*Chapter*

# A BRIEF HISTORY ON SOIL MICROCOSMS AS AN EXPERIMENTAL APPARATUS FOR BIOGEOCHEMICAL RESEARCH

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## ABSTRACT

Most biogeochemical processes in soils involve changes in oxidation and reduction (redox) status, which can be characterized by redox potential (Eh). Determination of aeration status represented by Eh measurement can reasonably predict the stability of various compounds of biogeochemical interests. One experimental approach is to determine and to closely control the redox potential and pH at which various redox couples function. Such a redox potential – pH controlling device was originally designed half a century ago. Since then, it was named a "soil microcosm", because the incubated soil represents a miniature of the soil in the natural environment. Modifications have been made to suit specific research needs and to improve the performance of soil microcosms. The applications of soil microcosm techniques have significantly contributed to our understanding of biogeochemical processes among soil/sediment, plants, water and atmosphere, especially in wetland ecosystem.

## 1. INTRODUCTION

Following World War II, the rapid increase in global population resulted in unprecedented social, economic and environmental challenges. One of the most important challenges was food production for world population growth to meet basic needs and demands for rising living standards. Rice (*Oryza sativa*) is a staple food for a large part of the world population (FAO, 2003). As a part of the world-wide effort for the "Green Revolution" movement during the post WWII era, the International Rice Research Institute (IRRI) founded in1960 made a significant contribution for increasing global rice production. In addition to developing new rice varieties, understanding the soil chemistry in flooded rice fields to meet rice production became IRRI's major science endeavor. Early research in flooded rice fields (artificial wetland) made a major contribution in developing the foundation of today's wetland biogeochemistry, due to the similar hydrological settings as in natural wetlands. A large majority of the fundamental knowledge and principles in soil biogeochemistry were obtained from soil microcosm experiments.

## 2. DEVELOPMENT OF SOIL MICROCOSM APPARATUS

Soil aeration status can cause fundamental changes in soil chemistry. Unlike aerobic upland soils, flooded soils are devoid of oxygen supply from the atmosphere, and anaerobic conditions develop shortly after consumption of remaining oxygen by soil microbes. To understand the factors governing the growth of rice plants in flooded environments and for weed control and fertilization necessary for maximum yield, rice culture became a major driver in studies of biogeochemical processes in soils.

Most soil biogeochemical processes involve oxidation and reduction (redox) reactions. Oxidation reactions are a process of losing electrons, while reduction reactions are a process of gaining electrons. Oxidation reactions must couple with reduction reactions, which are called redox coupled reactions, to maintain the system electrically neutral. At the end a reductant (electron donor) is oxidized, while an oxidant (electron acceptor) is reduced in a coupled redox reaction. Soil oxidation–reduction status can be conveniently determined by redox potential

(Eh). It has been observed that pH changes are associated with redox potentials when soils experience hydrological fluctuations. Due to the presence of oxygen and water, soil redox potentials are normally in a range from +700 mV (under fully drained conditions) to -300 mV (under prolonged flooding conditions) at near neutral conditions (Bohn, 1971; Ponnamperuma, 1972).

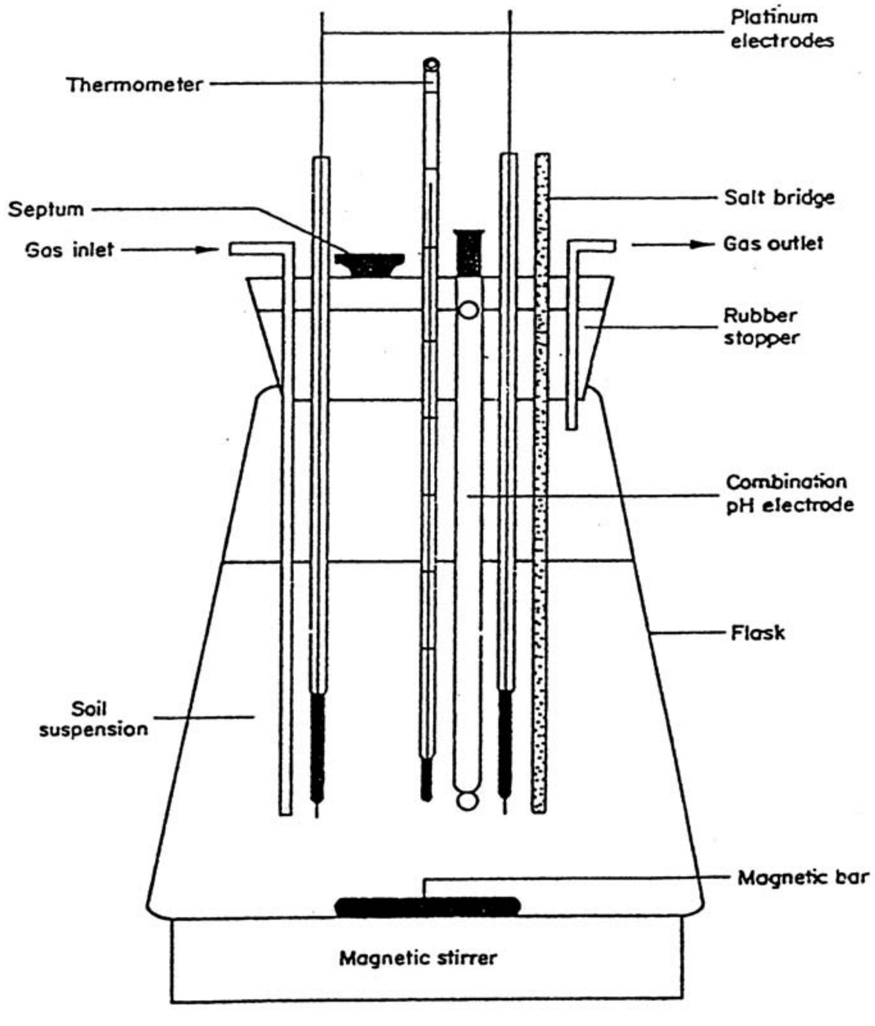

Figure 1. Schematic of a soil redox potential and pH controlling device.

Simultaneous measurement of both redox potential and pH in soil solutions were conducted at IRRI to study soil chemistry for rice production (IRRI, 1964). It was recognized that soil redox potential and pH are the two dominant factors controlling the stability of various compounds of biogeochemical interests. However, under the same hydrological conditions, soil redox potential and pH were found largely different due to the difference in soil chemical characteristics, such as organic matter and various redox active inorganic components. Thus there was a need to determine and to closely control the redox potential and pH at which various redox couples function. Such a redox potential – pH controlling device was originally designed several decades ago (Patrick, 1966; Patrick et al., 1973). Since then, it was named a “soil microcosm”, because the incubated soil represents biogeochemical conditions of the soil in the natural environment.

Typically, a soil microcosm involves redox potential and pH measurement in a homogenous soil suspension (Figure 1). Redox potential control (± 10 mV) can be achieved by adding either air (to raise Eh) or inert gases such as nitrogen or helium (to lower Eh) through a gas pump regulation system controlled by a meter relay. Most of biological and chemical processes tend to decrease the soil redox potential. When the designated redox potential is reached, the solenoid value is activated by the meter relay, allowing oxygen to enter the system to prevent the redox potential from decreasing below the designated value. In a rare case with a system where the redox potential tends to increase, redox potential control can be obtained using hydrogen-nitrogen mixtures. A similar system can be used for controlling pH (± 0.05 pH unit) in the soil suspension where pH electrode serves as the sensor. Acid or alkali is pumped into the system automatically through a solenoid valve to maintain the pH at the designated values. The system can be designed so that the amount of acid or alkali required is measured.

In such a soil microcosm, samples can be taken as soil solution, soil slurry, and gas from the headspace when needed. Gases in the headspace produced by the soil microbial processes can be flushed out by using an inert gas, and can also be accumulated if the flush stops.

## 3. MODIFICATION OF MICROCOSM FOR SPECIAL NEEDS

To improve the performance of the above soil suspension microcosm, several modifications can be made to suit specific research needs (Yu and Rinklebe, 2011). (1) To replace the rubber stopper with a screw cap where various openings can be prepared for electrodes and tubing through fitting unions. This helps the removal of the electrodes and tubing when needed. (2) To replace the magnetic stirrer bar with an overhead stirrer can make stirring the soil suspension more successful, but to maintain air tight for the system can be a challenge. (3) To replace the pH and redox meter with a data logger can significantly improve the frequency of data acquisition. (4) To provide temperature control for the microcosm can study the effect of temperature on biogeochemical processes. This can be done by using a heating blanket surrounding the incubation flask or by using a heating plate placed at the bottom of flask to reach above ambient temperature. To reach sub-ambient temperature (as well as above ambient temperature), a double-hull incubation vessel can be used where water of desired temperature can be pumped between the two layers of the vessel. (5) To install an automated gas sampling and analysis system allows the researcher to monitor the dynamics of gas concentration in a microcosm (Figure 2).

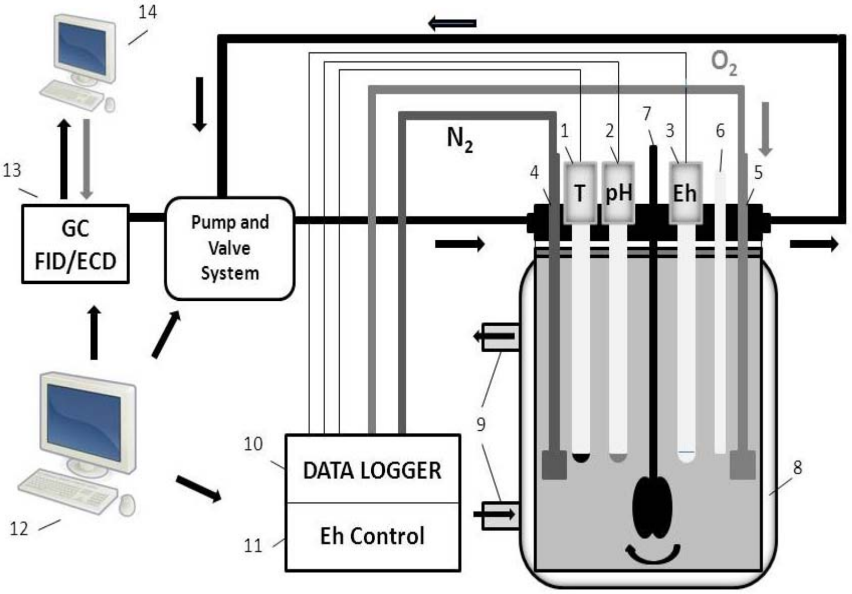

Figure 2. A fully automated soil microcosm with redox, pH, temperature control and gas analysis. (1) thermometer; (2) pH electrode; (3) redox potential (Eh) electrode; (4) dispersion tube for $N_2$; (5) dispersion tube for $O_2$; (6) sampling tube; (7) stirrer; (8) double-hull vessel; (9) temperature control by a thermostat and water circulation; (10) data logger for Eh, pH, and temperature; (11) automatic redox regulation by $N_2$ and $O_2$ valves; (12) control computer for data logger, pump, and valve system (gas sampling), and gas chromograph; (13) gas chromatograph (GC) with flame ionization detector// electron capture detector for trace gas measurements; (14) computer for GC control and GC data storage.

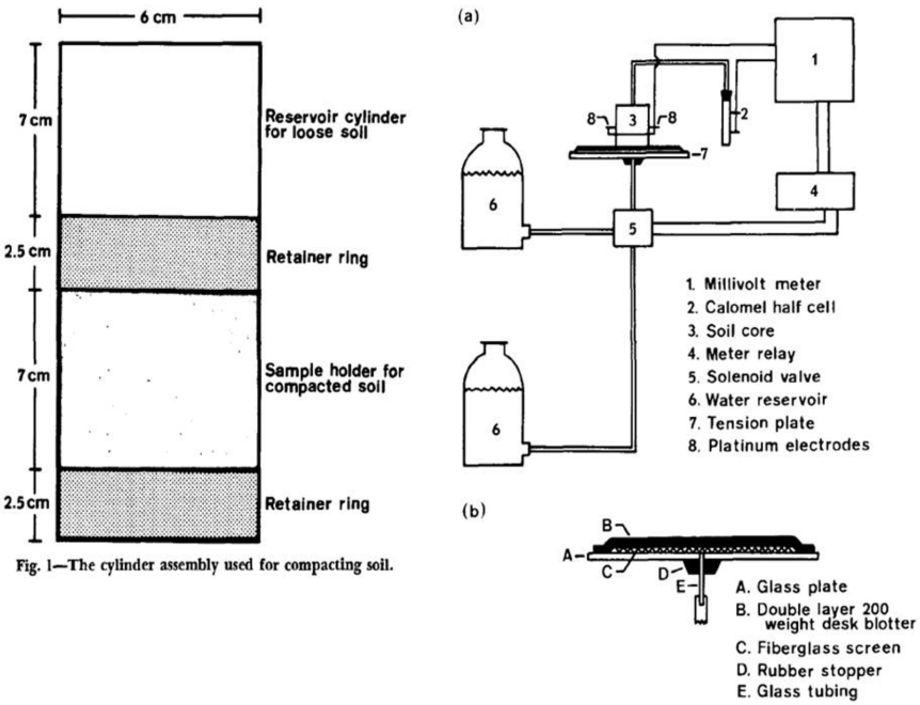

Figure 3. Schematic setup of a soil core microcosm.

A packed soil core can be controlled to reach a designated redox potential (± 20 mV). This is one step closer than a stirred soil suspension to a physically undisturbed soil (Patrick and Henderson, 1981). The principle of the soil core microcosm is to control the redox potential in soil cores by regulating the water tension. At a high water tension, the soil pore space is drained to let air enter to raise the redox potential. At a low tension, the soil pore space is filled up with water to lower the redox potential. A soil core can be prepared by packing the soil into a brass cylinder fitted with platinum electrodes on the side. The soil core is then placed on a moisture tension plate connected with two water reservoirs of different heights. Water tension control (redox control) can be reached by a two-way solenoid valve that is automatically controlled by a redox meter relay to connect to either of the two water reservoirs (Figure 3). Since rapid water capillary flow is essential for the soil redox potential control, this soil core microcosm can be readily applied only to coarse-textured soil. For a medium-textured and fine-textured soil, mixing the soil with sand is needed before packing the soil core. At the end of the study the soil core can be sectioned horizontally into slices for sample profile analysis.

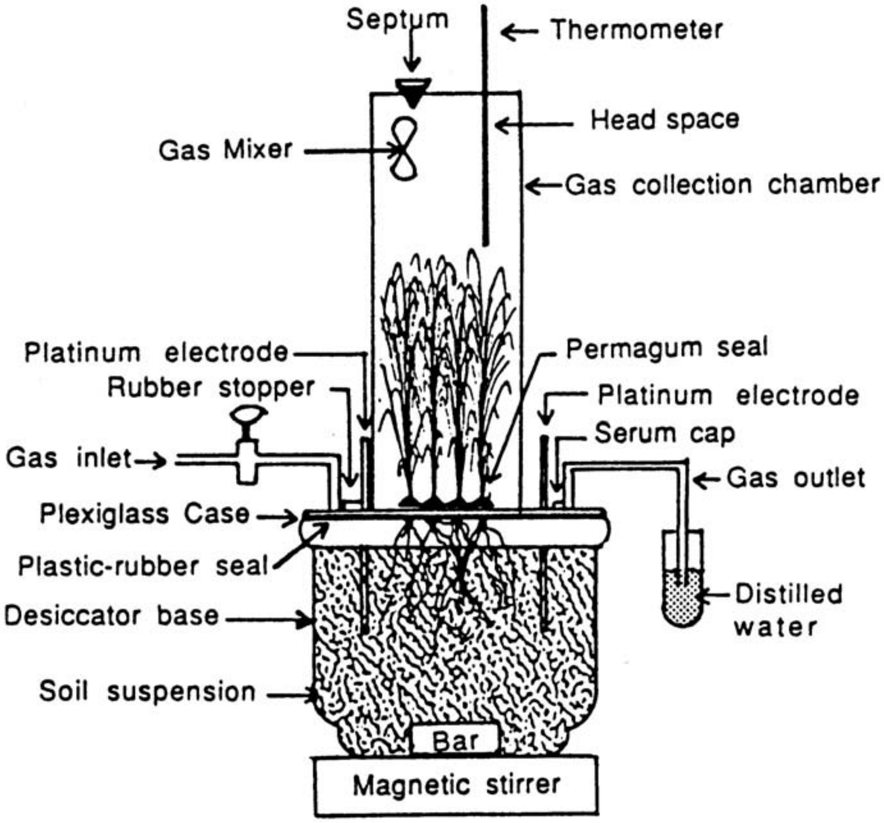

Figure 4. Schematic sketch of a soil-plant microcosm with gas chamber.

A soil-plant microcosm was also developed to simulate the interactions between soils and plants in nature (Reddy et al., 1976; Tolley et al., 1986). The principle is to transplant plant seedlings into a redox-pH controlled soil suspension. In this microcosm, a desiccator base is used as an incubation vessel for the soil suspension, and a plexiglass plate is used as the cover for the vessel sealed with black plastic rubber or black silicone rubber sealant to maintain air tight. The soil suspension can be controlled for a target redox potential and pH as a regular soil microcosm (Figure 1). Various openings can be prepared on the plexiglass plate for the tubing, electrodes and plants to get through. Plant seedlings can be sealed to the plexiglass plate with a soft gum material. To study the gas exchange between plant and atmosphere, a transparent closed chamber can be placed on top of the plexiglass plate. Gas samples can be taken from the headspace of the gas collection chamber through a rubber septum (Figure 4). Soil chemistry analysis can be conducted as in a regular soil suspension microcosm (Figure 1). At end of the incubation, plant samples can be taken for nutrients and growth analysis.

## 4. APPLICATION OF MICROCOSM IN BIOGEOCHEMICAL RESEARCH

It has long been observed that flooding can cause a pH increase in acidic soils but a pH decrease in calcareous soils (IRRI, 1963). Early research for rice production found significant accumulation of ammonium but rapid loss of nitrate in flooded soils. Flooding increased the content of soluble phosphorus and enhanced phosphorus uptake by the rice plants. Meanwhile toxic levels of heavy metals were also observed in flooded rice fields (IRRI, 1964).

The development of microcosm techniques significantly improves our understanding in soil biogeochemical processes of biological, ecological and environmental interests. The critical redox conditions for the reductions of major soil oxidants upon flooding are summarized in Figure 5 (Patrick and Jugsujinda, 1992; Yu et al., 2007). Two aerobic/anaerobic interfaces exist in flooded rice fields (1) flooded soil surface layer maintained by $O_2$ dissolved in the standing water, and (2) plant rhizosphere maintained by $O_2$ diffusing through the rice plant (Reddy et al., 1989). In addition to flooding and drainage soil management practices, these aerobic/anaerobic interfaces play an important role in the physiology and nutrition of rice plant. Lack of aerobic conditions in the flooded rice soils limits microbial nitrification activity, while dominant moderately reducing conditions favor strong denitrification activity resulting in rapid nitrate loss. Significant phosphorus release upon the flooding of rice fields has been associated with the soil iron reduction (Figure 5). Accumulation of carbon dioxide in the flooded soil may help to reduce the mobility of heavy metals and attenuate their toxic effects on rice plants. Similar results have been observed in natural wetlands.

Understanding various soil redox processes under different hydrological conditions makes fairly reliable prediction on the stability of redox active compounds in soil/sediment, water and atmosphere. This is not only important for fertilization and irrigation management for agricultural production, but also important for water quality in surface and ground water, as well as in marine environment (Reddy and Patrick, 1977; Masscheleyn et al., 1991). In recent years, global climate change becomes the most important environmental challenge where interdisciplinary and multidisciplinary research is urgently needed. Production of greenhouse gases and carbon sequestration in soils contributes to the long term sustainability of soil-plant ecosystem. Critical redox conditions have been identified for major greenhouse gases in terms of global warming potential by using soil microcosm technique (Masscheleyn et al., 1993; Yu and Patrick, 2004).

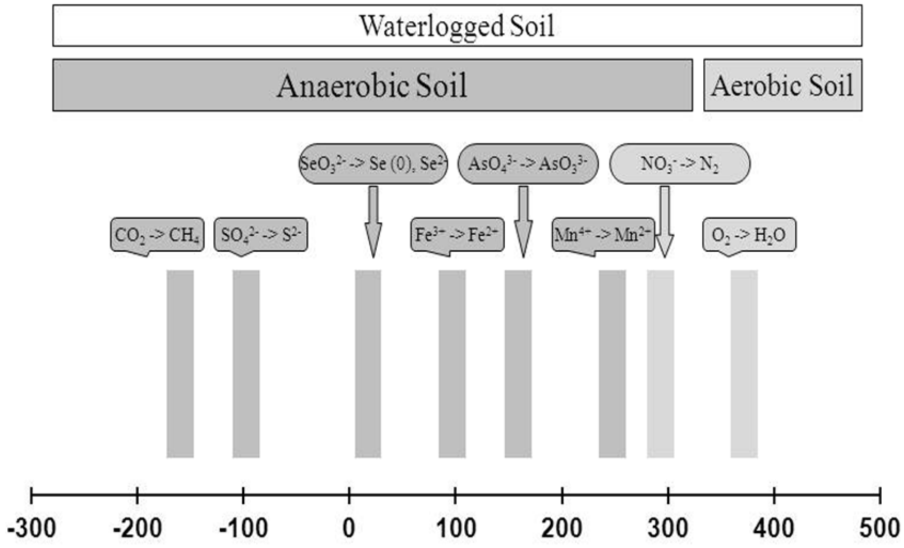

Figure 5. Critical redox potential conditions for major biogeochemical process in soils.

Advancement and modifications of microcosms makes possible a wide range of application of these techniques in ecological and environmental research, which contribute to understanding functions and services provided by the real world natural ecosystem.

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