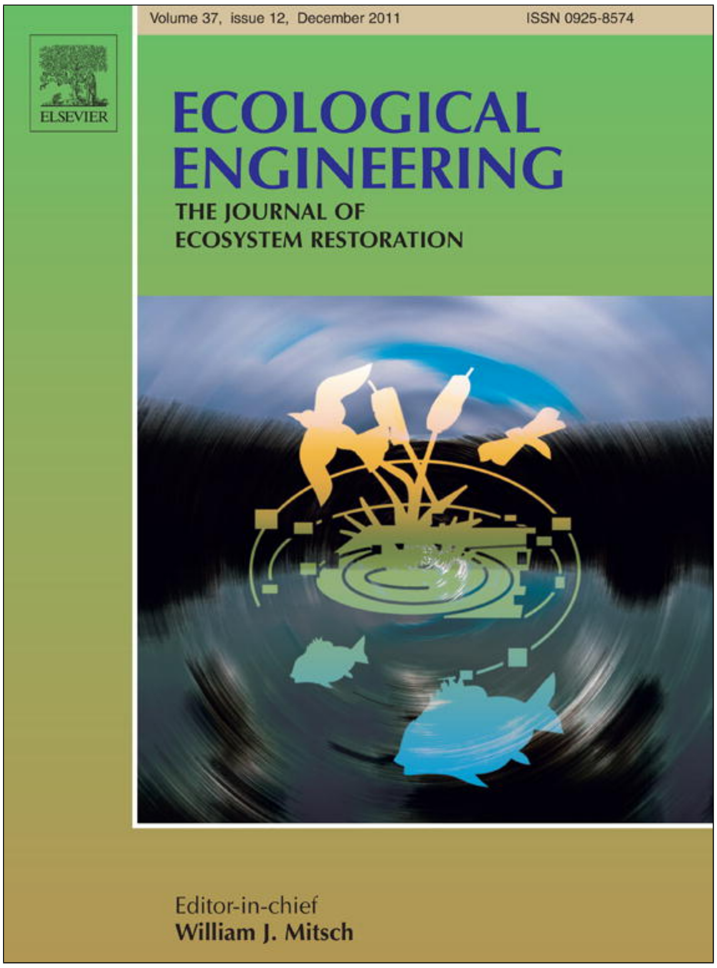
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Short communication

Advancement in soil microcosm apparatus for biogeochemical research

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ABSTRACT

Application of soil microcosms has largely improved our understanding in biogeochemical processes, because all major environmental factors can be independently controlled. Recent advancement to improve the performance of soil microcosm has been made. The modifications include using a different incubation vessel and cap, replacing a magnetic stirrer with an overhead stirrer, providing temperature control for the microcosm, using data logger for continuous measurements of redox potential (Eh), pH and temperature, and applying automatic gas analysis. The modifications can be made in any combination to suit an individual's needs and budget.

1. Introduction

Most biogeochemical processes in soils and sediments involve changes in oxidation and reduction (redox) status, which can be characterized by redox potential (Eh). Typically redox potential can vary from +700 mV (under well-drained conditions) to −300 mV (under prolonged flooding conditions). With Eh changing from high (aerobic conditions) to low (anaerobic conditions), a series of reactions can sequentially take place according to their thermodynamic order, including reduction of oxygen (O_2), nitrate, manganese (IV), iron (III), sulfate, and carbon dioxide (CO_2). The electron donors driving these reduction reactions are reduced compounds, commonly organic matters. Meanwhile, the systems also experience dramatic pH changes (Bohn, 1971; Ponnamperuma, 1972).

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 several decades ago (Patrick, 1966; Patrick et al., 1973). Since then, it was named a "soil microcosm", because the incubated soil represents a miniature of the soil in the natural environment. The application of soil redox potential–pH controller (soil microcosm) has significantly contributed to our understanding in biogeochemical processes, especially in wetland ecosystem (Rupp et al., 2010). In general, the application involves soil solution and soil suspension samples from the homogenous soil–water slurry (Patrick and Jugsujinda, 1992; Antic-Mladenovic et al., 2011), and gas samples from the headspace of the microcosm (Matheson et al., 2002; Yu and Patrick, 2004). After some modification, vegetation growth under different Eh–pH conditions can also be studied (DeBusk et al., 1995; Yang et al., 2001).

2. Basic components of a soil microcosm

Typically, a soil microcosm involves an incubation of homogenous soil–water suspension where its redox potential and pH can be closely monitored and controlled during the study. A schematic showing its basic components and a photo of the actual apparatus is illustrated in Fig. 1.

In this soil microcosm, the soil suspension can be made by mixing soil and water in a certain ratio to form a soil slurry. For mineral soils, a soil-to-water ratio of 1:4 is appropriate, and for organic soils the ratio can be as low as 1:10. The soil suspension can be kept homogenous by using a magnetic stirrer at bottom of the flask with a magnetic stirring bar inside. The microcosm remains air-sealed by a rubber stopper with various openings for installation of electrodes, probes and tubing (Fig. 1). Soil temperature can be monitored by a thermometer, and soil pH can be monitored by a combination pH electrode connected with a pH meter. Soil redox potential can be measured by a commercially available combination oxidation–reduction potential (ORP) electrode, or by a home-made platinum (Pt) electrode (Faulkner et al., 1989) and a calomel or a silver–silver chloride (Ag/AgCl) reference electrode

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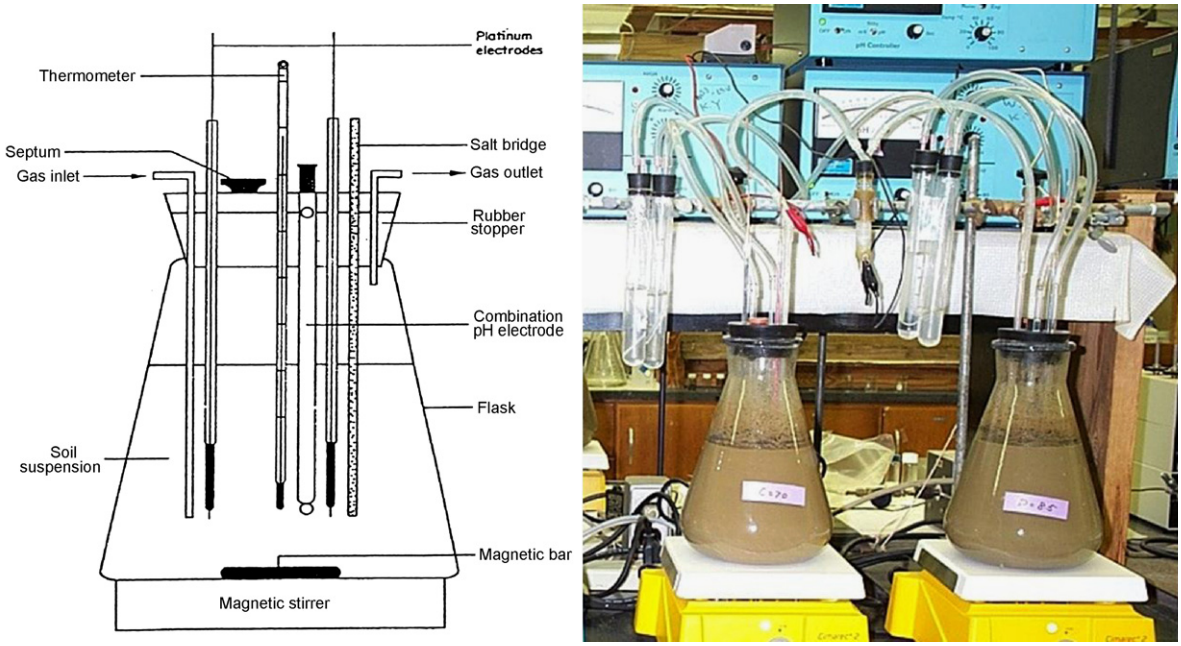

Fig. 1. Basic components of a soil microcosm, a schematic (left) and a photo (right).

that are connected to a millivolt meter. For accuracy purposes, two Pt electrodes are commonly used for each soil microcosm. The flasks should be wrapped with aluminum foil or other appropriate material to protect them against light, preventing algae growth and photo-oxidation reactions.

Gas inlets and outlets are installed in the rubber stopper to purge the microcosm system when needed. Redox potential in the soil microcosm can be controlled by two approaches. One approach is to start the soil under aerobic conditions and to prevent the redox potential from falling below a set value. The other approach is to start the soil under anaerobic conditions and then to raise the redox potential to a selected higher value. A bench pH/ORP controller (Industrial & Chemical Measurement, Oregon, USA) with an internal relay is used to activate the redox potential control (Fig. 1). The soil redox potential can be maintained within a certain range by adding either air (with oxygen to raise Eh) or inert gases such as nitrogen (N_2) or helium (to lower Eh) through an automatic gas regulation system. When the set redox potential is reached, the solenoid value is activated by the meter relay, allowing gas to purge the microcosm so that the target Eh level can be reached. The two Pt electrodes can be wired together to yield an average reading. By this system, the redox potential in the microcosm can be controlled within ±5 mV of the target level for several days.

A similar system can be used for controlling pH in the soil suspension. In this case, a pH electrode, instead of a Pt electrode, serves as the sensor. To maintain the pH at the designated values, a very slow flow of acid (0.5 N HCl) or alkali (0.5 N NaOH) from a burette or other container can be controlled using a solenoid valve. Control of pH within ±0.05 pH unit of the target value can be easily maintained for days. The system can be designed to measure the amount of acid or alkali required.

3. Potential problems and modifications

Several modifications can be made to improve or to correct some potential problems for the above basic soil microcosm apparatus. The following modifications can be made in any combination to suit an individual's needs and budget.

3.1. Replacement of rubber stopper with a screw cap

The rubber stopper used for the basic microcosm serves as a holder for electrodes, salt bridge, and several tubing for gas and liquid exchange (Fig. 1). Since air-tight is essential for the microcosm, the openings in the rubber stopper need to be determined carefully to prevent leaking. The glass electrodes and tubing need to be moistened with water and then to be carefully inserted through the rubber stopper. In practice, it is not an easy task to retrieve the electrodes and tubing after use or for replacement, even the rubber stopper has been soaked in water for hours. To avoid such difficulty, a wide-mouth or straight-sided bottle and a screw cap sealed with Teflon tape can be used to replace the original incubation flask. Openings can be prepared on the screw cap, and electrodes and tubing can be inserted through a Nylon buck-head compression fitting union. Air-tight can be maintained by tightening the union. The fitting unions can be sealed with the screw cap by a silicon rubber washer or an O-ring (Fig. 2, bottom left). Retrieval of electrodes and tubing can be easily made by unscrewing the fitting union.

3.2. Replacement of magnetic stirrer with an overhead stirrer

The magnetic stirring system frequently encounters problems with the stirring bar, such as difficulty to start rotation, inappropriate rotation and cease of rotation. This causes heterogeneity of the soil slurry during incubation (Fig. 1). Moreover, the stirring bar can be worn out after prolong period of use and the magnetic materials can be released, causing potential contamination to the incubated soil slurry. In an extreme case, a hole can eventually be drilled at bottom of the glass flask due to long-lasting rotation of a stirring bar, resulting in leaking of the soil slurry. Using an overhead stirrer (i.e. IKA RW 16 basic) instead can prevent this problem (Fig. 2). The overhead stirrer consists of a stirrer motor connected to a stainless steel stirring shaft and a low-speed blade with large surface. A solid Teflon stirrer bearing can be used for the stirring shaft getting through the cap of incubation vessel into the soil suspension. No lubricant is needed when the stirrer is in low-speed motion, because Teflon material has small friction with stainless steel. The above mechanical Teflon stirrer bearing

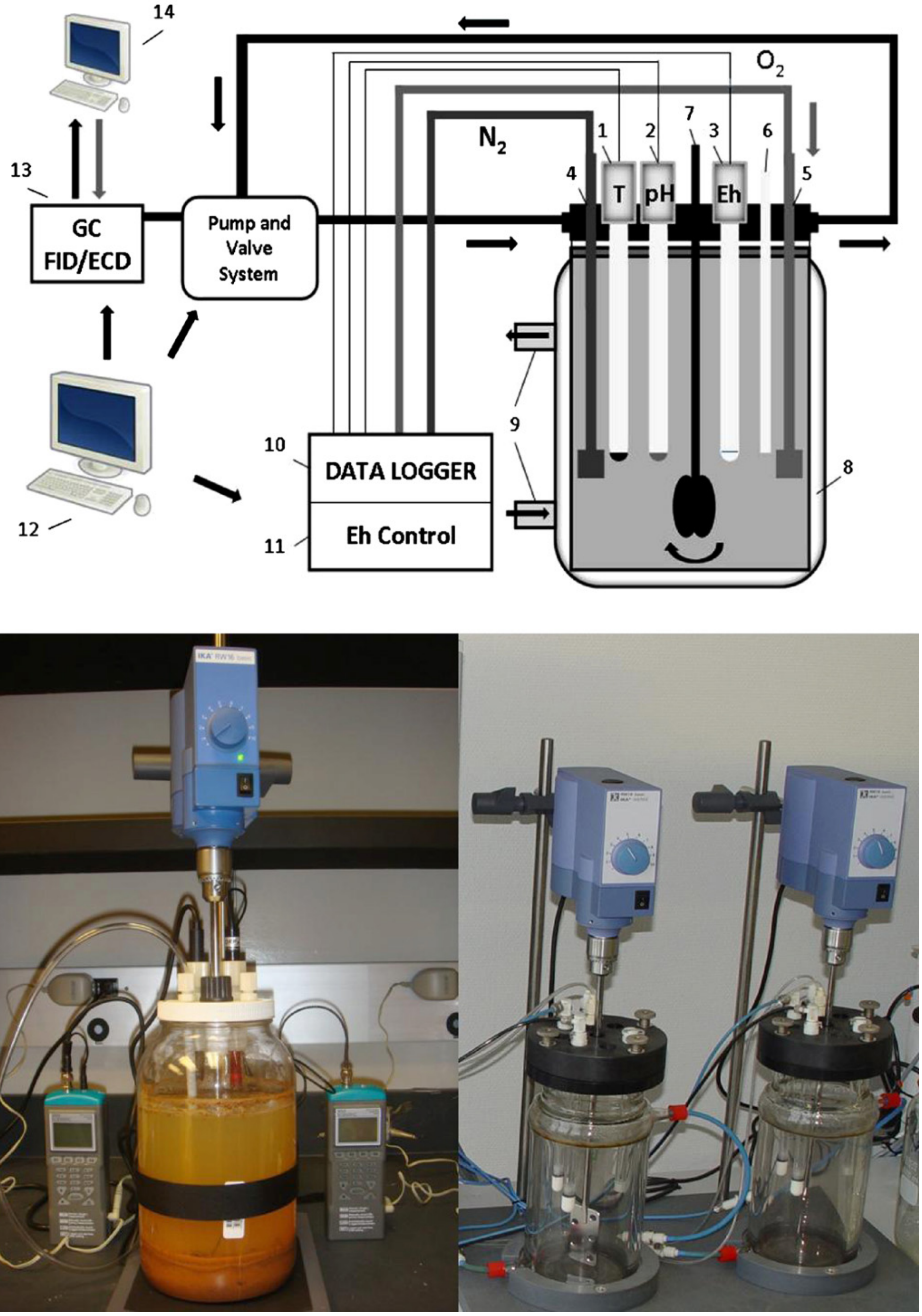

Fig. 2. A schematic of a modified soil microcosm (above), and a photo of microcosm with automatic Eh and pH measurement (bottom left), and a photo of microcosm with temperature control and automatic gas analysis system (bottom right). Components (above) include (1) thermometer; (2) pH electrode; (3) redox potential (Eh) electrode; (4) dispersion tube for N_2; (5) dispersion tube for O_2; (6) sampling tube; (7) overhead stirrer; (8) double-hull incubation 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 chromatograph (start signal); (13) gas chromatograph (GC) with flame ionization detector/electron capture detector for trace gas measurements (CO_2, CH_4, and N_2O); (14) computer for GC control and GC data storage.

alone does not guarantee a perfect air-seal. Therefore, an air-tight seal for the stirrer can be maintained by two options. The less expensive (<$100) option requires some hand work before each experiment using dentist material commonly called a-gum body (Fig. 3, top). The chemical name for the dentist material is vinyl-polysiloxan–silicone (Dentsply, Konstanz, Germany). This material is on one hand very enduring and tough but on the other hand also very flexible. The other option is to use a costly bearing to make a prefect seal (Fig. 3, bottom). A customized cover for the microcosm vessel, including the air-tight rotating unit and various openings for the electrodes, sampling tubes, and gas connections is currently about 1000 Euros (Fig. 2, bottom right).

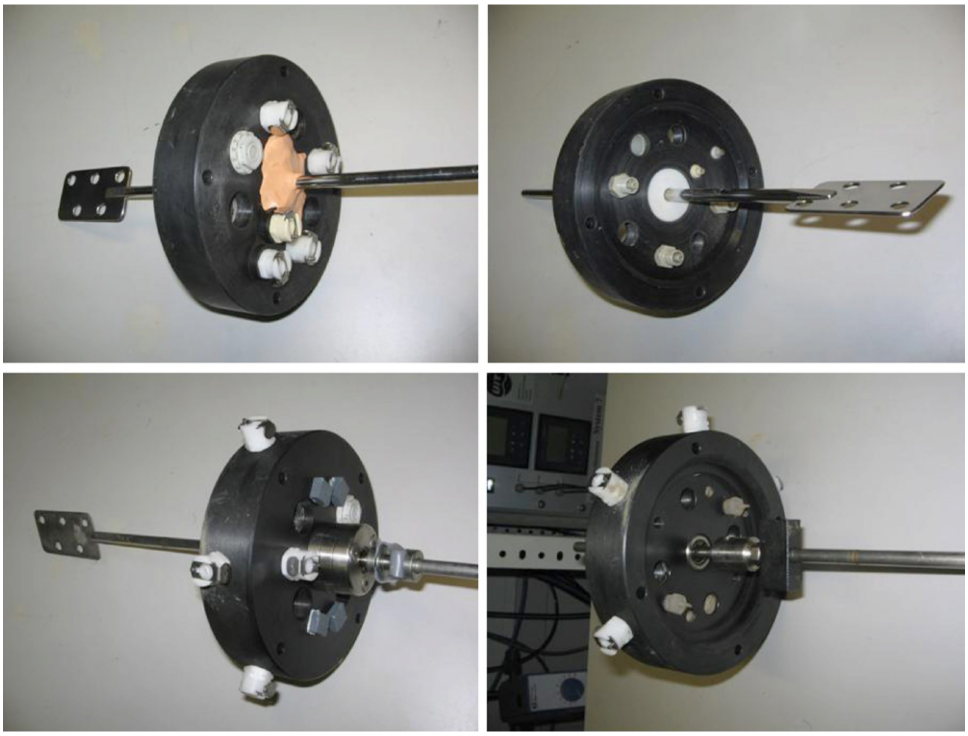

Fig. 3. Two options for an overhead stirrer to reach an air-tight seal. Top two photos illustrate a less expensive (<$100) option, and the bottom two photos illustrate an expensive (>$1000) option.

3.3. Installation of data logger for recording redox potential and pH

The pH/ORP controller used in Fig. 1 has no function of data logging. Manual reading of redox potential and pH is limited in capturing rapid changes of redox potential and pH during incubation. Installing a data logger for automatic recording can significantly improve the frequency of data acquisition. For example, an inexpensive (about $300) pH/mV data logger 850059 (Sper Scientific, Arizona, USA) can memorize up to 4000 records at steps between 1 s and 2 h (Fig. 2, bottom left). Resolution of at least 5 min is suggested, since the data logger may cause some error when recording in seconds. The pH electrode is combined with a temperature probe for this data logger. A more complex data logger (for instance LogTrans 16-GPRS, UIT, Dresden, Germany) can be used to store data from several microcosms. The recorded data of Eh, pH and temperature can be simultaneously downloaded to a computer. Both the pH electrode and the Eh electrodes need to be calibrated before application according to standard procedure (Faulkner et al., 1989).

3.4. Temperature control of a microcosm

All biogeochemical processes are temperature dependent, and so are both redox potential and pH measurement. An isothermal condition for an experiment can be achieved in a temperature controlled laboratory. The basic microcosm has no temperature control mechanism designed to study the effect of temperature on biogeochemical processes in soils and sediments (Fig. 1). Application of an incubator with temperature control is not practical for a microcosm due to the associated accessories and instruments. To reach higher than ambient temperature, a heating blanket surrounding the incubation flask can be used. Another similar option is to use a heating plate placed at bottom of the flask if an overhead stirrer is used for the microcosm. Reaching below ambient temperature can be a challenge. A double-hull incubation vessel (KGW-Isotherm, Germany) can be used for a full-range temperature control of the incubation (Fig. 2). Water of desired temperature can be pumped between the two layers of the vessel to maintain temperature control of the system. Various refrigerating and heating water circulator are commercially available with temperature range between −20 to 80 °C.

3.5. Automatic analysis of gas samples

By integrating all the above modifications, a fully automated microcosm has been developed (schematic as in Fig. 2, top). The system can simultaneously control several microcosms for Eh, pH and temperature. Each microcosm consists of a double-hull glass vessel with an air-tight lid. It is equipped with an overhead stirrer, a Pt electrode with a silver-silver chloride (Ag/AgCl) reference electrode (EMC 33), a pH electrode (EGA 153), and a temperature electrode (Pt 100) (all Meinsberger Elektroden, Ziegra-Knobeldorf, Germany). Data collected by the sensors can be stored in a data logger (LogTrans 16-GPRS, UIT, Dresden, Germany), and can be downloaded to a computer. The system is equipped with an automatic-valve regulation system which allows automatic control of Eh and pH. Redox potential can usually be kept within Eh-windows of approximately 30–40 mV of the target values by automated supply of O_2 or N_2. The system also has an automated microcosm headspace gas sampling and gas analysis system. Gas concentrations are analyzed using a gas chromatograph (GC) instrument with an electron capture detector (ECD, for N_2O), and a flame ionization detector (FID, for CH_4 and CO_2) coupled with a methanizer for transformation of CO_2 into CH_4 after separation.

4. Applications

This article summarizes some recent advancement in soil microcosm apparatus for better application of this valuable technique in

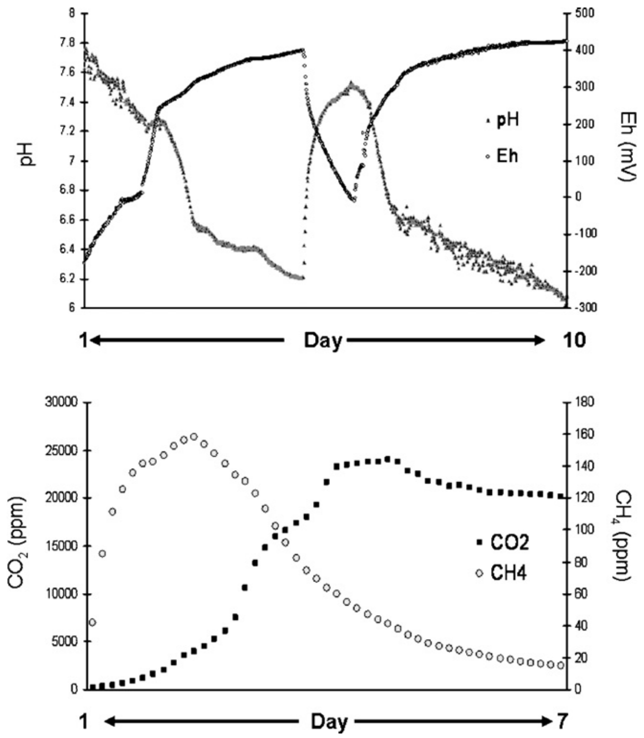

Fig. 4. In a study using a modified microcosm, 960 continuous readings of Eh and pH were recorded in 10 days (top), and 50 continuous measurements of CO_2 and CH_4 concentration were made in 7 days (bottom).

various biogeochemical researches. An example was given in Fig. 4 (top) where a data logger was used for automatic recording of soil redox potential (Eh) and pH in a soil microcosm. The measurements were continuously made every 15 min, which captured a complete dynamics of Eh and pH in the soil slurry without any data gap caused by operator's absence. This high-resolution measurement can be critical when soil slurry experienced dramatic changes in Eh and pH within a short period of time (Vorenhout et al., 2004).

Application of automatic gas analysis was made to study three greenhouse gases, CO_2, CH_4 and N_2O at the same time (Yu et al., 2007). Continuous measurements allow the researcher to monitor the dynamics of gas concentration in a microcosm (Fig. 4, bottom). At any certain point, gas concentration represents the balance between gas production and consumption processes that are commonly taking place simultaneously. Gas consumption generally increases with ambient gas concentration. However, gas production is normally independent of the product concentration, especially for trace gases. Thus, a compensation point is reached when gas concentration stabilizes due to its production equals consumption (Conrad, 1994).

By using the automatic microcosm system (Fig. 2), compensation points for CH_4 under different Eh-pH conditions were determined for the first time (Yu et al., 2007). This is largely due to the technical improvement in frequency of Eh, pH measurements and gas analysis, which is not available in the basic setup of the microcosm. The recent advancement in soil microcosms definitely provides more opportunities for future biogeochemical research.

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