Field methods

10

The measurements of microbial biomass and activity under laboratory conditions supply useful information on the physiological state of microbial populations in soil. Such measurements, however, do not reflect the real situation under natural conditions. Generally, the objectives of field measurements are to quantify mineralization processes, and thereby to gain insight into how the nutrient minerals and organic matter of the soil can be more efficiently utilized and conserved. Furthermore, field methods provide information on the effect of environmental factors on soil microflora. In this chapter, up-to-date field methods are presented and discussed.

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Estimation of soil respiration

K. Alef

Measurement of CO_2 evolution rates (long-term assay)

(Anderson 1982)

Principle of the method

The method is based on the determination of CO_2 evolved from undisturbed soils. The NaOH solution is placed in an open glass jar above the soil surface and the area to be measured is covered with a metal cylinder closed at the upper end. After the incubation, the NaOH solution is removed and the CO_2 concentration is measured by titration.

Materials and apparatus

Metal cylinders with one sealed end (Fig. 10.1)
Screw-capped glass jars (7 cm high, 6.5 cm diameter)
Tripods made of heavy metal or plastic

Chemicals and reagents

NaOH solution (1.0 M)
Barium chloride ($BaCl_2$, 3 M)
HCl (1.0 M)
Phenolphthalein indicator

1 g of phenolphthalein is dissolved in about 80 ml ethanol (95%) and brought up with ethanol (95%) to 100 ml.

Procedure

Pipette 20 ml of NaOH solution into a glass jar and place on a tripod located on the soil surface of the selected site. Immediately place the metal cylinder over the NaOH solution and press the edges about 2 cm into the surface of the soil. The system should be shielded from direct sunlight (e.g. using aluminium foil). After an incubation time of 24 h or more, the glass jar is removed (cap the jar for transport to the laboratory) and the NaOH solution is titrated as described earlier (see Chapter 5). Controls are performed by incubating the jars (containing NaOH solution) in the field in completely sealed metal cylinders.

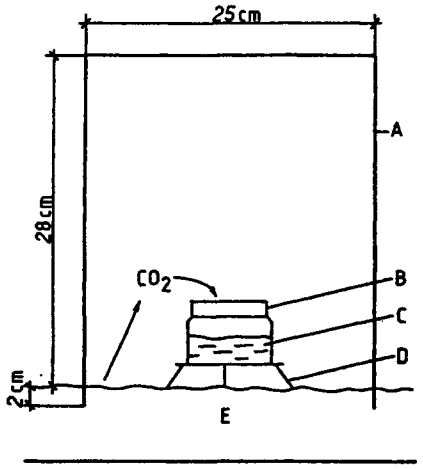

Figure 10.1. Long-term estimation of CO_2 production from the soil surface (Alef 1991; Anderson 1982): (A) Metal cylinder; (B) glass jar with screw cap; (C) NaOH solution; (D) tripod and (E) soil.

Calculation

The CO_2 evaluation rates can be calculated as follows:

$$C \text{ or } CO_2 \text{ (mg)} = (B - V)\, NE \quad (10.1)$$

where B is the HCl (ml) needed to titrate the NaOH solution from the control, V is the

HCl (ml) needed to titrate the NaOH solution in the jars exposed to the soil atmosphere, $N = 1.0$ (HCl normality) and E is the equivalent weight (22 for CO_2 and 6 for C). The data are expressed as milligrams of CO_2 per square metre per hour.

Discussion

A full standardization of this method has not yet been achieved. Results obtained by this method are controversial due to many modifications used in the assay. Underestimations and overestimations of the amount of CO_2 evolved have been reported (Wanner 1970; Kucera and Kirkhham 1971; Edwards and Sollins 1973; Edwards 1974).

The CO_2 evolution rates depend very strongly on the physical and chemical properties of the studied soils. Furthermore, the temperature as well as the water content affected these rates.

CO_2 evolution in the field assay is due to the respiration of microorganisms, animals, plant roots and abiotic CO_2 production.

Destruction of soil structure should be avoided. This causes temporary increases in CO_2 evolution.

Measurement of CO_2 evolution rates (short-term assay)

(Anderson 1982)

Principle of the method

The method is based on the collection of CO_2 evolved from known areas of the soil surface by the means of an apparatus (Fig. 10.2; Richter 1972), which consists of four small gas collectors, a gas analysis tube and a small bellows pump. In the gas analysis tube, CO_2 reacts with a hydrazine compound ($CO_2 + N_2H_2 \rightarrow NH_2COOH$), whose consumption is shown by a change in the redox indicator.

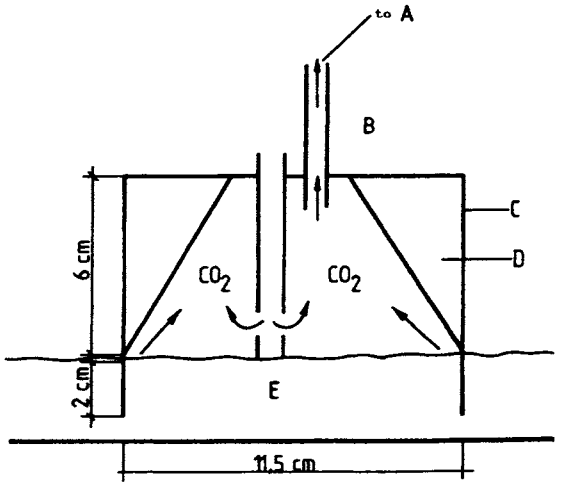

Figure 10.2. Short-term estimation of CO_2 production from the soil surface (Alef 1991; Anderson 1982): (A) pump; (B) copper tubes; (C) metal can; (D) plastic funnel; (E) soil.

Materials and apparatus

Gas collectors

The four gas collectors cover a surface area of 400 cm^2 and have a total volume of about 1.1 l. The cans of each collector are shown in Fig. 10.2

Hand-operated bellows pump (100 ml volume, Drägerwerk AG Lübeck, Germany)
Stopwatch
Thermometer

Procedure

After selecting an appropriate site, press the edges of the four gas collectors 1–2 cm into the soil, then draw 1 l of the air (10 strokes of the pump) through the system. Break off the fused ends of a gas analysis tube, and insert it between the collectors and the pump. An arrow on each tube shows the proper direction of the gas flow. Draw 2 l of air through the system (20

strokes, about 400 s), read the volume of CO_2 present in the 2 l of gas directly from the tube by comparing the leading edge of coloration with the markings on the tube's surface. Then repeat the process to determine the volume of the ambient air. To do this disconnect the gas collectors and, using a fresh gas analysis tube, draw an air sample (2 l) from a height of 1 m above the soil surface. During the measurement, record the air temperature.

Calculation

Example

A 2 l sample of soil air and ambient air drawn within 400 s at 20°C contained 0.15% of CO_2 by volume. Under the same conditions, 2 l of ambient air contains 0.07% of CO_2 by volume. The total surface under the four collectors is 400 cm^2 (100 cm^2 under each collector).

Then 0.08% of CO_2 by volume or a total 0.0008×2000 ml = 1.6 ml CO_2 400 s^{-1} was evolved from the soil. This means 14.4 ml CO_2 h^{-1} 400 cm^{-2} or 360 ml CO_2 m^{-2} h^{-1}. With the use of simple gas laws, this value can be corrected to standard temperature (0°C) and pressure (101.3 kPa = 760 Torr) as follows:

$$\frac{(360 \text{ ml } CO_2 \text{ m}^{-2} \text{ h}^{-1}) \times (101.3 \text{ kPa}) \times (273°C)}{(101.3 \text{ kPa}) \times (273°C + 20°C)} = 335.4 \text{ ml } CO_2 \text{ m}^{-2} \text{ h}^{-1} \quad (10.2)$$

Since at standard conditions, 1 ml of CO_2 is equal to 1.96 mg of CO_2

$$(335.4 \text{ ml } CO_2 \text{ m}^{-2} \text{ h}^{-1}) \times \frac{1.196 \text{ mg } CO_2}{1 \text{ ml } CO_2} = 657 \text{ mg } CO_2 \text{ m}^{-2} \text{ h}^{-1} \quad (10.3)$$

Discussion

The measurements can be carried out with minimal disturbance of soil and within a short time.

Automatic bellows pumps are commercially available (Dräger Werk AG, Lübeck, Germany).

Measurement of CO_2 and O_2 concentrations at various soil depths

(Richter 1972; Anderson 1982)

Principle of the method

The method is based on the gas chromatographic analysis of small samples of the soil atmosphere drawn from the desired depths by means of gas sampling probes and gas-tight syringes.

Materials and apparatus

Sampling probe
Gas-tight syringes (5 ml); needles and rubber stoppers into which the needles can be inserted
Gas chromatograph equipped with TCD; molecular sieve of 5 Å column (2 m) for analysis of O_2; Porapak R column (2 m) for analysis of CO_2.

Procedure

The probe is pushed into the soil to the desired depth. Two millilitres of air are withdrawn and discarded by using a syringe (Richter 1972; Anderson 1982) without a needle inserted into the opening of the cannula within the probe. An additional 2 ml of air are withdrawn and the needle is fitted to the syringe. Immediately after that a 1 ml sample of air is discharged (to flush out the ambient air from the needle) and the needle is sealed by inserting it deep into a rubber stopper. The analysis of soil atmospheric samples is carried out by a gas chromatograph according to standard analytical

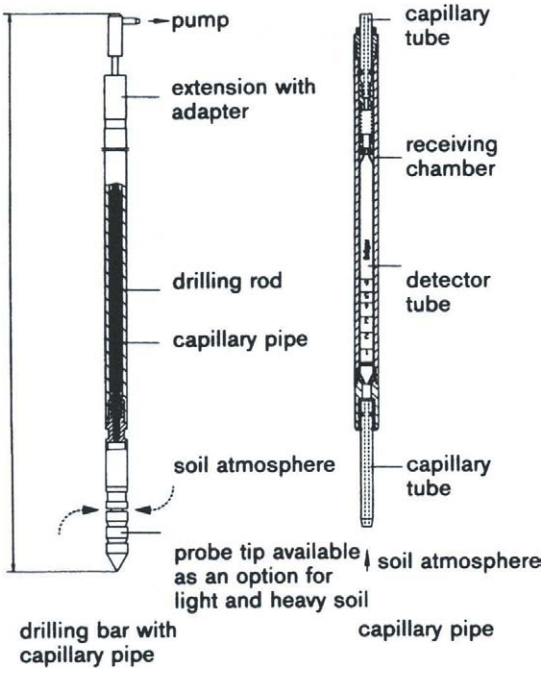

Figure 10.3. Dräger probe for collecting gas samples from soil.

procedures (He as carrier gas at flow rate of 60 ml min^{-1}, column temperature 25°C, detector temperature 50°C). A standard curve can be prepared from standard gases containing either 0.1 or 1% of CO_2 by volume or 10% of O_2 by volume.

Similarly, a probe (Fig. 10.3, Dräger, Lübeck, Germany) can also be used. Instead of the syringe, air samples are withdrawn using a small manual pump. The Dräger probe is usually applied for collecting air samples in contaminated sites. Chemical compounds present in the air sample are adsorbed on an active coal column. These compounds can then be analysed in the laboratory.

Discussion

The most difficult part of this technique is to obtain samples from the desired depths without contaminating them with the atmosphere from other depths or the surface.

In compacted soils a pilot hole should be made (use the slide hammer) and the probe inserted into the hole as quickly as possible to prevent excess atmospheric air from entering the hole (Roulier et al 1974).

The gas chromatographic determinations of CO_2 and O_2 are very sensitive.

Automated monitoring of biological trace gas production and consumption

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Soil acts as source or sink of many trace gases and plays an important role in global atmosphere/biosphere interactions (Andrae and Schimel 1989; Bouwman 1990). The quantification of the contribution of soil to the biogeochemical cycling of different trace gases requires automatic monitoring of the fluxes (Loftfield et al 1992), because temporal (diurnal as well as seasonal) and spatial variations prevent reasonable estimates of overall annual fluxes with simple approaches (Brumme and Beese 1992). Another purpose of automated field measurements is to improve or verify our knowledge about factors controlling trace gas emission.

Principle of the method

The method is based on the estimation of the gas concentration under field conditions in a covered soil (boxes, Fig. 10.4). The increase or decrease in the gas concentrations during the closure of the boxes can be used to calculate gas fluxes. An automated system, in which a personal computer controls the closing and opening of the chamber, the gas sampling in the chambers, the analysis of CO_2, N_2O and CH_4, and the calculation of the gas fluxes, enables the monitoring of diurnal, day-to-day and seasonal fluctuations.

Materials and apparatus

The chamber

The double-walled chamber is shown in Fig. 10.4. It is made from Plexiglass (10 and 6 mm thick), and consists of an inner and outer part, and a lid. The area of each part is 0.25 m^2 corresponding to a gas volume of 50 l each. The inner part is the zone where the measurements are made. The outer part acts as a buffer zone to reduce disturbances of the gas

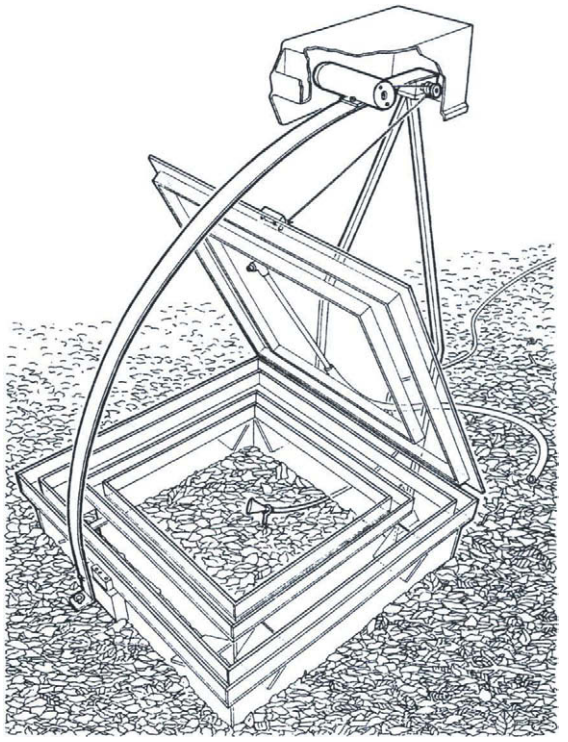

Figure 10.4. Double-walled chamber with motor driven lid (Loftfield et al 1992).

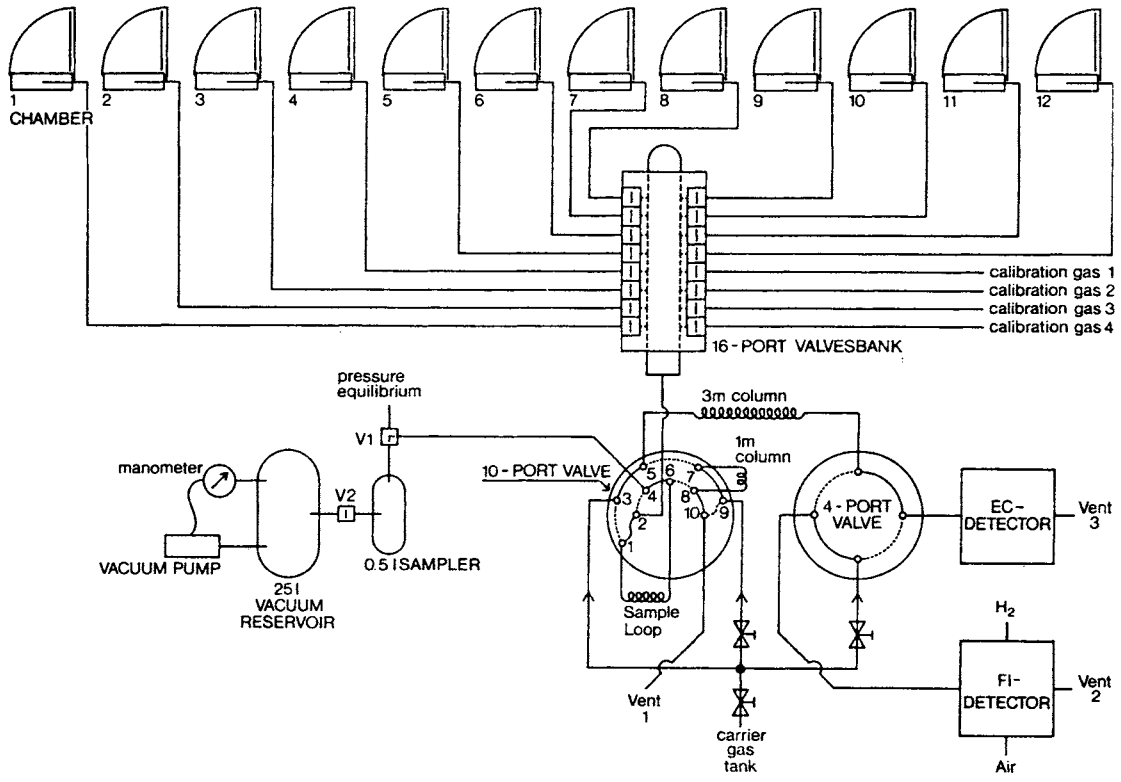

Figure 10.5. Gas plumbing for chamber sampling and gas chromatography analysis.

fluxes caused by wind action (Matthias et al 1980). To avoid damage to the root system, the chambers are placed on top of the forest floor or only inserted deep enough to achieve the horizontal placement required for the 2 cm water trap to seal the lid. Driven by a 12 V motor, the lid can be closed and opened at given time intervals. The lid has an orifice to ensure that no pressure or vacuum can be built up during the process of closing and opening. A magnetic contact controls the movement of the lid (see Fig. 10.6). Twelve chambers are connected to a gas chromatograph by heatable sampling tubes over a 16-port valve bank (Fig. 10.5). They consist of an inner Teflon tube (3 mm i.d.) and an outer polyethylene hose (9 mm i.d.). A heating wire is wound around the Teflon tube to prevent condensation of water.

Sixteen-port valve bank

Besides the ports of the 12 chambers, four ports for calibration gases are linked to the 16-port valve bank (Fig. 10.5).

Gas sampling

The gas in the chambers is sampled in a 0.5 l flask by opening a valve of the 16-port valve bank. The flask has been evacuated before by the opening of V2. In this way, 0.5 l of gas are drawn into the flask over a 5 ml sample loop within the gas chromatograph. This amount is enough to exchange the gas in the tube system five times. After the sample loop has been filled, the tension is equalized to the atmosphere by turning V1. The gas sample now is introduced into the carrier gas stream of the gas chromatograph by shifting the 10-port valve.

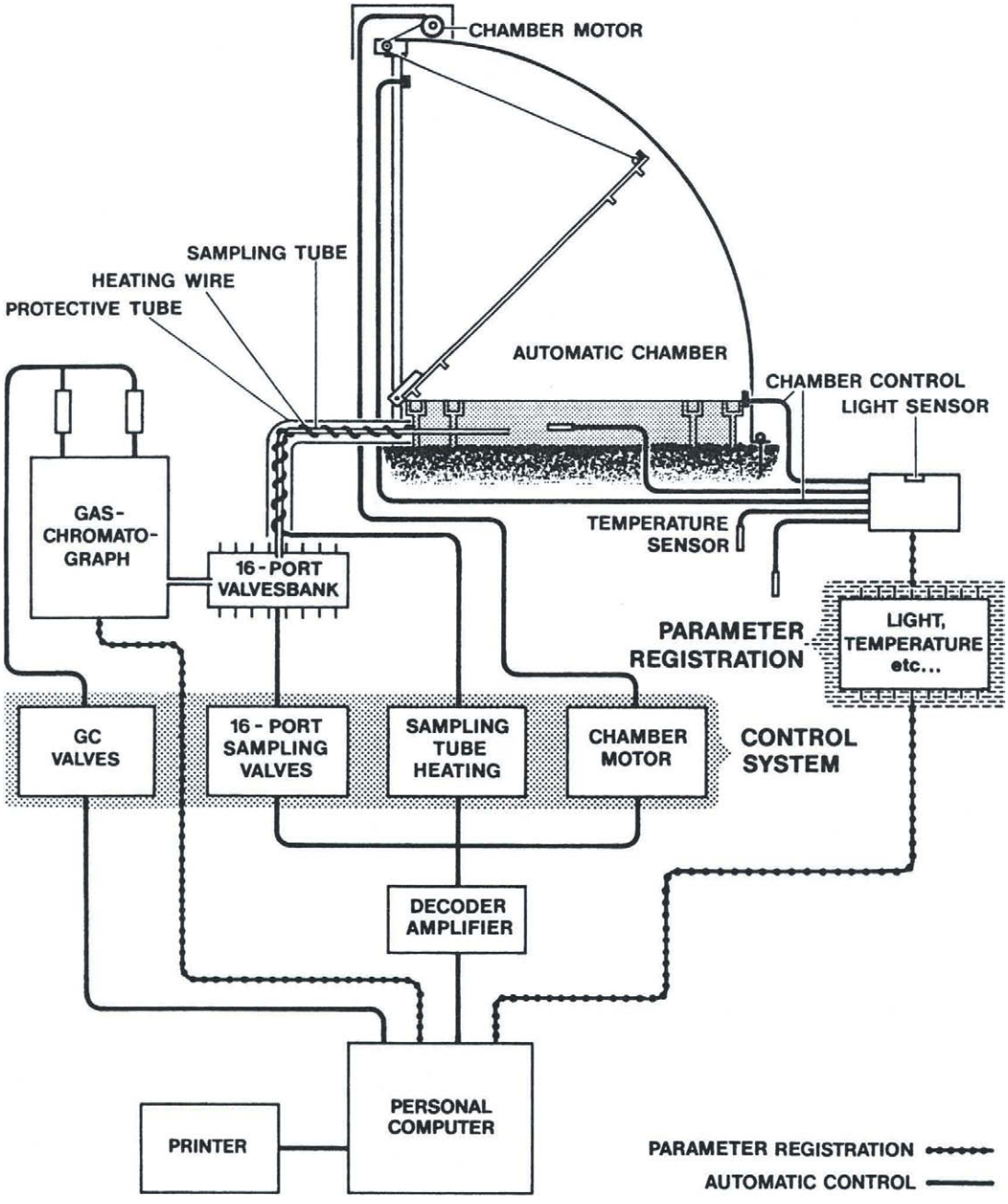

Figure 10.6. Schematic diagram of the personal computer supported control and data acquisition. ●–●–●, parameter registration; ———, automatic control (Loftfield et al 1992).

Gas chromatography

The air samples are analysed by a gas chromatograph, equipped with a pneumatic 10- and 4-port valve, a ^{63}N electron capture detector (ECD) and a flame ionization detector (FID) (Fig. 10.5). A plumbing system (1/16 inch stainless steel) described by Mosier and Mack (1980) was chosen because it has the

advantage of removing dirt and vapour by a precolumn back-flush-system. Both columns, the precolumn (1 m) and the analytical column (3 m), are filled with 150–200 µm Porapak Q (Millipore, Milford, MA). The electron capture detector, the valves and the column oven temperatures are 280, 65 and 65°C, respectively. The flow rate of the carrier gas was 18 ml min^{-1}. In contrast to Mosier and Mack (1980), N_2O, CO_2 and CH_4 are measured in the same gas sample.

AT-compatible personal computer and printer

The interface was achieved by using a 16-channel A/D card (no. 2814) and a 32-port I/O card (no. 2817), obtained from Data Translations (Marlborough, MA).

Hardware

All chambers are equipped with one light and three temperature sensors (Fig. 10.6). One temperature sensor is located in the chamber and the other two are in the soil. Hardware was developed for connecting the PC with the GC valves, the 16-port valve bank, the 12 sampling tube heatings and the 12 chamber motors.

Software

The system is controlled by a personal computer (Fig. 10.6). Special software, written in Turbo Pascal (Umweltfreundliche Energieanlagen, Göttingen, Germany), operates the 16-port valve bank, the V1 and V2 valves for collecting the samples, the 12 chamber motors to close and open the chambers, the 12 sampling tube heatings and the 10- and 4-port valves of the gas chromatograph. The gas analysis sequence for the calibration gases and the air from the chambers is variable and can be adapted to the problems under investigation. All important operations, errors in operation and results are displayed both on the screen and on the printer. In addition to the control of the sampling operations, the PC monitored the GC detector signals, the lid position of each chamber, the light intensity and the temperatures.

Gases

Nitrogen 5.0, ECD quality
Hydrogen 5.0, ECD quality
Hydrocarbon-free air
Different concentrations of calibration gases

Procedure

The operation of the system is described by a typical analysis cycle (Table 10.1). First the heating for the sampling tubes is turned on. Next, the 0.5 l flask, the 16-port valve bank, the sample lines and the GC sample loop are evacuated by opening valve V2. Following this step, valve V2 is closed and a single port of the 16-port valve bank is opened to clean the system. A gas sample (495 ml) from one of the open chambers is drawn into the evacuated system and the 5 ml GC sample loop of the 10-port valve (solid lines in Fig. 10.5) is filled. Thereafter, the sample valve is closed and the whole procedure is repeated for gas analysis with two extensions. Before closing the valve V2 and the sample valve, the pressure is measured and a malfunction is noted if the given values were not obtained (Table 10.1). Afterwards the lid is closed and the tube heater is turned off before the sample valve is closed. Next, the system is vented to the atmosphere (valve V1) and the pressure is measured for calculation of the sample volume.

The 10-port valve then acts (dotted lines in Fig. 10.5) for the injection of a 5 ml gas sample into the carrier gas stream. After N_2, O_2, CH_4, CO_2 and N_2O have left the precolumn, the 10-port valve turns back to the initial position (solid lines in Fig. 10.5). Water vapour remains on the precolumn

Table 10.1. Sequence of timed events for a typical operation cycle of the automated soil gas chamber system.

Time (s)	*Event*
0	Turn on tube heater
30	Open valve V2
90	Close valve V2
91	Open sample valve (V1–V16) (for cleaning)
151	Close sample valve (V1–V16)
152	Open valve V2
210	Measure pressure If > 1 kPa, note *error*, continue If < 1 kPa, continue
212	Close valve V2
213	Open sample valve (V1–V16) (for 1st gas analysis)
273	Close lid Is lid closure verified? No: Note *error*, turn motor off, turn heater off, continue Yes: turn off lid motor, continue
274	Measure pressure If < 80 kPa, note *error*, continue If > 80 kPa, continue
275	Turn tube heater off
276	Close sample valve (V1–V16)
277	Open valve V1 to equalize pressure in the system
287	Measure pressure for sample volume calculation
289	Close valve V1
293	Actuate 10-port valve to "inject"
368	Reset 10-port valve to "load" (solid lines in Fig. 10.5)
370	Open sample valve (V1–V16) (for 2nd gas analysis)
387	Open integration window for FID signal
421	Close window, turn 4-port valve to ECD (solid lines in Fig. 10.5), integrate CH_4 signal
440	Open integration window for ECD signal
540	Close window, return 4-port valve to FID (dotted lines in Fig. 10.5), integrate CO_2 and N_2O signal

and is displaced by the back flush. At the same time the sample loop is refilled with the next sample while the gases are separated in the analytic column. CH_4 is led to the FID (4-port valve, dotted lines in Fig. 10.5) and, after closing the 4-port valve, CO_2 and N_2O are led to the ECD. Then the 4-port valve is reset. The automatic registration and analysis of the gas measurements are performed by an integration subroutine. The chromatograms are saved for a later control as well as the calculated peak areas. Errors during the measurements are indicated and noted. One cycle lasted 6 min and was a part of an overall cycle in which all 12 chambers were closed for 1 h (variable) and samples were taken and analysed at 0, 30 and 60 min (variable) after closure of the chambers. Calibration gases and samples from the chambers are analysed alternatively. The order of gas analysis has to be chosen at the beginning. The temperature and light intensities were collected at 5-min intervals and recorded as hourly averages. During the opening and closing of the chambers a control subroutine is activated. If the operation is not finished within a fixed time of 50 s (variable), the system indicates a malfunction and the chamber is taken out of operation until the next check up.

Discussion

The automatic chamber system has been developed by two manufacturers, Umweltfreundliche Energieanlagen (UfE) in Göttingen and Loftfields Analytische Lösungen (LAL) in Neu Eichenberg, Germany.

An adjustable chamber system has been developed to measure gas fluxes from crops and soil. With increasing height of the crop, additional extensions can be installed between the basal chamber and an upper chamber with the lid.

The GC system has to be installed in an isothermal room because of the extreme sensitivity of the detector signal to changes in temperature.

By using N_2 as carrier gas, the calibration curve for CH_4 and CO_2 is not always linear. Therefore it is recommended that four different calibration gases are used.

Quantification of total denitrification losses from undisturbed field soils by the acetylene inhibition technique

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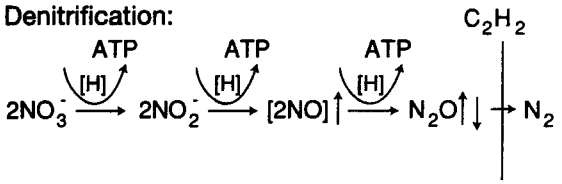
Denitrification (nitrate respiration) is probably the major source of nitrogen loss from terrestrial and aquatic ecosystems (Benckiser and Syring 1992; Körner et al 1993). Because of the lack of reliable direct measurements under natural conditions, the estimates of total global denitrification losses range from 83 to 390 g N a^{-1}. Such estimates have little significance so far, because basic data on denitrification measurements from soils and aquatic ecosystems are essentially missing and insufficient to allow reliable calculations.

Denitrification:



(10.4)

Denitrification is an aerobic energy-conserving process (ATP synthesis by means of cytochromes). It may occur alternatively to or simultaneously with respiration and nitrification in a great number and variety of taxonomically aerobic microorganisms (1–10% of the culturable bacteria) (Burth et al 1982; Ottow and Fabig 1985; Abou Seada and Ottow 1985, 1988; Schmider and Ottow 1986; Hooper et al 1990). The need to use nitrate (or nitrite and N_2O) alternatively or even simultaneously with O_2 occurs when the demand for electron acceptors cannot be met during intensive mineralization processes in the water films of so-called hot spots (Ottow 1992; Simarmata et al 1993). Consequently, denitrification losses can be recorded even at high oxygen partial pressures in the soil air (Prade and Trolldenier 1990; Benckiser 1994; Schwarz et al 1994). All soil treatments (such as using organic manure, soil tillage, fertilization, grassland conversion, etc.), which enhance microbial activity will stimulate nitrate respiration, especially at high soil moisture, temperature (5–65°C) and nitrate availability or supply (von Bischopinck and Ottow 1985; Benckiser and Warneke-Busch 1990). At the various microsites in the field the specific favourable conditions for denitrification may change rapidly, diurnally, and locally as well as temporarily (Arah 1990; Aulakh et al 1991; Benckiser and Syring 1992; Ottow 1992). For every field method developed to quantify total denitrification losses during a longer (e.g. vegetation) period, consideration should be taken of the large temporal and spatial variability of dentitrification caused by the inhomogeneous distribution of the carbon sources, diffusional

constraints of nitrate, O_2 and/or N_2O imposed by the ever-changing soil water content as well as by the delayed N_2O fluxes as a result of its entrapping in pores and water films, and adsorption to soil colloids (Becker et al 1990; Rolston 1990; Benckiser 1994). Thus, the spatial and temporal variability of denitrification in the field can vary by up to 300% and more (Benckiser et al 1986, 1987; Rolston 1990; Smith 1990, Aulakh et al 1991). This high variability, however, provides the statistical framework explaining both the interactions of essential ecological factors controlling denitrification as well as the wide range of gaseous nitrogen losses recorded in the field. The reliability of field measurements is consequently limited by the previously mentioned variability in denitrification. The results obtained by the acetylene inhibition techniques can only be considered as estimates of the magnitude of gaseous nitrogen losses.

Principle of the method

The acetylene inhibition technique (AIT), used to quantify total denitrification losses from soils, is based on a complete blockage of the N_2O reductase activity by HC≡CH, which has a structure similar to that of N=N−O (Federova et al 1973; Balderston et al 1976; Yoshinari and Knowles 1976; Yoshinari et al 1977; Ryden et al 1979; Ryden and Dawson, 1982; Benckiser et al 1986; Kapp et al 1990). Concentrations in the range of 0.2–1.0% v/v exhibited complete inhibition of N_2O reduction in denitrifying organisms in soils (Balderston et al 1976; Yoshinari and Knowles 1976; Yoshinari et al 1977; Kapp et al 1990).

Consequently, the N_2O fluxes collected from C_2H_2-treated soils can provide a measure of the overall denitrification rate, which is equal to the N_2O-N and N_2 release (Benckiser et al 1986; Klemedtsson and Hansson 1990; Kapp et al 1990, 1993). At present, there are two AIT-based approaches to quantify total denitrification losses from undisturbed soils:

1. Soil core incubation method (Parkin et al 1985; Robertson et al 1987; Ryden et al 1987; Aulakh et al 1991).

2. Flow-through soil cover method (Ryden et al 1979; Ryden and Dawson 1982; Benckiser et al 1986; Kapp et al 1990, 1991; Lehn-Reiser et al 1990; Schwarz et al 1994).

Materials and apparatus

Soil core incubation method

Airtight anaerobic jars (Fig. 10.7)
Glass bridges with tubes, airtight double-way glass cocks, rubber ball and one-way valves
Rubber septum seals fitting to the tubes of the glass bridges
Core samplers (varying volumes)
Airtight syringes fitted with a lock.

Flow-through soil cover method

Self-constructed PVC soil covers (of variable dimensions) equipped with a sharpened steel frame at the bottom, an air inlet and outlet (PVC tubes, 10 mm i.d.), and fixed or removable Plexiglass lids
Glass or polyethylene columns for CO_2 and H_2O trapping
Glass tubes (straight, 20 cm length, 2 cm i.d., or U-shaped, 50 cm length, 2 mm i.d., for trapping N_2O) + fitting rubber stoppers
Glass tubes (0.5 mm i.d.)
Rubber tube (0.5 mm i.d.) flow meter (0–70 l min^{-1})
Membrane pump
PVC tubes (1 m length, 6 mm i.d.) for C_2H_2 contribution
Polyethylene tube (6 and 10 mm i.d.)
Electric borer
Borer (10 mm × 60 cm)
Erlenmeyer flasks with tubes and fitting rubber stoppers
Glass containers with glass tubes as outlets and fitting rubber stoppers
Tube-clamps and double-way cocks

Rubber septum seals fitting to the necks of the Erlenmeyer flasks
Airtight syringes fitted with a lock
PVC or steel tubes (1 m length, 6–10 mm i.d.) with soil air inlets and outlets for soil air sampling
Micro-PVC tube (0.5 mm i.d.)
Septum seals (8–12 mm i.d.)
Double-sided needles
Evacuated vacucontainer (5 ml)

Gas chromatography

A gas chromatograph equipped with an ECD and/or a thermal conductivity detector (TCD) is required. N_2O can be separated from CO_2 and C_2H_2 using a Porapak Q column (120 mesh, 2 m long), and N_2 from O_2 using a 0.5 mm molecular sieve column (60/80 mesh, 2 m long). For the detection of low N_2O-N concentrations an ECD detector is essential (carrier gas N_2, flow rate 30 ml min^{-1}, make up gas at 40 ml min^{-1}; instead of N_2, a mixture of Ar and CH_4 at 95:5% can be used). Proper conditions for separating and quantifying N_2O are an oven, injector and ECD temperature of 40°C, 60°C and 300°C, respectively. The range of detection is 0.1–400 ng N_2O-N. Higher N_2O-N concentrations as well as CO_2, C_2H_2, N_2 and O_2 can be quantified by a TCD detector (carrier gas He, flow rate 30 ml min^{-1}). Proper conditions for separating and quantifying N_2, O_2, CO_2 and C_2H_2 are an oven, injector and TCD temperature of 40°C, 60°C and 150°C, respectively. The range of N_2O-N detection is 0.1–50 µg N_2O-N. The high sensitivity for N_2O detection by GC makes the AIT a favourable tool for an accurate determination of denitrification losses. The heterogeneity of soils and soil samples, as well as the high spatial and temporal variability in denitrifying activities are the major restrictions.

Gases and chemicals

Acetylene (technical grade)

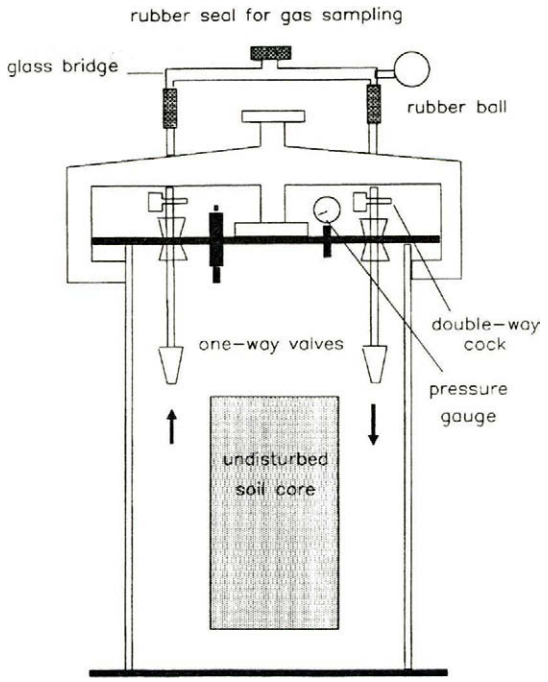


Figure 10.7. A modified anaerobic jar (4.5 l) used to incubate intact soil cores in the presence of acetylene for total denitrification measurements. Commercially available anaerobic jars (Gössner Company, Hamburg, Germany) are modified by introducing a glass bridge, gas taps, suba seal and rubber ball (Ottow et al 1985; Abou Seada and Ottow 1985, 1988).

Nitrogen or Ar and CH_4 (ECD grade)
Helium (purity 99.996% v/v)
N_2O diluted in N_2 (100 parts per million by volume; purity 99.995 v/v, calibration gas), CO_2, O_2 and N_2O (purity 99.995 v/v, calibration gases)
Granulated $CaCl_2$
$NaOH + Ca(OH)_2$ (tablets with indicator)
Molecular sieve, 0.5 nm (2 mm pellets)
Concentrated H_2SO_4
Distilled water

Procedure

The soil core incubation method

Using a rubber hammer, steel corers (metal rings generally used by soil physicists, 6–11 cm diameter × 7.5–20

cm) are randomly and carefully driven in the soil in order to obtain almost undisturbed soil cores. Cores damaged by the presence of stones or organic debris are discarded. Some compacting of about 10% of the volume may occur during sampling (Ryden et al 1987). The collected cores (at least five replicates) are placed immediately into modified, *ca.* 4.5 l anaerobic jars (Fig. 10.7). The jars and the cores are flushed with air or He (Ar, N_2) gas (for about 15 min), depending on whether aerobic or anaerobic incubation is desired. After syringe injection of C_2H_2, using airtight PVC syringes (Becton and Dickinson, Germany), through the seal, the total air phase is carefully mixed using the rubber ball (Fig. 10.7) to give a final acetylene concentration of 5–10% v/v. The jars are incubated in darkness (20–25°C) or at the actual soil temperature directly in the field in prepared holes (Ryden et al 1987). Daily (up to 14 days) 1 ml gas samples are taken after gas mixing from the jar with a gas-tight syringe, and the N_2O, CO_2 and O_2 concentrations are determined by GC. The respired O_2 from the atmosphere of the jars is adjusted to 20% v/v by adding O_2 with a syringe through the rubber seal and mixed with the gas phase using the rubber ball. Samples may be run without acetylene, if the N_2O part of total denitrification is of interest. This soil core method is suitable for evaluating soil denitrification rates of almost undisturbed soil under nearly natural conditions. Denitrification losses are generally expressed in kg N ha^{-1} day^{-1}.

In situ determination of total denitrification losses

Self-constructed (Fig. 10.8) open cover boxes (50 cm × 10 cm × 15 cm), equipped with a sharpened steel base and a removable Plexiglass lid (to enable photosynthesis) are inserted carefully about 5 cm deep into the tilled soil between the plant rows or into the sward (four replicates). Six holes (*ca.* 60 cm deep and 10 mm diameter) are established by an electric borer around the cover boxes. One day before measuring the N_2O fluxes from the soil, each box is flushed (without pressure) for 4 h with 40 l of acetylene (Messer-Griesheim, Germany) through six perforated probes (PVC tubes, 8 mm in diameter; Benckiser et al 1986; Lehn-Reiser et al 1990; Schwarz et al 1994). Three hours prior to the N_2O collection, an additional 10 l of acetylene is introduced into the soil for 2 h. The acetylene distribution and concentration in the soil air are checked by special gas sampling tubes inserted carefully in prebored holes (Fig. 10.8). Appropriate acetylene concentrations of 0.2–1.0% (v/v) should be present in the soil air and are easily ascertained even at relatively high water tensions up to 6 kPa (*ca.* field capacity) (Benckiser et al 1986; Kapp et al 1990). In order to eliminate acetone contamination of the soil, acetylene is passed through flasks containing concentrated sulphuric acid before introducing it into the soil (Benckiser et al 1986). During the equilibration period (2 h), the gas lids of the cover boxes are removed to avoid the accumulation of acetylene gas and an increase in temperature of the chamber. During the actual period of N_2O sampling, the open chambers are flushed continuously with an air stream of 20 l h^{-1} using a vacuum pump (Vacubrand M2), needle valves and flow meters (Platon, Germany; Fig. 10.8). The N_2O released in the air stream is led through a CO_2 and H_2O filter (a flask containing granules of $CaCl_2$ and NaOH) and collected in three traps (19 cm long, 2.5 cm in diameter) filled with 0.5 nm molecular sieve pellets (2 mm, Merck, Germany). N_2O absorbed over 4–8 h periods in the traps is transported to the laboratory. Concomitantly, with the same procedure, the N_2O in the surrounding air used for flushing the cover boxes is collected. In order to

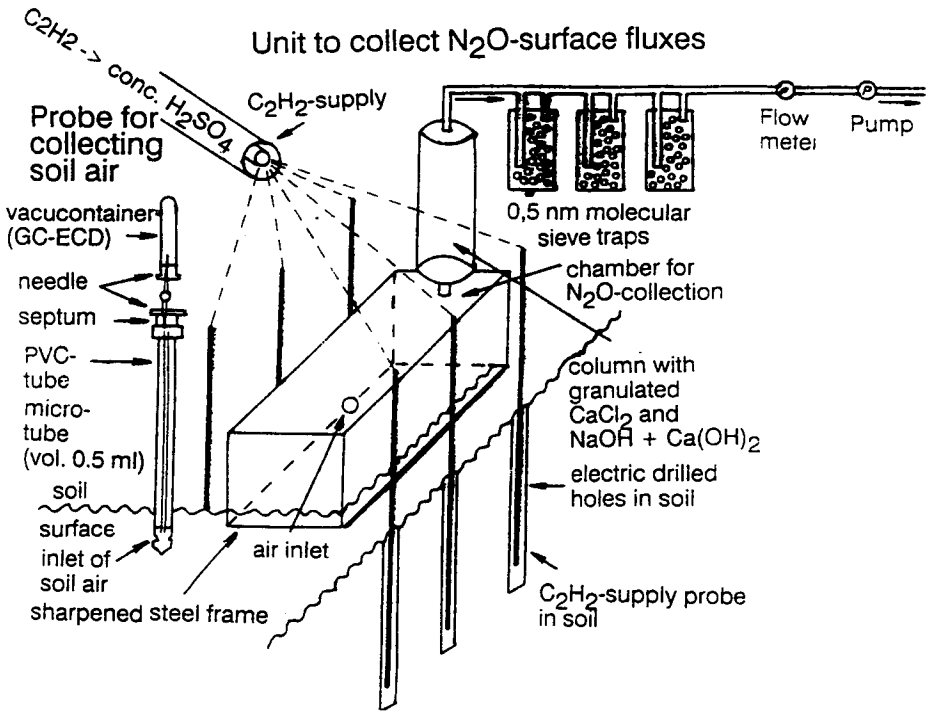


Figure 10.8. Schematic diagram of the open soil cover method to quantify total denitrification losses in undisturbed fields with the acetylene inhibition technique (Benckiser et al 1986; Kapp et al 1990; Lehn-Reiser et al 1990; Schwarz et al 1994).

avoid microorganisms adapting to acetylene as a substrate or bypassing the inhibition effect, the sampling sites are changed every 4 days. The N_2O absorbed on the 105 g of the 0.5 nm molecular sieve is liberated from the pellets in the evacuated Erlenmeyer flasks (ca. 1 l) containing 150 ml water (Fig. 10.9); Ryden and Dawson 1982; Benckiser et al 1986; Kapp et al 1990). After 2 h the vacuum is replaced by air, and the N_2O concentration in each flask is determined by GC. The amount of N_2O dissolved in water is calculated as proposed by Moraghan and Buresh (1977). After subtraction of the N_2O in the air used for flushing the cover boxes, the N_2O surface fluxes are calculated in g N_2O-N ha^{-1} day^{-1}. Before starting AIT measurements, the actual nitrate concentrations in the soil should be determined because soils are sinks rather than N_2O sources, if the nitrate concentration is low with respect to the amount of easily decomposable carbon (Knowles 1990; Simarmata et al 1993).

Discussion

Estimates of total denitrification losses suffer from high variability even within short distances of a field. A temporarily high denitrification activity at the microsite level is essentially determined by the amount and availability of the easily decomposable organic matter in the soils (Ottow 1992; Benckiser 1994; Schwarz et al 1994). The higher the inhomogeneity of the soil with respect to the distribution of the organic debris, the greater is the variability in denitrification. Homogeneously tilled (arable) soils with a low tortuosity will give a

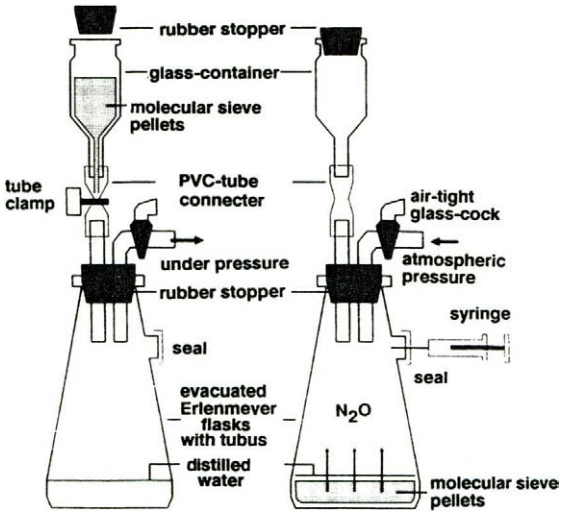


Figure 10.9. Units required to release the N_2O absorbed to the 0.5 nm molecular sieve pellets in the field (Ryden and Dawson 1982; Benckiser et al 1986; Kapp et al 1990; Lehn-Reiser et al 1990; Schwarz et al 1994).

uniform diffusion and the lowest variability, and thus the most reliable results with the AIT (Benckiser et al 1986, 1987). At present, the AIT is the most suitable and probably the most reliable method for routine determinations of total denitrification losses in undisturbed fields (Knowles 1990).

The main advantages of the AIT are:

1. The application in undisturbed as well as in disturbed, fertilized ecosystems.
2. The relatively simple and accessible equipment required.
3. The high sensitivity of the N_2O measurements by gas chromatography.

Another approach, the ^{15}N method, is mainly restricted to the evaluation of fertilizer nitrogen losses and is less reliable for measurements of total denitrification losses from undisturbed soils (grassland, forests and zero-tilled arable land). The use of ^{15}N needs (a) a homogeneous incorporation of ^{15}N in the (top) soil, and (b) expensive materials and equipment (mass spectrometer). If denitrification is calculated indirectly by difference, all ^{15}N pools have to be considered (^{15}N mass balances; Aulakh et al 1991). Denitrification losses deriving from the nitrogen out of the native organic nitrogen pool are not considered sufficiently. Despite this, there is good agreement between the denitrification losses estimated by the AIT and the ^{15}N method at comparable field conditions (Parkin et al 1985; Aulakh et al 1991). The major restriction of the AIT is caused by the inhibition of nitrification even in the presence of small C_2H_2 concentrations (Mosier 1980; Knowles 1990; Mosier and Schimel 1993). Consequently, during the denitrification measurements, the nitrate supply by nitrification is interrupted and total nitrogen losses by nitrate respiration refer only to the amount of nitrate present in the soil at the moment of measurement. Frequent changes of the measuring site is one way to try to minimize this limitation. Further, at low nitrate concentrations and a relatively high availability of easily decomposable organic matter, N_2O will be respired in soils to N_2 even in the presence of C_2H_2 (Simarmata et al 1993). Therefore, frequent nitrate monitoring during denitrification measurements by the AIT is essential for reliable interpretations of field data. Additional effects on denitrification by the metabolism of C_2H_2 under aerobic and particularly under anaerobic conditions may occur, but should be neglected in all soils containing 1–2% of total carbon. However, C_2H_2 may increase the rate of soil carbon mineralization or added glucose, if nitrate is not limiting (Haider et al 1983). This mechanism remains to be clarified. Acetylene concentrations of 1–2% (v/v) should be present in the soil air to establish C_2H_2 gradients that allow the N_2O reductase activity in all aggregates to be blocked sufficiently (Becker et al 1990). Even at such high concentrations acetylene does not affect the general soil metabolism (Kapp et al 1990; Simarmata et al 1993).

Lysimeter

P. Burauel
W. Steffen
F. Führ

Principle of the lysimeter

The lysimeter type that is described here enables experiments to be carried out under natural environmental conditions. Questions concerning nutrient turnover as well as the fate of xenobiotics can be studied in the soil plant system. This also includes research activities in soil microbiology such as questions about microbial ecology and soil biochemistry. The relationship between laboratory and field studies can be drawn using data from lysimeter experiments. The lysimeter units in use are composed of a square or round cylindrical casing (0.5 or 1 m^2 surface area) filled with an undisturbed soil monolith of 1.10 m of depth. The casing, which is placed in a rack with a perforated bottom, is inserted in a completely watertight container embedded in the ground. All equipment is made from stainless steel (Führ et al 1976, 1991; Führ 1985; Steffens et al 1990, 1992; Brumhard 1991; Pütz 1992).

The lysimeter station

The lysimeter station at the Institute of Radioagronomy was designed and constructed to perform experiments closely matching natural environmental conditions to evaluate, for example, the fate of ^{14}C-labelled pesticides in the soil/plant system in accordance with good agricultural practice. Using the lysimeter as a test system, mass balances can be drawn

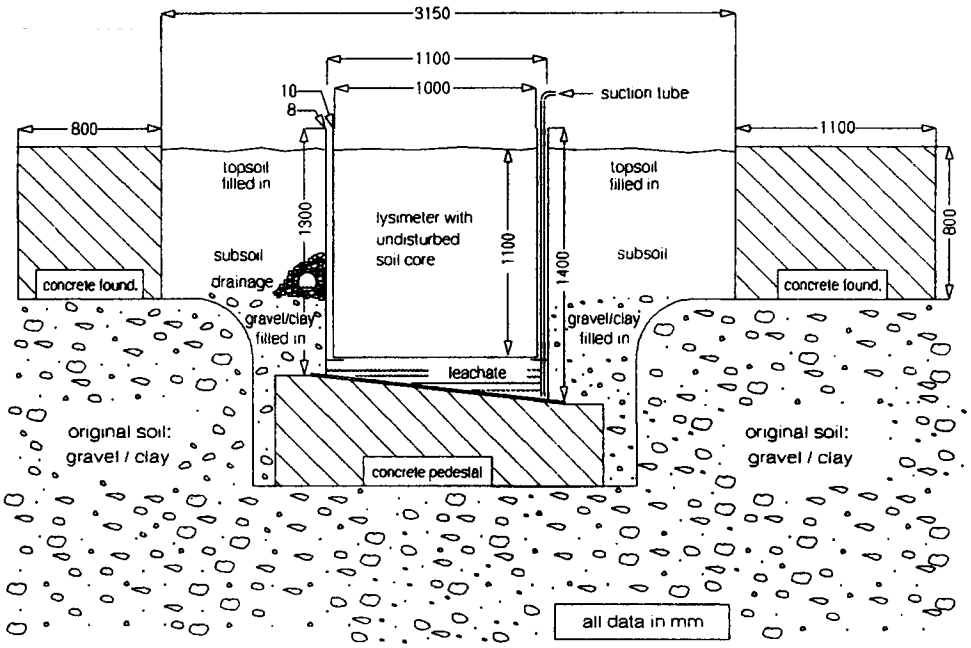


Figure 10.10. Cross-section of the lysimeter system (dimensions are in millimetres).

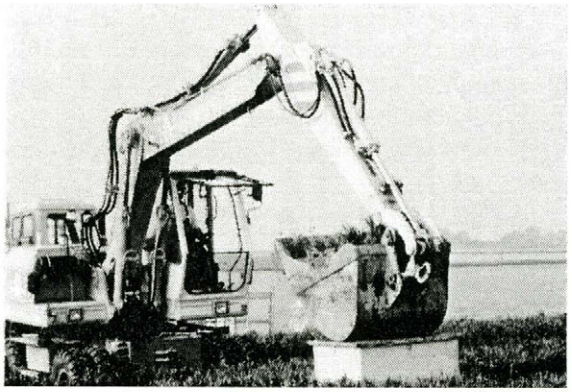
up considering almost all processes of dissipation. A cross-section of the lysimeter installation is shown in Fig. 10.10. A concrete bed serves as a basement to place the lysimeter unit in the ground. The lysimeter casing itself stands in a second watertight container on four arms fastened to the walls at a depth of 120 cm. This container is attached to the concrete basement. In total it is 140 cm deep so that there is enough storage space to collect water percolating through the soil monolith. The percolating drainage water is sampled by a suction tube inserted into a pipe fixed to the higher wall of the container. Figure 10.11 shows a single lysimeter embedded in a control plot to minimize possible side effects. Fifty lysimeters (20 of 0.5 m^2 and 30 of 1.0 m^2) are distributed in 10 strips of five lysimeters, each covering in total approximately 1000 m^2.

Filling of the lysimeters

The filling of a lysimeter is an important procedure in order to obtain undisturbed soil monoliths. To collect an undisturbed soil monolith, the lysimeter casing is covered at the top with a steel plate (30 mm thick). At the bottom, the walls of the casing (8–10 mm thick) have sharpened edges for cutting the soil. The casing is pressed into the soil by the shovel of an excavator to a depth of 110 cm (Fig. 10.12).

This technique guarantees that the soil monolith is pressed close to the walls of the casing and that there are no gaps between the walls and the soil. This is very important when studying the movement of xenobiotics in the soil. After the casing is pressed into the soil, the surrounding soil is removed and the bottom of the rack is pushed under the casing, cutting the soil with the sharpened edge at the front of the

Figure 10.11. The lysimeter station at the Institute of Radioagronomy.



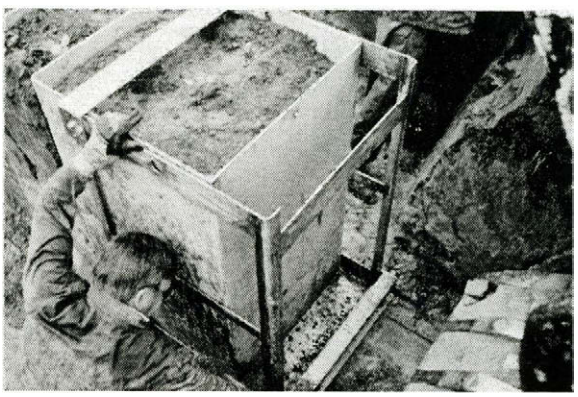


Figure 10.12. Filling of the lysimeter with an undisturbed soil monolith.

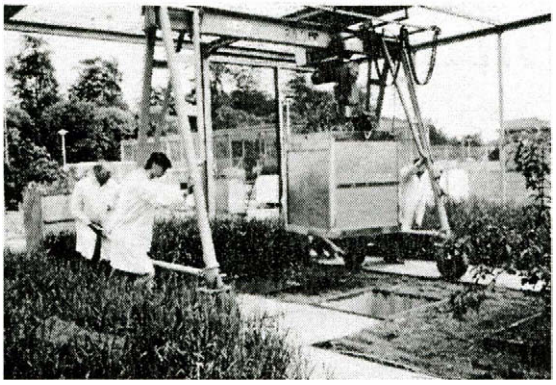


Figure 10.13. Placement of the lysimeter casing in the lysimeter station.

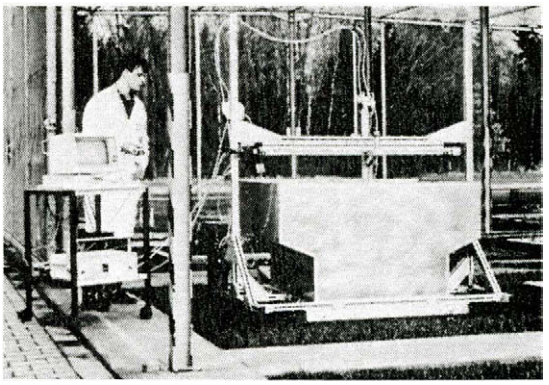


Figure 10.14. Automatic spraying apparatus for applying ^{14}C-labelled agrochemicals in lysimeter experiments.

base (Fig. 10.12). Then the lysimeter casing with the undisturbed soil monolith standing in the rack is lifted from the hole and transported to the lysimeter station. There the lysimeter casings are inserted into the containers, which are permanently installed in the ground (Fig. 10.13). With this technique, up to 15 lysimeters can be filled per day (9 h). In principle, any soil with a deep profile can be used. However, it will be very difficult, if not impossible, to collect undisturbed monoliths from heavy clays and stony soils using the technique described above.

Application of the lysimeter

Lysimeter experiments offer the opportunity to run experiments under farming conditions with the ability to change the type of crops, soil type and tillage. In the case of rotations, the crop treated with a ^{14}C-labelled pesticide is always tested first. If appropriate, intermediate crops such as mustard plants, phacelia or clover are also grown, Fertilization matches agricultural practice and questions of nutrient supply strategies can also be included in the design of the study, as long as sampling dates and procedures do not interfere with each other. In order to control weeds, fungal pests and insects within the lysimeter, and the surrounding plots, agrochemicals are applied in accordance with integrated pest control management. A meteorological station registers the air temperature and humidity, precipitation and wind velocity – all factors governing the fate of a pesticide in the system. Temperature and water content in the soil are also recorded due to their effects on the microbial activity of soil. The homogeneous application of the labelled pesticide or fertilizer is an important step in lysimeter experiments, especially for sampling reasons. Realistic rates of the tested pesticide and spray solution volumes (20–40 ml m^{-2}) as in agricultural practice should be followed to provide results that are transferrable to the field. Two different spraying techniques can be used: a hand-operated garden sprayer and an especially designed automatic spraying apparatus with nozzles, used in agricultural practice (Fig. 10.14). Before spraying, the lysimeter is surrounded by thin aluminium plates covered with foil in order to avoid contamination of the surrounding area and to control for balance purposes – the amount of the ^{14}C-labelled pesticide not reaching the lysimeter area. Several treatments have shown that up to 20% of the pesticide is lost during application and it mainly remains attached to the tin foil (Mittelstaedt et al 1992). This has to be taken into account when planning the application of definite rates of a particular agrochemical.

Extraction of soil solution with porous suction cups

H. Deschauer

In environmental studies the importance of the soil solution has continued to increase over the last decade. By reflecting the dynamic processes in the soil system, the soil solution is one of the most important components of the ecosystem used for studying the temporal and spatial distribution of nutrients and pollutants, the biological availability of all soil constituents, and the biological and chemical turnover of mineral and organic soil material. Several techniques are used to extract the soil solution. Extracts from bulk soil material are made by centrifugation, percolation, with immiscible liquids or by pressure filtration. All these methods produce a severe disturbance of the physicochemical properties of the soil as well as of the soil sampling site, and are not suitable for long-term and continuous investigations. *In situ* methods used are extraction with porous suction cups or sampling by resin bags. Because of their easy handling and the suitability for continuous sampling, suction cups are the most widely used method for ecological studies (Liator 1988).

Principle of the method

For sampling of the soil solution, suction is applied to a porous suction cup, which is in contact with the soil material. In this way capillary-bound soil water is extracted by the induced pressure gradient when the suction generated is lower than the soil water potential. The solution is stored in a sample chamber or in a collection vessel.

Three collection strategies are used (Grossmann and Udluft 1991)

The soil solution can be extracted continuously over a long period (1 week) with an electrical pump; or discontinuous sampling (regular or episodic) can be done either using a hand pump or an electrical pump. While the continuous sampling integrates over a long period, both discontinuous methods sample a solution from a single water-transport event.

Materials and apparatus

Many materials have been tested for their suitability for collecting soil solution. The hydrological and physical properties, the chemical resistance, and the exchange capacity as well as the hydrophilic properties of a material are relevant to its use in long-term field experiments. Table 10.2 summarizes the properties of the materials most frequently used for suction cups.

Field installation and maintenance

Installation procedure is similar to soil tensiometers. Installation is possible horizontally as well as vertically (Fig. 10.15). All compartments of the suction system containing soil solution should be placed below ground to prevent freezing. For most suction cup materials and with simple electrical pumps a maximum suction of

Table 10.2. Comparison of different materials tested as suction cups.

	Pore size (μm)	*Air entry (kPa)*	*Physical resistance*	*Chemical resistance*	*Exchange capacity*	*Hydrophilia*
P80 ceramic[1]	1	400	++	+	+	++
Frited glass[2, 4]	10–16	200–300	−	++	++	++
PTFE[3, 4]	2–10		+	++	++	−
Stainless steel[4]	0.5*	300	++	+	+	++
Sintered nickel[1]	n.a.	100	++	−	−	++
PVDF[5]	0.22	350	+	+	n.a.	+
Nylon[5]	0.45	210	++	++	n.a.	−

References: [1]Hetsch et al (1978); [2]Long (1978); [3]Beier and Hansen (1992); [4]Köhler (1993); [5]Grossmann et al (1985).
n.a., no data available; −, poor; + good; ++, very good; * irregular porosity.

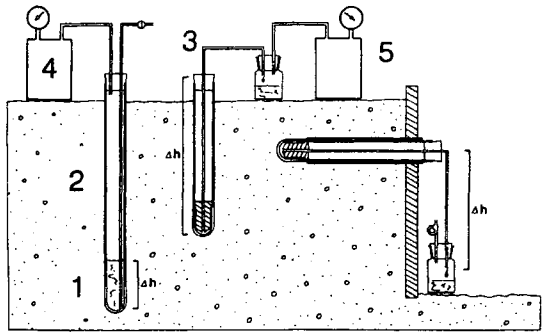


Figure 10.15. Different arrangements of soil solution samplers (Grossmann 1988): (1) suction cup; (2) hard or flexible cover; (3) capillary; (4) sampling vessel; (5) pump.

about 80 kPa may be reached, resulting in the extraction of water preferentially bound in mesopores. The equipment used is summarized in Grossmann and Udluft (1991) and shown in Fig. 10.12. The suction cup itself consists of the porous cup (1), a hard or flexible cover (2) and a capillary (3) for sampling soil solution. The system is completed by a sampling vessel (4) and a pump (5). Suction cup covers and capillaries are mostly made of PVC and polyethylene, but stainless steel, Teflon and glass are also used. The energy supply is provided either by batteries or power cables. Small membrane pumps are often used because of the low energy requirement.

For some of the materials mentioned (P-80, sintered Ni), a prewash with dilute acid and demineralized water is recommended to prevent bleeding of metals. Teflon cups are produced using fatty acids and they should therefore be cleaned with an organic solvent before use. Furthermore, all materials should be preconditioned with a solution comparable to the soil solution. In laboratory experiments, sorption of trace metals has been shown for many materials (P-80, PVDF, sintered nickel), which can be reduced by preconditioning with soil solution. Guggenberger and Zech (1992) showed a marked effect from preconditioning on the sorption of dissolved organic carbon (DOC) with new P-80 suction cups. The sorption of P and NO_3^- has also been reported for ceramic suction cups.

Besides the chemical sorption of trace metals, P and nitrate, a filter effect has also been found. Colloidal or particular organic constituents of the soil solution may be filtered or precipitated on the surface of the suction cup. For sintered nickel cups, the precipitation of nickel phosphates is discussed as a reason for the reduced permeability after percolation of the soil solution. On the other hand, P-80 cups have been used for several years in field experiments with no effects on permeability.

Sorption of mineral or organic substances on the suction cup surface or filter effects may change the surface properties of the cup material. Köhler and Deschauer (unpublished results) found an increased sorption of hydrophobic organic compounds on several suction cup materials after preconditioning with dissolved organic material. Hence, the results of laboratory investigations on sorption or filtering on cup surfaces should be evaluated critically and, after installation, the suction cups should be conditioned in the field for several weeks.

In fine and medium-textured soils under unudic moisture regime, the suction cups are not influenced by the climatic conditions over a period of several years. In winter, suction cups may be destroyed by mechanical forces due to soil freezing and thawing. Therefore, all constituents of the suction cup system containing soil solution should be placed below ground to prevent freezing. In coarse-textured soils or under dry climatic conditions, permanent contact of the suction cup with the soil may be disturbed by drying and shrinking of the soil (clay) or the silty material used for installation. Both problems require re-installation of the suction cups. The system should be tested for leaks at intervals of several months. For suction systems with a battery supply, fast depletion of the batteries is an indicator of small leaks.

Discussion

The use of suction cups for the extraction of soil solution is a well-established method in long-term field investigations. Despite their suitability for many experiments and questions, some problems with the method should be taken into consideration. Due to the suction generated, cups sample the soil solution from mesopores and fail to include the water transported in the macropores and fine pores. Hence, in structured soils with large and deep soil cores a combination of suction cups and suction-free lysimeters is necessary to collect a "real" soil solution. Due to the size of the cups, only a small part of the soil is in contact with the suction cups, resulting in a large number of replications being required to cover the soil heterogeneity. Depending on the number of replicates and the soil under investigation, a large error must be accepted. Differences in the inflow rate of suction cups, and the problems of filtering and adsorption increase this error.

Water volumes sampled by suction cups are not applicable for calculating the mass of water transported in the soil system. Hence a separate determination of soil water transport (soil tensiometers) is necessary to calculate water and substance transport. Besides the problems of adsorption and filtering, degassing of the soil solution is further influenced by the suction cups. The result of degassing by suction generation is changes in the buffer properties and the pH of the soil solution as well as losses of volatile organic compounds.

Considering the problems of suction soil solution samplers, the user has to solve this problem by choosing the appropriate suction cup with respect to the following questions

1. Which constituents of the soil solution are of particular interest for the investigation?
2. What level of precision is needed?
3. Which physical properties of the soil are to be expected?
4. How much will it cost?

As stated by Liator (1988), "no single and simple solution to soil solution collection at most soil conditions" is available.

Litterbag method

H.A. Verhoef

The litterbag method is commonly used to study the decomposition of organic matter in terrestrial ecosystems. In this method a large number of bags is placed in the field and a randomly chosen set of replicate bags is retrieved at predetermined time intervals, and analysed for loss of mass and/or changes in the chemical composition of the organic matter. The method was firstly used by Falconer et al (1933) and is often attributed to Bocock and Gilbert (1957). The litterbag is an open structure that allows a free exchange of air, water and solutes. The mesh size of (parts of) the bags is essential. By using bags with different mesh sizes, particular groups of organisms (soil animals) can be excluded, while those entering the bags can be extracted, and an idea of the relative contribution of various animal groups can be obtained.

An interesting modification of the traditional litterbag methodology of putting all the bags in the soil simultaneously, followed by subsequent sampling, is the method of Herlitzius (1983). The aim of this method is to elucidate whether, and to what extent, the decomposition of leaf litter is controlled by the date or duration of the exposure of the material in the soil.

Recently, the information available from using litterbags has been increased by the use of stratified litterbag sets (Faber and Verhoef 1991). These have been applied in stratified organic soil layers typical of coniferous forest soils.

Principle of the method

This is the determination of the temporal changes in a specific amount of soil organic matter, concerning mass and chemical composition, often combined with determination of temporal changes in root biomass, and soil biota densities.

Materials and apparatus

The material for the construction of litterbags can be nylon, fibre glass, polyester, polyvinyl and Terylene. Some bag types have closed plastic walls. These have the advantage of preventing compression of the enclosed litter and keeping the enclosed litter in a more or less fixed position. It has the disadvantage that vertical abiotic and biotic influences have greater impact than horizontal ones.

Procedure

For pine forests the procedure presented here discriminates for L, F and H layers, including fresh litter. Fresh litter is sampled at abscission time from the tree. Differentiation between the L, F and H layers is made in the field, and all organic material is taken to the laboratory and air-dried at room temperature for 1 week. To reduce heterogeneity, the L layer is sorted by hand for intact needles, material from the F layer is sieved until 2–5 mm in length, whereas the H layer is sieved with a 0.35 mm mesh sieve.

All material is stored at room temperature until required for replacement. One week before introduction the water content of the different organic layers is adjusted to the water content of the specific layer at the period of introduction. The weight and thickness of the L, F and H litterbags is in agreement with the thickness of the organic layer in the field, in such a way that the litterbags can be fitted into the organic layer. The "fresh" layer is put on top of the organic layers, and the amount is comparable to the needle fall at that time of the year. The size of the litterbags is 10 × 10 cm but may vary depending on the size of the experimental plot. The mesh sizes

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As stated by Liator (1988), "no single and simple solution to soil solution collection at most soil conditions" is available.

based on Herlitzius (1983), can be analysed in accordance with Gunadi (1993).

The combination of the data from the "from" and the "to sequence" treatment in one figure gives two possible mirror symmetries: (1) the axis of symmetry can be parallel to the *y*-axis, which means that the duration or the length of the exposure is important in relation to litter quality; and (2) the axis of symmetry can be parallel to the *x*-axis, which means that the date or seasonality effects are more important in the decomposition process. To test whether the axis of symmetry can be found parallel to the *x*- or *y*-axis, one should first change the direction of the "to sequence" treatment to become one direction with the "from sequence" treatment. If the two-way ANOVA result is not significant in the first-order interaction and in the main effect, then the duration or length of the exposure is important. Then one should change the value of the "to sequence" treatment to become negative. If the ANOVA result is not significant in the first-order interaction, the data of exposure or seasonality is important.

Discussion

The litterbag method is commonly used to study the decomposition of organic matter at different sites or under different treatments.

Apart from changes in weight and chemical composition of organic matter, one can use litterbags to follow root ingrowth and population dynamics of the soil inhabitants.

Using the "to" and "from sequence" method one can get information about the importance of the date or duration of exposure of the material for its breakdown.

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