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ORIGINAL PAPER

Soil Nitrous oxide and Carbon dioxide emissions following incorporation of above- and below-ground biomass of green bean

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Abstract Information on soil N_2O and CO_2 emissions from above- and below-ground biomass of legume crops is limited in scientific literature. Therefore, a laboratory study was conducted to evaluate the differences in soil N_2O and CO_2 emissions from the above- and below-ground biomass of green bean. Leave and shoot (LS) and root nodule (Nod) of green bean were incorporated into soil and incubated for 53 days. N_2O and CO_2 emissions were measured throughout the 53-day study period. Incorporation of organic residues significantly ($p \leq 0.05$) increased N_2O and CO_2 emissions. However, the Nod treatment yielded higher emissions of N_2O as compared to LS treatment. Cumulative N_2O emissions were 13-fold and fourfold in Nod and LS treatment as compared to the control, respectively. CO_2 emissions were higher in LS treatment than that of Nod treatment. Cumulative CO_2 emissions were 2.15-fold and 1.15-fold in LS and Nod treatments as compared to the control, respectively. The results of current study suggest that below-ground biomass of legume crops produces higher N_2O emissions while CO_2 emissions were higher in above-ground biomass.

Keywords Carbon dioxide · Crop straw · Dissolved organic carbon · Nitrous oxide · Organic matter · Root residue

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Introduction

Incorporation of crop residues into the soil has been widely accepted to maintain soil fertility and to increase crop productivity. Residues of crops also enhance N_2O and CO_2 emissions (Baggs et al. 2000; Liu et al. 2014). Crop residues may affect N_2O and CO_2 emissions from soils in different ways, such as (1) supply of easily available organic carbon (OC), (2) supply of mineral N, (3) stimulation of microbial activities (Wu et al. 2015) and (4) increase in the oxygen consumption in the soil locally, creating optimal conditions for N_2O production via denitrification (Lin et al. 2013). Soil incorporation of crop residues with easily mineralizable organic C and N may result in increased N_2O and CO_2 emissions into atmosphere (Velthof et al. 2002; Lou et al. 2007). However, the content of easily mineralizable organic matter differs significantly between crop residues (Henriksen and Breland 1999).

The magnitudes of soil N_2O and CO_2 emissions vary with the easily mineralizable organic contents, chemical composition and quality of the organic residues incorporated (Millar and Baggs 2004; Liu et al. 2014). Several studies have revealed the emissions of N_2O and CO_2 following different species of organic residue incorporation into soil (Jianwen et al. 2004; Millar et al. 2004; Novoa and Tejeda 2006: Liu et al. 2014). However, N_2O and CO_2 emissions from above- and below-ground biomass of crops, especially legume crops, are still unclear. Some researchers reported that the decomposition of root residues was slower than the shoot residues of the same plant due to the differences in their chemical compositions such as cellulose, organic carbon, nitrogen and lignin contents (Puget and Drinkwater 2001; Lu et al. 2003). Millar and Baggs (2004) stated that N_2O emissions from soils were mostly related to the quality of the plant litter. Mineralization of plant residues and the emission of N_2O were found to be dependent upon the composition of the residues (Németh et al. 1996; Shelp et al. 2000; Laville et al. 2011).

Legume crops are supposed to stimulate N_2O emissions by increasing N inputs into soils. Therefore, legume crops can attribute largely N release into atmosphere upon decomposition of crop residues, including nodules of roots. Upon decomposition, root nodules of legume crops can release substantial amounts of ammonium and nitrate concentrations in the soil (Rochette et al. 2004). Nitrate (NO_3^-–N) and ammonium (NH_4^+–N) can contribute N_2O emissions during microbial nitrification and denitrification in soil (Mahvi et al. 2005). Therefore, legume root nodules should not be neglected as concern to emissions of greenhouse gases. Similarly, Al-Kaisi and Yin (2005) demonstrated that incorporation of crop residues in corn–soybean field markedly increased CO_2 emissions. Soil organic C was increased in crop residues incorporated soil which enhanced CO_2 release into atmosphere.

To the best of our knowledge, there is less information about soil N_2O and CO_2 emissions from above- and below-ground biomass of legume crops. We hypothesized that the differences in N_2O and CO_2 emissions from crop residues are based on contents of mineralizable N and C and depend on the type of crop residue. Therefore, we planned a laboratory study to evaluate the differences in N_2O and CO_2 emissions from root nodules residue and straw of green bean (*Phaseolus vulgaris* L.).

Materials and Methods

Soil sampling and analysis

The soil was sampled at 0–20 cm depth from a cultivated paddy field in Qianjiang city of Hubei province, China (30°25′17.6″N, 112°50′81.7″E; 50 m above sea level). After manual removal of visible plant residues and roots, air-dried soil samples were ground to pass through a 2-mm sieve for incubation and soil analysis. Soil is classified as loam alluvial soil. The main characteristics of these soils are shown in Table 1.

The particle size distribution was analyzed using the pipette method. Soil pH was measured using a combined electrode and pH meter (PB-10; Sartorius AG, Germany) in a 1:2.5 (soil: distilled water) mixture (de Santiago-Martín et al. 2013; Shaaban et al. 2013a). Soil bulk density was determined by retrieving cores of known volume which were subsequently oven-dried at 105 °C for 24 h (Shaaban et al. 2013b). Soil NH_4^+–N and NO_3^-–N were extracted from 1:5 (soil: 1 M KCl) mixture and analyzed using an auto sampler and analyzer (AutoAnalyzer-3, Seal, Germany) (Shaaban et al. 2014a). Total organic C and N in the residues were analyzed using dichromate oxidation method and Kjeldahl procedure, respectively (Page 1982). Dissolved organic carbon (DOC) was extracted by deionized water, and the supernatant was filtered through a filter membrane of 0.45 µm (Shaaban et al. 2014b). The DOC was analyzed using a C/N Elemental Analyzer (Vario-Max CN, Germany). Microbial biomass carbon (MBC) was estimated using the chloroform fumigation extraction method (Lin et al. 2013). Both the non-fumigated and fumigated soil extracts were filtered through a filter membrane of 0.45 µm (PES®, USA). The contents of MBC were analyzed using C/N Elemental Analyzer (Vario-Max CN, Germany) and estimated using the following factor:

$$MBC = 2.22\ E_c$$

where E_c is the difference between organic C extracted of fumigated and non-fumigated soils.

Experimental setup

Root nodule residue and straw of green bean (*Phaseolus vulgaris* L.) were obtained from a cultivated field after the harvest of green bean crop. The straw and root residues were oven-dried and ground to pass through a 1-mm sieve. The treatments were arranged as follows: control (CK), root nodule residue: 0.5 g 100 g^{-1} of soil (Nod), leaves and shoot straw: 0.5–100 g^{-1} soil (LS). Each treatment comprised three replicates. One-liter glass bottles (depth × height = 10 × 14 cm) were used for incubation. The entire experiment was carried out under aerobic conditions at 25 °C for 53 days.

One set of glass bottles was used to measure N_2O and CO_2 emissions with 100 g soil in each bottle, while the other set was used for soil analysis with 400 g soil in each bottle. The soil was incubated in the bottles for 1 week (pre-incubation) to initiate the microbial activity prior to addition of organic residues. After 1 week of pre-incubation, distilled water was added to obtain 55 % water-filled pore space (WFPS) in each glass bottle. After that, the organic amendments were added and mixed thoroughly with soil. Moisture content was maintained at 55 % WFPS by weighing the glass bottles twice a week and adding distilled water if needed.

Gas sampling and analysis

The lids of glass bottles were fitted with a gas-tight rubber septum and a three-way stopcock to allow sampling the trace gases. Glass bottles were stored in an electric incubator at controlled temperature of 25 °C in the dark under aerobic conditions. A thin polythene sheet was placed over the tops of the bottles. Approximately, 40 pinholes per bottle were pierced to allow gaseous exchange but prevent

Table 1 Characteristics of soil and organic residues used in the experiment

Properties	Soil	Leave and shoot	Root nodules residue
pH	7.93		
Total carbon (%)	1.91	40.10	32.35
Total nitrogen (%)	0.16	2.28	3.00
C:N ratio	11.94	17.59	10.78
Total organic carbon (%)	1.84		
Dissolved organic carbon (mg kg^{-1})	126.77		
NH_4^+–N (mg kg^{-1})	4.38		
NO_3^-–N (mg kg^{-1})	3.06		
Bulk density (g cm^{-3})	1.20		
Clay (%)	12.10		
Silt (%)	47.66		
Sand (%)	40.22		

moisture loss. Polythene sheets were removed before sampling, and bottles were exposed to open air for 20 min to ensure that bottles were filled with ambient air. The bottles were closed for 2 h, and two gas samples were taken from headspace with a 30-ml plastic syringe immediately after closure (T_0) and after 2 h (T_1). The T_0 sample was subtracted from T_1 to get actual flux of trace gases. Over the incubation period, gas samples were taken from head space of glass bottles on day 1, 2, 3, 4, 5, 6, 7, 8, 11, 18, 25, 32, 39, 46 and 53.

N_2O and CO_2 concentrations were simultaneously detected using a gas chromatograph (Agilent-7890A, USA). Concentrations of N_2O and CO_2 were analyzed by an electron capture detector (ECD) and a flame ionization detector (FID), respectively. The temperature of column was maintained at 40 °C, while ECD was set at 300 °C. The oven and FID were operated at 50 and 300 °C, respectively. N_2O and CO_2 concentrations were calculated by comparing the peak area with those of standard reference gasses (Beijing special gas factory). The fluxes were calculated as μg N_2O–N kg^{-1} and mg CO_2–C kg^{-1} by using the ideal gas law and linear regression model at a temperature of 25 °C (of incubation) and an average air pressure during the specified period. Soil samples were taken concurrently with gas samples to analyze soil NH_4^+–N, NO_3^-–N, DOC and MBC.

Statistical analyses

All the statistical analyses were performed using the SPSS statistics package (SPSS 16.0). Statistically significant differences were identified by analysis of variance (ANOVA). Tukey's post hoc tests were utilized to identify the significant differences $p \leq 0.05$ and $p \leq 0.01$. Pearson's correlations between the trace gases and soil variables were calculated using the same statistical package.

Results and Discussion

Effect of organic residues on soil N_2O emissions

Incorporation of organic residues significantly ($p \leq 0.05$) influenced and increased N_2O emissions. However, Nod treatments yielded greater N_2O emissions as compared to LS treatment (Table 2). N_2O emissions increased rapidly and peaked at 9.23 and 3.94 μg N_2O–N kg^{-1} h^{-1} on day 5 in Nod and LS treatments, respectively (Fig. 1). The emissions of N_2O then decreased gradually until the end of the study. However, N_2O emission in control (no organic residue) was constantly low throughout the study period. The cumulative N_2O fluxes were 13-fold and fourfold in the Nod and LS treatments as compared to the control, respectively (Table 2).

Research has demonstrated that application of organic amendments increases soil organic C which further accelerates microbial processes such as nitrification and denitrification responsible for N_2O production (Baggs et al. 2000). Incorporation of various organic residues to yellow-brown soil in central China also demonstrated the enhanced N_2O emissions but varied among crop residues (Lin et al. 2013). The differences in magnitudes of N_2O emissions varied depending on the composition, type and C/N ratios of the crop residues. Flessa and Beese (1995) indicated that the effect of crop residue application on N_2O emission was dependent on the C and N contents of different crop residues. The C and N contents of crop residue play an important role in N_2O emissions (Frimpong and Baggs 2010). The optimum C/N ratio for soil microbial activities is thought to range from 25 to 30. Nitrogen release is facile from mineralization of organic compounds with C/N ratios lower than 20, neither release nor immobilization prevails for C/N ratios between 25 and 30, and microbes immobilize inorganic N when the C/N ratio is higher than 30 (Flessa and Beese 1995). Therefore, organic residues with

Table 2 N_2O and CO_2 emissions from above- and below-ground biomass of green bean

Treatments	N_2O–N			CO_2–C		
	Range ($\mu g\ kg^{-1}\ h^{-1}$)	Mean ($\mu g\ kg^{-1}\ h^{-1}$)	Cumulative ($\mu g\ kg^{-1}$)	Range ($mg\ kg^{-1}\ h^{-1}$)	Mean ($mg\ kg^{-1}\ h^{-1}$)	Cumulative ($mg\ kg^{-1}$)
CK	0.02–0.65	0.32 c	198.51 c	0.03–1.69	0.92 b	724.72 b
Nod	0.18–9.23	3.61 a	2598.10 a	0.12–2.19	1.07 b	836.66 b
LS	0.00-3.94	1.46 b	808.61 b	0.12–6.40	2.45 a	1560.43 a

Data are means of three replicate samples. Different letters within columns indicate significant differences at $p \leq 0.05$ by Tukey's test

CK control, *Nod* residue of root nodules, *LS* leave and shoot straw

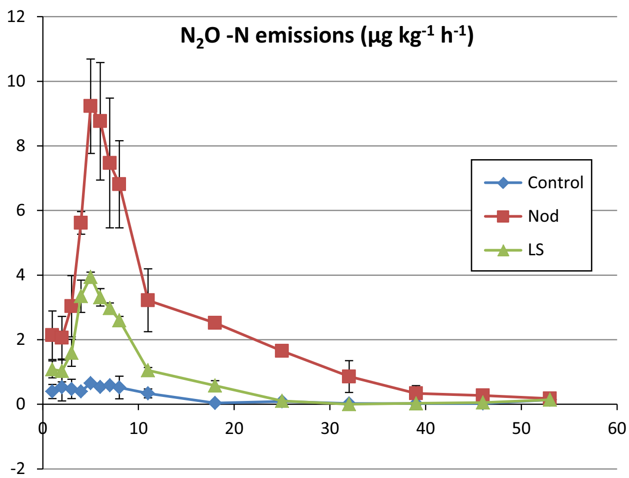

Fig. 1 N_2O emission from control, Nod (root nodules) and LS (leave and shoot straw), *vertical bars* represent standard deviations ($n = 3$)

lower C/N ratios are generally decomposed faster than the residues with higher C/N ratios. In the present study, the root nodule residue was characterized with a lower C/N ratio of 10.78 as compared to LS which had a C/N ratio of 17.59 (Table 1). The root residues (in Nod treatment) therefore more rapidly decomposed and yielded higher N_2O emissions as compared to the LS treatment.

The increased N_2O emissions in Nod treatment could be explained by the N contents in soil following incorporation of organic residues. The mineralization of root nodules generated higher mineral N contents in soil as compared to the LS treatment (Fig. 3, 4). Ammonium (NH_4^+–N) concentration increased rapidly and reached at 61.04 and 43.95 mg kg^{-1} on day 2 in Nod and LS treatments, respectively. Afterward, NH_4^+–N concentration gradually decreased and reached to the values almost same as in control on day 18 of incubation (Fig. 3). Nitrate (NO_3^-–N) concentration continuously increased during the entire incubation and reached at 52.10 and 49.52 mg kg^{-1} on day 53 in Nod and LS treatments, respectively (Fig. 4). Higher mineral N contents in Nod treatment yielded higher soil N_2O emissions. These results are in accord with the earlier studies where decomposition of plant residues with low C/N ratio released higher soil N_2O emissions (Huang et al. 2004; Millar et al. 2004). Faster decomposition of root nodule as compared to LS treatment led to the increased NH_4^+–N and NO_3^-–N concentrations which are N_2O precursors in the soil. Therefore, it appeared from the results of our study that below-ground residues with low C/N ratio contributed to higher N_2O emissions in the Nod treatment when compared to the LS treatment. Moreover, N_2O emissions and soil N contents were higher at early stage and lower at later stage of incubation. This concurrent occurrence of higher N_2O emissions and soil N contents implies that the N contents were also critical in controlling soil N_2O emissions.

Another explanation of higher N_2O emissions in Nod treatment is the increased oxygen (O_2) consumption and denitrifiers activity (Millar and Baggs 2004). We conjecture that the decomposition of organic residues consumed oxygen, which created feasible conditions for denitrification stimulating N_2O production. The findings of Lin et al. (2013) also support our results as they reported that enhanced soil C availability in plant residue incorporated soil accelerated the activities of microbes which during respiration decreased oxygen in the soil, resultantly higher N_2O emissions. Microbial activities in soil can demonstrate trend of N_2O release from soil to the atmosphere (Dambreville et al. 2006). Microbial biomass C (MBC) is one of the most active components of the soil organic C that indicates the activities and growth of microbes and regulates a variety of biochemical processes in the soil. Paul (2006) found an increased N_2O emission relative to increased MBC contents. In the present study, positive relationships ($r = 0.43$, $p \leq 0.01$) were observed between N_2O emissions and soil MBC contents (Table 3), indicating that enhanced microbial activity following incorporation of organic residues yielded high N_2O emissions. Therefore, higher N_2O emission at early stage of incubation was associated with higher contents of MBC, which was later on decreased with the decrease of MBC. This is in agreement with earlier observations where higher N_2O emissions were associated with higher MBC contents in soils (Lou et al. 2007).

Table 3 Pearson correlations among variables

Variables	N_2O	NH_4^+–N	NH_3^-–N	CO_2	DOC	MBC
N_2O	–					
NH_4^+–N	0.69*	–				
NO_3^-n–N	−0.25*	−0.66*	–			
CO_2	0.28*	0.45*	−0.62*	–		
DOC	0.40*	0.68*	−0.74*	0.82*	–	
MBC	0.43*	0.68*	0.64*	0.79*	0.89*	–

* Correlation is significant at $p \leq 0.01$ ($n = 135$)

The amount of dissolved organic C (DOC) can also affect N_2O emissions from soils (Lin et al. 2013). The contents of DOC reflect easily available C to microbes as a substrate for growth and metabolism (Jensen et al. 1997). The increased availability of DOC stimulated N_2O emissions (Kaiser et al. 1998). Martin-Olmedo and Rees (1999) revealed that poultry manure increased DOC in soil which further increased N_2O emissions. In our study, DOC contents positively correlated with N_2O emissions. These results are supported by the findings of Sanchez-Martin et al. (2010) as they observed a positive correlation between soil DOC and N_2O emissions. Furthermore, the majority of microbial denitrification relies on heterotrophic bacteria which require an organic carbon source (Wang and Wang 2012). The positive relationship between N_2O emissions and DOC implies that enhanced activity of denitrifying bacteria contributed to higher N_2O emissions after decomposition of organic residue.

Soil CO_2 emissions

Incorporation of organic residues to soil significantly ($p \leq 0.05$) influenced and enhanced CO_2 emissions (Table 2). The magnitudes of CO_2 emissions were greater in the LS treatment as compared to the Nod treatment. CO_2 emissions increased and peaked at 6.40 and 2.19 mg CO_2–C kg^{-1} h^{-1} on day 3 in LS and Nod treatments, respectively (Fig. 2). The emissions of CO_2 then gradually decreased until the end of the study. Compared to the control, the cumulative CO_2 fluxes were increased by up to 2.15-fold and 1.15-fold in the LS and Nod treatments, respectively (Table 2). Similar results were observed in an earlier study where higher CO_2 emissions were found from the soils amended with the residues composed of high C contents (Millar and Baggs 2004). Higher C contents in LS treatment attributed to higher emissions of CO_2 than that of Nod. The results show that CO_2 emissions were highly dependent on the C contents of amended crop residues.

Incorporation of organic residues significantly ($p \leq 0.05$) increased DOC in the present study and higher DOC contents were found in the LS treatment as compared

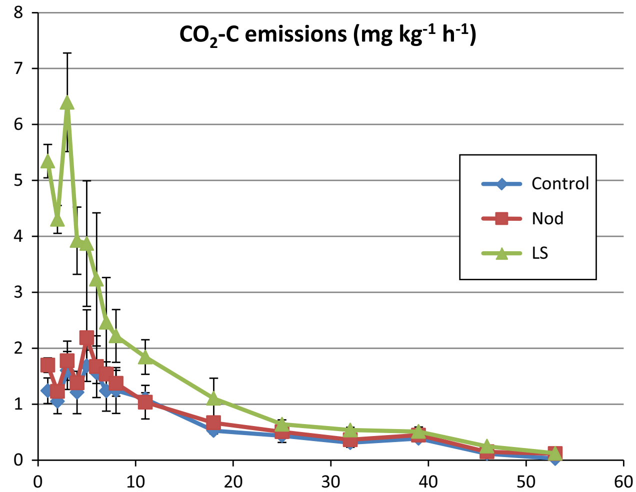

Fig. 2 CO_2 emission from control, Nod (root nodules) and LS (leave and shoot straw), *vertical bars* represent standard deviations ($n = 3$)

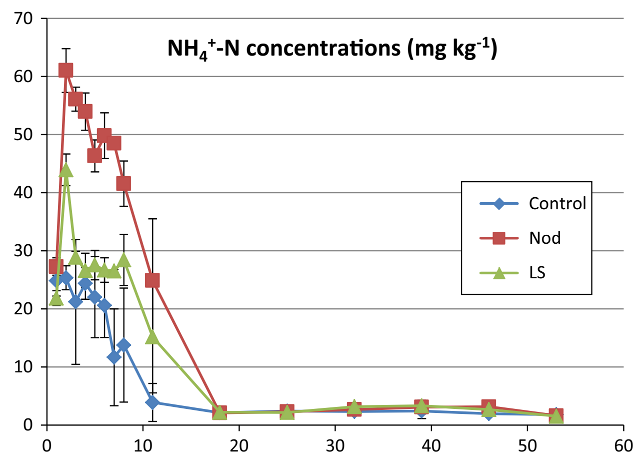

Fig. 3 Ammonium concentrations in control, Nod (root nodules) and LS (leave and shoot straw), *vertical bars* represent standard deviations ($n = 3$)

to the Nod treatment (Fig. 5). Upon incorporation of organic residues, DOC contents were peaked at 253.53 and 171.15 mg kg^{-1} on day 2 in LS and Nod treatments, respectively. After that, DOC gradually decreased until the end of the incubation period. Pearson's correlation analysis also showed that soil CO_2 emissions were strongly correlated with DOC ($r = 0.82$, $p \leq 0.01$, Table 3), indicating that increased C supply after residue incorporation promoted soil respiration. Changes in DOC contents may potentially cause fluctuations in the magnitude of CO_2 emissions (Davidson et al. 2000). As a specific fraction of organic C, dissolved organic carbon (DOC) represents an easily degradable and available substrate to microorganisms (Boyer and Groffman 1996; Huang et al. 2014). Soil respiration has been revealed to be reliant on C supply

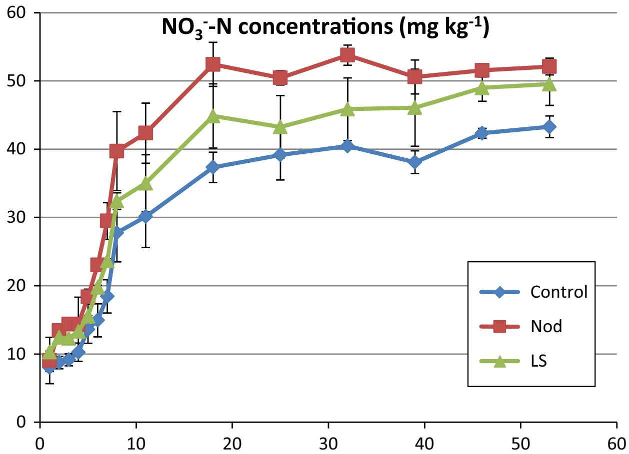

Fig. 4 Nitrate concentrations in control, Nod (root nodules) and LS (leave and shoot straw), *vertical bars* represent standard deviations ($n = 3$)

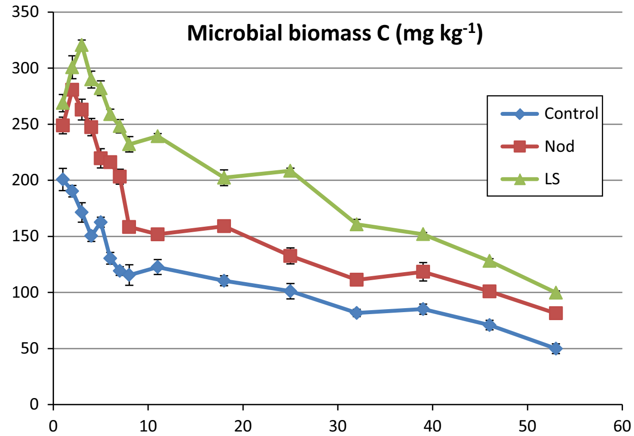

Fig. 6 Microbial biomass carbon in control, Nod (root nodules) and LS (leave and shoot straw), *vertical bars* represent standard deviations ($n = 3$)

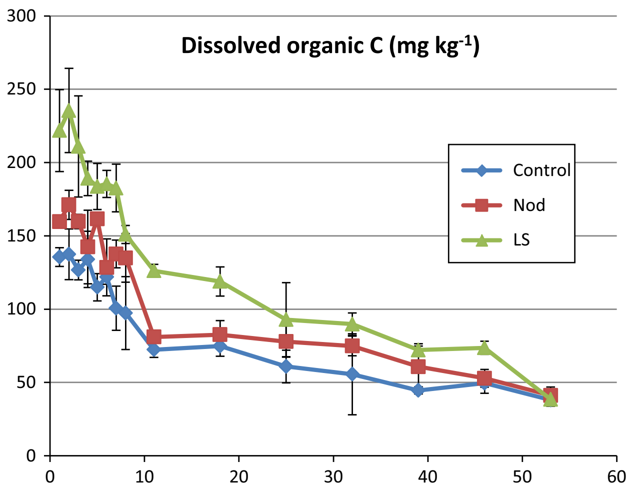

Fig. 5 Dissolved organic carbon in control, Nod (root nodules) and LS (leave and shoot straw), *vertical bars* represent standard deviations ($n = 3$)

(Silva et al. 2009; Sevilla-Perea et al. 2015). In an earlier study, rice straw addition enhanced availability of organic C and microbial activities which accounted for the increase in soil CO_2 emissions (Bhattacharyya et al. 2012). Our results showed that CO_2 emissions were higher in LS treatment that had higher C contents of 40.10 % than that of Nod treatment with 32.35 % C contents. Therefore, the larger magnitudes of CO_2 were released from LS treatment as compared to Nod treatment.

CO_2 emission was high at the beginning of the incubation and maximum on day 2 of the commencement of experiment. DOC contents were also high at the early stage and lower at later stage of incubation (Fig. 5). The increase in carbon supply stimulated microbial activities in LS treatment and thereby higher CO_2 emissions at the early stage of incubation. Similar results were observed by Duong et al. (2009) as they reported that the maximum CO_2 evolved immediately after 1 day of the onset of incubation with wheat straw at an application dose of 2 % in a sandy loam soil. The authors explained that the mechanism of the high respiration rates at the beginning of experiment was due to easily available organic compounds following wheat straw addition. In another study, the incorporation of rice straw increased CO_2 emissions within 10 days and then gradually decreased (Lou et al. 2007). Results of our study are in agreement with the above-discussed studies as CO_2 emissions were higher at high levels of DOC contents (Figs. 2, 5).

Microbial activities are considerably influenced by incorporation of organic residues depending on the type of incorporated material (Lin et al. 2013). Microbial biomass carbon (MBC) was increased significantly ($p \leq 0.05$) by the incorporation of organic residues. MBC was higher in LS treatment as compared to Nod treatment. MBC increased rapidly and reached at 320.51 and 280.65 mg kg^{-1} in LS and Nod treatments, respectively, on days 3 and 2 of the study period (Fig. 6). Afterward, MBC decreased steadily until the end of the study. Positive and significant correlation between MBC and CO_2 emissions ($r = 0.79$, $p \leq 0.01$) also confirmed that microbial respiration increased by the available C from organic residues. Our results are in agreement with Lou et al. (2007) as they found that soil CO_2 fluxes positively correlated with DOC and MBC after 55 days of the experiment commencement. Bhattacharyya et al. (2012) also found significant correlations between CO_2 emissions, DOC and MBC after rice straw and green manure application in a paddy soil. Overall, it is worthwhile to mention from our results that leave and shoot straw of green bean could regulate more CO_2 emissions as compared to root nodules.

Conclusion

The current study demonstrated the effects of above-ground (LS treatment) and below-ground biomass (Nod treatment) of green bean on N_2O and CO_2 emissions. Mineralization of both LS and Nod residues differed depending on the C/N ratio. Root nodule residue showed higher mineralization rate and mineral N contents because of low C/N ratio and thereby enhanced soil N_2O emissions. Nevertheless, leave and shoot straw yielded higher CO_2 emissions as compared to the root nodule residue. The findings of this study suggest that below-ground biomass of legume crops is capable of producing high soil N_2O emissions while high soil CO_2 emissions are released from above-ground biomass. Such information from laboratory study can open a window of exploring further research regarding decomposition of different crop biomass under various conditions and their impact on greenhouse gas emissions. Further research is needed to investigate N_2O and CO_2 emissions from above- and below-ground biomass of a variety of crops.

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