Influence of Contrasting Soil Moisture Conditions on Carbon Dioxide and Nitrous Oxide Emissions from Terminated Green Manures

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ABSTRACT

Carbon dioxide (CO₂) and nitrous oxide (N₂O) emissions from decomposing legume green manures largely depend on soil moisture. A potential management to mitigate N₂O 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 CO₂ and N₂O emissions. Two timings of rainfall were simulated as early and late rainfall that received 80 mm deionized water at or 1 wk after incorporation of the legumes. Additional 20 mm water was added after 2 wk of the first simulated rainfall. Gas fluxes of CO₂ and N₂O were measured using closed chamber method for 28 d incubation assay. Soil concentrations of NH₄⁺ and NO₃⁻, concentrations of N in undecomposed biomass, and abundances of denitrifier bacterial genes (nirK, nirS, and nosZ) and arbuscular mycorrhiza fungi (AMF) were determined as weekly intervals. Carbon dioxide emissions increased immediately after the first simulated rainfall events and peaked around Day 2 to 3, whereas N₂O emissions reached peak level around Day 8 to 10 from both legume treatments. After the first rainfall simulations, soil NH₄⁺ and NO₃⁻ concentrations increased, whereas biomass N concentrations decreased rapidly. Abundance of nirK, nosZ, and AMF was positively correlated (P < 0.05) to N₂O 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 N₂O emissions.

Abbreviations: ADF, acid detergent fiber; ADL, acid detergent lignin; AMF, arbuscular mycorrhiza fungi; qPCR, quantitative polymerase chain reaction; SGP, Southern Great Plains; WFPS, water-filled pore space.

The interest in cultivating legumes as green manures is increasing in the US Southern Great Plains (SGP) (Bergtold et al., 2017; Foster et al., 2017; Kandel et al., 2018). The growing periods of summer crops such as corn (Zea mays L.), sorghum (Sorghum bicolor (L.) Moench.), 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 (Vicia villosa Roth) or various annual clovers (Trifolium spp.) or short-growing-season spring legumes such as grass pea (Lathyrus sativus L.), singletary pea (Lathyrus hirsutus L.), or field pea (Pisum sativum L.) can be cultivated as a green source of nitrogen (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 N₂O, particularly after termination and incorporation into the soil (Kandel et al., 2018). Rates of N₂O 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 CO₂ and N₂O (Blanco-Canqui et al., 2012; Sims, 1986; Whalen...
Elevated concentrations of mineral N in soil after incorporation of legumes may lead to large emissions of N₂O during fallow periods.

Soil moisture greatly affects N₂O 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 N₂O 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 N₂O during the fallow period between termination of green manures and active growth of recipient crops is crucial for mitigating N₂O 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 N₂O would be to incorporate legume biomass during dry periods based on short-term rainfall forecasts. The effects of soil moisture on N₂O emissions have been extensively studied. However, there is limited information in the US SGP on the effects of available soil moisture on N₂O 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 post-soil incorporation on the emissions of CO₂ and N₂O from fall- and spring-planted legumes. Therefore, we undertook a mesocosm study to examine the impact of simulated rainfall at different times post-soil incorporation on the emissions of CO₂ and N₂O from fall- and spring-planted legumes. The hypotheses of the study were (i) emissions of CO₂ and N₂O 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 CO₂ and N₂O 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% water-filled pore space (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, acid detergent fiber (ADF), and acid detergent lignin (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 filled only with soil. For legume treatments, legume biomass was cut to 1-cm pieces and thoroughly mixed with the soil 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 rainfall. 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 to 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 CO₂ and N₂O 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 N₂O and CO₂ were measured at 40-s intervals. Fluxes were calculated by linear regression using the
Table S1. Each sample was quantified in duplicate using the RotorGene 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.

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 nitrate (NO3–), ammonium (NH4+), pH, and electrical conductivity (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 (FIStar 5010 Analyzer, Foss North America, Inc.) to determine concentrations of nitrate (NO3–) and ammonium (NH4+) 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 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 SoAdvanced Universal SYBR Green Supermix (Bio-Rad Laboratories); genus-specific primers and PCR conditions are provided in Supplemental Table S1. Each sample was quantified in duplicate using the Rotor-Gene 6000 Real-Time PCR Detection System and Rotor-Gene Q Software. Each qPCR run was performed with 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 CO2 and N2O 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 N2O 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.

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Hairy vetch</th>
<th>Grass pea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture, %</td>
<td>76.3</td>
<td>87.7</td>
</tr>
<tr>
<td>C concentration, % of DM†</td>
<td>46.1</td>
<td>46.6</td>
</tr>
<tr>
<td>N concentration, % of DM</td>
<td>3.2</td>
<td>4.8</td>
</tr>
<tr>
<td>C/N ratio</td>
<td>14.4</td>
<td>9.8</td>
</tr>
<tr>
<td>Total N in biomass, kg ha–1</td>
<td>256</td>
<td>382</td>
</tr>
<tr>
<td>Cellulose, % of DM</td>
<td>25.1</td>
<td>20.2</td>
</tr>
<tr>
<td>Hemicellulose, % of DM</td>
<td>14.8</td>
<td>8.3</td>
</tr>
<tr>
<td>Lignin, % of DM</td>
<td>7.0</td>
<td>4.1</td>
</tr>
</tbody>
</table>

† Dry matter.
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 Electrical Conductivity

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 wk 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 wk 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 NH₄⁺ 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 NH₄⁺ concentrations increased within 1 wk of the first simulated rainfall in both legume treatments. Overall, NH₄⁺ concentrations remained higher with grass pea treatment than with hairy vetch.
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 wk. 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$_2$O from the untreated control remained close to zero throughout the 28-d incubation period (Fig. 5b). For the early rainfall simulation, N$_2$O 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 from hairy vetch treatment. The second simulated rainfall slightly increased N$_2$O emissions from the grass pea treatment. For the late rainfall simulation, N$_2$O 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$_2$O emissions ($P < 0.05$), some approaching 5.4 kg N$_2$O–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$_2$O 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$_2$O were higher from the late rainfall simulation for both the grass pea (16.9 kg N$_2$O–N ha$^{-1}$) and hairy vetch treatments. Statistical differences ($P < 0.05$) of total cumulative emissions among treatments are indicated by different letters.
vetch (20.6 kg N\textsubscript{2}O–N ha\textsuperscript{-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). For late rainfall simulation, AMF abundance on Day 7 in the legume treatments was lower than 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 compared with grass pea, except on Day 28.

There were moderate correlations between N\textsubscript{2}O 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\textsubscript{2}O emissions was not significant. However, correlation between N\textsubscript{2}O 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\textsubscript{2} and N\textsubscript{2}O 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\textsubscript{2}O emissions. Higher CO\textsubscript{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\textsubscript{2}O 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\textsubscript{2}O emissions (Kandel et al., 2018). Higher N\textsubscript{2}O 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...
N2O 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 N2O 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 (Käaniez et al., 2010; Šimek and Cooper, 2002). Previous studies have shown strong effects of soil acidity in N2O production because reduction of N2O to N2 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 N2O 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 N2O 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 N2O emissions (Kandel et al., 2019).

The strong correlations between abundance of AMF and N2O emissions were also reported previously, indicating different fungal taxa are also responsible for N2O 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 (Tanci Zivanov 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 N2O emissions. Therefore, it can be suggested that N2O production from soil fungi should not be neglected.

CONCLUSIONS

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 CO2 and N2O 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 CO2 and N2O 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 N2O were increased after simulated rainfall at the time of legume incorporation and 1 wk 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 N2O after incorporation of green manures. Therefore, further research is required to evaluate other management techniques to lower the loss of N as N2O, like maturity level of the crops at time of incorporation or different types of the cover crops (legumes or nonlegumes etc.).

SUPPLEMENTAL MATERIAL

Supplemental Table S1. Primers used and qPCR parameters for evaluation of microbial community abundance.

REFERENCES


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