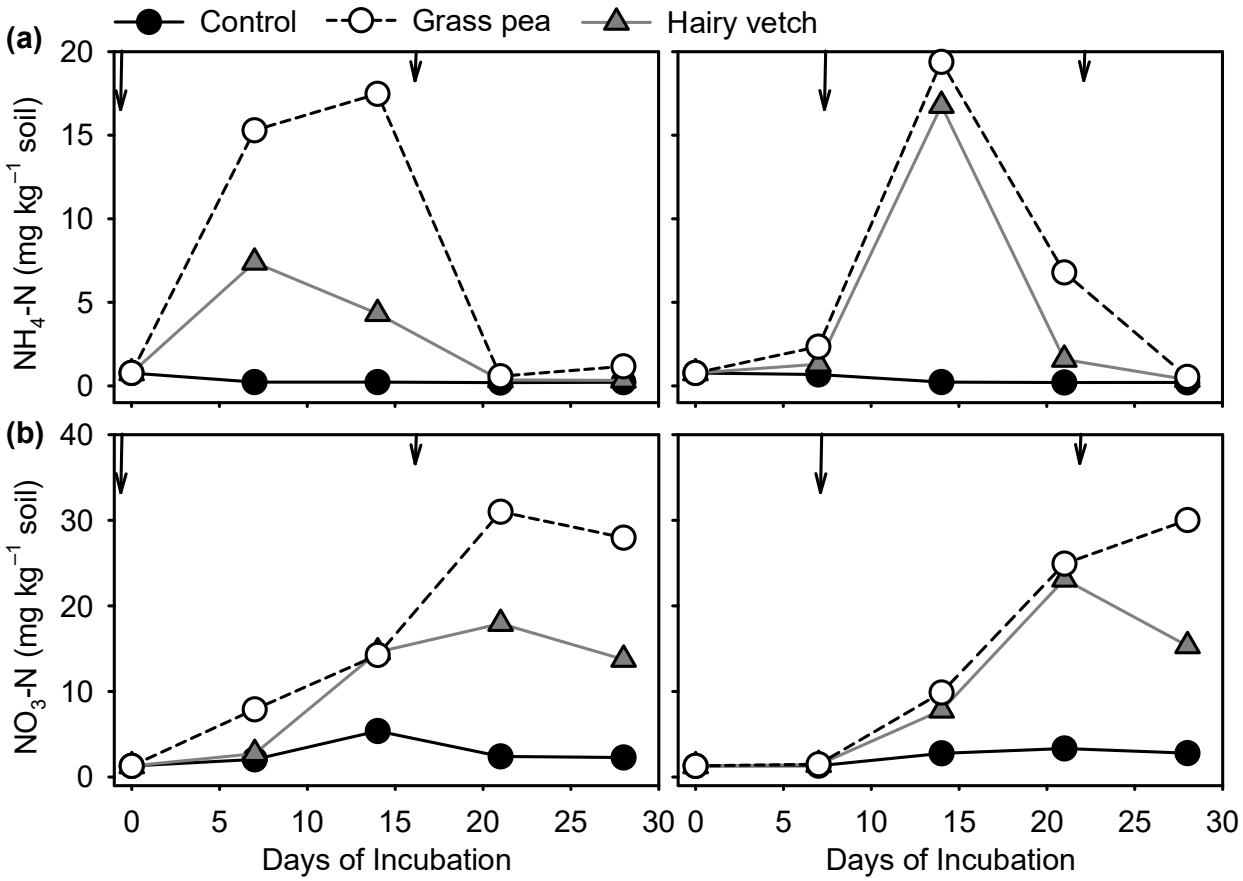


Figure 4. Dynamics of soil (**a**) soil pH and (**b**) electrical conductivity (EC) in the 0–10 cm soil depth. The first and last measurements are shown as mean and standard error of individual cylinders (n = 3). Other points represent measurements from a cylinder from each treatment combinations. Left panels are measurements from early rainfall simulation and right panels are measurements from late rainfall simulation treatments. Long arrows indicate the times of the first simulated rainfall (80-mm) and arrows short arrows indicate the times of the second simulated rainfall (20-mm).

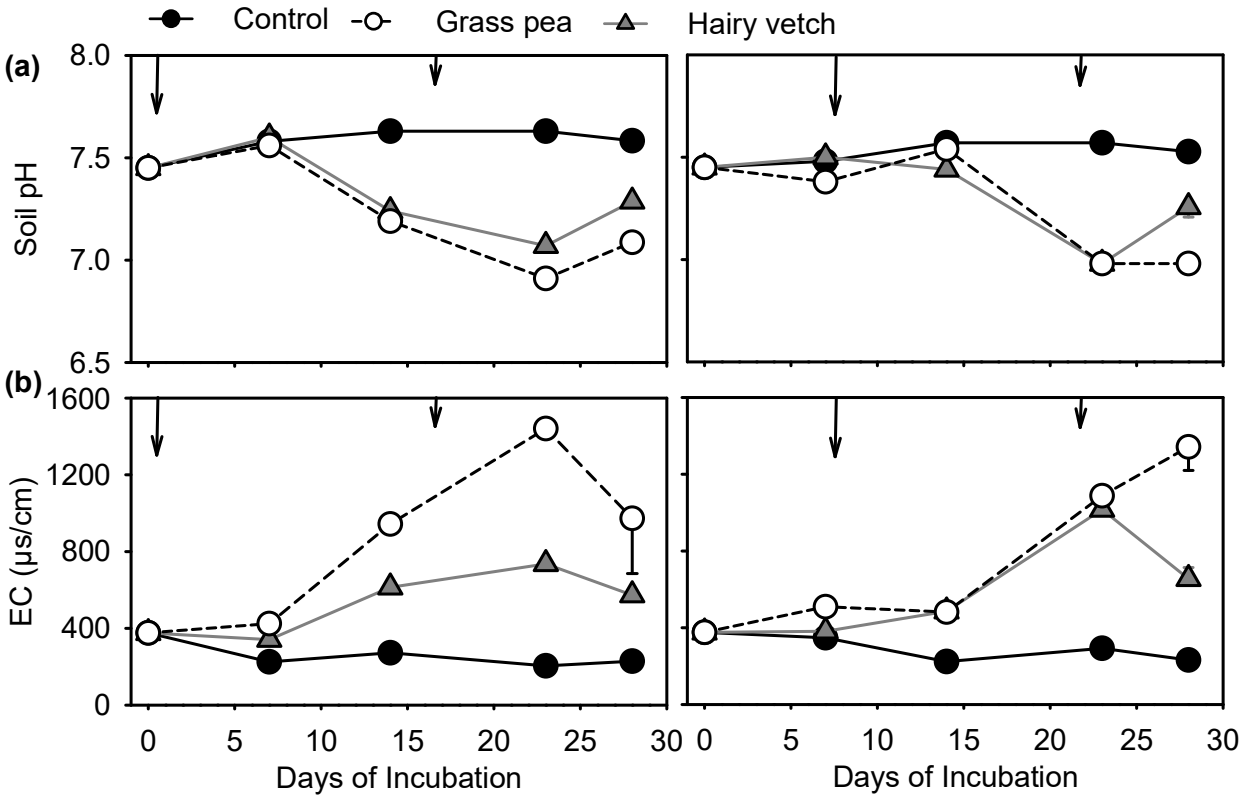


Figure 5. Time series of fluxes of (**a**) CO_2, and (b) N_2O. Data are shown as mean and standard error of individual cylinders (n = 3). Unidirectional error bars are shown for clarity. Left panels are measurements from early rainfall simulation and right panels are measurements from late rainfall simulation treatments. Long arrows indicate the times of the first simulated rainfall (80-mm) and arrows short arrows indicate the times of the second simulated rainfall (20-mm).

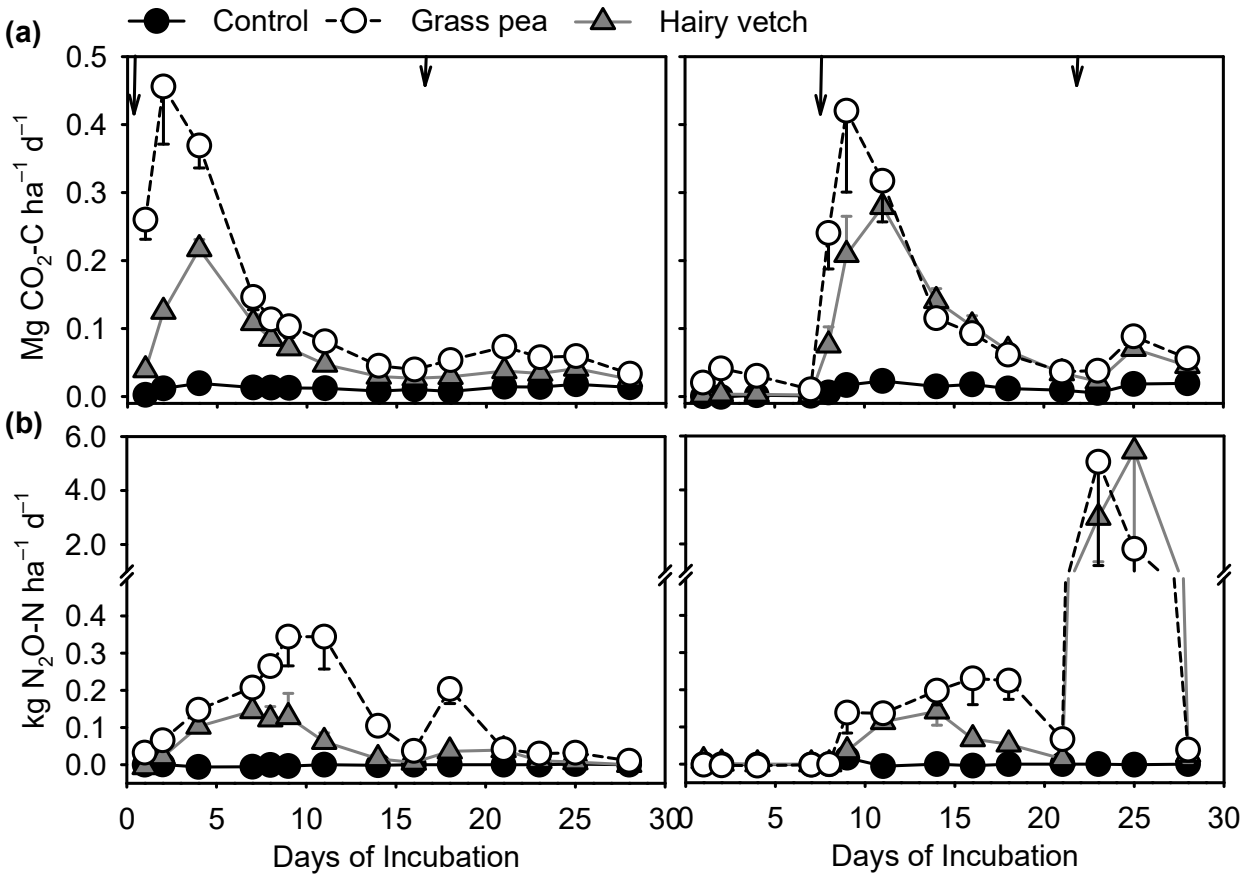


Figure 6. Cumulative estimates of (a) CO_2 and (b) N_2O emissions during 28-day incubation period. Data are shown as mean and standard error of individual cylinders (n = 3). The statistical differences ($P < 0.05$) of total cumulative emissions among the treatments are indicated by different letters on the top of bars.

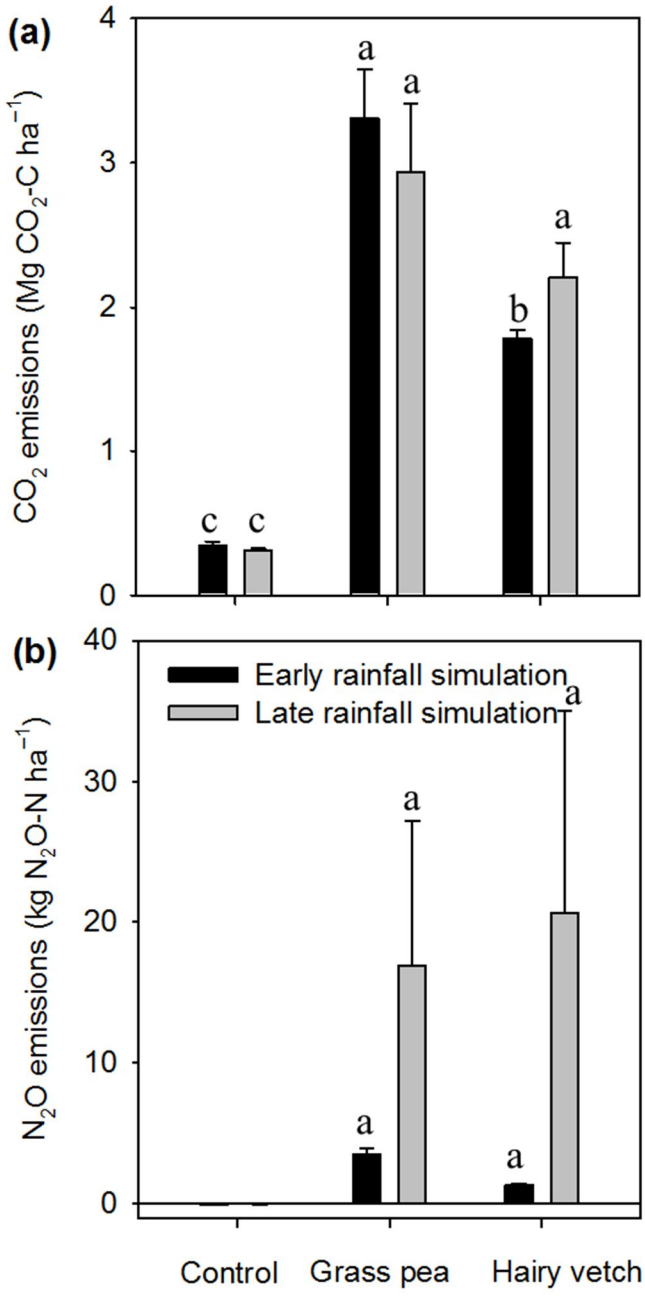
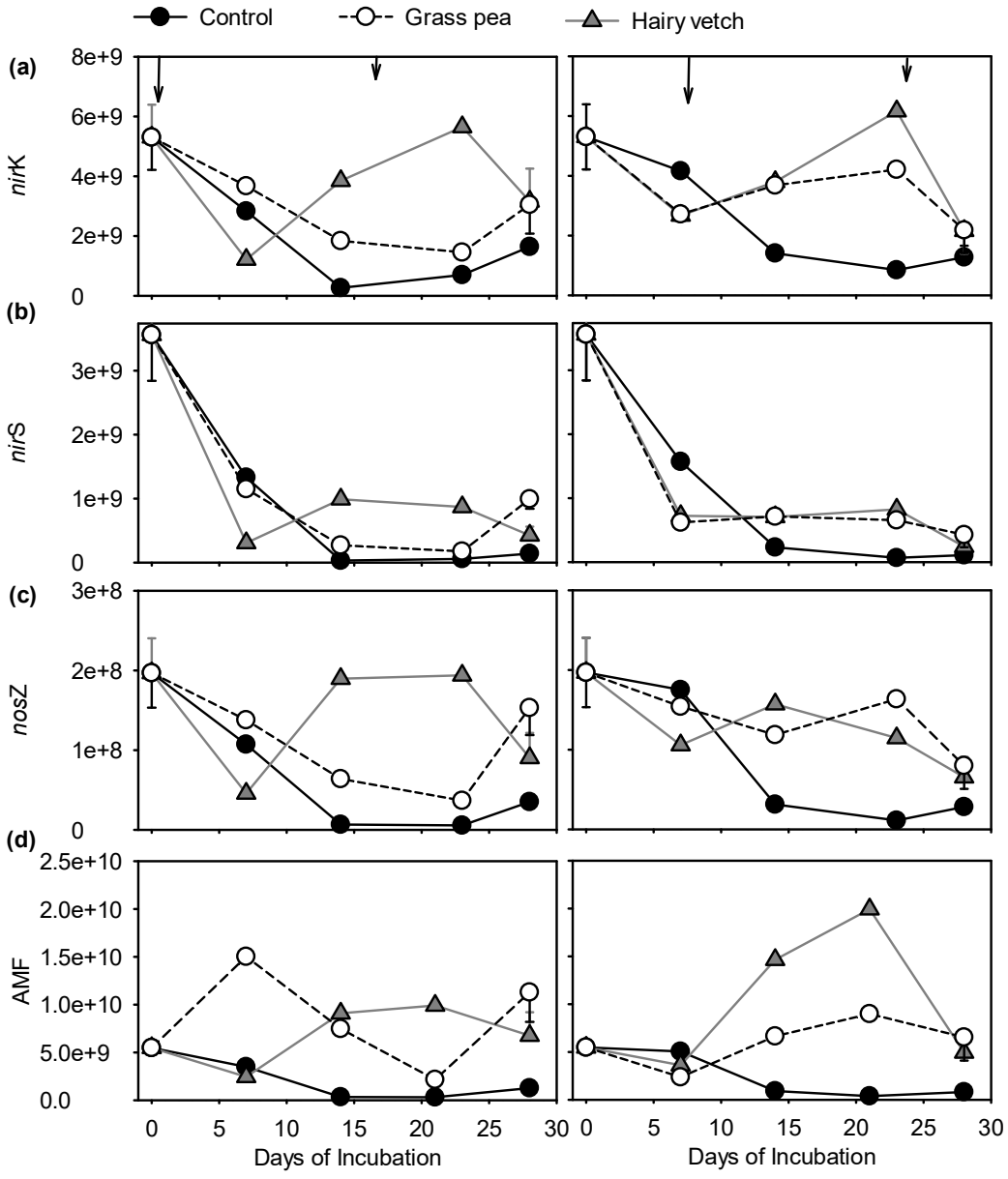


Figure 7. Dynamics in abundance of denitrifier genes (**a**) nirK, (**b**) nirS, (**c**) nosZ, and (**d**) AMF (Arbuscular Mycorrhizal Fungi) in the 0–10 cm soil depth. The first and last measurements are means and standard errors of n=3 cylinders. Other points represent measurements from individual cylinders from each treatment combination. Left panels are measurements from early rainfall simulation and right panels are measurements from late rainfall simulation treatments. Long arrows indicate times of first simulated rainfall (80-mm) and arrows short arrows indicate times of second simulated rainfall (20-mm).



CHAPTER V

OVERALL CONCLUSION

It can be concluded that incorporating cover crops at specific maturity levels could help in mitigating N_2O emissions, as significantly greater (30–35%) cumulative N_2O emissions were recorded post-incorporation from both leguminous and non-leguminous cover crops that were terminated at the reproductive stage than the vegetative stage. Additionally, properties of the biomass of cover crops underwent complex interactions with environmental variables (e.g., timing of rainfall events), and influenced cumulative emissions of N_2O. The incorporation of non-leguminous (oats) cover crops resulted in significantly greater post-incorporation N_2O emissions compared to emissions from an incorporated legume (grass pea) cover crop. Management of the residues of cover crops can help mitigate N_2O emissions. The 90-day cumulative emissions from grass pea plots where aboveground biomass was harvested as forage were two times lower than from grass pea plots where biomass was incorporated, showing incorporated aboveground biomass was a major source of N_2O emissions. Soil incorporation of legumes biomass, based on a short-term rainfall forecast may not be an effective tool to avoid large N_2O emissions as emissions were not significantly different between early and late simulated rainfall treatments. Replacing spring fallow with cover crops also had negative impact on growth and development of the future crop, as growth and yield of crabgrass and finger millet were lower compared to the control treatments. Future research should consider economic analysis on harvested biomass of cover crops as a tradeoff for yield loss of future crops.

Future research should also examine interactions between the properties of biomass generated by cover crops and different environment variables affecting growth, such as timing and frequency of rainfall events, soil temperature, and abundance of denitrifying communities in relation to N_2O emissions

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