Research Article

Soil CO₂ Emissions and Drivers in Rice–Wheat Rotation Fields Subjected to Different Long-Term Fertilization Practices

Soil CO₂ emissions are key components of global carbon cycling. Hence, knowledge of their drivers is important for estimating and modifying carbon storage pools. To extend knowledge of these drivers, results of a long-term (31-year) experiment in a rice–wheat rotation field in subtropical Central China were analyzed to determine effects on soil CO₂ flux (F_{CO₂}) of various fertilization practices and related environmental factors including soil organic matter (SOM), microbial biomass carbon (MBC), total nitrogen (TN), total phosphorus (TP), and total potassium (TK) contents; activities of three soil enzymes (acid phosphatase, urease, and catalase); and soil temperature, pH, cation exchange capacity (CEC), bulk density and porosity. The results clearly show that F_{CO₂} was sensitive to changes in soil nutrient conditions under different long-term fertilization practices. Notably, it was significantly higher in plots receiving organic manure applications than in those receiving only chemical fertilizers (N, NP, and NPK) and unfertilized plots. Correlation analysis showed that F_{CO₂} was significantly correlated with the soil’s SOM, TN, TP, and MBC contents, acid phosphatase and urease activities, and several physicochemical soil properties including pH, CEC, bulk density and porosity. However, no significant correlations were observed between F_{CO₂} and either TK content or catalase activity. The findings suggest that at given temperature and soil moisture contents, variations in soil CO₂ emissions associated with the fertilization practices are strongly related to SOM, and the nutrient contents, microbial and enzyme activities, and physicochemical properties of the soil.

**Keywords:** Physicochemical soil properties; Soil CO₂ fluxes; Soil enzyme activity; Soil nutrients; Soil organic matter

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### 1 Introduction

Soil CO₂ fluxes (F_{CO₂}) are major contributors to total carbon emissions and significantly influence soil carbon stocks. Annual global CO₂ emissions from soils have doubled in recent decades, and anthropogenic soil emissions are now ca. 11 times higher than emissions from fossil fuel combustion [1]. At an annual scale, it has been reported by Balogh et al. [2] that the contribution of soil respiration reached 60–80% of the gross primary production and 40–60% of the ecosystem respiration. Hence, minor changes in soil CO₂ emissions have major impacts on annual increases in the atmospheric CO₂ concentration [3], and it is essential to understand the processes involved (in all major terrestrial ecosystems) to formulate appropriate management policies and technological procedures to reduce soil CO₂ emissions and enhance carbon sequestration.

However, soil CO₂ emissions are affected by complex interactions among diverse ecological processes, including (inter alia) the decomposition and mineralization of soil organic matter (SOM), and respiration of roots, associated microorganisms, and fauna [4, 5]. These (and other important biochemical processes in soils) are strongly influenced by temperature and water regimes. Hence, temperature is widely recognized as a key regulator of soil F_{CO₂}. It is exponentially correlated with F_{CO₂} [6], and the proportional change in respiration with a 10°C increase in temperature (Q_{10}) is an important variable, which reportedly ranges from 1 to 12 and is strongly affected by soil microbial community composition and plant phenology [7]. Similarly, soil moisture intensely affects plants’ photosynthetic and growth rates, ecosystem productivity, microbial activities, SOM decomposition, diverse soil variables including gas diffusion and chemical reaction rates, and (hence) soil CO₂...
emissions [8]. Generally, $F_{\text{CO}_2}$ tends to be maximal, or close to maximal, when the soil moisture content is around 50% of the water-holding capacity [9].

The cited studies, and numerous others, have provided abundant information on effects of soil temperature and moisture on CO$_2$ fluxes, but far less information is available on effects of other potentially important variables (e.g., soil microbial biomasses, nutrient contents, and enzyme activities). There is clearly a need to address this lack of knowledge, since (for instance) SOM is a major substrate for soil organisms, thus SOM contents and potential soil respiration rates are highly correlated [10]. Numerous soil microbial and enzyme activities are involved in SOM decomposition [11], and thus play significant roles in environmental control of $F_{\text{CO}_2}$. For instance, soil microbial biomass carbon (MBC) accounts for ca. 1.4% of total soil organic carbon and its turnover significantly contributes to global CO$_2$ emissions [12]. Moreover, growth rates and activities of soil microbes and plant roots are highly influenced by their nutrient (especially nitrogen, phosphorus, and potassium) status, and increasing nutrient supplies can strongly increase rates of numerous relevant processes in the soil [13]. For example, high nutrient contents are generally associated with dense microbial populations and hence high $F_{\text{CO}_2}$ [14]. However, much greater understanding of effects of nutrient contents on $F_{\text{CO}_2}$ (particularly in the vast areas covered by agricultural soils, where nutrient levels are generally high) is required [15].

We hypothesized that in addition to soil temperature and moisture, other biotic and abiotic factors, including SOM, and soil nutrient contents, microbial and enzyme activities, and physico-chemical factors may strongly influence $F_{\text{CO}_2}$ and its seasonal variations, under given climatic conditions. Moreover, adding fertilizers to soils can strongly stimulate microbial activities, and may thus induce increases in CO$_2$ emissions from agro-ecosystems. Thus, there is clear need to assess long-term effects of fertilization practices on diverse soil properties, associated emissions, and the sustainability of the systems. Hence, in the study presented here, seasonal $F_{\text{CO}_2}$ trends in plots under eight long-term fertilization treatments during wheat–rice rotations in central China were examined. We also explored associated changes in soil physical and chemical properties, and their correlations with the observed $F_{\text{CO}_2}$ trends.

## 2 Materials and methods

### 2.1 The long-term fertilization trial

The focal long-term trial was located at the Nanhu Agricultural Research Station (30°28’N, 114°25’E, 20 m above sea level), Hubei Academy of Agricultural Sciences, Wuhan, Hubei province, China. Soils were classified as hydromorphic paddy soils developed from yellow-brown soils. The experimental field is located in a typical area of the Yangtze River valley of subtropical China and has a humid mid-subtropical monsoon climate. The mean annual temperature, frost-free period, and precipitation are 17°C, 276 days, and 1300 mm, respectively. Most of the precipitation at the experimental site occurs between April and August.

Since initiation of the long-term trial, in 1981, a rice–wheat rotation cropping system has been applied in the experimental field, in which rice (*Oryza sativa* L.) has been grown from June to October each year, then wheat (*Triticum aestivum* L.) from November to May in the following year. At the start of the experiment, the soil had a pH of 7.8, and 1.1% organic matter, 1.8 g kg$^{-1}$ total nitrogen (TN), 5.0 mg kg$^{-1}$ available phosphorus, and 87.0 mg kg$^{-1}$ available potassium contents. Eight treatments with three replicates have been consistently applied in 24 plots ($8 \times 5 \text{m}^2$) at the site, involving applications of: no fertilizer (control) (CK), nitrogen (N), nitrogen and phosphorus (NP), nitrogen, phosphorus and potassium (NPK), manure (M), inorganic nitrogen and manure (N + M), nitrogen, phosphorus and manure (NP + M), and nitrogen, phosphorus, potassium, and manure (NPK + M). The average annual amounts of nitrogen, phosphorus, potassium, and manure applied in each treatment from 1981 to 2012 are given in Tab. 1. The manure (local commercial organic fertilizer) consisted of straw bedding impregnated with liquid and solid pig manure, which had 15.08 g kg$^{-1}$ nitrogen, 20.84 g kg$^{-1}$ P$_2$O$_5$, 13.56 g kg$^{-1}$ K$_2$O, and ca. 50% water content. The manure was applied in a 1:1 ratio (as basal dressings) and the mineral fertilizers in a 60:40 ratio during the rice and wheat cultivation periods. The phosphorus, potassium, and manure fertilizers were applied once as basal dressings during each of the wheat and rice cultivation periods, by evenly broadcasting them onto the soil surface and immediately incorporating them into the ploughed soil (0–20 cm depth) by tillage before sowing. The nitrogen fertilizer was applied with irrigation water at three stages of the rice cultivation period (basal, tillering, and panicle initiation) and three stages of the wheat period (basal, overwintering, jointing) using a hole-sowing machine in the furrows, in both cases in 2:1:1 splits.

### 2.2 Soil respiration measurement and temperature sensitivity analysis

$F_{\text{CO}_2}$ was determined using a cylinder static chamber of 22.5 cm diameter and 30 cm height. The rate of increase in CO$_2$ concentration within the chamber was monitored with an ACE (ADC Bioscientific Ltd.) automated soil CO$_2$ flux system. The ACE has a highly accurate CO$_2$ infrared gas analyzer housed directly inside the soil chamber, with no long gas tubing connecting the soil chamber and no separate analyzer. This ensures accurate and robust measurements, and the fastest possible response times to fluxes in gas exchange. $F_{\text{CO}_2}$ was measured at two locations in each plot on 23 clear days, between 8:00 and 12:00 a.m., from June 18, 2011 to May 30, 2012. Thus there were six replicate measurements per treatment on each occasion. To acquire these measurements, cylindrical collars were carefully installed to avoid disturbing roots and soil structure 2 weeks before the first determination, and left in situ during the entire study period. Furthermore, living plants (including their roots) within them were manually removed a day before the first determination.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Annual amount of nutrient input</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td>No fertilizer application</td>
</tr>
<tr>
<td>N</td>
<td>150 kg N ha$^{-1}$ year$^{-1}$</td>
</tr>
<tr>
<td>NP</td>
<td>150 kg N and 75 kg P$_2$O$_5$ ha$^{-1}$ year$^{-1}$</td>
</tr>
<tr>
<td>NPK</td>
<td>150 kg N, 75 kg P$_2$O$_5$ and 150 kg K$_2$O ha$^{-1}$ year$^{-1}$</td>
</tr>
<tr>
<td>M</td>
<td>22 500 kg manure ha$^{-1}$ year$^{-1}$</td>
</tr>
<tr>
<td>N + M</td>
<td>150 kg N and 22 500 kg manure ha$^{-1}$ year$^{-1}$</td>
</tr>
<tr>
<td>NP + M</td>
<td>150 kg N, 75 kg P$_2$O$_5$ and 22 500 kg manure ha$^{-1}$ year$^{-1}$</td>
</tr>
<tr>
<td>NPK + M</td>
<td>150 kg N, 75 kg P$_2$O$_5$, 150 kg K$_2$O and 22 500 kg manure ha$^{-1}$ year$^{-1}$</td>
</tr>
</tbody>
</table>

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before each flux measurement to avoid the respiration of aboveground plant parts confounding the results. Soil temperature was recorded regularly by the ACE analyzer unit at a depth of 2 cm.

The sensitivity of $F_{CO_2}$ to temperature was assessed by fitting data collected during the rice and wheat cultivation periods to the equation

$$F_{CO_2} = a e^{bT}$$

where $F_{CO_2}$, a, b are the soil CO$_2$ flux rate (µmol m$^{-2}$ s$^{-1}$), soil temperature (°C) at 2 cm depth, basal $F_{CO_2}$ (the intercept of the $F_{CO_2}$ vs. temperature curve at 0°C), and the temperature sensitivity of $F_{CO_2}$, respectively. $Q_{10}$ was calculated to describe the proportional rate of increase of $F_{CO_2}$ with a 10°C increase in temperature as follows:

$$Q_{10} = e^{10b}$$

### 2.3 Soil sampling and measurements

After the rice harvest in 28 September 2011, 24 samples of the 0–20 cm deep soil layer were taken from randomly chosen positions in each plot using a 5-cm diameter stainless steel soil sampler. All samples from each plot were carefully mixed to form a composite. A portion of each fresh composite sample was passed through a 0.25 mm sieve for MBC content and enzyme activity analyses. The remaining portions were air-dried in the laboratory then ground to pass through a 0.25 mm sieve for SOM, TN, total phosphorus (TP), and total potassium (TK) content analyses.

The soil chemical and physical properties were determined using routine analytical methods [16], briefly as follows. SOM was determined using the dichromate oxidation method, while TN was measured using a continuous flow analyzer (Skalar, the Netherlands) after Kjeldahl digestion. TP and TK contents were determined by the molybdate blue and KMnO$_4$ in the presence of H$_2$SO$_4$. The soil chemical and physical properties were determined using routine analytical methods [16], briefly as follows. SOM was determined using the dichromate oxidation method, while TN was measured using a continuous flow analyzer (Skalar, the Netherlands) after Kjeldahl digestion. TP and TK contents were determined by the molybdate blue and KMnO$_4$ in the presence of H$_2$SO$_4$.

### 2.4 Statistical analyses

One-way analysis of variance, ANOVA, was used to assess between-treatment differences in the measured variables. Then, correlation coefficient and regression analysis were applied to evaluate associations between $F_{CO_2}$ and the other measured variables, in conjunction with least significant difference, LSD, tests to detect significant ($p < 0.05$) between-treatment differences. For all these analyses, SPSS 16.0 software was used and all significant differences mentioned hereafter refer to the $p < 0.05$ level unless otherwise stated.

### 3 Results

#### 3.1 Seasonal variations in soil CO$_2$ emissions

During the rice cultivation period, $F_{CO_2}$ varied widely across the treatments, ranging from $-0.15$ to $10.71$ µmol m$^{-2}$ s$^{-1}$, but was generally low at the rice seedling stage, then gradually increased and peaked at the tillering stage (Fig. 1A; negative values indicate carbon sequestration and are associated with flooded, anaerobic conditions). During the wheat cultivation period, mean measured flux rates were consistently around $1–3$ µmol m$^{-2}$ s$^{-1}$ at the wheat seedling stage, then markedly increased from March and peaked in May (ranging from $3.36$ to $6.70$ µmol m$^{-2}$ s$^{-1}$). However, as shown in Fig. 1B, they were higher from plots receiving organic manure (M, N + M, NP + M, and NPK + M) than from plots receiving only chemical fertilizers (N, NP, and NPK) or the CK treatment (Fig. 1B). The fluxes from NP and NPK plots were also significantly stronger than those from CK plots. However, there was no significant difference in $F_{CO_2}$ between the N and CK treatments.

#### 3.2 Effects of long-term fertilization on the temperature sensitivity of soil $F_{CO_2}$

Exponential regression was applied to describe the relationship between $F_{CO_2}$ and temperature under each treatment throughout the rice and wheat cultivation periods (Tab. 2). Significant relationships between $F_{CO_2}$ and temperature during the wheat cultivation period under all eight treatments ($p < 0.05$) could be detected, but not under any treatment during the rice cultivation period, possibly because draining and flooding cycles strongly influence CO$_2$ emissions from paddy soils. Soil temperature accounted for 53.72–88.24% and 2.10–13.40% of the observed seasonal variations in $F_{CO_2}$ during the wheat and rice cultivation periods, respectively. Thus, since soil temperature appeared to influence $F_{CO_2}$ far more strongly under wheat cultivation conditions than under rice cultivation conditions, only $F_{CO_2}$ measurements taken during the wheat cultivation period were used to parameterize the $Q_{10}$ models. As shown in Tab. 2, the resulting models demonstrated that the fertilization treatments had differing effects on the $F_{CO_2}$ temperature sensitivity: mean $Q_{10}$ values were highest under the N and CK treatments (2.42 and 2.30, respectively; a non-significant difference), and significantly lower than the CK value under all the other treatments (1.94–2.10). The lack of a significant difference between the N and CK treatments in this respect may be at least partly due to nitrogen loading increasing the temperature sensitivity of fluxes before planting and during early growth stages, by increasing the decomposition rate of native SOM [15].

### 3.3 Correlations between soil $F_{CO_2}$ and soil nutrient contents

As shown in Tab. 3, mean SOM contents of the soil in plots receiving only chemical fertilizers (N, NP, and NPK) or no fertilizer (CK)
ranged from 31.23 to 34.87 g kg\(^{-1}\), while those of soils in plots receiving manure were significantly higher: 42.00–43.93 g kg\(^{-1}\). Similarly, TN and TP contents were significantly higher under the manure treatments, but there was no significant difference in TK contents between plots receiving fertilizer and control (CK) plots. In addition, simple correlation analyses detected significant positive correlations between \(F_{\text{CO}_2}\) rates and SOM (Fig. 2A, \(R^2 = 0.9320, p = 0.0001\)), TN (Fig. 2B, \(R^2 = 0.8941, p = 0.0004\)), and TP (Fig. 2C, \(R^2 = 0.8755, p = 0.0006\)), but not TK contents (Fig. 2D). These findings clearly indicate that soil SOM, TN, and TP contents influence \(\text{CO}_2\) emissions more strongly than TK contents at the study site.

Table 2. Parameters for the exponential equation (\(F_{\text{CO}_2} = ae^{bT}\)) describing the relationship between soil \(\text{CO}_2\) flux (\(F_{\text{CO}_2}\)) and soil temperature, and apparent \(Q_{10}\) values (\(Q_{10} = e^{10b}\)) associated with all fertilization treatments, during the rice and wheat cultivation periods

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rice cultivation period</th>
<th></th>
<th>Wheat cultivation period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(a)</td>
<td>(b)</td>
<td>(R^2)</td>
</tr>
<tr>
<td>CK</td>
<td>0.1346</td>
<td>0.0792</td>
<td>0.0909</td>
</tr>
<tr>
<td>N</td>
<td>0.0836</td>
<td>0.1022</td>
<td>0.1340</td>
</tr>
<tr>
<td>NP</td>
<td>0.2997</td>
<td>0.0650</td>
<td>0.0293</td>
</tr>
<tr>
<td>NPK</td>
<td>0.1677</td>
<td>0.0817</td>
<td>0.0545</td>
</tr>
<tr>
<td>M</td>
<td>0.1362</td>
<td>0.1040</td>
<td>0.1185</td>
</tr>
<tr>
<td>N + M</td>
<td>0.6417</td>
<td>0.0438</td>
<td>0.0210</td>
</tr>
<tr>
<td>NP + M</td>
<td>1.1098</td>
<td>0.0189</td>
<td>0.0380</td>
</tr>
<tr>
<td>NPK + M</td>
<td>0.5824</td>
<td>0.0513</td>
<td>0.0341</td>
</tr>
</tbody>
</table>

*\(p < 0.05\), **\(p < 0.01\).
3.4 Correlations between soil F_{CO2} and biological soil factors

To assess the potential biological contribution of fertilization to CO2 emissions, mean F_{CO2} values recorded during the experimental period were plotted against MBC and activities of three soil enzymes (acid phosphatase, catalase, and urease). Microbial biomass is the living, active fraction of the SOM, and accumulates together with SOM during soil development. Hence, the 30-year applications of inorganic fertilizer and manure had resulted in strong increases in the soil MBC contents: 110.7, 133.2, 136.0, 148.8, 22.5, 68.4, and 61.5% increases under the M, N + M, NP + M, NPK, NP, and NPK plots, relative to the CK treatment, respectively (Tab. 4). In addition, correlation analysis showed a significant relationship between F_{CO2} and MBC (Fig. 3A, R^2 = 0.9386, p < 0.0001).

### Table 3. Soil organic matter (SOM) and total nitrogen (TN), phosphorus (TP), and potassium (TK) contents under the eight fertilizer treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SOM (g kg^{-1})</th>
<th>TN (g kg^{-1})</th>
<th>TP (g kg^{-1})</th>
<th>TK (g kg^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td>31.24 ± 0.97e</td>
<td>1.82 ± 0.15f</td>
<td>1.27 ± 0.23g</td>
<td>24.58 ± 2.00a</td>
</tr>
<tr>
<td>N</td>
<td>33.33 ± 2.25h</td>
<td>2.09 ± 0.13e</td>
<td>1.68 ± 0.31d</td>
<td>24.71 ± 1.51a</td>
</tr>
<tr>
<td>NP</td>
<td>34.87 ± 0.94b</td>
<td>2.06 ± 0.13e</td>
<td>2.45 ± 0.26c</td>
<td>24.68 ± 1.75a</td>
</tr>
<tr>
<td>NPK</td>
<td>34.41 ± 0.91b</td>
<td>2.35 ± 0.25d</td>
<td>2.60 ± 0.44bc</td>
<td>26.37 ± 1.82a</td>
</tr>
<tr>
<td>M</td>
<td>43.37 ± 1.01d</td>
<td>2.68 ± 0.37c</td>
<td>2.82 ± 0.41b</td>
<td>25.46 ± 2.93a</td>
</tr>
<tr>
<td>N + M</td>
<td>42.00 ± 0.74a</td>
<td>2.88 ± 0.44bc</td>
<td>2.79 ± 0.51b</td>
<td>25.20 ± 2.02a</td>
</tr>
<tr>
<td>NP + M</td>
<td>43.23 ± 0.99a</td>
<td>2.94 ± 0.30ab</td>
<td>3.34 ± 0.37a</td>
<td>24.67 ± 1.05a</td>
</tr>
<tr>
<td>NPK + M</td>
<td>43.93 ± 2.49a</td>
<td>3.09 ± 0.06a</td>
<td>3.46 ± 0.62a</td>
<td>24.94 ± 1.66a</td>
</tr>
</tbody>
</table>

Values are means ± standard error of means (n = 3). Different letters within columns indicate significant differences between treatments at p < 0.05.

**Figure 2.** Dependence of soil CO2 flux on soil organic matter (A), and total nitrogen (B), phosphorus (C), and potassium (D) contents under the eight treatments. Error bars indicate twice the standard error of the mean.
Soil acid phosphatase, urease, and catalase activities were measured partly because they have potential utility as bioindicators of soil fertility [13]. Acid phosphatase activities associated with the eight treatments declined in the following order: NPK > NP > M > N > CK, but some of these differences were non-significant (Tab. 4). Similarly, mean urease activities (which ranged from 1.44 to 2.99 NH₃-N mg g⁻¹ h⁻¹) were higher under M, N + M, NP + M, and NPK + M treatments than under treatments including no manure application (Tab. 4). In contrast, there was no clear treatment-related pattern in measured catalase activities (Tab. 4). Significant positive correlations were detected between FCO₂ and activities of both acid phosphatase (Fig. 3B, \(R^2 = 0.8445, p = 0.0008\)) and urease (Fig. 3D, \(R^2 = 0.8479, p = 0.0012\)), but no significant correlations were detected between FCO₂ and catalase activity (Fig. 3C). The apparently strong positive effects of phosphatase and urease activities on FCO₂ may be related to their Table 4. Microbial biomass carbon (MBC) and activities of acid phosphatase, catalase, and urease under the eight fertilizer treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MBC (mg kg⁻¹)</th>
<th>Acid phosphatase (P₂O₅ mg kg⁻¹ h⁻¹)</th>
<th>Catalase (0.1 M KMnO₄ g⁻¹ h⁻¹)</th>
<th>Urease (NH₃-N mg g⁻¹ h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td>1148 ± 111²</td>
<td>75.04 ± 12.76³</td>
<td>14.63 ± 2.08⁵</td>
<td>1.51 ± 0.16⁶</td>
</tr>
<tr>
<td>N</td>
<td>1406 ± 48⁴</td>
<td>78.72 ± 2.55⁶</td>
<td>14.48 ± 5.41⁵</td>
<td>1.44 ± 0.12⁷</td>
</tr>
<tr>
<td>NP</td>
<td>1933 ± 80⁸</td>
<td>114.33 ± 16.74⁹</td>
<td>15.44 ± 5.73⁷</td>
<td>1.73 ± 0.19⁸</td>
</tr>
<tr>
<td>NPK</td>
<td>1854 ± 12⁰</td>
<td>125.14 ± 10.21²</td>
<td>13.67 ± 1.25⁵</td>
<td>1.45 ± 0.26⁶</td>
</tr>
<tr>
<td>M</td>
<td>2417 ± 125²</td>
<td>143.81 ± 9.20⁶</td>
<td>18.06 ± 2.5²b</td>
<td>2.73 ± 0.24¹</td>
</tr>
<tr>
<td>N + M</td>
<td>2676 ± 151¹b</td>
<td>165.50 ± 15.32⁶</td>
<td>21.72 ± 1.51¹b</td>
<td>2.78 ± 0.31¹a</td>
</tr>
<tr>
<td>NP + M</td>
<td>2708 ± 28¹a</td>
<td>186.13 ± 13.00⁴</td>
<td>28.69 ± 1.51¹a</td>
<td>2.75 ± 0.31¹a</td>
</tr>
<tr>
<td>NPK + M</td>
<td>2855 ± 177⁷</td>
<td>188.58 ± 18.41⁴</td>
<td>19.31 ± 3.02²bc</td>
<td>2.99 ± 0.35³a</td>
</tr>
</tbody>
</table>

Values are means ± standard error of means (n = 3). Different lowercase letters within columns indicate significant differences between treatments at \(p < 0.05\).

Soil acid phosphatase, urease, and catalase activities were measured partly because they have potential utility as bioindicators of soil fertility [13]. Acid phosphatase activities associated with the eight treatments declined in the following order: NPK + M > NP + M > N + M > M > NPK > N > CK, but some of these differences were non-significant (Tab. 4). Similarly, mean urease activities (which ranged from 1.44 to 2.99 NH₃-N mg g⁻¹ h⁻¹) were higher under M, N + M, NP + M, and NPK + M treatments than under treatments including no manure application (Tab. 4). In contrast, there was no clear treatment-related pattern in measured catalase activities (Tab. 4). Significant positive correlations were detected between FCO₂ and activities of both acid phosphatase (Fig. 3B, \(R^2 = 0.8445, p = 0.0008\)) and urease (Fig. 3D, \(R^2 = 0.8479, p = 0.0012\)), but no significant correlations were detected between FCO₂ and catalase activity (Fig. 3C). The apparently strong positive effects of phosphatase and urease activities on FCO₂ may be related to their activities.
respective roles in increasing the availability of phosphorus and nitrogen substrates for soil biota.

### 3.5 Correlations of soil FCO2 with soil pH, CEC, bulk density and porosity

As shown in Tab. 5, under the eight treatments mean soil pH values ranged from 6.9 to 7.3, CEC from 20.24 to 23.68 cmol kg⁻¹, bulk density from 1.23 to 1.39 mg cm⁻³, and porosity from 47.47 to 53.58%. Soil CEC and porosity were significantly higher under the manure treatments than under the control treatment, but there were no significant between-treatment differences in either soil pH or bulk density. Significant negative correlation were found between FCO2 and both soil pH (Fig. 4A, \( R^2 = 0.7916, p = 0.0031 \)) and bulk density (Fig. 4C, \( R^2 = 0.7230, p = 0.0075 \)), and significant positive correlations between FCO2 and both soil CEC (Fig. 4B, \( R^2 = 0.8723, p = 0.0007 \)) and porosity (Fig. 4D, \( R^2 = 0.7231, p = 0.0075 \)).

### 4 Discussion

The main factors explaining seasonal variations in FCO2 during the rice–wheat rotation at the site evaluated in this study were the crop regime, soil temperature, and water management practices. During the rice cultivation period, flooding and draining cycles showed the highest potential for controlling CO2 emissions. FCO2 was severely restricted during the submerged period of paddy rice cultivation, particularly at the rice seedling stage, presumably because the flooding severely reduced oxygen supplies from the atmosphere and hence respiration. These changes in FCO2 are consistent with previously reported effects of intermittent flood-drainage cycles in paddy fields [20]. In the cited study, CO2 emissions were highest during the rice tillering stage, seasonal increases in CO2 fluxes were closely related to increases in root production and biomass, and FCO2 was maximal during a pulse of emissions shortly after removal of the floodwater (a strong diffusion barrier). During the wheat cultivation period, across the eight treatments mean FCO2 values ranged from 1 to 6.7 μmol m⁻² s⁻¹, and were largely governed by time (and thus the crop’s developmental stage), moisture, and temperature (all of which were positively correlated with the fluxes). The strong correlation between crop development and FCO2 is consistent with expectations, as it is closely linked to photosynthesis and respiration rates [15].

The temperature sensitivity of FCO2 (\( Q_{10} \)) is known to be one of its main determinants and is influenced (inter alia) by soils’ nutrient status, water contents, prevailing climatic conditions, and ecosystem type [6, 7]. In order to obtain robust estimates of the variable, many researchers have calculated \( Q_{10} \) values for various soils. For example, Jia et al. [21] found that \( Q_{10} \) of FCO2 ranged from 1.54 to 2.08 in temperate semiarid grasslands of northern China, and Tu et al. [22] recorded values of 2.87, 3.09, and 3.19 in the litter layer, root-free soil, and rhizosphere, respectively, in a subtropical bamboo ecosystem. In the present study, it could also be shown that FCO2 was significantly exponentially correlated with soil temperature under wheat cultivation conditions under all eight treatments (Tab. 2). However, no significant relationships were detected between FCO2 and temperature under rice cultivation conditions. Soil temperature accounted for 53.7–88.2% and 2.10–13.4% of the variations in FCO2 during the wheat and rice cultivation periods, respectively. According to the derived exponential equations, \( Q_{10} \) values during the wheat cultivation period ranged between 1.94 and 2.42. It could also be seen that \( Q_{10} \) values strongly differed among treatments, and were significantly lower under all of the fertilization treatments, except N, than under the control (CK) treatment. The results of this study, together with previous findings, indicate that fertilization practices generally reduce the \( Q_{10} \) of FCO2, but as Ding et al. [15] suggested, adding nitrogen fertilizers may induce strong increases in \( Q_{10} \) values of unplanted soils by increasing native SOM decomposition rates and slightly reduce the dependence of FCO2 on soil temperature.

The effects of long-term fertilization on FCO2 might be due to its effects on soil quality factors that affect carbon transformations in the soil. For example, associated increases in nutrient contents and availability could enhance CO2 fluxes by inducing increases in root respiration rates and microbial activities [13, 15, 23]. In the present study, FCO2 rates were 89–118% higher from plots under manure treatments than from unfertilized plots. The enhancement of FCO2 rates by manuring could be explained by associated increases in both the quality and quantity of substrates for soil respiration, induced by the release of nutrients and biological activities in soils [12]. It could also be shown in this study that applications of chemical fertilizers (N, NP, and NPK) increased FCO2 by increasing soil nutrient contents and biological activities, including activities of enzyme involved in nitrogen and phosphorus cycles. Similarly, Lijierhoft et al. [24] showed that high doses of nitrogen fertilizers may stimulate soil CO2 fluxes in a loamy sand soil planted with wheat. In contrast, adding nutrients such as nitrogen, phosphorus, potassium,
calcium, magnesium, and sulfur through mineral fertilizers may reportedly diminish soil $F_{CO_2}$ in boreal forests, by reducing microbial biomass and fine root production [25, 26]. Thus, the effects are complex, and influenced by numerous factors.

Soil nutrients reportedly influence $F_{CO_2}$ by affecting both heterotrophic and autotrophic respiration processes [14, 25], but these variables have been considered less frequently than soil temperature and moisture in analyses of environmental controls of $F_{CO_2}$. However, general linear regression analysis of the data showed that SOM, TN, and TP accounted for about 93, 89, and 87% of the annual $F_{CO_2}$ variation, respectively. In contrast, no significant correlations were observed between $F_{CO_2}$ rates and TK. The findings indicate that the presence of organic matter with nitrogen and phosphorus could induce higher increases in $F_{CO_2}$ than carbon alone, possibly through the inorganic nutrients stimulating root respiration [27], and the organic matter stimulating the release of native substrates and soil nutrients through its decomposition [12]. Accordingly, the increases in soil CO2 emissions associated with long-term fertilization observed here in the wheat–rice rotation field could be attributed to its direct stimulatory effects on plant root and soil biota activities.

All of the measured biological properties, except catalase activity, correlated significantly with $F_{CO_2}$ (Fig. 3), supporting the prediction that the soil MBC content and activities of soil enzymes involved in carbon, nitrogen, and phosphorus cycles could be important drivers of $F_{CO_2}$ emissions. These positive correlations are consistent with the findings cited above regarding effects of organic and inorganic fertilizer applications [12, 27]. They are also consistent with indications that adding organic matter (manure or plant residues) can strongly increase soil microbial biomass and enzyme activities [28], through the links between SOM levels, availability of carbon and nitrogen substrates, microbial and enzyme activities, and (hence) $F_{CO_2}$ [13]. For example, strong positive correlations have been found between acid phosphatase and urease activities, soil microbial biomass, and $F_{CO_2}$ [29], partly because secretion of the enzymes by microorganisms and plants is regulated by soil phosphorus, nitrogen, and carbon availability. Thus, activities of both of these enzymes have been frequently used as indicators of overall microbial activity [30]. In contrast, no significant correlations between $F_{CO_2}$ and catalase activity was observed, but this is also consistent with expectations as catalase is an intracellular oxidoreductase [31] that it is not directly involved in soil carbon and nitrogen cycling.

Long-term fertilization at this study site also influenced $F_{CO_2}$ by affecting soil pH, CEC, and the chemical stability of organic matter. Negative correlations between $F_{CO_2}$ and pH were found because the

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**Figure 4.** Dependence of soil CO2 flux on soil pH (A), cation exchange capacity (CEC) (B), bulk density (C), and porosity (D) under the eight treatments. Error bars indicate twice the standard error of the mean.
plots with high SOM content (positively related to $F_{\text{CO}_2}$) were associated with low pH values. Indeed, the soil pH was slightly (but not significantly) lower under the manure treatments than under the control treatment, probably due to release of organic acids from the manure. In contrast, $F_{\text{CO}_2}$ was significantly ($p = 0.0007$) and positively correlated with CEC, because the increases in organic matter contents induced by the fertilization treatments also increased CEC. This is also consistent with expectations since the treatments could provide numerous cation binding sites through both the decomposition of added organic matter [32], and stimulation of the decomposition of native organic matter [33]. These significant correlations also suggest that the accumulation and stabilization of organic matter influence $F_{\text{CO}_2}$.

In soils, $\text{CO}_2$ migrates to soil pores before emission into the atmosphere. Thus, soil bulk density, porosity, and both air permeability and gas diffusion coefficients also influence $F_{\text{CO}_2}$ [34]. The $F_{\text{CO}_2}$ values recorded here were negatively correlated with soil bulk density but positively correlated with total porosity, corroborating previous findings. Furthermore, the long-term fertilization treatments reduced the soil’s bulk density, and the manure treatments both reduced its bulk density most strongly, and increased total porosity more than the mineral fertilizer and control treatments. Increases in biological activities associated with high porosity and low density also probably contributed to increases in $\text{CO}_2$ fluxes from the soil.

5 Concluding remarks

It can be concluded that variations in $F_{\text{CO}_2}$ in the studied rice–wheat rotation field were strongly affected by the long-term fertilization practices, through associated and highly interactive changes in the soil’s nutrient contents, physicochemical properties, and biological activities. The results showed that $F_{\text{CO}_2}$ was significantly correlated with the soil’s TN, TP, and MBC contents, acid phosphatase and urease activities, physicochemical factors (e.g., pH, CEC, bulk density and porosity), and SOM. However, no significant correlations were detected between $F_{\text{CO}_2}$ and either the soil’s TK content or catalase activities. The findings suggest that suitable modification of long-term fertilization practices could potentially be used to ameliorate $\text{CO}_2$ emissions and increase soil carbon stocks via their effects on these variables.

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