Major Biogeochemical Processes in Soils—A Microcosm Incubation from Reducing to Oxidizing Conditions

Kewei Yu*
Wetland Biogeochemistry Institute
School of the Coast and Environment
Louisiana State Univ.
Baton Rouge, LA 70803

And:
Institute of Applied Ecology
Chinese Academy of Sciences
Shenyang 110016
China

Frank Böhme
Helmholtz Centre for Environmental Research–UFZ
Dep. of Soil Chemistry
Theodor-Lieser-Str. 4
06120 Halle/Saale
Germany

Current Address:
KataLeuna GmbH Catalysts
Am Haupttor
06237 Leuna
Germany

Jörg Rinklebe
Helmholtz Centre for Environmental Research–UFZ
Dep. of Soil Chemistry
Theodor-Lieser-Str. 4
06120 Halle/Saale
Germany

Current Address:
Univ. of Wuppertal
Dep. D, Soil and Groundwater Management
Pauluskirchstraße 7
42285 Wuppertal
Germany

Heinz-Ulrich Neue
Helmholtz Centre for Environmental Research–UFZ
Dep. of Soil Chemistry
Theodor-Lieser-Str. 4
06120 Halle/Saale
Germany

Ronald D. DeLaune
Wetland Biogeochemistry Institute
School of the Coast and Environment
Louisiana State Univ.
Baton Rouge, LA 70803

Six soils used for rice (Oryza sativa L.) production were incubated using an automatic microcosm system. Production of trace gases (CO₂, CH₄, and N₂O) and transformation of N, S, and metals (Fe and Mn) were studied in soil suspensions incubated from reducing to oxidizing conditions. Results show that soil pH variation was inversely correlated to soil redox potential (Eₐ) change (P < 0.01). Soil CO₂ production exponentially increased with soil Eₐ increase. In contrast, soil CH₄ production and DOC showed an exponential decrease with soil Eₐ increase. Without the presence of soil oxidants, methanogenesis occurred across the entire Eₐ range, with probable H₂-supported methanogenesis at higher soil Eₐ conditions constituting up to 20% of total CH₄ production. The CH₄ compensation point, where CH₄ concentration became constant due to equilibrium between CH₄ production and consumption, exponentially decreased with soil Eₐ increase. At pH 7, the critical Eₐ above which soils consumed atmospheric CH₄ varied among the soils, but was generally >400 mV. Significant N₂O production was observed between 200 and 500 mV. Nitrification could also contribute to N₂O production when Eₐ is >500 mV, a possible critical Eₐ for the initiation of nitrification. The critical Eₐ for substantial immobilization of Fe and Mn was estimated to be around 50 and 250 mV, respectively. The intermediate Eₐ range (approximately –150 to 180 mV) provided optimum conditions for minimizing cumulative global warming potential resulting from CO₂, CH₄, and N₂O production in soils. Our results have implications in interpreting the overall benefits of soil C sequestration efforts.

Abbreviations: DOC, dissolved organic carbon; Eₐ, redox potential; GC, gas chromatograph; OM, organic matter.

Oxidation and reduction reactions regulate many biogeochemical reactions in Earth surface environments. The intensity of soil reduction can be rapidly characterized by soil oxidation–reduction (redox) potential (Eₐ), which allows the prediction of the stability and availability of various nutrients and metal elements in soils and sediments. Soils tend to undergo a series of sequential redox reactions in a homogenous environment when soil redox status changes from aerobic (high Eₐ) to anaerobic (low Eₐ) conditions. Major reactions include, in order of Eₐ from high to low, nitrification, denitrification, Mn(IV) reduction, Fe(III) reduction, SO₄²⁻ reduction, and methanogenesis (Patrick and DeLaune, 1972; Ponnampерuma, 1972; Smith and DeLaune, 1984; Reddy et al., 1989; Patrick and Juguindia, 1992). Meanwhile, soil respiration going from aerobic to anaerobic conditions results in CO₂ production across the entire Eₐ range. Although the reduction reactions proceed in a thermodynamic order (Ponnampemura, 1972; Patrick and Reddy, 1978), the given oxidation–reduction system is only partially applicable to field conditions, because the mineral phases present in soils are mixed and often unknown. Changes in pH and activities of reactants and products can also alter the order of redox reactions. As a consequence, reduction potentials of a given redox reaction can span a wide range along the redox scale. Chemical reactions that are favored thermodynamically are not necessarily favored kinetically. The lack of effective coupling and the slowness of redox reactions mean that catalysis is required if equilibrium is to be attained. In soils, the catalysis of redox reactions is mediated by microorganisms. Equilibrium
depends entirely on the growth and ecological behavior of the soil microbial population and the degree to which the reactants and products can diffuse and mix. Most of the information on soil redox processes has been obtained from flooded rice systems, but applies to natural wetland soils, and probably upland soils as well (Yu et al., 2001).

Wetland rice ecosystems are a unique aerobic and anaerobic environment. In wetland rice soils, two distinct aerobic–anaerobic interfaces have been identified: (i) the water–soil interface that receives sufficient O2 from the floodwater (Patrick and DeLauney, 1972)—the thickness of the layer may range from several millimeters to several centimeters depending on perturbation by soil fauna and the percolation rate of water; and (ii) the plant rhizosphere maintained by O2 diffusing through the aerenchyma of rice plants (Reddy et al., 1989). Redox processes play an important role in soil nutrient availability, biogeochemical cycling of elements, and ecological functions of rice ecosystems. Carbon dioxide, CH4, and NO3 are the most important atmospheric trace gases that contribute to the global greenhouse effect. Biological NO3 can be produced from nitrification under aerobic conditions, and denitrification under moderately reducing conditions where the reducing condition is not intense enough to completely reduce NO3 to N2 gas. Denitrification is the final step of the N cycle by which atmospheric N2 fixed in the biosphere returns to the N2 pool. Significant CH4 formation (methanogenesis) in soils generally occurs under strictly reducing conditions when soil redox potential decreases below a critical point. Rice fields have been the most studied methanogenic ecosystem because of their economical importance and high potential as an atmospheric CH4 source. Culturable microorganisms associated with CH4 and NO3 dynamics were found to be strongly related with key edaphic soil properties (i.e., pH, C/N ratio) in soils (Kravchenko and Yu, 2006). Transformation of Mn, Fe, and S between their oxidized and reduced forms can significantly affect N2O and CH4 dynamics in soils (Yu and Patrick, 2004).

Almost all reported information on major redox processes has been obtained by incubating soils in a direction going from oxidizing to reducing conditions. This is analogous to flooding a thoroughly drained soil where all soil redox-active components are in their oxidized forms. During the incubation, however, soil microbial communities and enzyme activities for anaerobic processes may develop progressively, which ultimately influences the dynamics of various redox reactions. For example, N2O production is significantly affected by the development of denitrifying enzymes during the incubation, especially the N2O reduction enzyme that controls the N2O/N2 ratio (Rudaz et al., 1991; Dendooven and Anderson, 1995). In this study, the soil incubation was initiated under oxidizing conditions, continuing until reducing conditions (Phase I) developed, which allowed anaerobic microbial activities to fully function and soil redox-active components to be transformed to their reduced forms. In the subsequent reducing to oxidizing phase of the incubation (Phase II), major soil biogeochemical processes were analyzed at different soil redox conditions, including nitrification, denitrification, methanogenesis, methanotrophy, and transformation of Fe, Mn, and S. This was an analog to draining a long-flooded rice field, with the results complementing the previous information obtained by incubating soils from oxidizing to reducing conditions.

MATERIALS AND METHODS

Soil Sampling

Six soils (surface 20 cm) were collected from four major rice-cultivating states in the USA (Arkansas, California, Louisiana, and Texas), and from two Asian regions: Hangzhou (China) and Java (Indonesia). The soils were air dried, sieved (1-mm sieves), thoroughly mixed, and stored at room temperature (20°C) before the experiment. Major soil characteristics were analyzed and are provided in Table 1.

Description of the Soil Microcosm System

Soils were incubated using an advanced microcosm system, which allows continuous monitoring and control of soil Eh, pH, and temperature in soil suspensions (Fig. 1). Soil Eh was maintained within a specific range by adding N2 (to lower Eh) and O2 (to raise Eh) through an automatic-valve gas regulation system. All microcosm systems were connected to a gas chromatograph (GC) via a computer-operated valve–pipe system. Thus, gas concentration in the headspace of each microcosm was automatically quantified.

Soil Incubation and Measurement

In total, 12 microcosm systems were used, allowing for two replicates of each soil except for the Louisiana and China soils (not replicated due to limited amounts of soil sample). Soil suspension was established by adding 200 g dry soil to a 2.88-L microcosm vessel with 1.6 L of deionized water. To each soil suspension, 5 g

<table>
<thead>
<tr>
<th>Soil</th>
<th>Classification†</th>
<th>pH</th>
<th>OM‡</th>
<th>Total N</th>
<th>Sand</th>
<th>Silt</th>
<th>Clay</th>
<th>Fe§</th>
<th>Mn§</th>
<th>S§</th>
<th>Na§</th>
<th>K§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arkansas</td>
<td>Alfisols</td>
<td>6.0</td>
<td>14.6</td>
<td>0.7</td>
<td>41</td>
<td>809</td>
<td>150</td>
<td>134</td>
<td>105</td>
<td>13</td>
<td>88</td>
<td>142</td>
</tr>
<tr>
<td>California</td>
<td>Entisols</td>
<td>6.7</td>
<td>40.8</td>
<td>1.6</td>
<td>33</td>
<td>366</td>
<td>601</td>
<td>224</td>
<td>107</td>
<td>45</td>
<td>431</td>
<td>305</td>
</tr>
<tr>
<td>Louisiana</td>
<td>Alfisols</td>
<td>7.3</td>
<td>16.7</td>
<td>0.7</td>
<td>143</td>
<td>731</td>
<td>126</td>
<td>68</td>
<td>19</td>
<td>11</td>
<td>185</td>
<td>104</td>
</tr>
<tr>
<td>Texas</td>
<td>Alfisols</td>
<td>5.1</td>
<td>25.4</td>
<td>1.1</td>
<td>75</td>
<td>284</td>
<td>641</td>
<td>115</td>
<td>35</td>
<td>38</td>
<td>140</td>
<td>291</td>
</tr>
<tr>
<td>China</td>
<td>Ultisols</td>
<td>5.6</td>
<td>46.4</td>
<td>2.7</td>
<td>122</td>
<td>470</td>
<td>408</td>
<td>211</td>
<td>280</td>
<td>65</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Indonesia</td>
<td>Andisols</td>
<td>5.3</td>
<td>23.7</td>
<td>1.0</td>
<td>12</td>
<td>408</td>
<td>408</td>
<td>211</td>
<td>280</td>
<td>65</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

† U.S. Soil Taxonomy classification: the Arkansas soil is a Stuttgart soil (fine, smectitic; thermic Albaquultic Hapludalf); the California soil is a Willows clay (fine, smectitic, thermic Sodic Endoaquert); the Louisiana soil is a Crowley silt loam (fine, smectitic, thermic Typic Albaqualf); the Texas soil is a Beaumont clay (fine, smectitic, hyperthermic Chronic Dystraquef); No classification information is available for the Chinese and Indonesian soils.

‡ Organic matter.

§ Mn, Fe, S, K, and Na contents were analyzed after extracting the soils with diethylene triamine pentaacetic acid (DTPA) solution.

¶ Not determined.
min. A single Pt electrode with a Ag–AgCl reference electrode was used for the E_h measurement. Original soil NO_3^− was completely denitrified during this earlier phase of the incubation. In the subsequent oxidizing phase (Phase II) of the incubation, soil E_h was stepwise elevated to a specific E_h value by providing O_2 to the microcosms. Soil E_h and pH were continuously monitored and recorded. To provide a N source for denitrification, 6 mL of 0.1 M NO_3−N as KNO_3 was added to each microcosm when soil E_h reached moderately reducing conditions (E_h approximately 0 mV at pH 7). Nitrate was added only once because it can significantly buffer soil redox conditions. Concentrations of CO_2, CH_4, and N_2O in the microcosm headspace were quantified every 2 h. At selected E_h levels, soil suspension was withdrawn (20 mL each time) from each microcosm, and was immediately filtered in an N_2 atmosphere through a 0.45-µm Millipore membrane (Whatman Inc., Florham Park, NJ) into two 10-mL test tubes. One subsample was used immediately to monitor concentrations of dissolved organic carbon (DOC), NO_3−, and NH_4^+. For the other subsample, three drops of 2 M HNO_3 was added to preserve the solution for later analysis of Na^+, K^+, soluble Mn^{2+}, Fe^{2+}, and S (mainly in form of SO_4^{2−}). The microcosm was flushed with N_2 after a series of gas measurements and after sampling the soil suspensions. Changes in soil mass, water, and headspace volume in the microcosm were considered in calculations while soil/water ratio remained the same in the microcosms.

Sample Analysis

Initial soil pH was measured in a soil/water (1:1) slurry. Soil OM was measured colorimetrically after oxidizing with K_2Cr_2O_7 and concentrated H_2SO_4. Soil total N was analyzed in dry combustion by a Leco N analyzer (Leco Corp., St. Joseph, MI). Initial soil Mn, Fe, S, K, and Na contents were analyzed by inductively coupled plasma (ICP) after extracting with diethylene triamine pentaacetic acid (DTPA) solution. All metal elements and S concentrations in the soil solution were analyzed directly on ICP–mass spectrometry using an ELAN 5000 (PerkinElmer, Wellesley, MA). Nitrate and NH_4^+ were colorimetrically measured using a FLAstar 5000 Analyzer (FOSS Analytical, Hillerød, Denmark). Dissolved organic C was analyzed after combustion of the finely sprayed solution with a micro N/C analyzer (Analytik Jena AG, Jena, Germany). Gas concentrations were analyzed using a Shimadzu GC-14BPFE (Shimadzu Corp., Kyoto, Japan) with an electron capture detector (for N_2O), a flame ionization detector (FID, for CH_4), and another FID detector (for CO_2) coupled with a methanizer for transformation of CO_2 into CH_4. The GC columns were filled with Haysep Q (80/100 mesh).

Calculation and Statistical Analysis

The experiment was conducted at room temperature (20 ± 1°C). Gas production rate was calculated by linear regression of three consecutive analyses with time after flushing the microcosm with N_2. The amount of gas dissolved in the liquid phase was determined by using the mole fraction solubility of 5.07 × 10^{−4} for N_2O, 2.81 × 10^{−5} for CH_4, and 7.07 × 10^{−4} for CO_2 (Lide, 1991). Soil E_h was adjusted to the standard H_2 electrode by adding 210 mV (correction factor for the Ag–AgCl electrode) to the recorded instrument reading. All E_h data were reported as their corresponding values at pH 7 that were calculated according to the inverse relationship of E_h and pH as described by the Nernst equation. Redox potential change per pH unit may vary from 59 to 177 mV, depending on redox couples and kinetics of the reaction (Bohn, 1971). Since E_h values represent mixed potentials, a simple correction of 59 mV per pH unit (assuming equal numbers of protons and electrons involved in the reactions) was used.

Statistical analysis was conducted using SAS 9.1 (SAS Institute, Cary, NC). Simple linear regressions using PROC REG were conducted. Multiple regressions were conducted when more than one variable was considered in the model, with stepwise analysis to identify the most significant factor(s). Variables were considered statistically significant at P ≤ 0.05 (α = 0.05).

RESULTS AND DISCUSSION

Relationship between Soil Redox Potential and pH

The measured E_h in soils generally represented a composite value that reflects...
tion occurs under oxidizing conditions (high $E_H$) and metha-
† Measurements from both Phase I (from oxidizing to reducing conditions) and Phase II (from reducing to oxidizing conditions) were used.

In this study, only

a weighted average contributed by all redox couples present. In aerobic soils where the $O_2$–$H_2$O redox couple functions, the $E_H$ range is between 300 and 700 mV. Soil $E_H$ and pH were recorded in both the earlier oxidizing to reducing (Phase I) and later reverse (Phase II) phases of the incubation. The soil $E_H$ generally ranged from −200 to 600 mV in this study, a typical $E_H$ range that occurs in wetland soils under natural conditions. At the extreme two ends of this $E_H$ range, nitrification occurs under oxidizing conditions (high $E_H$) and methanogenesis under strictly reducing conditions (low $E_H$). Soil pH fluctuated with changes in soil $E_H$ conditions. All major soil redox reactions (such as denitrification, and reduction of Mn, Fe, and $SO_4^{2−}$) increase soil pH. The pH increase, however, is limited by the precipitation of Fe(II) and Mn(II) carbonates occurring at about pH 7, and production of $CO_2$ and organic acids from decomposing OM. Under reducing conditions, all soils tend to reach near-neutral pH values for either originally acidic or alkaline soils (Ponnamperuma, 1972). Dynamics of the soil $E_H$ and pH measurement are summarized in Table 2. Statistical analysis showed a significant ($P < 0.01$) negative correlation between the soil $E_H$ and pH. The activities of many biogeochemical processes will be significantly altered due to such pH shifts with redox fluctuations.

### Carbon Dioxide Production

All major reduction reactions of soil oxidants generate $CO_2$ (soil respiration), with OM as the electron donor. Soil oxidants [mainly Mn(IV), Fe(III), and $SO_4^{2−}$] can be regenerated by introducing $O_2$ into a reducing system, as was the case in Phase II in this study. Thus, aerobic respiration by soil microbes using $O_2$ as an electron acceptor also probably contributed to $CO_2$ production in this study.

Dissolved organic C, as an active soil electron donor, was measured by sampling soil suspensions at different stages of the Phase II incubation, because of its direct relationship with soil $CO_2$ and $CH_4$ production. Carbon dioxide production rates, for all studied soils, increased with soil $E_H$. The same tendency has been reported in incubations from oxidizing to reducing conditions (Yu and Patrick, 2003, 2004). In this study, only $CO_2$ production rates at times of DOC measurements are included in Fig. 2. Higher $CO_2$ production rates were found at higher redox conditions, despite decreasing DOC content in the soil microcosms with increasing $E_H$ (Fig. 3-i). The DOC content decrease found at higher redox conditions indicates that formation of DOC from soil OM could not balance mineralization to $CO_2$ during the incubation. Multiple regression analysis of all soils ($n = 52$) indicated that both the soil $E_H$ and DOC were positively correlated with the logarithm of $CO_2$ production rates ($r^2 = 0.42$, $P < 0.01$). Stepwise regression analysis indicated that the soil $E_H$ was more highly related to $CO_2$ production (for $E_H$ analysis: $r^2 = 0.41$, $P < 0.01$; for DOC analysis: $r^2 = 0.06$, $P = 0.08$). For each microcosm, DOC-adjusted $CO_2$ production rates were calculated by assuming the $CO_2$ production rate is proportional with DOC content (DOC-adjusted $CO_2$ production rate = measured $CO_2$ production rate × DOC content). With this adjustment, the calculated $CO_2$ production rates are independent of DOC content in each microcosm. The $CO_2$ production rates and corresponding DOC measurements are summarized in Table 3. Without DOC interference of $CO_2$ production rates (after adjustment), $CO_2$ production rates tended to increase exponentially with the soil $E_H$. For all soils, regression of the $CO_2$ production rates (after adjustment) with the soil $E_H$ showed a significant relationship ($r^2 = 0.57$, $P < 0.01$).

Soil $CO_2$ production rates are generally low during anaerobic respiration, with less energy yield for soil microorganisms. Such inefficient respiration is a principle mechanism for soil $C$ sequestration, as found in wetland ecosystems (Smith et al., 1983) and no-till agroecosystems (Kessavalou et al., 1998). Conversely, significant increases in respiration rates after flooding were reported in paddy soils (Bossio and Scow, 1995) and floodplain soils (Rinklebe and Langer, 2006), possibly due to an unspecific stress to aerobic microorganisms. The overall benefit of $C$ sequestration, however, deserves careful evaluation. Some of the $C$ sequestration benefit may be offset, in terms of contribution of total soil radiative forcing, by enhanced soil $N_2$O production (Batjes, 1998), and especially by significant $CH_4$ production (Roulet, 2000; Yu et al., 2006).
Methane Production

Dissolved organic C is a relatively mobile and labile form of soil C. In flooded soils, DOC may serve as a C source for CH₄ production. Methane emission rates have been found to be positively correlated with the dynamics of DOC in the rice root zone (Lu et al., 2000). Significant CH₄ production was found under relatively low E_H conditions in this study (Fig. 3-ii). As a substrate for methanogenesis, soil DOC is directly responsible for the observed CH₄ production. Following the same assumption as for CO₂ production, CH₄ production rates were adjusted with the corresponding DOC content in each microcosm. After adjusting the CH₄ production rates with the measured DOC, the results showed similar CH₄ production patterns under different E_H conditions (Fig. 3-iii). Increasing soil E_H by supplying O₂, however, may exert a toxic effect on the methanogenic bacteria along with increasing the soil redox status. In fact, experiments with cultures of methanogenic bacteria showed that O₂ had a greater adverse effect on methanogenic activity than high redox potentials, and that methanogens were able to initiate CH₄ production at E_H values up to 420 mV (Fetzer and Conrad, 1993). Other studies in which soils were not treated with O₂ showed initiation of CH₄ production at E_H values around 0 to 100 mV (Peters and Conrad, 1996; Ratering and Conrad, 1998). Nevertheless, soil redox potential is generally a good indicator for the onset of soil methanogenesis, but should be combined with careful characterization of the soil and its CH₄ production behavior.

Statistical analysis (n = 37) indicated that there was no significant correlation between the CH₄ production rates (both before and after DOC adjustment) and DOC contents (P > 0.05) in the studied soils. Methane production tended to exponentially increase with decreasing soil E_H (r² = 0.27, P < 0.01). After adjusting the CH₄ production rates by the soil DOC contents, the correlation between the CH₄ production rates and soil E_H remained significant (r² = 0.15, P < 0.01).

The results of this incubation study from reducing to oxidizing conditions suggest that soil redox potential may not be a good indicator for the cessation of ongoing soil methanogenesis. The relative poor relationship (low r² values) between the CH₄ production rates and soil E_H may be due to two mechanisms involved in CH₄ production. Past studies have shown that, when soil incubations were initiated under oxidizing conditions, small amounts of CH₄ production were observed at the beginning of the incubation with high E_H (Fetzer and Conrad, 1993; Roy et al., 1997; Yao and Conrad, 1999; Yu and Patrick, 2003). Such initial methanogenesis was H₂ dependent and was generally insignificant (2–6% of total CH₄ production) compared with the vigorous acetate-dependent methanogenesis under strictly anaerobic conditions (Yu and Patrick, 2003). Under conditions where soil goes from oxidizing to reducing, redox-active soil oxidants, such as NO₃⁻, Mn(IV), Fe(III), and SO₄²⁻, can significantly reduce H₂ production, limiting early CH₄ production. Significant CH₄ production can only take place when these oxidants are reduced into their reduced forms. At the same time, soil oxidants can contribute to oxidation of the existing CH₄ in the system without using O₂ (Iversen et al., 1987; Miura et al., 1992; Kumaraswamy et al., 2001). The critical E_H for significant CH₄ production has been determined to be about −150 mV or less in most studies (Neue et al., 1995; Yu et al., 2001; Yu and Patrick, 2003). In this study, measurement of CH₄ production started under strictly reducing conditions when all soil redox-active oxidants had been transformed into their reducing forms. There was no limitation for methanogenesis in such a reducing environment. In transition from reducing to oxidizing conditions, inhibition

Fig. 2. Carbon dioxide production rates under different redox potential (E_H) conditions in a microcosm incubation study with six soils. Only results when soil dissolved organic carbon (DOC) measurements were conducted are included.
mechanisms for methanogenesis (by redox-active soil oxidants, and even increase of O₂ partial pressure) developed gradually. No clear Eₐ boundary could be found for the two phases of methanogenesis in this study. With less competition of various soil oxidants for H₂, CH₄ production under higher Eₐ conditions was significant (up to 20% of total CH₄ production), compared with the CH₄ production under strictly reducing conditions. A reported field study concluded that H₂-dependent methanogenesis contributed about 25 to 30% of the CH₄ produced in soils (Conrad and Klose, 1999a, 1999b). Methane production tended to terminate only when soil Eₐ was >400 mV in this study. Measurement of CH₄ production under different redox conditions provides valuable guidance for managing rice fields to mitigate CH₄ emission and also helps to understand CH₄ dynamics in natural wetlands under different hydrological conditions.

**Methane Compensation Point under Different Redox Potential Conditions**

Compensation occurs when consumption is balanced by simultaneous production. Trace gas consumption generally increases with ambient trace gas concentration. Trace gas production, however, is normally independent of the product concentration. Therefore, there exists a concentration level for a specific gas at which its production equals consumption, the so-called compensation point (Conrad, 1994). In this study, no compensation point could be determined for CO₂ due to lack of consumption activity in the system, or for N₂O due to the limited number of measurements.

Mechanisms of CH₄ production have been discussed above. The major soil CH₄ consumption mechanism is aerobic oxidation using O₂. The significance of anaerobic CH₄ oxidation has not been quantified, but the occurrence has been reported in marine sediments and in saline inland waters (Iversen and Joergensen, 1985; Iversen et al., 1987), and also in soils coinciding with reduction of Fe(III) (Kimura et al., 1992; Miura et al., 1992). Compensation points for CH₄ have so far not been determined in either field or laboratory conditions. The automatic microcosm system used in this study made detailed monitoring of CH₄ concentration in the microcosm possible. In such a closed system, two approaches for measuring CH₄ compensation point were implemented. One was to monitor CH₄ concentration increasing in the headspace of microcosms until it reached a steady state (Fig. 4-i), and the second was to monitor CH₄ concentration decreasing to a steady state (Fig. 4-ii). Under low- Eₐ conditions, a high CH₄ compensation point was found due to strong CH₄ production and weak CH₄ oxidation capacity. The CH₄ compensation point was low under high-Eₐ conditions due to strong methanotrophic activity and weak methanogenesis activity. This analysis was applied to each soil across the entire Eₐ range studied, and the results...

---

**Fig. 3.** Soil CH₄ production rates and dissolved organic carbon (DOC) concentrations in soil suspensions under different redox potential (Eₐ) conditions in a microcosm incubation study with six soils: (i) DOC measurement; (ii) original CH₄ flux rate; and (iii) DOC adjusted CH₄ flux rate. Only results when soil DOC measurements were conducted are included. Adjusted CH₄ flux rates were calculated by: DOC adjusted CH₄ flux rate = measured CH₄ flux rate × DOC content.
showed that the CH$_4$ compensation point decreased exponentially with increasing soil E$_H$ (Table 4). For all studied soils, Fig. 4-iii clearly shows the reverse relationship between the CH$_4$ compensation point and soil E$_H$. The critical E$_H$ where the CH$_4$ compensation point equals the ambient atmospheric CH$_4$ concentration, above which soils start to consume atmospheric CH$_4$, is important. Such critical E$_H$ values varied among the different soils studied. Regression analysis indicated that the critical E$_H$ value was 414 mV for all soils combined, above which the soils functioned as a sink of atmospheric CH$_4$ (Table 4).

The CH$_4$ compensation point largely depends on the CH$_4$ oxidation capacity. In rice fields, variations in CH$_4$ emission have been primarily attributed to variations in methanotrophic activities (Sass et al., 1990; Schütz et al., 1989). Similar results have been reported in a Florida swamp where the CH$_4$ emission increase associated with a decrease in environmental oxidation was not due to stimulation of methanogenesis but due to a decrease in the methanotrophic activity (King et al., 1990). Therefore, CH$_4$ oxidation strength determines not only the potential of soils to act as a sink of atmospheric CH$_4$, but also the CH$_4$ emission strength of soils.

**Nitrous Oxide Production and Associated Ammonium and Nitrate Content**

With no addition of NO$_3^-$ at the beginning of the incubations (Phase I), NO$_3^-$ was essentially depleted when soils reached strongly reducing conditions (data not shown). During the reducing to oxidizing phase of the incubation, the soils occasionally reached higher E$_H$ conditions when excess O$_2$ was introduced, and N$_2$O formation was detected when the soil E$_H$ reached 500 mV or higher (Fig. 5). Without external NO$_3^-$ addition, soil nutrient analysis showed an elevated level of NO$_3^-$ in the soil suspensions when the soil E$_H$ was <300 mV. This could be attributed to a redox condition too high for denitrification but favorable for nitrification. Nitrous oxide production in soil suspensions under lower E$_H$ conditions remained low under such oxidizing conditions of the incubation. Initiation of nitrification at E$_H$ >500 mV was evidenced by NO$_3^-$ concentration elevation during the Phase I incubation without adding NO$_3^-$ (Fig. 5). The prolonged soil incubation under high- E$_H$ conditions in this study provides corroborating data for previous incubation studies where soil E$_H$ remained above 500 mV for just a few hours (Yu and Patrick, 2003, 2004).

Following a soil E$_H$ increase during the incubation, there was a parallel and substantial pH decrease in soil suspensions (Table 2). Microorganisms involve in soil N transformations generally function optimally under near-neutral pH conditions (Paul and Clark, 1996); however, nitrification can proceed rapidly at low pH. Significant net nitrification rates at pH 5 have been observed in a tropical forest soil (Neill et al., 1995). Nitrous oxide production depends mainly on denitrification intensity and the N$_2$O/N$_2$ ratio (higher under acidic conditions) in denitrification products. Small pH shifts may have little effect on total N$_2$ + N$_2$O produced, but the relative effect of pH on N$_2$O reductase may regulate the N$_2$O/N$_2$ ratio (Burford and Bremner, 1975; Firestone et al., 1980; Klemmedtsson et al., 1997).

### Table 3. Variations of CO$_2$ production rates and associated dissolved organic carbon (DOC) contents, and exponential regression analysis of DOC-adjusted CO$_2$ production rates with soil redox potential (E$_H$) in a microcosm incubation study with six soils.

<table>
<thead>
<tr>
<th>Soil</th>
<th>DOC range (mg L$^{-1}$)</th>
<th>CO$_2$ range (mg kg$^{-1}$ h$^{-1}$)</th>
<th>Equation</th>
<th>$r^2$</th>
<th>$P$ (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Original</td>
<td>DOC adjusted†</td>
<td></td>
</tr>
<tr>
<td>Arkansas-1</td>
<td>16.7–94.5</td>
<td>1.3–5.3</td>
<td></td>
<td>1.3–25.7</td>
<td>0.92</td>
</tr>
<tr>
<td>Arkansas-2</td>
<td>13.0–85.3</td>
<td>0.9–6.2</td>
<td></td>
<td>0.9–40.9</td>
<td>0.88</td>
</tr>
<tr>
<td>California-1</td>
<td>10.5–161.3</td>
<td>0.5–8.7</td>
<td></td>
<td>0.5–30.5</td>
<td>0.56</td>
</tr>
<tr>
<td>California-2</td>
<td>11.1–15.4</td>
<td>1.9–9.3</td>
<td></td>
<td>7.4–42.3</td>
<td>0.98</td>
</tr>
<tr>
<td>Louisiana</td>
<td>23.2–105.5</td>
<td>0.1–2.7</td>
<td></td>
<td>0.5–12.1</td>
<td>0.86</td>
</tr>
<tr>
<td>Texas-1</td>
<td>9.9–53.8</td>
<td>0.8–23.7</td>
<td></td>
<td>0.8–44.1</td>
<td>0.79</td>
</tr>
<tr>
<td>Texas-2</td>
<td>8.3–53.4</td>
<td>0.9–4.4</td>
<td></td>
<td>0.9–8.7</td>
<td>0.94</td>
</tr>
<tr>
<td>China</td>
<td>70.6–230.0</td>
<td>0.5–5.0</td>
<td></td>
<td>1.1–16.3</td>
<td>0.47</td>
</tr>
<tr>
<td>Indonesia-1</td>
<td>13.7–61.4</td>
<td>0.8–6.8</td>
<td></td>
<td>0.8–9.8</td>
<td>0.12</td>
</tr>
<tr>
<td>Indonesia-2</td>
<td>7.7–62.0</td>
<td>0.7–6.7</td>
<td></td>
<td>0.7–53.8</td>
<td>0.59</td>
</tr>
</tbody>
</table>

† DOC-adjusted CO$_2$ production rate = measured CO$_2$ production rate × DOC content.
Relation between Metals and Sulfur Transformation and Soil Redox Potential Conditions

When soils reached strongly reducing conditions \((E_H < -200 \text{ mV})\), all soil redox-active species were transformed into their reducing forms, \(\text{Fe(III)}\) to \(\text{Fe(II)}\), \(\text{Mn(IV)}\) to \(\text{Mn(II)}\), and \(\text{SO}_4^{2-}\) to \(\text{S}^2-\), resulting in observed higher soluble Fe and Mn concentrations in soil solutions (Fig. 6-i and 6-ii). Elevating soil redox status from reducing to oxidizing conditions in this study generated a reverse order of Fe and Mn immobilization (Fe earlier than Mn), compared with the reported order of Fe and Mn mobilization (Mn earlier than Fe) when soil was incubated from oxidizing to reducing conditions (Patrick and Jugsujinda, 1992). In this study, the critical \(E_H\) value for substantial immobilization of Fe and Mn was estimated to be about 50 and 250 mV, respectively. Previous studies showed that 100 mV at pH 7 was the critical value for Fe reduction and consequent dissolution (Gotoh and Patrick, 1974). Another study showed that, at pH between 6 and 8, most of the Mn conversion was found to take place at \(E_H\) of 200 to 300 mV (Gotoh and Patrick, 1972). Variations of critical \(E_H\) values for Fe and Mn transformations exist among different soils and different studies, due to the composite nature of redox reactions in the system. With drying in fields (soil pH will decrease), Fe and Mn carbonates will dissolve and oxidize to form oxides, amorphous oxides, and hydroxides that slowly recrystallize to stable Fe(III) oxides. Not much is known about this transformation, but pure Fe(OH)₃ and MnO₂ are not likely to form. At \(E_H\) values between that typically found in flooded soils and that of well-aerated soils, the stable form of Fe is \(\text{Fe}_5(\text{OH})_8\) (Schwab and Lindsay, 1983). At higher \(E_H\), \(\text{Fe}_5(\text{OH})_8\) is not stable, although some \(\text{Fe}_5(\text{OH})_8\) may persist during short, dry falls in rice fields. Under aerobic conditions, ferricydrite may also be formed (Neue, 1991).

The most common form of soluble S in soils is \(\text{SO}_4^{2-}\). Since most S in soils occurs in the organic state, reactions are closely associated with organic matter transformations and the activity of microorganisms. Upon flooding, sulfates are reduced to sulfides, and proteins are dissimilated after hydrolysis to \(\text{H}_2\text{S}\), mercaptans, \(\text{S}_2\text{O}_3^2-\), \(\text{NH}_3\), and fatty acids (Neue and Mamaril, 1985). No clear relation between soluble S (mostly in the form of \(\text{SO}_4^{2-}\)) content and soil \(E_H\) \((r^2 = 0.004, P = 0.59, n = 84)\) could be found in this study (Fig. 6-iii). Precipitation of certain metal ions as sulfides in flooded soils or sediments is an important mechanism regulating the solution concentrations of toxic \(\text{S}_2\text{O}_3^2-\) and metal ions (\(\text{Fe}^{2+}\), \(\text{Mn}^{2+}\), \(\text{Zn}^{2+}\), \(\text{Cu}^{2+}\), and \(\text{Hg}^{2+}\)). For the metal ions involved, the toxicity to plants is inversely related to the solubility of their sulfide salts, with \(\text{Hg}^{2+}\) being the most toxic and \(\text{Fe}^{2+}\) the least toxic (Engler...
When a flooded soil or sediment is drained and subsequently aerated, sulfides are transformed to more soluble $SO_4^{2-}$, while the metal ions become insoluble oxidized forms. For acid soils, a further increase in soil acidity may dissolve these metal oxides by chemical reactions.

Ponnamperuma (1972) reported that the specific conductance of the soil solution first increases after flooding by 1 to 2 dS m$^{-1}$ as a result of production of $NH_4^+$, $HCO_3^-$, $RCOO^-$, $Mn^{2+}$, and $Fe^{2+}$, followed by displacement of $Na^+$, $K^+$, $Ca^{2+}$, and $Mg^{2+}$ from soil colloids. The subsequent decrease in conductance is the result of $HCO_3^-$ removal, degradation and transformation of organic constituents, changes in the pH-dependent charges, and precipitation. In this study, the non-redox-active metal species, such as Na and K, showed no relation with soil $E_H$. Concentrations of $Na^+$ and $K^+$ in soil microcosms tended to strongly correlate with the duration of incubation. For all soils, $Na^+$ and $K^+$ concentration significantly increased during the incubation, probably due to both dissolution and ion exchange mechanisms (Table 5). During the study period, $Na^+$ and $K^+$ concentrations apparently did not reach saturation status. Breaking down the soil mineral structure under fluctuating redox conditions due to ferrolysis (Brinkman, 1979) may enhance the dissolution processes. Exchange reactions may also be important in regulating the behavior of water-soluble Fe and Mn (Gotoh and Patrick, 1972).

### CONCLUSIONS

The microcosm incubation and measurement of changes in soil biogeochemical processes under reducing to oxidizing conditions has several advantages: (i) it allows all possible microbial communities and enzymes to be fully developed during the preincubation from oxidizing to reducing conditions, (ii) all soil redox-active oxidants are converted into their reduced forms, creating an environment representing prolonged flooded soils and sediments, (iii) the information obtained on trace gas dynamics and nutrient transformation by elevating soil redox status is more similar to conditions occurring in draining flooded fields, and (iv) it provides an extended period of aerobic conditions by elevating soil $E_H$ with $O_2$.

The $E_H$ range of $-150$ to $180$ mV (corresponding value at pH 7) represents optimum soil conditions for minimizing the cumulative global warming poten-

### Table 4. Exponential regression analysis of CH$_4$ compensation point and soil redox potential ($E_H$) in a microcosm incubation study with six soils.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Relation between CH$_4$ (µL L$^{-1}$) compensation point and $E_H$ (V)</th>
<th>Critical $E_H$ †</th>
<th>$r^2$</th>
<th>$P$ (n)</th>
<th>$E_H$ (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arkansas</td>
<td>$\log_{10} CH_4 = -1.55 E_H + 1.18$</td>
<td>0.90</td>
<td>&lt;0.01</td>
<td>(7)</td>
<td>606</td>
</tr>
<tr>
<td>California</td>
<td>$\log_{10} CH_4 = -4.06 E_H + 1.73$</td>
<td>0.90</td>
<td>&lt;0.01</td>
<td>(14)</td>
<td>367</td>
</tr>
<tr>
<td>China</td>
<td>$\log_{10} CH_4 = -1.82 E_H + 1.06$</td>
<td>0.83</td>
<td>0.27</td>
<td>(3)</td>
<td>451</td>
</tr>
<tr>
<td>Indonesia</td>
<td>$\log_{10} CH_4 = -2.68 E_H + 1.49$</td>
<td>0.80</td>
<td>&lt;0.01</td>
<td>(14)</td>
<td>466</td>
</tr>
<tr>
<td>Louisiana</td>
<td>$\log_{10} CH_4 = -2.83 E_H + 1.74$</td>
<td>0.78</td>
<td>0.05</td>
<td>(5)</td>
<td>530</td>
</tr>
<tr>
<td>Texas</td>
<td>$\log_{10} CH_4 = -4.03 E_H + 1.96$</td>
<td>0.94</td>
<td>&lt;0.01</td>
<td>(5)</td>
<td>427</td>
</tr>
<tr>
<td>All soils</td>
<td>$\log_{10} CH_4 = -3.02 E_H + 1.56$</td>
<td>0.77</td>
<td>&lt;0.01</td>
<td>(48)</td>
<td>437</td>
</tr>
</tbody>
</table>

† $E_H$ above which soils would consume atmospheric CH$_4$ was calculated from the regression equation taking CH$_4$ concentration as 1.75 µL L$^{-1}$. Lack of statistical significance was found only in the Chinese soil due to the limited number of measurements (n).
tial from CO₂, CH₄, and N₂O (Fig. 2, 3, and 5), which is in good agreement with previous studies (Yu and Patrick, 2003, 2004). The results have a significant implication in evaluating the overall benefits of soil C sequestration efforts, because part of the C captured in soils may be substantially offset by enhanced soil CH₄ production at lower Eₜ conditions (Roulet, 2000), and enhanced N₂O emission under higher Eₜ conditions (Li et al., 2005). Optimum Eₜ conditions for minimizing soil global warming potential are mainly due to three factors: (i) the reduction potential in this Eₜ range is favorable for complete denitrification, with N₂ as the end product, but is still not strong enough to initiate significant CH₄ production, (ii) a slightly acidic pH condition limits methanogenesis, and the N₂O/N₂ ratio of denitrification is relatively smaller than more acidic conditions (Eₜ > 180 mV), and (iii) soil oxidants (mainly Fe and Mn) significantly lower the CH₄ compensation point by competing with H₂ produced from OM decomposition, and possibly by anaerobically oxidizing CH₄.

The comprehensive analysis of soil nutrient and metal transformations and trace gas dynamics in this study integrates the main environmental factors regulating major biogeochemical processes in soils. Fluctuation in soil Eₜ status represents changes occurring in aerobic and anaerobic environments on scales as small as soil aggregates (Tiedje et al., 1984) to as large as riparian zones and wetland ecosystems (Yu et al., 2006). The achieved quantification of the CH₄ compensation point under different Eₜ conditions provides a better understanding of the role of soils as a source or sink of atmospheric CH₄. Biological CH₄ oxidation is an important mechanism in controlling the CH₄ emissions from anoxic soils and sediments, because up to 90% of the produced CH₄ is consumed before being released to the atmosphere (Frenzel et al., 1992). A prolonged period of incubation under high soil Eₜ conditions provides some evidence of soil nitrification activity, which is normally studied in a soil slurry system. Our analysis indicates that 500 mV is probably a critical Eₜ value for soil nitrification to take place, where NO₃⁻ (also possibly part of N₂O) is formed from the oxidizing NH₄⁺.

Fig. 6. Soluble (i) Fe, (ii) Mn, and (iii) S concentrations in soil suspensions under different redox potential (Eₜ) conditions in a microcosm incubation study with six soils. Data represent the cumulative results from all six soils. The vertical lines represent the approximate Eₜ conditions at which (i) Fe and (ii) Mn become immobilized when Eₜ further increases. To use the same scale for the y axis, values of each analyte are standardized by multiplying by a standardized factor. For Fe concentration (soil × standardized factor): Arkansas × 10, California × 1.67, Louisiana × 10, Texas × 1, China × 1.5, Indonesia × 3. For Mn concentration (soil × standardized factor): Arkansas × 1, California × 2, Louisiana × 4, Texas × 4.8, China × 2.4, Indonesia × 1. For S concentration (soil × standardized factor): Arkansas × 3.56, California × 1.33, Louisiana × 3.56, Texas × 1.78, China × 1, Indonesia × 1.

ACKNOWLEDGMENTS
This paper is dedicated to Dr. William H. Patrick, Jr. (1925–2004) who participated in collecting soil samples and planning for this collaborative study.
Table 5. Variations of Na and K concentrations in soil suspensions, and their liner regression analysis with incubation time in a microcosm incubation study with six soils.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Day</th>
<th>Range Na (mg L⁻¹)</th>
<th>Equation</th>
<th>r²</th>
<th>P (n)</th>
<th>Range K (mg L⁻¹)</th>
<th>Equation</th>
<th>r²</th>
<th>P (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arkansas-1</td>
<td>68</td>
<td>14.8–27.4</td>
<td>Na = 0.16Day + 13.0</td>
<td>0.71</td>
<td>&lt;0.01 (8)</td>
<td>51.2–186.5</td>
<td>K = 1.84Day + 52.9</td>
<td>0.99</td>
<td>&lt;0.01 (8)</td>
</tr>
<tr>
<td>Arkansas-2</td>
<td>68</td>
<td>14.5–29.6</td>
<td>Na = 0.17Day + 13.0</td>
<td>0.67</td>
<td>0.24 (7)</td>
<td>65.9–266.5</td>
<td>K = 2.86Day + 69.6</td>
<td>1.00</td>
<td>&lt;0.01 (7)</td>
</tr>
<tr>
<td>California-1</td>
<td>71</td>
<td>49.7–72.7</td>
<td>Na = 0.32Day + 46.1</td>
<td>0.83</td>
<td>&lt;0.01 (8)</td>
<td>41.04–150.0</td>
<td>K = 1.39Day + 42.7</td>
<td>0.89</td>
<td>&lt;0.01 (8)</td>
</tr>
<tr>
<td>California-2</td>
<td>71</td>
<td>50.7–71.3</td>
<td>Na = 0.31Day + 47.8</td>
<td>0.92</td>
<td>&lt;0.01 (10)</td>
<td>40.4–152.1</td>
<td>K = 1.49Day + 42.1</td>
<td>0.96</td>
<td>&lt;0.01 (10)</td>
</tr>
<tr>
<td>Louisiana</td>
<td>68</td>
<td>19.7–33.6</td>
<td>Na = 0.17Day + 19.0</td>
<td>0.77</td>
<td>&lt;0.01 (7)</td>
<td>73.0–239.2</td>
<td>K = 2.41Day + 89.3</td>
<td>0.96</td>
<td>&lt;0.01 (7)</td>
</tr>
<tr>
<td>Texas-1</td>
<td>54</td>
<td>14.7–26.8</td>
<td>Na = 0.24Day + 14.7</td>
<td>0.96</td>
<td>&lt;0.01 (6)</td>
<td>27.5–96.8</td>
<td>K = 1.09Day + 30.3</td>
<td>0.93</td>
<td>&lt;0.01 (6)</td>
</tr>
<tr>
<td>Texas-2</td>
<td>44</td>
<td>15.9–26.0</td>
<td>Na = 0.22Day + 15.6</td>
<td>0.99</td>
<td>&lt;0.01 (4)</td>
<td>35.2–91.6</td>
<td>K = 1.06Day + 40.4</td>
<td>0.93</td>
<td>0.03 (4)</td>
</tr>
<tr>
<td>China</td>
<td>68</td>
<td>11.6–28.2</td>
<td>Na = 0.20Day + 10.0</td>
<td>0.61</td>
<td>0.04 (7)</td>
<td>57.2–176.3</td>
<td>K = 1.50Day + 59.0</td>
<td>0.95</td>
<td>&lt;0.01 (7)</td>
</tr>
<tr>
<td>Indonesia-1</td>
<td>63</td>
<td>17.8–30.6</td>
<td>Na = 0.19Day + 16.7</td>
<td>0.75</td>
<td>&lt;0.01 (8)</td>
<td>33.9–155.7</td>
<td>K = 1.84Day + 37.9</td>
<td>0.98</td>
<td>&lt;0.01 (8)</td>
</tr>
<tr>
<td>Indonesia-2</td>
<td>68</td>
<td>18.0–33.0</td>
<td>Na = 0.19Day + 16.4</td>
<td>0.69</td>
<td>0.01 (8)</td>
<td>37.8–188.6</td>
<td>K = 2.06Day + 41.0</td>
<td>0.98</td>
<td>&lt;0.01 (8)</td>
</tr>
</tbody>
</table>

REFERENCES


