# EFFECTS OF COVER CROPPPING ON NITROGEN DIOXIDE, CARBON DIOXIDE AND METHANE FLUXES AND SOIL ENZYME ACTIVITIES IN CORN-SOYBEAN ROTATION SYSTEM

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## ABSTRACT

The effect of cover crops (ryegrass, hairy vetch, and oilseed radish) in terms of microbial biomass carbon (MBC), C and N mineralization, and enzymatic activities in a corn-wheat-soybean cropping systems under a Mollisol was evaluated. The distributions of total organic C (TOC), total Kjeldahl N (TKN), microbial biomass C (MBC), readily mineralizable C and N, and five enzyme activities (β-glucosidase, β-glucosaminidase, acid phosphatase, arylamidase, and fluorescein diacetate hydrolysis) involved in the cycling of C, N, P and S were studied in three soil depths (0-5. 5-10, 10-20 cm) while soil surface fluxes of carbon dioxide (CO2), methane (CH4), and nitrous oxide (N2O) were estimated. Ryegrass showed higher activity in acid phosphatase, β-glucosidase and β-glucosaminidase. Ryegrass and hairy vetch significantly increased organic C and N, and MBC. Level of mineralized C and N were the same in ryegrass and hairy vetch. There was no clear variation in CO2, N2O and CH4 fluxes from the cover crop treatments. N2O fluxes increased with an increase in soil moisture. The negative CH4 fluxes manifest the soil as CH4 sink. No significant differences among cover crop treatments in terms of CO2-C, N2O-N and CH4-C emissions, a reflection that their emissions are highly variable. Empirical data on carbon dioxide (CO2), methane (CH4) and nitrous oxide (N2O) fluxes are important in management systems to evaluate mitigation strategies, while microbial biomass and enzyme activities can be used as sensitive indicators of ecological stability.

**Keywords:** Carbon sequestration; Soil Quality; Microbial Activity

## 1. INTRODUCTION

Climate change is one of the pressing issues today and is considered the biggest environmental, social and economic threats the world is experiencing at the moment. The causes and challenging issues of climate change, particularly its negative impacts to crop production have become the interest of research and development-oriented groups and organizations. The emission of greenhouse gases such as $CO_2$, $CH_4$ and $N_2O$ are believed to contribute much to the rapid increase in earth's temperature and presumed to induce global warming. Beside fossil fuel combustion, the agriculture industry particularly land use change and soil cultivation have been identified as contributory to the global emission of these gases in the atmosphere (USEPA 2009). Agriculture productivity depends upon the soil which serves as the reservoir of nutrients and water necessary for plant growth; however, it also produces $CO_2$ as a result of aerobic respiration from soil microbes and plant roots. The conversion of soil N thru the processes of nitrification and denitrification

release the $N_2O$ in the atmosphere (Sahrawat & Keeney 1986), while intensive rice farming releases $CH_4$ in the environment (Sommer et al 2004b).

The potential of soil to sequester carbon is now being looked upon to offset global climate change. Soil carbon sequestration implies removal of atmospheric $CO_2$ by plants, and storage of fixed carbon as soil organic matter, where one strategy for carbon stock build up is the addition of crop residue and decrease the rate of soil organic matter decomposition (Lal 2004). With improvement of soil organic matter, it lessens the potential to release the carbon in the atmosphere. Other than acting as reservoir for atmospheric $CO_2$, soil organic matter (SOM) is an important attribute of soil quality as it controls the many key functions of the soil (Doran & Parkin 1994). Soil organic matter affects soil structure, water retention, nutrient availability, and soil microorganisms (Aparicio & Costa 2007; Leite et al. 2007). The increase of organic matter both in terms of quality and quantity can have beneficial effects on soil quality because it is related to aggregation, water infiltration and availability for crop production (Doran & Parkin 1994; Franzluebbers 2002).

The adoption of good agricultural practices (GAP) that improves soil organic matter content can reduce the emission rate of atmospheric $CO_2$ while leaving positive impacts on soil quality. One of these recommended GAP is cover cropping that has been widely recognized and promoted as a practical way to enhance soil productivity and environmental quality (Walsh et al.1996; Baumann et al. 2001). The practice involves the use of cover crops, which adds organic matter to soil and release available nutrients as the organic matter breaks down for uptake of crop (Sanchez et al. 2007). Microbial communities play a role in the decomposition of these organic materials and plant residues incorporated into the soil, leading to energy flow, nutrient cycling and organic matter build up or soil carbon sequestration (Lynch & Bragg 1985; Kandeler et al. 1996). Soil microorganisms mediate the mineralization of soil organic matter and nutrients. The microbial biomass is a small but important reservoir of nutrients, and many transformations of nutrient occur in the biomass (Dick 1992). Soil microbes respond quickly to any stress affecting the ecosystem; hence are used as a biological indicator in the evaluation of agricultural management practices with respect to soil quality.

The transformation of nutrients in the soil by the microbial communities are mediated by enzyme reactions (Kandeler et al. 1996), which can be evaluated through simple, sensitive, and relatively rapid protocols (Ndiaye et al. 2000; Nannipieri et al. 2002). Enzymatic activities in the soil can also be used as an early indicator of change in the dynamics of soil organic matter (Andersson et al. 2004). When soil organic matter increases, enzyme activities increase, which implies larger microbial communities and improved stabilization of humic materials (Bending et al. 2002).

Measurements of microbial biomass and enzyme activities can assess the effects of a certain agricultural practice on soil earlier than the usual total organic C or N measurements (Powlson & Jenkinson 1981; Carter 1986; Powlson et al. 1987; Saffigna et al. 1989; Balota et al. 1998) and therefore they may be an indicator of potential C sequestration (Sa et al. 2001). The microbial biomass and enzyme activities become a valuable tool for understanding changes in soil properties and in the degree of soil degradation or soil quality (Smith & Paul 1990; Doran & Parkin 1994; Brookes 1995; Sparling 1997).

The study was undertaken to evaluate the effect of cover crops (ryegrass, hairy vetch, and oilseed radish) in terms of microbial biomass carbon (MBC), C and N mineralization, and enzymatic activities in a corn-wheat-soybean cropping systems grown under a Mollisol. The distributions of total organic C (TOC), total Kjeldahl N (TKN), microbial biomass C (MBC), readily mineralizable C and N, and five enzyme activities (b-glucosidase, b-glucosamidase, acid phosphatase, arylamidase, and fluoresce diacetate hydrolysis) were compared among the three cover crops at three soil depth (0-5, 5-10 and 10-20 cm). Furthermore, estimate soil surface fluxes of carbon dioxide ($CO_2$), methane ($CH_4$), and nitrous oxide ($N_2O$) and the cumulative greenhouse gas emission as affected by cover cropping practices were estimated.

## 2. MATERIALS AND METHODS

### 2.1 Experimental Site and Design

The field experiment was established at the Purdue Agricultural Center, Lafayette, Indiana. Plots which have grown to corn--soybean rotation for 10 years were chosen for the study. There were a total of eight blocks; each having four plots assigned for the three cover crop treatment (hairy vetch, oilseed radish and rye grass), and a control (no cover crop). Each plot measured 10 by 20 meters. The experiment was arranged in a completely randomized block design with four replicates per treatment.

### 2.2 Soil sampling

Soil samples were collected in September 2013 from the different plots. Twelve core samples were taken using a one inch-diameter soil probe to a depth of 20 centimetres at one meter interval along two transects within each plot. The cores were divided into three layers, 0–5, 5–10, and 10–20 cm. Composite samples from each replicates were placed in plastic bags, sealed, and held in coolers until they could be transported to the laboratory for analysis. In the laboratory, the soil was passed through an 8-mm sieve, removing plant material and rocks larger than the mesh openings. A representative 200-g subsample was set aside, placed in a plastic bag, properly labelled and stored at 4°C for soil microbial biomass C determination, while another representative 200-g subsample portion was hand sieved to pass a 2-mm sieve, air dried, and stored at 4°C until use for measurement of enzymatic activities, potentially mineralizable C and N, soil organic C and total organic N.

The field moisture content of the samples collected was determined using the gravimetric method (Topp & Ferré 2002), while a field estimate of soil bulk density was calculated (Grossman & Reinsch 2002). All analyses were conducted at the USDA-ARS National Soil Erosion Research Laboratory.

### 2.3 Enzyme Activities

The *β-glucosidase, β-glucosaminidase* and *Acid phosphatase* activities were determined by the method of Eivazi and Tabatabai (1988), Parham and Deng (2000), and Tabatabai (1994), respectively. The methods are based on colorimetric determination of the *p-nitrophenol* (PNP) released by β-glucosidase, *β-glucosaminidase* and *Acid phosphatase* when soil is

incubated, and are expressed as milligrams of ρ-nitrophenol (PNP) released per gram of soil per hour.

Arylamidase activity was measured using the method of Acosta-Martínez and Tabatabai (2000) which is based on colorimetric determination of β-naphthylamine produced by arylamidase activity when the soil is incubated. The extractant is specified as milligrams of $\beta$-naphthylamine released per gram soil per hour. Fluorescein diacetate (FDA) hydrolysis was measured using the Green et al. (2006) procedure, modified by using tris (hydroxymethyl) aminomethane rather than a phosphate buffer (Prosser et al. 2011), and reported as milligrams of fluorescein released per kilogram soil during the 3 hours of incubation.

All enzyme activities were assayed on 1.0 gram, air-dried; 2-mm sieved soil and was performed in triplicate with the appropriate controls and standards. The results are expressed on a moisture-free basis. Moisture content was determined by drying the soil samples at 105°C for 24 hours.

## 2.4 Soil Microbial Biomass

A modification of the chloroform-fumigation procedure of Jenkinson and Powlson (1976) was used to determine the soil microbial biomass carbon. Twenty-five (25) gram-dry weight equivalent of soil sample was weighed in a 50 ml beaker and the samples were brought to 60% water-filled pore space (WFPS). Three samples were fumigated with chloroform for 24 hours, and another three samples were non-fumigated which served as controls. The soil samples were placed in sealed mason jars along with a 5-ml KOH as base trap, and aerobically incubated in a dark chamber for the standard 10 and 20-day periods. At the end of 10 days, respiration readings were taken on both the non-fumigated (control) and the fumigated samples to determine the amount of $CO_2$ evolved with a gas chromatograph. Microbial biomass was calculated by subtracting $CO_2$ evolved during the 0-10 and 10-20-day incubation period of the control from the $CO_2$ evolved from the fumigated samples. A k-value of 0.41 was used for conversion of $CO_2$-C to biomass C (Anderson & Domsch 1989).

## 2.5 Soil Organic Carbon and Nitrogen

Carbon mineralization was determined from a 10 gram 2-mm air-dried soil which was re-wetted to achieve a 60% WFPS and to further re-activate microbial activity. The soil in a snap vial was placed in a sealed mason jar with the presence of a 2-ml KOH base trap and distilled water to maintain humidity level in the jar. The soil was incubated for 28 days in a dark chamber. Base traps are replaced after 1, 3, 7, 10, 14, 21 and 28 days. At each sampling period, KOH was collected and replaced. Aliquots of the base trap were acidified with 0.1 M HCl and the $CO_2$ concentration was measured using a gas chromatograph (Varian Model 3800) equipped with a CombiPal auto sampler (CTC Analytics) and a thermal conductivity detector. All analyses were ran in triplicate with appropriate controls and standards.

Nitrogen mineralization was determined from the 28-day incubation of 10 gram 2-mm air-dried soil that was extracted with 150 ml 2-M KCl solution (Hart et al. 1994). Extraction was done before and after the incubation to determine the baseline level of N and the amount of N that has been mineralized. The concentrations of $NO_3^-$ and $NH_4^+$ in the extracts were determined with QuikChem methods 10–107–04–1–A and 10–107–06–2–A,

respectively, on a Lachat flow injection analyzer (Lachat Instruments, Milwaukee, WI, USA). The net mineralized N in soil was the difference between the extractable inorganic N contents before and after incubation.

Total organic C and N was determined by dry combustion with a Dohrman DC-80 total C analyzer (Santa Clara, CA).

## 2.6 GHG Gas Sample Collection

Gas sampling was done using the vented chamber technique. Chamber anchors, which measured 73.7 cm x 35.4 cm x 12.0 cm, were inserted 10 cm into the soil. A U-shaped channel, measuring 1.8 cm wide by 1.9 cm deep, was welded to the outer edge of the chamber anchor. During flux measurements, the U-shaped channel was filled with water, and the chamber lid, measuring 75.8 by 38 by 13 cm, was placed into the U-shaped channel, forming an airtight seal. The chamber anchors were placed at the center two rows of each plot, roughly 3 m from the end of the plot, such that a single row of corn would be located in the center of the chamber. Roughly the same chamber anchor placement was used for the soybean plots. The chamber anchors were left in the ground semi-permanently and were only removed when field operations occurred. The anchors were placed back in the ground as soon as possible after each field operation was completed, and at least 24 h passed when the anchors were placed in the ground and when flux measurements were taken. A small area of bare soil was set aside to estimate gas emission from a non-cropped soil.

To determine $CO_2$, $N_2O$, and $CH_4$ fluxes, a vented aluminium flux chamber lid was placed in the channel around the anchor. Individual gas samples were withdrawn from the chamber at 0, 15, and 30 minutes following chamber placement using a 30-mL syringe. From this syringe, 20 mL of the gas sample was injected into a 10-mL evacuated vial, which was over pressurized so that if any gas leaks existed, there would not be contamination from ambient air. To minimize the variability associated with diurnal effects of gas emissions from the soils, flux measurements were made between 1200 and 1500 h.

In general, gas flux measurements were made weekly from July to September 2013. There were 11 gas flux measurements undertaken in the whole duration of the study. At the time of sample collection, soil moisture, air and soil temperature were measured in each plot. Soil moisture was measured using time domain reflectometry (Fieldscout TDR 300, Spectrum Technologies, Plainfield, IL) with 7.6-cm-long probes. Soil temperature was measured using a digital thermometer (Fisher Scientific, Pittsburgh, PA) with the probe inserted 5 cm into the soil, while air temperature was measured using the same digital thermometer with the probe set approximately 1.0 m above the soil surface.

## 2.7 Greenhouse Gas Analysis

Upon arrival at the laboratory, gas samples were analysed for $CO_2$, $N_2O$, and $CH_4$ using a Varian Model 3900 gas chromatograph (Agilent Technologies, Santa Clara, CA), equipped with a thermal conductivity detector, electron capture detector, and flame ionization detector (Hernandez-Ramirez et al. 2009). Cumulative gas emissions or uptake was calculated by linear interpolation between sampling dates. Cumulative soil respiration was calculated for each of the two phase of crop rotation within the four cover crop treatment and bare soil.

## 3. RESULTS AND DISCUSSION

### 3.1 Enzymatic Activities

The five enzymes responded differently on the treatments. With the exception of FDA, all other enzyme activities showed significantly different among each other with cover crop treatments. A significant cover crop effect was noted in the acid phosphatase, arylamidase, β-glucosidase and β-glucosaminidase with higher enzyme activity observed in the ryegrass cover crop regardless of depth. At the top 5-cm soil layer, the activities of the four enzymes were significantly higher in soil with ryegrass, compared with hairy vetch and oilseed radish which showed comparable enzyme activities. Further data analysis, however, showed that hairy vetch was at par with ryegrass in terms of arylamidase activity. This holds true at subsurface layers (5-10 and 10-20 cm depth) where the three cover crops showed comparable effects on enzyme activities. Ryegrass and hairy vetch showed similar effects in terms of acid phosphatase activity; while ryegrass and oilseed radish for arylamidase. At the 10-20 cm depth, the three cover crops did not vary significantly.

The differences in the responses of the enzymes to the three cover crops suggest that there may be qualities unique to each cover crop that affects the specific enzyme activity. Ryegrass which showed enhanced enzymatic activities than hairy vetch and oil seed radish at 0-5 cm depth could be attributed to the fact that ryegrass has been used as cover crop in the same plots for almost 10 years while hairy vetch and oilseed radish were used as cover crop only in 2012. It could have been affected by rhizosphere which is the zone of the soil with high microbial and enzyme activity. In the case of ryegrass, the presence of its extensive root system increased its rhizosphere effect compared to hairy vetch and oil seed radish. Studies also showed that annual ryegrass residues in cotton stimulated total and Gram-negative bacteria, fluorescent pseudomonads, and total fungi for all sampling periods under both tillage systems (Wagner 1995). Another reason could be the differences in the amount and type of organic matter these cover crops inputted to the soil. The study of Pancholy and Rice (1973) revealed that the type of organic matter influence activities of invertase, cellulase and amylase, while the study of Nannipieri et al (1983) showed that ryegrass residue contains enzymes phosphatase, urease and protease.

The impact of hairy vetch and oilseed radish on enzyme activities was quite impressive having been introduced in the plots as cover crops later than ryegrass. The non-significant variation of the impact of hairy vetch and ryegrass in the arylamidase activity implies that continuous use of these cover crops would soon impact enzymatic activities similar to ryegrass. In a cover crop study with soybean, ryegrass and hairy vetch cover crops were noted to stimulate soil microbial population in the surface soil (Wagner 1995). They further revealed that both cover crops in soybeans enhanced surface soil esterase and phosphatase activities for the first 21 days after planting, with hairy vetch initially enhancing activity more than rye. Both cover crops enhanced microbial biomass/populations and soil enzyme activity, thereby improving soil quality.

In general, the activity of the five enzymes decreased with increasing soil depth. Some factors contributory to the higher enzyme activity at the surface soil layer may include the effect of the no tillage system employed in the study area. Some studies reported that enzyme activities decreased with soil cultivation (Gupta & Germida 1988; Dick 1984; Klein & Koths 1980; Doran 1980; Angers et al. 1993). Using NT and/or cover crop systems can alter enzymatic activity (Bandick & Dick 1999; Dick 1994), microbial biomass (Linn & Doran 1984; Wagneretal 1995; Kirchner et al. 2003; Zablotowicz et al. 1998), microbial community

structure (Lupwayietal 1998; Feng et al. 2003), and macroflora diversity (Gaston et al. 2003; Reelederetal 2006).

## 3.2 Soil Total Carbon, Total Nitrogen and C:N Ratio

The total organic carbon content of the soil varied significantly among cover crop treatments. The continuous cover cropping with ryegrass since 2003 resulted in an increase of soil organic carbon by 38 percent when compared with the control. Meanwhile, the introduction of hairy vetch as cover crop enhanced soil organic carbon by 20 percent. This indicated that both ryegrass and hairy vetch manifested rapid degradation of the cover crop residues when incorporated into the soil surface (0-5 cm depth). There is no significant difference among the treatments in terms of total C contents in the subsoil (5-10 and 10-20 cm). According to Brye et al. (2002), this happens in most short-term soil C studies as small gradual changes in SOM are generally difficult to detect because of high background C level and natural variability of soils.

Large quantities of organic materials are supplied to soils from the roots of cover crops during their growing periods (Goodfriend et al. 2000). The major source of the organic matter is root senescence and exudation (Shamoot et al. 1968; Goodfriend et al. 2000; Lu et al. 2002). The exudates and other organic constituents result in the production of large amounts of active polysaccharide binding agents in the surface soil (Haynes et al. 1991; Degens 1997). The hairy vetch grows slowly in fall, but root development continues over winter, while annual ryegrass remained vegetative over the winter months and continued growth in early spring. Ryegrass develops a densely ramified root system (Gaborcik et al. 2000; Isse et al. 1999).

As expected, total organic carbon (TOC) substantially decreased with increasing soil depth. Averaged across all treatments, TOC contents were 22, 17, and 15 g $kg^{-1}$ at 0–5-, 5–10-, and 10–20-cm soil depths, respectively. The decrease in SOC is likely due to less plant-derived organic material in the deeper soil layers.

Similar trend of result is observed for total organic N (TON). At the top soil layer (0-5 cm), the long-term use of ryegrass as cover crops significantly improved soil TON with mean of 2.24 g N $kg^{-1}$ soil, which is 31 percent higher than the control plots. Cover cropping with hairy vetch indicated a TON value of 1.98 g N $kg^{-1}$ soil. Soil total N levels were the same in both cover crops relative to oilseed radish and the control plot. This is likely due to greater N input from ryegrass derived from its roots and residues which have been in the area for a long time, while hairy vetch, being a legume which was planted as forage cover crop have increased N level through its N-rich litter (Sanchez et al. 2007). The study of Hargrove (1986) and Kuo et al. (1997) proved that rye, hairy vetch, and crimson clover increased organic C and N concentrations.

Similar to soil total C, the main effect of depth was significant for soil total N level which decreased with increasing soil depth at respective mean of 1.92, 1.58, 1.43 g N $kg^{-1}$ for 0-5, 5-10 and 10-20 cm. The no till management employed in the area could have been the reason for the greater soil organic C and N concentrations in the surface soil layer. No till resulted from the placement of plant residue at the soil surface, thereby reducing residue contact with soil microorganisms in the subsurface soil layers for decomposition (Havlin et al. 1990; Franzluebbers et al. 1995a; and, Salinas-Garcia et al. 1997). Indeed, many other studies indicate inorganic soil N is concentrated near the soil surface in no-till cover crop-

corn systems (Ebelhar et al. 1984; Frye et al. 1988; Hargrove 1986; Hoyt & Cole 1989; Huntington et al. 1985; McCracken et al. 1989; Utomo et al. 1990; and, Varco et al. 1989).

The C/N ratio of soils with three cover crop treatments and control plot. The C:N ratio of soils with ryegrass were higher than the control plots, but did not differ significantly with hairy vetch and oilseed radish, with respective mean values of 11.67, 11.45 and 11.46 g C $g^{-1}$ N in the top soil layer (0-5 cm). At the subsoil, no significant differences on C:N ratio were observed among the four treatments. Across all treatments, the C:N ratio have average value of 11.4, 10.8 and 11.0 g C $g^{-1}$ N at the 0-5, 5-10 and 10-20 cm depth, respectively. The C:N ratio in all the treatments was narrow which range from 10.73 to 11.67 g C $g^{-1}$ N. This indicates that the crop residues and resulting soil organic matter fractions were low in C and high in nitrogen. The total quantity of biomass produced by the cover crop, its C:N ratio, and how it is managed will determine how much soil organic matter is likely to increase. Cover crop residue with a low C:N ratio will decompose much faster than residue with a high C:N ratio.

## 3.2 Soil microbial biomass

Soil microbial biomass (SMB) ranged from 853 to 2,720 mg C $kg^{-1}$ soil. Relative to the control treatment, cover cropping with ryegrass significantly enhanced SMB-C by 35% at the 0–5 cm depth. Although hairy vetch was introduced in the area, the SMB-C value was at par with ryegrass. However, it did not vary significantly with oilseed radish. At 5-10 and 10-20 cm depth, the SMB-C levels were distinguishable among the three groundcover treatments and the control plots. Higher microbial biomass was obtained in soils with cover crop due to the presence of plant biomass (e.g., above- and below-ground) inputs to the soil from the treatments. Perhaps, weeds were allowed to grow in the control plots and mowed similarly, which explains why the soil microbial biomass from the control treatment approximates that of the oilseed radish (0-5 cm) and hairy vetch (5-10 cm) cover crop plots. As the cover crops were mowed and residues left on the soil, SOM accumulated in the topsoil and substrates of different quality and quantity were made more available to soil microorganisms improving conditions for their growth and reproduction in the top soil layer (Govaerts et al. 2007).

Averaged across treatments, SMB decreased with increasing depth. The surface soil layer (0-5 cm) had a mean SMB-C of 2,126 mg C kg-1, while 1,419 and 1,014 mg C $kg^{-1}$ in the 5-10 and 10-20 cm depth, respectively. This could be attributed to the no tillage management employed in all the treatments. The accumulation of crop residues at the surface provides substrates for soil microorganisms, which accounts for the higher MBC at the soil surface layer. Many studies were done and observed an increase in the total soil microbial biomass under no-tillage in the surface layer of the soil (Collins et al. 1992; Alvarez et al. 1995). No tillage practice increased the microbial biomass by increasing labile carbon in soil. This protects soil aggregates and does not break fungal networks that are important habitats for the microbial biomass in soil.

## 3.4 C and N Mineralization

The value of C mineralization ($C_{min}$) released as $CO_2$ during the 28-day incubation varied from 185 to 549 mg C $kg^{-1}$ soil. Cover cropping treatments indicated significant differences in terms of C mineralization in the three soil depths. At top soil surface (0-5 cm depth), rye grass treatment obtained $C_{min}$ value of 516 mg C $kg^{-1}$ while hairy vetch had 549 mg C $kg^{-1}$. Mineralizable C in these two cover crops was significantly higher than in oilseed radish with

$C_{min}$ value of 416 mg C $g^{-1}$. At lower soil depth, rye grass cover treatment consistently obtained greater $C_{min}$ values with 387 and 246 mg C $kg^{-1}$ soil in the 5-10 and 10-20 cm depth, respectively. Carbon mineralization decreased with increasing soil depth. There was a marked stratification in between sampling depths as shown by the average $C_{min}$ mean values across treatments. Carbon mineralization in the surface soil layer with 498 mg C $kg^{-1}$ soil is higher by 71 and 139% in the 5-10 and 10-20 cm depth, respectively. In general, C mineralization decreases with depth because of the lower total carbon in the deeper soil layers, which are generally observed in most agricultural soils.

Ryegrass and hairy vetch showed comparable effects on N mineralization at surface soil. Non-legume cover crops, such as rye, have been known to increase soil organic C and N (Kuo et al. 1997a and Kuo et al. 1997b). Legume cover crops, such as hairy vetch, have been known to enrich soil N compared with non-legume or no cover crop (Abdul-Baki & Teasdale 1993, Teasdale & Abdul-Baki 1995, and Sainju et al. 1999).

No significant differences could be detected in the amounts of N mineralized during the 28-day incubation among treatment at 5-10 and 10-20 cm depths. The three cover crops treatments imposed similar effect on N mineralization which values ranged from 18.92 to 21.68 mg N $kg^{-1}$ and 13.18 to 15.82 mg N $kg^{-1}$ at 5-10 and 10-20 cm-depth, respectively.

## 3.5 Greenhouse Gas Emission

### 3.5.1 Soil Moisture, Soil and Air Temperature

The mean volumetric soil moisture content from the plots measured on the days of gas flux measurements ranged from 23 to 42%. Plots with cover crops tend to have higher moisture than the no cover crop plot. The soil and air temperature measured in plots at the time of flux measurements, ranged from 21 to 36°C and 22 to $27^0$C, respectively. The soil and air temperature was almost constant and had a similar pattern. The mean soil and air temperature from the five plots were 25.76 and 25.41 $^0$C, respectively.

### 3.5.2 Greenhouse gas fluxes

The calculated greenhouse gas fluxes throughout the study period. A positive value indicates gas emission from the soil, while a negative value indicates gas uptake. There was no clear variation in soil $CO_2$, $N_2O$ and $CH_4$ fluxes from the cover crop treatments. The soil respiration rate increased gradually from the start of gas sampling on June 10 and kept relatively high levels for 4 weeks. The respiration rate then decreased to relatively low levels from July 15 to July 24, and then again increased gradually on August 5. This later it decreased until the last period of gas flux measurement on September 17. Highest soil respiration rate was noted in the oilseed radish at 144 mg C $m^{-2}$ $h^{-1}$ while lowest level was recorded in the bare plot at 26.12 mg C $m^{-2}$ $h^{-1}$.

A close examination of the flux revealed that $N_2O$ fluxes peaked on the $5^{th}$ and the $7^{th}$ gas flux measurement (August 8 and 22) and then decreased to relatively lower values towards the end of the study period. Nitrous oxide is emitted by soils as a result of denitrification in anaerobic soil and nitrification in aerobic soil with the anaerobic production considered more important. Thus, emissions generally increase with increasing soil moisture. This explains the increase in $N_2O$ flux at the $5^{th}$ and $7^{th}$ gas flux measurement due to sudden increase in the soil moisture.

$CH_4$ fluxes were very low except for the no cover crop treatment which suddenly reached its peak at 0.010 mg $CH_4$-C $m^{-2}$ $h^{-1}$ during the 7th reading (July 22) and the oilseed radish with 0.010 $CH_4$-C $m^{-2}$ $h^{-1}$ in the 10th reading (August 12). Uptake of $CH_4$ was observed as manifested by the negative fluxes. The results jive with Goulding et al. (1995) where they found out that $N_2O$ and $CO_2$ are emitted from the soil whereas $CH_4$ is normally oxidised by aerobic soils making them sinks for atmospheric $CH_4$.

No significant differences on mean fluxes were observed between the cover crop treatments. The plots with and without cover crop registered significantly higher $CO_2$ and $N_2O$ fluxes were relative to the bare plot, while mean $CH_4$ fluxes were relatively the same in all plots.

### 3.5.3 Greenhouse gas emission

The total $CO_2$ emission obtained from June to September from plots with cover crop treatments were 1.97, 1.94 and 1.91 Mg C $ha^{-1}$ in hairy vetch, oilseed radish and ryegrass, respectively. These values obtained did not significantly differ from the total $CO_2$ emission from plots without cover crop at 1.89 Mg C $ha^{-1}$. In this study, the bare plot generated the least amount of $CO_2$ at 1.0 Mg C $ha^{-1}$. When comparing all treatments, there were no significant differences among treatments in terms of cumulative $N_2O$ emission. The plots with or without cover crops emitted relatively the same quantity of $N_2O$ from 261 to 442 kg $CO_2$ equivalent ha-$^{1}$. There was no significant difference among cover crop treatments for $CH_4$ uptake by soils in this study which could be attributed to the very low $CH_4$ fluxes obtained in the experiment. The soils in all plots were a sink for $CH_4$ with a range of uptake from 1.37 to 2.18 kg $CO_2$-equivalent $ha^{-1}$.

## 4. SUMMARY AND CONCLUSION

1. Of the five enzymes tested, rye grass showed higher activity in acid phosphatase, β-glucosidase and β-glucosaminidase; while the three cover crops were similar in terms of arylamidase and FDA. In general, the activity of the five enzymes decreased with increasing soil depth due to the effect of the litter fall that stays in the surface soil, a characteristic of a non-tilled soil.

2. Rye grass and hairy vetch cover crops have significantly higher organic C and N compared to oilseed radish. The concentrations of organic C and N are good indicators of soil quality and productivity due to their favorable effects on physical, chemical, and biological properties. The rye grass and hairy vetch cover crops in the no-tillage system can conserve and/or maintain organic C and N concentrations in the soil, thereby improving soil quality and productivity. The mean soil C:N ratio between the three cover crops was relatively constant and very narrow which implies that the crop residues and resulting soil organic matter fractions from these cover crops are low in C and high in nitrogen.

3. Incorporating cover crops (rye grass and hairy vetch) significantly increased soil microbial biomass C in the top (0-10 cm) soil layer. The greater soil microbial biomass from cover crop treatments were probably related to the increased level of plant organic matter inputs. The net effects of cover cropping on soil microbial biomass were much less clear at deeper soil layer (10-20 cm). The accumulation of crop residues at the

surface provides substrates for soil microorganisms, which accounts for the higher MBC at the soil surface layer.

4. The levels of mineralized C and N were the same in rye grass and hairy vetch cover crops. This is likely due to greater C input from rye grass derived from its roots and residues which have been in the area for a long time, while hairy vetch, being a legume have increased N level through its N-rich litter. In general, C and N mineralization decreases with depth because of the lower total carbon and nitrogen in deeper soil layers, which are generally observed in most agricultural soils.

5. There was no clear variation in soil $CO_2$, $N_2O$ and $CH_4$ fluxes from the cover crop treatments. Mean $CO_2$-C flux ranged from 78.97 to 85.65 mg C $m^{-2}$ $h^{-1}$ higher than the bare plot with mean $CO_2$ flux of 46.30 mg C $m^{-2}$ $h^{-1}$. The oilseed radish obtained the lowest average $N_2O$ flux, relative to other cover crop treatments and the bare plot. $N_2O$ fluxes have noted to increase with an increase in soil moisture. The negative $CH_4$ fluxes manifest that the soil in the study area were sometimes sink of $CH_4$.

6. There were no significant differences among cover crop treatments in terms of $CO_2$-C, $N_2O$-N and $CH_4$-C emissions. The lack of cover cropping effect on the cumulative emission of these greenhouse gases is a reflection that their emissions are highly variable. Besides, the gas flux measurement was only done for a short period of four months.

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