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Measurement of actual and potential denitrification and soil respiration with an automated gas chromatographic system

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(With 4 figures)

Summary

The described instrument was developed out of the demand to get a most versatile and effective gas analyser with limiting amounts of investment money and analysis time. It consists of a gas chromatograph with a Thermal Conductivity Detector and an Electron Capture Detector, three electromagnetic valves, a Porapak and a Molecular Sieve analysis column. An automatic Head Space Sampler can be attached. The described setup can be used for several methods concerning gas metabolism in soils but it is focused on the routine measurement of ambient nitrous oxide concentrations as well as of high nitrous oxide concentrations as obtained in potential denitrification experiments. Carbon dioxide is measured simultaneously with nitrous oxide. Neon can be used as internal standard. Oxygen can be measured simultaneously with carbon dioxide after a slight alteration of the setup. Frontflush of acetylene and backflush of oxygen are installed to promote baseline stability and a long lifetime of the ECD.

Key-words: soil microbiology, methods, gas chromatography, denitrification, respiration.

Messung der aktuellen und potentiellen Denitrifikation und der Bodenatmung mit einem automatisierten gaschromatographischen System

Zusammenfassung

Die Nachfrage nach einem möglichst vielseitig verwendbaren und effektiven Gasanalysator, bei gleichzeitiger Limitierung von Investitionsgeldern und Analysenzeit, führte zu der Entwicklung des beschriebenen Gerätes. Es besteht aus einem Gaschromatographen mit Wärmeleitfähigkeitsdetektor und Elektroneneinfangdetektor, drei elektromagnetischen Ventilen und einer Porapak sowie einer Molekularsieb-Analysensäule. Ein automatischer Dampfrauminjektor kann angegliedert werden. Der beschriebene Aufbau kann für mehrere Methoden im Zusammenhang mit Gasumsetzungen im Boden verwendet werden. Insbesondere eignet er sich für Routinemessungen von atmosphärischen Distick-

stoffoxidkonzentrationen sowie von hohen Distickstoffoxidkonzentrationen, wie sie bei der Untersuchung der potentiellen Denitrifikation erreicht werden. Kohlendioxid wird gemeinsam mit Distickstoffoxid gemessen. Neon kann als interner Standard verwendet werden. Nach einem geringfügigen Umbau des Systems kann Sauerstoff simultan mit Kohlendioxid gemessen werden. Eine Vorspüleinrichtung für Acetylen und ein Rückspülsystem für Sauerstoff wurden eingebaut, um die Stabilität der Basislinie und die Lebensdauer des ECDs zu fördern.

Schlüsselworte: Bodenmikrobiologie, Methoden, Gaschromatographie, Denitrifikation, Respiration.

1. Introduction

In recent years measurements on the metabolism of trace gases in the soil have gained increasing interest (ANDRAE and SCHIMEL 1989, BOUWMAN 1990). Concerning gaseous nitrogen losses from soil there are four paths of interest:

1. Denitrification as measured via the acetylene-inhibition-method. This method can be used to determine either actual denitrification under field conditions or potential denitrification under optimised laboratory conditions (anaerobiosis, addition of substrates, optimum temperatures). Acetylene is used to block the conversion from nitrous oxide to dinitrogen, which means that all the denitrified nitrogen can be measured as nitrous oxide by gas chromatography. Thus this method is valuable for the determination and prediction of fertilizer losses in various soils. For this method the accurate determination of very low as well as very high levels of nitrous oxide (0.5 vpm to 10,000 vpm) with high background levels of acetylene (10 %) is needed (1 vpm = 1 ppm per volume).

2. Nitrous oxide emissions from soil. Nitrous oxide can be produced during denitrification as well as during nitrification. The latter process cannot be measured during the acetylene-inhibition-method as acetylene blocks nitrification. The net nitrous oxide produced in the soil is important for its effects on atmospheric chemistry, as it promotes global warming and ozone depletion in the stratosphere. There is still a lack of knowledge concerning the effects of various agricultural practices on nitrous oxide emissions from soil. For this method the accurate determination of ambient nitrous oxide concentrations (0.5 vpm) plus/minus minimal differences is needed.

3. Carbon dioxide release from soil. Carbon dioxide production can also be measured using the Isermeyer assay (trapping of carbon dioxide in alkaline solution) or Infrared Gas Analysis. The advantages of gas chromatography are the small samples required and the speed and accuracy of analysis (GARDINI et al. 1991). Concerning gaseous nitrogen losses it is of interest to detect the respirational activity of the soil biomass and to determine possible side effects of acetylene application on soil microbial activity. Carbon dioxide is also interesting as a greenhouse gas itself. For this method the gas chromatographic determination of carbon dioxide concentrations rising from ambient (0.03 %) to the percent level is necessary.

4. Oxygen uptake from soil. Gas chromatographic determination of oxygen can be conducted as alternative to Sapromat measurements. It is used to monitor the respirational activity of the soil biomass as well as to get a value for the aerobic status of the soil. The latter is of particular interest in long laboratory incubations. For this method concentrations of 21 % oxygen down to 0.1 % oxygen should be measureable.

Concluding from the previous statements there are several requirements for an optimised gas analysis system, which is most effective regarding financial input and analysis time:

- The four above mentioned methods should be conducted in one GC-system.
- Nitrous oxide should be measured in very low and very high concentrations.
- A large number of samples should be measured without dilution or concentration of gas samples.
- There should be a contrivance to inject small (100 µl) to big (5 ml) sample amounts.
- Automation of sample injection should be possible.
- Storage vessels for gas samples should be combined with automatic injection.
- The separation of neon and oxygen from carbon dioxide and nitrous oxide should be possible in one analysis.
- The electron capture detector should be prevented from damage by oxygen and acetylene using a frontflush and a backflush system.
- Leak detection and gas volume calculation in the assay vessel could be conducted using neon as internal standard.

The abovementioned requirements led to the adaptation of a system described by KLEMEDTSSON et al. (1986) and modified for our applications.

2. Material and Methods

Our system consists of a Shimadzu GC-14B gas chromatograph connected to a dual channel data processor C-R4A Chromatopac. The GC is equipped with a thermal conductivity detector (TCD) and an electron capture detector (ECD) which are connected in series. The instrument contains two columns which are connected in series with two intermittent valves which permit alternative use of one column or both (fig. 1). The two columns are a Porapak QS, 80–100 mesh, 5 m × 1/8 in. O. D. stainless-steel column and a Molecular Sieve 5A, 3 m × 1/8 in. O. D., stainless-steel column. In total three electromagnetic 8-port valves (Valco instruments E45) are integrated into the system. The first valve serves as sample valve and is operated manually. The other two valves are controlled by a Basic program over the data processor.

The operating conditions are as follows: Oven temperature 80 °C, injector temperature 120 °C, ECD temperature 330 °C, current 1 nA, TCD temperature 100 °C, current 120 mA. Carrier gas 1 = Helium, gas flow 20 ml/min, carrier gas 2 = Helium, gas flow 20 ml/min, carrier gas 3 = Helium, gas flow 20 ml/min. Reference gas for TCD = Helium, gas flow 20 ml/min, scavenger gas for ECD = nitrogen ECD quality, gas flow 8 ml/min (fig. 1).

The glass liner of the injector is filled with anhydrous $Mg(ClO_4)_2$ (technical grade) and serves as water trap. The sampling valve (I) contains a 1 ml sample loop, which can be exchanged for a 100 µl or a 5 ml sample loop according to sample sizes and concentrations. The sample can also directly be injected into the injector.

There are different successions of valve settings depending on which gases should be determined in one sample. First the measurement of neon, carbon dioxide and nitrous oxide is described:

During analysis of one sample the valve setting is changed four times (fig. 1). During phase 1 all three valves are in the "inject" mode. The sample is carried from the sample loop to the injector, which contains the water filter, and into the Porapak column where it is separated into dinitrogen/oxygen/neon and carbon dioxide and nitrous oxide and acetylene. Dinitrogen/oxygen/neon travels

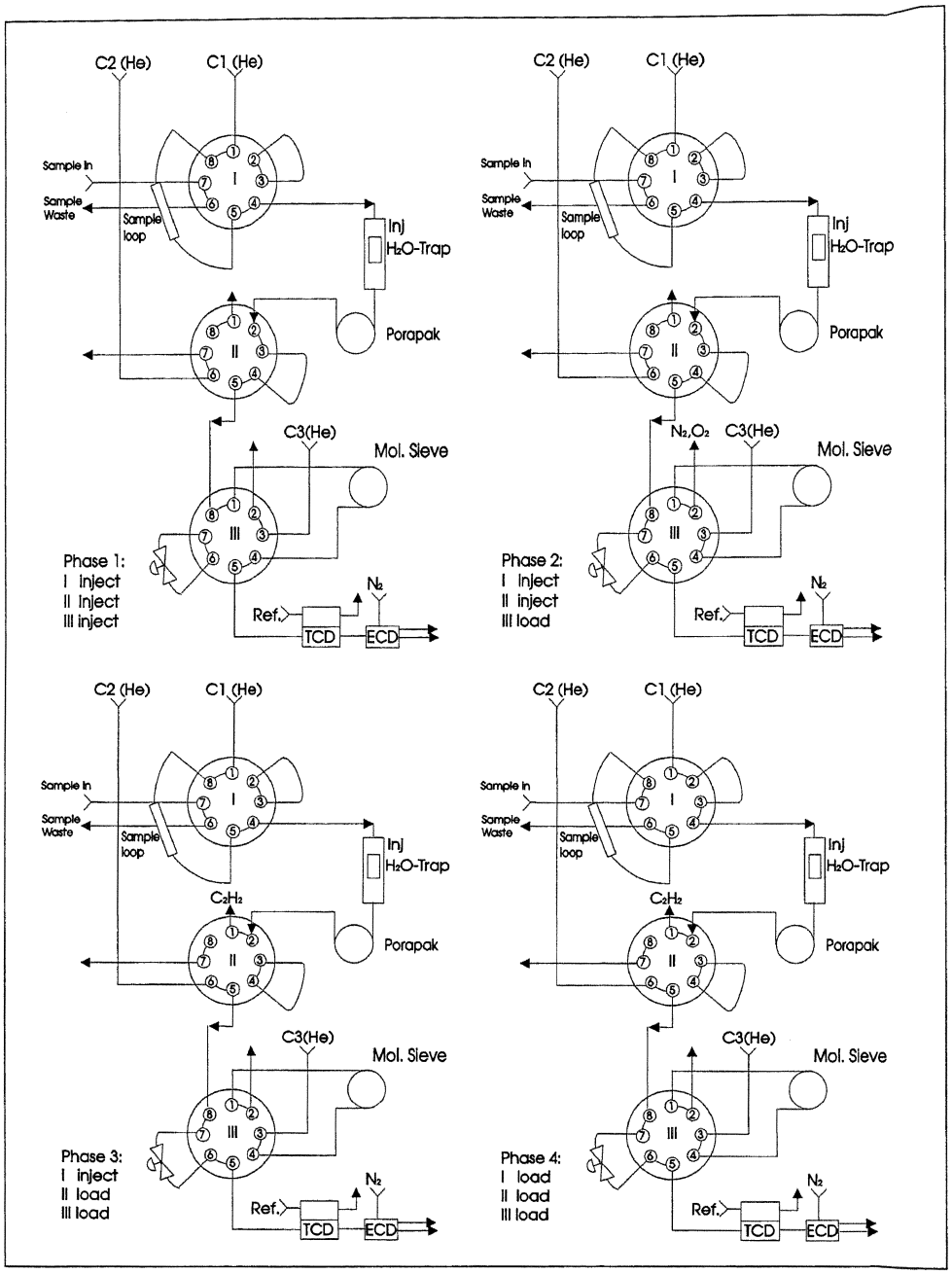

Fig. 1: Valve switching program for the gas chromatographic analysis of neon, carbon dioxide and nitrous oxide in air containing 10 % acetylene
Abbreviations: C1–C3 (He)= carrier gas 1–3 (Helium), Inj=Injector, I=sample valve, II and III=automatically operated 8-port valves, Porapak and Mol. Sieve=analytical columns, TCD=thermal conductivity detector, ECD=electron capture detector, Ref.=reference gas, N_2(=dinitrogen, scavenger and make up gas for ECD)

quickly into the Molecular Sieve column, where the three compounds are separated from each other. Valve III is switched when neon has reached the detectors while dinitrogen and oxygen are still in the Molecular Sieve column from where they are now backflushed by carrier gas 3 (phase 2). Carbon dioxide and nitrous oxide travel directly from the Porapak column to the detectors. Before acetylene comes off the Porapak column, valve II is switched to the "load" position so that acetylene is frontflushed and does not reach the detectors (phase 3). In phase 4 the sample valve (valve I) is loaded and with the switch to the "inject" position the Basic program for the automatic valve setting resumes from the start.

If neon is not used as internal standard the analysis of carbon dioxide and nitrous oxide can be speeded up by omitting the Molecular Sieve column (fig. 2, third chromatogram). After loading of the sample loop (phase 4, fig. 1) the sample is injected in phase 3 with the result that oxygen and dinitrogen are vented out of the system. After three minutes valve II is switched to the "inject" position (phase 2) and carbon dioxide and nitrous oxide are carried to the detectors. 7 minutes and 10 seconds after the start valve II is switched back to "load" and acetylene is flushed out to the air, which takes 1 minute (phase 3). For baseline adjustment phase 2 can be resumed before the injection of the next sample. The analysis of one sample takes 8 minutes.

The analysis of oxygen can be conducted in the valve setting mode of phase III. For this measurement the ECD can be uncoupled from the TCD to prevent oxygen damage. In this mode carbon dioxide is absorbed by the Mol. Sieve column and cannot be measured. The carbon dioxide can also be flushed from the Porapak column through valve II after oxygen has reached the Mol. Sieve column (Valve II "load", Valve III "inject"). This prevents progressive deterioration of the Mol. Sieve column by carbon dioxide (JEFFERY and KIPPING 1972).

For combined measurement of oxygen and carbon dioxide the carrier gas 3 and the vent on valve III have to be interchanged and the flow rate of carrier gas 3 has to be reduced. When oxygen/dinitrogen have entered the Mol. Sieve column valve II is switched to the "load" position and carbon dioxide is reaching the TCD while oxygen and dinitrogen are slowly separated on the Mol. Sieve. When the valve III is switched back to "inject", oxygen and dinitrogen can be determined.

3. Results and Discussion

The described GC system is designed for the measurement of nitrous oxide, carbon dioxide, oxygen, and neon.

Furthermore it can be used for the detection of the following gases: Hydrogen, dinitrogen, carbon monoxide, methane, acetylene, ethylene and ethane.

All the mentioned gases are either produced or decomposed in the soil and therefore their measurement is of importance for ecological studies.

For carbon dioxide and nitrous oxide the concentration limits for detection are as follows:

With the TCD, carbon dioxide can be detected at concentrations below ambient (0.033 %) to pure, whereas nitrous oxide is only detected at concentrations above 300 vpm. With the ECD, carbon dioxide could be detected if the detector temperature was lowered to 250 °C (CHRISTENSEN 1983), which is not done in our experimental setup as this would reduce the detector sensitivity for nitrous oxide. In case that only an ECD is available it can be recommended to

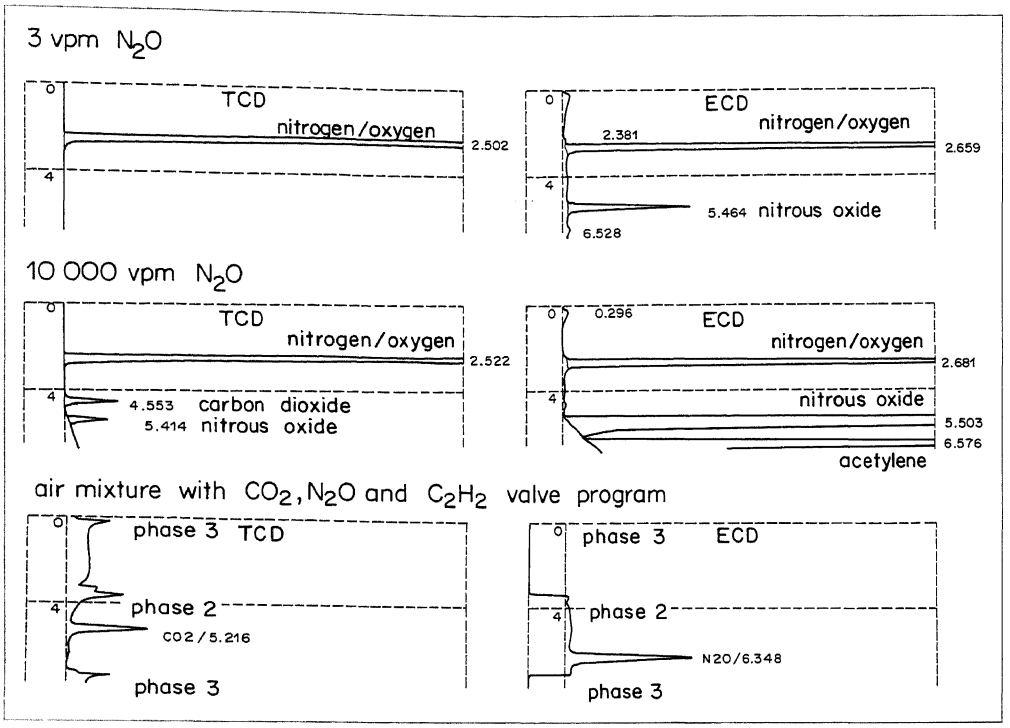

Fig. 2: Parallel chromatograms showing the separation of gases and the sensitivity of TCD (thermal conductivity detector] and ECD (electron capture detector) to different concentrations of nitrous oxide. The first two analyses are conducted without valve switching on the Porapak column

detect both carbon dioxide and nitrous oxide at 250 °C but to use a different carrier gas (argon plus 5 % methane).

In our system nitrous oxide can be detected at the ECD at levels from below ambient (0.3 vpm) to 400 vpm. The calibration curve shows a linear response from 0.3 to 25 vpm. Between 25 vpm and 400 vpm a two point calibration over a response curve is necessary as the detector signal decreases at higher concentrations (KLEMEDTSSON et al. 1986, SMITH and ARAH 1991).

Fig. 2 shows the analysis of three gas samples with simultaneous detection at the TCD and the ECD. The first two measurements are conducted without valve switching. Oxygen and dinitrogen (retention time 2.5 minutes) and acetylene (r.t. 6.5 minutes) reach the detectors, which would be unfavourable on the long run. In the first chromatogram 3 vpm nitrous oxide is only detected on the ECD. In the second chromatogram 10,000 vpm is detected by TCD and is too much to be accurately determined by ECD.

The third chromatogram shows routine measurement with frontflush of acetylene and backflush of dinitrogen and oxygen. The carbon dioxide peak shows up at the TCD and the nitrous oxide peak at the ECD.

Fig. 3 shows the measurement of an air sample. The TCD plot is overlayed by the ECD plot. There is a short lag time of 0.13 minutes between the two detectors, which can be seen at the oxygen/nitrogen peak. Carbon dioxide gives a positive peak at the TCD and a small negative peak at the ECD. Nitrous oxide at ambient concentrations is only detected by ECD.

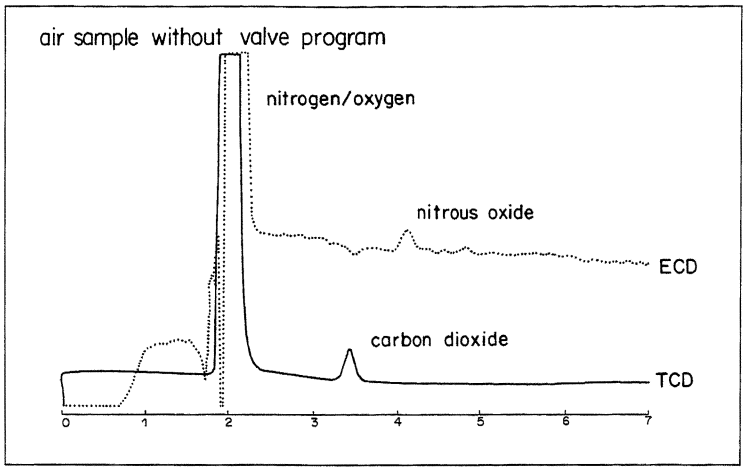

Fig. 3: Overlay of TCD (thermal conductivity detector) and ECD (electron capture detector) chromatograms from the analyses of an air sample conducted on the Porapak column

Fig. 4 shows the operation of the Shimadzu Headspace Sampler HSS-3A/2B, which can be combined with the GC and has the advantage that the serum vials are neither evacuated nor flushed during measurement. Therefore it is possible to use serum vials as incubation vessels for soil samples which can directly be inserted into the sampler and can be measured repeatedly. The same serum vials can also be evacuated, used for gas storage in field experiments and analysed automatically in the laboratory.

In conclusion it can be remarked that there are many applications in agricultural research for the described instrument. For the Austrian inventory of soils potential denitrification measurements were proposed for the microbiological soil monitoring (KANDELER et al. 1993). Such a soil monitoring would need a large number of routine analysis which could only be conducted with an automated GC system with a wide range of detectable nitrous oxide concentrations.

Actual emissions of nitrous oxide from soils are nowadays of special interest in connection with their involvement in the greenhouse effect. There are yet hardly

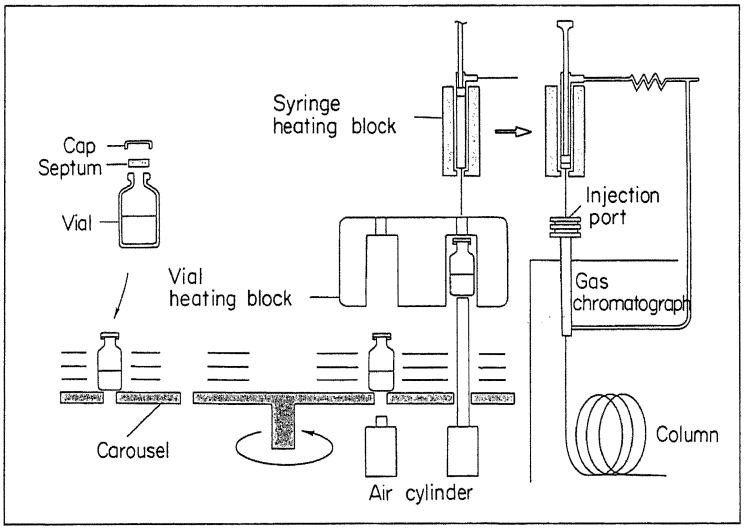

Fig. 4: Operation of the Shimadzu Headspace Sampler HSS-3 A/2 B

any experimental data on the influence of agricultural management on nitrous oxide emissions in Austrian soils. However such data are urgently needed as a basis for political strategies. The high temporal and spatial variability of nitrous oxide fluxes imply the measurement of many samples repeatedly over a long period of time in order to develop reasonable estimates of total annual fluxes (LOFTFIELD et al. 1992). Denitrification rates are highly dependent on soil structure, soil moisture/oxygen tensions, organic carbon, mineral nitrogen contents, temperature and the presence of roots. Thus seasonal patterns of denitrification can be observed as well as a log normal distribution of denitrification rates in space which is due to high activities associated with favourable soil microsites, so called "hot-spots" (ARAH 1990). Many efforts are made to overcome the methodological difficulties associated with the high spatial variation of nitrous oxide release. These include remote sensing by satellites, measurements of gas concentrations over large field plots involving infrared or laser techniques and micrometeorological methods (ROBERTSON et al. 1993).

However gas chromatographic analysis of air samples drawn from the soil headspace or the soil atmosphere is still the most widely applied method to calculate denitrification and nitrous oxide release associated with different soils and vegetation. These gas chromatographic measurements need automation as well as high detector sensitivity. With the described instrument it is tried to meet these diverse needs of some of the problems in modern agricultural research.

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