Effect of Nitrogen Fertilizer Application on Growing Season Soil Carbon Dioxide Emission in a Corn–Soybean Rotation

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Nitrogen application can have a significant effect on soil carbon (C) pools, plant biomass production, and microbial biomass C processing. The focus of this study was to investigate the short-term effect of N fertilization on soil CO2 emission and microbial biomass C. The study was conducted from 2001 to 2003 at four field sites in Iowa representing major soil associations and with a corn (Zea mays L.)–soybean (Glycine max L. Merr.) rotation. The experimental design was a randomized complete block with four replications of four N rates (0, 90, 180, and 225 kg ha⁻¹). In the corn year, season-long cumulative soil CO2 emission was greatest with the zero N application. There was no effect of N applied in the prior year on CO2 emission in the soybean year, except at one of three sites, where greater applied N decreased CO2 emission. Soil microbial biomass C (MBC) and net mineralization in soil collected during the corn year was not significantly increased with increase in N rate in two out of three sites. At all sites, soil CO2 emission from aerobically incubated soil showed a more consistent declining trend with increase in N rate than found in the field. Nitrogen fertilization of corn reduced the soil CO2 emission rate and season-long cumulative emission in two out of three sites, and increased MBC at only one site with the highest N rate. Nitrogen application resulted in a reduction of both emission rate and season-long cumulative emission of CO2–C from soil.

Increased levels of atmospheric CO2 have prompted research assessing the contributions of industrial, agricultural, and environmental practices to current and potentially continued increasing levels of CO2. Various management practices on agriculture land can have an impact on soil C content. Agricultural practices that affect soil C include tillage system, cropping system, N fertilization, and many other practices. It is estimated that soil contains approximately 1.4 × 10¹² Mg of C in organic matter and surface litter (Schlesinger, 1991). One of the pathways to lose soil C in a row cropping system as a result of management practices (i.e., tillage, N fertilization, etc.) is the emission of CO2 from soil. This soil C loss mechanism as affected by management practices can be determined and quantified by either measuring CO2 emission or through other indicators such as change in soil microbial biomass C or change in soil C fractions.

Generally, sources of CO2 from a soil system can be attributed to biological and chemical activity within the soil. Soil respiration involves organisms metabolizing substrates producing CO2 within the soil matrix (Anderson, 1982). Carbon dioxide loss from soil can also be associated with microbial decomposition of organic matter and root respiration (Witkamp and Frank, 1969; Edwards et al., 1970; Fritz et al., 1978; Singh and Gupta, 1977; Hanson et al., 2000). The mechanism of soil CO2 emission to the atmosphere, however, involves the movement of CO2 through soil pores, and release from the soil system can be measured at the soil surface (Rolston, 1986).

Factors such as soil temperature, soil moisture, cropping system, and N availability can all influence soil microbes and their activity. Previous research has shown inconsistent results, particularly in the case of N fertilization effect, on soil microbial biomass and CO2 emission. Kowalenko et al. (1978) found that soils fertilized with N demonstrated low soil CO2 emission in both field and laboratory studies, but those results were contrary to the findings by Willson et al. (2001), where N fertilization had no influence on soil microbial biomass in a corn–soybean rotation. Others have argued that soil microbial activity may increase due to N fertilization as a result of increased plant biomass production, which on incorporation, stimulates soil biological activity (Dick, 1992); or N fertilization reduces microbial biomass due to lowering soil pH (Smolander et al., 1994; Ladd et al., 1994). The inconsistency in N fertilization effects on soil CO2 emission in these field and labo-

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Abbreviations: CO2, carbon dioxide; MBC, microbial biomass carbon; V6, sixth-leaf stage of corn.
and other soil-atmospheric interactions have a great effect on most likely that soil moisture coupled with soil temperature affect plant growth and subsequently soil biological processes. The role of soil moisture in soil CO₂ emission as an indicator of C loss is not well defined. Kowalenko et al. (1978), Paul et al. (1999), and Bajracharya et al. (2000) reported that soil moisture alone had no strong effect on CO₂ emission. It is most likely that soil moisture coupled with soil temperature and other soil-atmospheric interactions have a great effect on CO₂ emission from soil (Pietikäinen et al., 2005).

The hypothesis for this research is that increasing N fertilizer application rate for corn production can increase soil CO₂ emission and increase soil microbial biomass.

Materials And Methods

Site Description

This study was conducted from 2001 to 2003 in producers’ fields having a history of a corn–soybean rotation in conservation tillage systems. The four sites in this study were in Boone, Floyd, Tama, and Warren counties in Iowa. The four locations represent four major soil associations. Site characteristics are summarized in Table 1. Planting, tillage operations, and pest control applications were conducted by the cooperating producers. All field measurements were performed by the research team. The plot size for each treatment was 15.2 m long by 4.6 m wide and the plots were located at an easily accessible area of the field. The experimental design was a randomized complete block with four replications. Nitrogen treatments were applied by hand broadcasting dry ammonium nitrate (NH₄NO₃) to the soil surface for each N rate just after corn emergence (2001 and 2003). The fertilizer N was not incorporated into the soil. The N rates used in this study were 0, 90, 180, and 225 kg N ha⁻¹. Nitrogen treatments were not applied in the soybean production year (2002).

Field Carbon Dioxide Emission Measurements

The soil CO₂ emission measurements were taken in 2002 when soybean was grown and in 2003 when corn was grown. The measurements started in the spring of 2002 (sites were planted to soybean), which was the year after N rate treatments had been applied to the previous corn crop in 2001. Carbon dioxide emission measurements were taken on the Warren site in 2002 only, the Boone and Floyd sites in 2002 and 2003, and the Tama site in 2003 only. Measurements began immediately after plant emergence each year, were completed at harvest in the fall of 2002 and continued until approximately 1 mo after harvest in the fall of 2003 as weather conditions permitted. The CO₂ measurements were collected during the daytime only due to the limitation of the automated CO₂ monitoring system availability. Five polyvinyl chloride (PVC) rings, 10 cm in diameter and 5 cm in height, were randomly placed and pressed 3 cm into the soil in each N rate treatment. The PVC rings were placed shortly after crop emergence. The five rings per plot were kept in the same location throughout the growing season and for the duration of CO₂ emission measurements. The random placement resulted in rings placed both in rows and between rows.

Carbon dioxide emission was measured using a LICOR-6400 (LICOR Inc., Lincoln, NE) infrared gas analyzer approximately every 7 to 10 d between 1000 and 1200 h. Before measuring soil CO₂ emission, the ambient CO₂ level was determined by leaving the chamber open sideways in an unobstructed area near the PVC ring until the registered reading of ambient CO₂ concentration by the LICOR-6400 was stable. The CO₂ emission rates were measured in μmol m⁻² sec⁻¹. The CO₂ emission rate values from all five PVC rings in each N rate treatment were averaged to determine the emission rate. Both soil temperature and field Carbon Dioxide Emission Measurements Temperature changes were monitored using a HOBO data logger (Onset, Pocasset, MA). The data logger generated daily temperature summaries for the entire period of measurement.

Table 1. Soil properties and classification of study sites.

<table>
<thead>
<tr>
<th>Site</th>
<th>Soybean planting</th>
<th>Soil series</th>
<th>Organic matter†</th>
<th>Soil pH†</th>
<th>Bulk Density</th>
<th>Soil classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boone</td>
<td>May 9, 2002</td>
<td>Clarion</td>
<td>44</td>
<td>6.0</td>
<td>19</td>
<td>1.4</td>
</tr>
<tr>
<td>Floyd</td>
<td>May 28, 2002</td>
<td>Clyde</td>
<td>92</td>
<td>6.4</td>
<td>32</td>
<td>1.2</td>
</tr>
<tr>
<td>Tama</td>
<td>May 14, 2002</td>
<td>Tama</td>
<td>37</td>
<td>6.7</td>
<td>49</td>
<td>1.5</td>
</tr>
<tr>
<td>Warren</td>
<td>May 4, 2002</td>
<td>Nevin</td>
<td>39</td>
<td>6.1</td>
<td>25</td>
<td>1.3</td>
</tr>
</tbody>
</table>

† Routine soil tests from 0- to 15-cm soil depth samples collected before planting. Organic matter determined by dry combustion using LECO CHN 2000 analyzer and P determined using 1:1 soil/water ratio. STP is soil test P determined by Mehlich-3 P and STK is soil test K by ammonium acetate.

Table 2. Planting date, plant population, and tillage operation for the soybean and corn production years.

<table>
<thead>
<tr>
<th>Site</th>
<th>Soybean planting date</th>
<th>Soybean population</th>
<th>Corn planting date</th>
<th>Corn population</th>
<th>Tillage operation for corn and soybean years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boone</td>
<td>May 9, 2002</td>
<td>450,000</td>
<td>Apr 26, 2003</td>
<td>77,000</td>
<td>Field cultivation before corn and chisel plow before soybean.</td>
</tr>
<tr>
<td>Floyd</td>
<td>May 28, 2002</td>
<td>445,000</td>
<td>Apr 29, 2003</td>
<td>84,000</td>
<td>No-tillage with both crops</td>
</tr>
<tr>
<td>Tama</td>
<td>May 14, 2002</td>
<td>420,000</td>
<td>Apr 23, 2003</td>
<td>73,000</td>
<td>No-tillage with both crops</td>
</tr>
<tr>
<td>Warren</td>
<td>May 4, 2002</td>
<td>371,000</td>
<td>Apr 28, 2001</td>
<td>73,000</td>
<td>No-tillage with both crops</td>
</tr>
</tbody>
</table>
(°C) and volumetric soil moisture (cm^3 cm^-3) were measured outside the PVC rings concurrently during the soil CO2 emission measurements in the top 5 cm of soil surface using a thermometer unit attached to the LICOR-6400 and a TRIME-FM (Mesa Corporation, Medfield, MA) time domain reflectometry (TDR) system. Carbon dioxide emission data was downloaded into an Excel spreadsheet from the LICOR-6400 using the WinFX program included in the LICOR-6400 software package. Carbon dioxide emission rates were converted from μmol m^-2 sec^-1 to kg ha^-1 d^-1. Cumulative soil CO2 emissions as a function of time throughout the growing season were calculated as follows:

\[
\text{Cumulative CO}_2(t) = \sum_{i=1}^{n} \frac{X_i + X_{i+1}}{2} (t_{i+1} - t_i)
\]

where \(X_i\) is the first week CO2 measurement and \(X_{i+1}\) is the following week CO2 measurement at times \(t_i\) and \(t_{i+1}\), respectively; and \(n\) is the final week of CO2 measurement during the study period.

**Microbial Biomass Measurements**

Soil samples for MBC determination were collected in June 2003 from the four N rate treatments used for CO2 emission measurements when corn reached the V6 growth stage (Ritchie et al., 1993). A composite sample of the 0 to 15 cm of soil from each treatment within each replication was collected and placed in a 3.75-L plastic bag, placed in a cooler at 4°C, and kept at field moisture content. Sample soils were removed from the cooler and sieved through an 8-mm and then a 4-mm sieve. Soil samples were prepared immediately for fumigation and inorganic N extraction to minimize any changes to the microbial populations due to removal and transport from the field environment.

Microbial biomass C was determined within 24 h of soil sample collection using the procedure described by Horwath and Paul (1994). Fifty g moist soil samples were fumigated with ethanol-free CHCl3 for 24 h in a Labconco vacuum desiccator (Kansas City, MO). Both fumigated and non-fumigated soil samples were extracted with 100 mL of 0.5 mol L^-1 K2SO4 and then filtered through Whatman No. 42 filter paper (Whatman International Ltd., Maidstone, UK). The extractate was then placed into a 0.9-L wide-mouth glass jar (Mason jar) along with 125 mL Nalgene bottle along with 50 mL of 2 mol L^-1 KCl. The Nalgene bottles were placed on an Eberbach shaker for 30 min and after that the extraction solution was filtered through Whatman No. 42 filter paper into 20-mL scintillation vials.

**Statistical Analysis**

Data were analyzed using the Statistical Analysis System package (SAS Institute, 2003). Soil CO2 emission rates in response to N rate were analyzed using Proc MIXED and repeated measurement procedures, where sample collection date was treated as a nonrandom variable over time. Similarly, soil CO2 emission differences between N rates in the laboratory incubation were analyzed using the PROC GLM for each measurement date. The GLM procedure was also used to analyze N mineralization and microbial biomass response to N rate. Cumulative CO2 emissions data over the growing season were analyzed using the GLM procedure. Significant differences were determined at \(p = 0.05\).

**Results And Discussion**

**Carbon Dioxide Emission from Soil during Soybean Production**

The effect of prior year N fertilizer application rate for corn on CO2–C emission from soil was examined from the soybean crop year 2002 (2002). The CO2–C emission in situ may include CO2–C from soil organic matter and crop residue decomposition, and root respiration. In this discussion we will refer to CO2–C regardless of the source as soil CO2–C. The immediate effect of the prior-year N rate on soil CO2–C emission during the soybean year at each site is summarized in Fig. 1. Across all sites the soil CO2–C emission rate peaked during the most active soybean growth stage, 60 to 80 d after planting, which may indicate greater root respiration and thus more CO2 contribution. Generally, different N rates had no significant effect on CO2–C emission rate, except on a few days at each site. The peak in CO2–C emission rate appeared to be highly related to soil moisture and temperature conditions. The progressive increase in soil temperature and soil moisture content led to increased CO2–C emission rates. The residual effect of N applied in the corn phase had no lasting effect on CO2–C emission during
the soybean year, even at the peak period of CO$_2$–C emission. The lack of significant differences in CO$_2$–C release during the growing season between all N treatments coincides with the lack of significant change in soil C fractions (data not presented). The effect of N rate greater than 0 kg N ha$^{-1}$ on cumulative CO$_2$–C emission was also inconsistent across sites, where the N rate of 180 kg N ha$^{-1}$ at the Boone site showed the lowest CO$_2$–C emission compared to other N rates (Fig. 1). In contrast, no significant differences in cumulative CO$_2$–C emission were observed at the Floyd or Warren sites (Fig. 1).

It appears that the effect of N fertilization on soil CO$_2$–C emission is inconsistent and it is highly site-specific, which can be attributed to differences in soil conditions. Two of the factors that may have an influence on CO$_2$–C emission are soil moisture and soil temperature. However, simple regression correlation of CO$_2$–C emission rates and soil moisture or temperature indicated that soil moisture or temperature was not well correlated with CO$_2$–C emission ($R^2 = 0.11$ and $R^2 = 0.32$ at $p = 0.05$, respectively). These findings agree with those reported by Lou et al. (2004) and Buyanovsky and Wagner (1998), where they reported poor relationship between CO$_2$–C emission and soil moisture content. This poor relationship was attributed largely to soil moisture spatial variability and a shallow depth (0 to 5 cm) for soil moisture measurement, which is not deep enough to include the effect of root and microbial activities on CO$_2$–C emission (Franzluebbers et al., 1994; Lou et al., 2004).

The influence of N fertilization on soil microbial activity and CO$_2$–C emission is not well understood and results reported by other investigators are inconsistent. For example, N fertilization had no influence on soil microbial biomass in corn–soybean systems (Willson et al., 2001). In contrast, it was reported that soil microbial activity may increase due to N fertilization as a result of increased plant biomass production, which on incorporation stimulates soil biological activity (Dick, 1992). However, the lack of a significant effect of N application in the prior corn crop on CO$_2$–C emission during the soybean growing season coincided with the limited effect N fertilization had on soil C change over 3 yr in this study (data not presented).

**Carbon Dioxide Emission from Soil during Corn Production**

The soil CO$_2$–C emission rate during the corn phase of the corn–soybean rotation was significantly different between N rates.
At certain times, especially in the mid part of the growing season (Fig. 2), but had no effect at most measurement dates. Most often the greatest \( \text{CO}_2 \) emission rate was with zero N applied. The effect of N rate on \( \text{CO}_2 \) emission was inconsistent across the growing season. This inconsistency appeared to be related to soil moisture and temperature variability within the fields (Fig. 2). The overall trend of soil \( \text{CO}_2 \) emission rate was similar to that found during the soybean phase, where the maximum \( \text{CO}_2 \) emission was observed during the rapid vegetative growth stage (60 to 80 d after planting), which may indicate greater root respiration during such time in the growing season.

The N rate effect on season-long cumulative \( \text{CO}_2 \) emission was site-specific. At the Boone site, the cumulative \( \text{CO}_2 \) emission was lowest with the 180 kg N ha\(^{-1}\) rate, similar to that measured in the soybean phase (Fig. 1 and 2). Other N rates had no effect on cumulative \( \text{CO}_2 \) emission at that site. As observed during the soybean year, the cumulative \( \text{CO}_2 \) emission with different N rates at the Floyd site showed no significant differences. Nitrogen application decreased cumulative \( \text{CO}_2 \) emission at the Tama site (Fig. 2). Increasing N fertilizer application rate was site specific and had inconsistent effect on depressing \( \text{CO}_2 \) emission during the corn year (Fig. 2). These results in general are consistent with other findings of field and laboratory studies that indicate soil \( \text{CO}_2 \) emission was depressed with N fertilization (Kowalenko et al., 1978). However, several studies concluded that change in soil C dynamics is a function of interrelated factors that include crop rotation, soil type, soil moisture, and soil temperature among many, which affect soil C loss (Rochette et al., 1999; Moore et al., 2000; Pietikäinen et al., 2005).

### Soil Incubation and Microbial Biomass Carbon

To eliminate the corn root system contribution to \( \text{CO}_2 \) production in the soil system and to evaluate the effect of N fertilization application on microbial biomass, a laboratory incubation study was conducted. Soil \( \text{CO}_2 \) emission during the laboratory incubation revealed more consistent and clear differences between N rates than the in situ soil \( \text{CO}_2 \) measurements (Fig. 3). The initial soil \( \text{CO}_2 \) emission rate 1 d after soil incubation was not significantly different for the soil collected from the four field study N rate treatments at any of the three sites (Fig. 3). The trend for soil \( \text{CO}_2 \) emission rate with all N rates for soil from each site had a similar rapid
decrease in \( \text{CO}_2 - \text{C} \) emission rate within 7 d of beginning incubation. The greatest \( \text{CO}_2 - \text{C} \) emission rate, when significantly different between N rates, was with the zero N treatment.

The cumulative soil \( \text{CO}_2 - \text{C} \) emission for the entire incubation period was greatest with zero applied N and decreased as N rate increased (Fig. 3). This occurred with soil from all three sites. These findings are surprisingly consistent with the field soil \( \text{CO}_2 - \text{C} \) emission measurements (Fig. 2), especially in the corn year.

The decrease in soil \( \text{CO}_2 - \text{C} \) emission with higher N fertilization rates has been found by other investigators (Kowalenko et al., 1978; Ma et al., 1999), which is in agreement with our findings of N fertilization effect in decreasing \( \text{CO}_2 \) emission. Soil \( \text{CO}_2 \) flux in general can be an indicator of microbial activity during the oxidation of organic matter. However, the impact of N fertilization on such activities and \( \text{CO}_2 \) release has been reported inconsistent, with decrease (Biederbeck et al., 1984; Ladd et al., 1994, Smolander et al., 1994) or increase (Entry et al., 1996; Conti et al., 1997). Other investigators found that N fertilization may decrease soil respiration due to a change in soil pH (de Jong et al., 1974; Smolander et al., 1994; Ladd et al., 1994). Nitrogen fertilization can decrease soil pH in the short-term and over time due to nitrification of ammonium. However, soil pH with the N rate treatments in our study was not changed significantly in the short period of this study (data not presented). As observed with the N rate effect on \( \text{CO}_2 - \text{C} \) emission during this study, the differences in MBC concentrations between sites were inconsistent, and only one site (Floyd) showed significant difference in MBC concentrations between N fertilization rates (Fig. 4A). The greatest MBC concentration was associated with the 225 kg N ha\(^{-1}\) rate compared to other N rates at that site. It has been suggested that factors such as soil temperature, soil moisture, and soil fauna, among many others, can have a significant effect on the overall soil respiration in the field (de Jong et al., 1974; Dick, 1992). Nitrogen fertilization effects on soil microbial biomass were also evaluated by determining the potential mineral N supply through organic N mineralization in each soil (Fig. 4B). Except for the Tama site N application significantly increased net N mineralization. However, N rates greater than 90 kg N ha\(^{-1}\) did not increase net N mineralization in soil from the Boone or Tama site, but did in soil from the Floyd site. Some studies have noted no effect of N fertilization on the net N mineralization (Franzluebbers et al., 1994; Carpenter-Boggs et al., 2000), while others found decrease in net N mineralization (McAndrew and Malhi, 1992; Wienhold and Halvorson, 1999). Studies have also shown that symbiotic and asymbiotic N fixation decline with increasing soil inorganic N (Knowles and Denike, 1974; Stacey et al., 1992).
Across all sites and the two crops, soil CO$_2$–C emission rates peaked during the most active crop growth period. The different N fertilization rates had no consistent significant effect on soil CO$_2$–C emission rate regardless of site, although the zero N rate was greatest when significant. At some measurement dates, especially early- to mid-season, application of N significantly depressed emission rates. Season-long cumulative soil CO$_2$–C emission in the soybean production year was not generally affected by N application in the prior corn crop. During the soybean year, although the trend in soil CO$_2$–C emission rate over time was similar to that in the corn year, the cumulative CO$_2$–C emission from all N treatments was not significantly different at two of three sites, and only decreased with application of 90 kg N ha$^{-1}$ at one site. However, in the corn year N application at all rates decreased cumulative soil CO$_2$–C emission.

Soil in the third crop year had no change in MBC with N application at two sites, and only a significant increase at one site with the highest N rate. Soil CO$_2$–C emission in the soil incubation experiment revealed more consistent differences between the N rate effect on CO$_2$–C emission than with the field measurements. The greatest cumulative amount of soil CO$_2$–C emission was with no applied N, and decreased significantly as N rate increased. This trend was consistent for soil collected from all sites. Also, the net N mineralization increased with increasing N rate applied to the corn crop. The results of the field and incubation study show no short-term effect of N fertilization within the season of N application during the corn production year, and in the following year for soybean on CO$_2$–C release from soil. If there is any effect, it is both a reduced emission rate and reduced season-long cumulative emission of CO$_2$–C with N application.

**References**


