Plant and Soil **181**: 145–151, 1996.

Influence of carbon availability on the production of NO, N_2O, N_2 and CO_2 by soil cores during anaerobic incubation.

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Key words: aerobic preincubation, available carbon, CO_2 production, denitrification, N_2O/N_2 ratio, water soluble carbon

Abstract

Net productions of permanent soil atmosphere gases (N_2, CO_2, O_2) and temporary gases (N_2O, NO) were monitored in soil cores using a non-interfering, fully automated measuring technique allowing highly time resolved measurements over prolonged periods. The influence of changes in available organic carbon on CO_2, N_2O, NO and N_2 production was studied by changing the soil carbon content through aerobic preincubations of different length, up to 21 days.

The aerobic preincubation caused an increase in NO_3^- concentration and a decrease in available carbon content. Available carbon content dominated both CO_2 and total N gas (N_2+N_2O+NO) production during anaerobiosis. Both CO_2 and total N gas production rates decreased with increasing length of the previous aerobic preincubation, this in spite of the higher initial NO_3^- concentration.

Total denitrification rates were closely related to the anaerobic CO_2 production rates. No relation was found between water soluble carbon content and total denitrification. The N_2O/N_2 ratio could be explained by an interaction of carbon availability, NO_3^- concentration and enzyme status. Net N_2O consumption was monitored. The balance between cumulative total N gas production and NO_3^- consumption varied according to the different treatments. Cumulative N_2O production exceeded cumulative N_2 production for 0 up to 5 days.

Introduction

Denitrification is a major source of N_2O, especially in intensively cultivated nitrogen rich soils (Bouwman, 1990). N_2O is implicated in the destruction of the stratospheric ozone layer (Crutzen, 1981) and N_2O is a long-lived infrared-absorbing greenhouse gas. Apart from its effects on the atmosphere, denitrification results in a decrease of available soil-N to plants. Both N_2 and N_2O result, in varying amounts, from denitrification. The major factors influencing the total denitrification rate are O_2-concentration, C-availability and NO_3^- concentration. The amounts of N_2O produced can vary from 0 to the majority of N reduced. The impact of several factors on the N_2O/N_2 ratio can largely be reduced to their influence on the relative availability of oxidant vs. reductant (Hutchinson and Davidson, 1993). Among the parameters explaining N_2O/N_2 ratios are N-oxide concentration, O_2 availability, organic C availability, ratio's of enzyme activity, time since initiation of denitrification etc. .

Most studies on denitrification use the acetylene inhibition method (Yoshinari et al., 1977), often with measurement intervals of one to several days. The acetylene inhibition method has however several well known disadvantages.

We studied denitrification under a He atmosphere to avoid the use of acetylene. The aim of the research was (1) to study the dynamics of the denitrification process, and (2) to monitor the influence of NO_3^- and C-availability on the N_2O/N_2 ratio of the denitrification products.

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Materials and methods

Soil

The soil used for all experiments was sampled from the upper 0–10 cm of a clayey silt loam soil (Mal, Belgium) with following characteristics : texture : 0–2 μm : 17%, 2–50 μm : 69%, >50 μm : 14%; pH_{H2O} : 6.7 (1:2.5 soil:water); pH_{KCl} : 5.6 (1:2.5 soil:KCl 1*N*); total organic carbon : 12.3 mg C g^{-1} dry soil. The bulked samples were air dried to a moisture content of 180 mg water g^{-1} dry soil, sieved to pass a 2 mm sieve, mixed and stored at 4 °C.

Measurement set-up

A fully automated set-up was used allowing measurement of net gas production from nine independent soil cores during prolonged periods under an artificial atmosphere. The nine circuits, each containing one soil core, are connected through a selection valve to an injection valve with two sample loops for simultaneous injection in two gaschromatographs (GC), one with electron capture detector (ECD) for the analysis of O_2, NO, CO_2 and N_2O, and one with thermal conductivity detector (TCD) allowing the analysis of O_2, N_2, CO_2 and N_2O. Gas is continuously recirculated through the soil cores with membrane pumps to avoid diffusional problems. The artificial atmosphere allows direct measurement of N_2-production. A complete description of the set-up, the analysis conditions and the accuracy of the method is given by Swerts et al. (1995).

Chemical analysis

Mineral N was analysed in a soil extract (25 g soil : 50 mL KCl 1 *N*). Nitrate, NO_2^- and NH_4^+ concentrations were determined colorimetrically on a Skalar-autoanalyser. Water soluble carbon was determined on a soil extract (35 g soil : 100 mL H_2O_{dest}) using a persulfate oxidation method according to McCardell and Fuhrmann (1992).

Experimental

Bulk soil was aerobically preincubated at 25 °C for 7, 13 or 21 days (experiments AER7, AER13, and AER21, respectively). Moisture content after incubation was 173.5 mg water g^{-1} dry soil. For experiment ADDNO3, 6.9 mg NO_3^--N kg^{-1} dry soil was added to the soil (40.5 mL kg^{-1} moist soil of a solution of 104 mg KNO_3 100 mL^{-1} H_2O) to reach an initial NO_3^- concentration comparable to AER13, but without aerobic preincubation. Moisture content after addition was 214.3 mg water g^{-1} dry soil. For experiment ADDH2O, 40.5 mL H_2O kg^{-1} moist soil was added to the soil. The achieved moisture content was 215.5 mg H_2O g^{-1} dry soil.

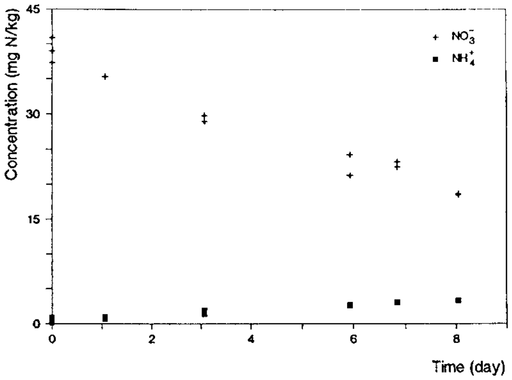

Figure 1. Time course of mineral N concentrations (mg N kg^{-1} soil) for experiment AER21.

For each experiment, 9 core holders (length 20 cm, diam. 6 cm) were packed with 450 g, for AER7, AER13 and AER21, or 468 g, for ADDNO3 and ADDH2O, carefully mixed moist soil and incubated at 25 +/- 0.1°C. Anaerobicity was obtained by flushing the soil cores for 10 min with He. The end of the He flushing was set as time 0 for the experiments.

Results

The different treatments resulted in initial NO_3^- concentrations of 31.3 +/- 1.3, 35.6 +/- 1.0, 39.0 +/- 1.0, 36.4 +/- 1.9 and 34.7 +/- 0.7 mg N kg^{-1} dry soil and initial water soluble carbon contents of 65 +/- 2, 57 +/- 6, 78 +/- 13, 64 +/- 8, and 76 +/- 16 mg C kg^{-1} dry soil for experiment AER7, AER13, AER21, ADDNO3 and ADDH2O, respectively. Nitrate concentrations declined linearly with time for all experiments. Changing NO_3^- and NH_4^+ concentrations are shown for experiment AER21 (Fig. 1). Nitrate concentrations reached zero at approximately day 9 for ADDNO3 and day 7 for ADDH2O. For all other experiments NO_3^- remained available throughout the experiment.

Patterns in water soluble carbon content changes were less obvious. In general an apparent initial decrease in soluble carbon content was followed by

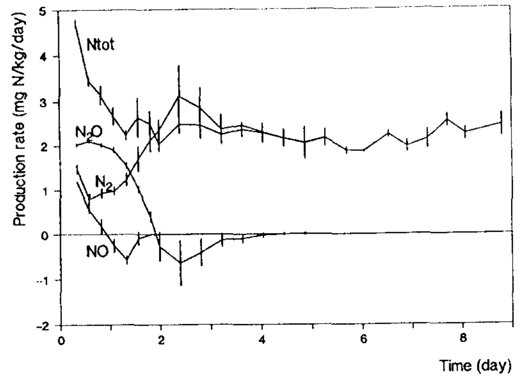

Figure 2. Time course of the N_2, N_2O, NO, and Ntot (N_2+N_2O+NO) production rates (average +/- standard deviation in mg N kg^{-1} day^{-1}) for experiment AER13.

a slight increase after day 5 (data not shown). Data for AER7 were extremely scattered.

The dynamic changes in CO_2 production rate and in evolution of several N gases were measured very accurately. As an example, Figure 2 shows the avg +/- std for production rates of the different N-gases against time for AER13. The average coefficient of variation for total N gas (N_2+N_2O+NO) production rates against time was 9.1% (0.9% to 36.1%) and 7.7% for CO_2 (2.2% to 20.2%).

The overall denitrification rates (total N gas production rates) of the different experiments are compared in Figure 3a. Total N gas production rates for AER13 and AER21 are clearly lower. The initially high rates for ADDNO3 and ADDH2O decline rapidly to reach a fairly constant level. At NO_3^- exhaustion (+/- day 7 for ADDH2O, +/- day 9 for ADDNO3) the production rates drop. For the N_2O production rates (Fig. 3b) AER7 and AER13 show a clear minimum with net consumption between day 2 and 3. AER21 has the same initial N_2O production rate but consumption is delayed. ADDNO3 and ADDH2O initially have higher N_2O production rates, after day 2 however there is no significant difference between AER21,ADDNO3 and ADDH2O. N_2 production rates (Fig. 3c) for AER7 and AER13 show a clear maximum at the time of maximal N_2O consumption. N_2 production rates for ADDNO3 and ADDH2O are quite constant until NO_3^- is exhausted. ADDNO3 and ADDH2O show a very fast reduction of the initially produced NO (Fig. 3d), for AER7, AER13, and AER21 consumption occurs later. CO_2 production rates (Fig. 3e) for AER13 and AER21 are significantly lower than for AER7, ADDNO3 and ADDH2O.

For experiments AER7, AER13, and AER21 there is a clear change in the balance between cumulative total N gas production and cumulative NO_3^- consumption (Fig. 4). For AER7 total amounts of NO_3^- consumed balance N gas production. For AER13 cumulative NH_4^+ production has to be subtracted from the cumulative NO_3^- consumption. In AER21 NO_3^- consumption clearly exceeds total N gas production. For ADDNO3 total N gas production is higher then NO_3^- consumption. In ADDH2O total N gas production is balanced by NO_3^- consumption minus NH_4^+ production.

Discussion

The main impact of aerobic preincubations of various length is expected to be an increase in NO_3^- concentration and a decrease in available carbon content with time. For NO_3^- this assumption is confirmed by the differences in initial NO_3^- concentration of AER7, AER13, and AER21. Although water soluble carbon content is considered to be a good indicator of available carbon content (Burford and Bremner, 1975; Katz et al., 1985), the measured water soluble carbon contents do not confirm the expected tendency in this study.

CO_2 production rates are considerably higher for AER7 compared to AER13. Production rates for AER13 are slightly higher than for AER21. Thus CO_2 production rates do confirm the decreasing C availability after aerobic preincubation. Perhaps the method to determine water soluble carbon content is not accurate enough to detect small differences at low C concentrations. Another possible explanation may be the extraction of carbon components which are in situ not readily available.

The higher availability of carbon in experiments AER7, ADDNO3, and ADDH2O compared to AER13 and AER21 is clearly reflected in the CO_2 production rates of Figure 3e. The easily available carbon is consumed throughout the experiment and CO_2 production rates decline accordingly, to approach about the initial CO_2 production rates of AER13 and AER21 by the end of the experiment.

Total denitrification rates (taken as the total N gas production or the sum of N_2O, N_2, and NO) (Fig. 3a) show, throughout the experiment a pattern parallel to CO_2 production rates. In spite of the increase in initial NO_3^- concentrations from AER7 over AER13 to AER21, their respective total N gas production rates decline. This points to available carbon as the most

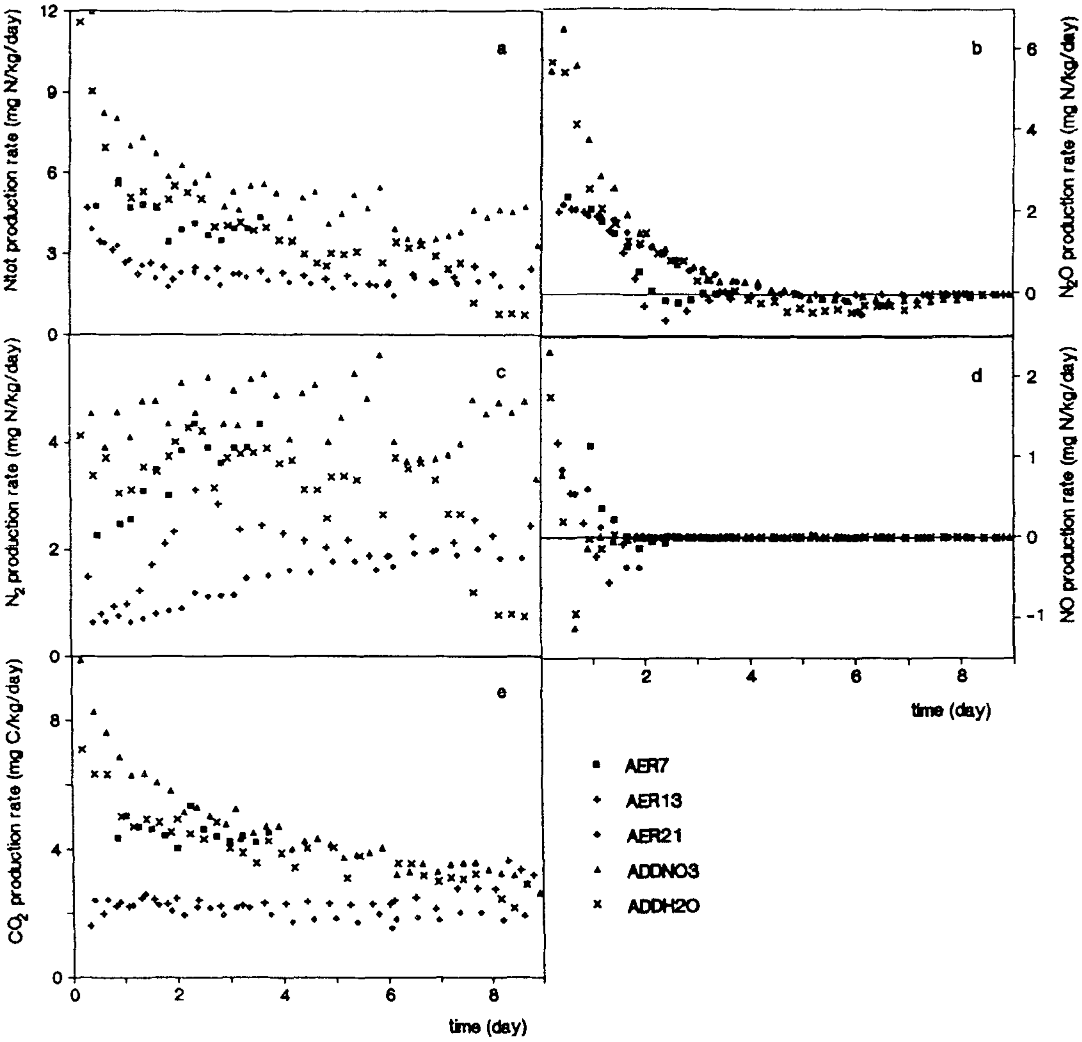

Figure 3. Time course of the average production rates of (a) Ntot (N_2+N_2O+NO), (b) N_2O, (c) N_2, (d) NO in mg N kg^{-1} day^{-1}, and (e) CO_2 in mg C kg^{-1} day^{-1} for the experiments AER7, AER13, AER21, ADDNO3, and ADDH2O.

influencing factor for the total denitrification rate under the given experimental conditions. This is conform to the findings of several other authors who concluded that carbon availability is the most limiting factor for denitrification under anaerobic conditions (Lalisse-Grundmann et al., 1988; Paul and Beauchamp, 1989; Weier et al., 1993). Most of those data, however, were obtained either through addition of carbon sources or through comparison of soil types differing in native carbon content. Our data, using one soil and aerobic preincubations of varying length, confirm the importance of available carbon.

In our study anaerobic CO_2 production was closely related to total denitrification. Water soluble carbon content however was a poor indicator. This is in accordance with Paul and Beauchamp (1989) and Bijay-Singh et al. (1988) as well as with data in the review papers of Sahrawat and Keeney (1986) and Aulakh et al. (1992).

The conversion of net N_2O production to N_2 production is generally attributed to differences in reaction rate for the reduction of NO_3^-, NO_2^-, NO and N_2O (Comfort et al., 1990; Tiedje, 1982). Because of the preferential acceptance of electrons by NO_3^- over N_2O, N_2O accumulates whenever NO_3^- supply exceeds the reducing demand of the denitrifying organisms. We do not expect NO_3^- to inhibit N_2O reductase as the maximum NO_3^- concentrations were low (less then 40 mg NO_3^--N kg^{-1}).

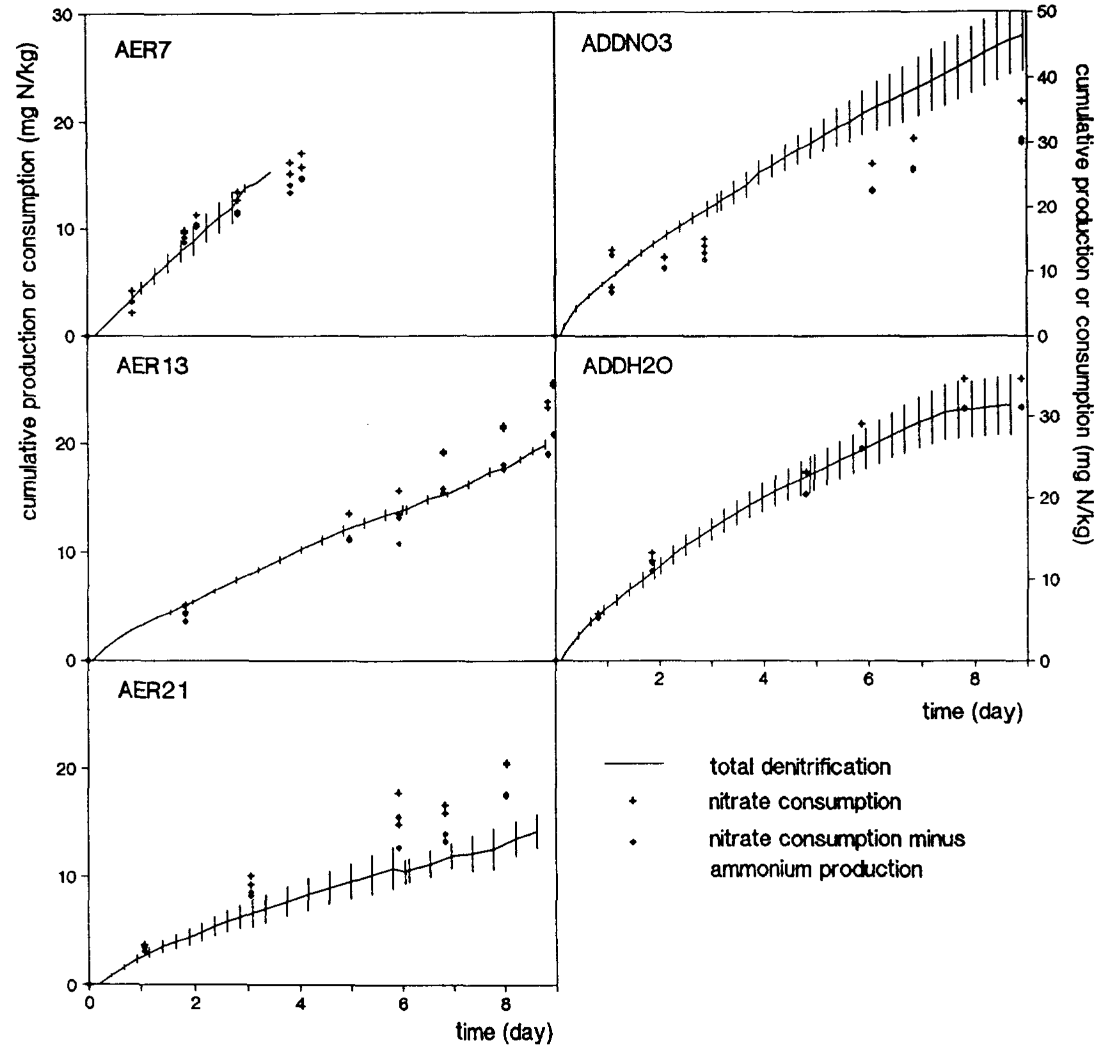

Figure 4. Time course of the cumulative denitrification (N_2+N_2O+NO) (average +/- standard deviation in mg N kg^{-1}) against cumulative nitrate consumption (mg N kg^{-1}) and cumulative nitrate consumption minus ammonium production (mg N kg^{-1}).

NO is reduced very quickly, in accordance with its high affinity for electron acceptance.

The sequence of increasing NO_3^- concentration against decreasing available carbon content of AER7 over AER13 to AER21 has a pronounced influence on the relative amounts of N_2O produced compared to N_2. This is a clear example of the influence of the relative availability of oxidant versus reductant. Hutchinson and Davidson (1993) state that when the available oxidant largely exceeds the reductant the substrate N-oxide will be incompletely reduced resulting in a larger N_2O/N_2 ratio. Analysis based on Michaelis-Menten enzyme kinetics predicts that the proportion of N_2O should increase whenever any of the other controllers slows the overall rate of reduction below the maximum that can be supported by existing enzyme. This is in accordance with Sahrawat and Keeney (1986) who state that an increase in NO_3^- concentration will cause an increase in N_2O/N_2 ratio while an increase in available carbon or in enzyme status will cause a decrease in the N_2O/N_2 ratio.

For AER7 and AER13 N_2 production rates increase to reach a maximum at day 2, at the time of maximum N_2O consumption (Fig. 3b,c). For ADDNO3 and ADDH2O, more C is available, but the N_2 production rates remain constant throughout the experiment until NO_3^- becomes limiting. Net N_2O production rates reach net consumption only between day four and seven. This apparent slower reduction of N_2O to N_2 under higher C availability conditions might be due to differences in initial enzyme concentrations. The denitrification rates during the first phase of denitrification are determined by the preexisting enzymatic capacity to denitrify, rather than by nitrate or carbon availability. Although the enzymes involved in denitrification are considered to be rather stable over prolonged periods, the aerobic preincubations at 25°C may have caused some decay, resulting in lower initial N_2O and N_2 production rates. Subsequent increases in reductase concentrations could explain the faster reduction of N_2O to N_2 for AER7 and AER13 compared to ADDH2O and ADDNO3. The slower N_2O reduction for AER21 could be due both, to a higher nitrate to available carbon ratio or, to a slower enzyme synthesis activity caused by the lower C-availability.

Cumulative N_2O production exceeds the cumulative N_2 production for 2 days in AER13, for 5 days in AER21, for 1 day in ADDNO3, and for 1.5 day in ADDH2O (data not shown). In AER7 the N_2O production does not exceed the N_2 production.

Cumulative total N gas production during denitrification is balanced by NO_3^- consumption in AER7 (Fig. 4). For AER13 to AER21 NO_3^- consumption increasingly exceeds total N gas production. Due to the decreasing availability of carbon an increasing part of the oxidant becomes available for incorporation in the biomass.

In ADDNO3 the total N gas production exceeds the NO_3^- consumption. This is often attributed to an unknown amount of NO_3^- originating from nitrification (Paul and Beauchamp, 1989; de Catanzaro et al., 1987). Indeed some trace amounts of O_2 were detected during this experiment, probably due to a leak in the system. Together with the O_2, N_2 will have intruded into the system as well, resulting in an overestimation of N-gases produced through denitrification. On the other hand, another N oxide source may have attributed to total N gas production. Addition of 6.9 mg NO_3^- N kg^{-1} soil only resulted in an increase of the initial NO_3^- concentration of +/- 2 mg NO_3^--N kg^{-1} soil. Part of the missing NO_3^- might have been available to denitrification.

For ADDH2O total N production is well balanced by NO_3^- consumption.

From the presented data, it can be concluded that available carbon was the most influencing parameter on total denitrification. Anaerobic CO_2 production was closely related to total denitrification. Water soluble carbon content, however, could not be correlated with the denitrification rates. The N_2O/N_2 ratio was influenced by the balance between NO_3^- concentration and available carbon. Hypothetical differences in enzyme status had to be taken into account to further explain N_2O/N_2 ratios. Cumulative N_2O production exceeded cumulative N_2 production for several days during the anaerobic incubations at 25°C. At lower temperatures in the field N_2O is likely to be the predominant gas produced during anaerobicity. For most experiments total N gas loss was well balanced by NO_3^- consumption.

Acknowledgements

This research was conducted in the frame of the Impulse Programme "Global Change" supported by the Belgian State - Prime Minister's Service - Federal Office for Scientific, Technical and Cultural Affairs. We thank Griet Uytterhoeven for excellent technical support.

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Section editor: R Merckx