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Laboratory kinetics of soil denitrification are useful to discriminate soils with potentially high levels of N_2O emission on the field scale

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Abstract – Emissions of N_2O , a greenhouse gas, were measured in field conditions on a Rendzic Leptosol, an Eutric Leptosol, a Haplic Calcisol, a Haplic Luvisol and two Gleyic Luvisols under cultivation, and on a Haplic Fluvisol and two Gleyic Cambisols under cultivation and grassland conditions. The kinetics of N_2O production and consumption during denitrification in these soils were studied during anaerobic incubations in the laboratory, with NO_3^- and N_2O addition in the presence or absence of acetylene. The soils with the highest “in situ” levels of N_2O emission, i.e. the Gleyic Luvisols under cultivation and the grassland soils, exhibited considerable transient accumulation of N_2O during denitrification studied in the laboratory. We therefore propose an empirical indicator, measured in the laboratory, of the soil’s potential to emit N_2O on the field scale. Carbon addition to one of the Gleyic Luvisols promoted N_2O reduction and limited the transient accumulation of N_2O during laboratory denitrification.

N_2O / soils / denitrification / empirical indicator / carbon

Résumé – Des cinétiques de dénitrification effectuées au laboratoire permettent de discriminer les sols qui émettent des niveaux importants de N_2O au champ. Les émissions de N_2O , gaz à effet de serre, ont été mesurées au cours d’essais au champ, sur des sols cultivés dont un Rendzic Leptosol, un Eutric Leptosol, un Haplic Calcisol, un Haplic Luvisol et deux Gleyic Luvisols, et sur des sols cultivés et en prairie, dont un Haplic Fluvisol et deux Gleyic Cambisols. En parallèle, les cinétiques de production et de consommation de N_2O au cours de la dénitrification ont été étudiées au laboratoire sur des suspensions de sol, au cours d’incubations anaérobies avec apport de NO_3^- et de N_2O , en présence et en absence d’acétylène. Les sols qui émettent le plus de N_2O « in situ », c’est-à-dire les Gleyic Luvisols cultivés et les sols de prairie, accumulent aussi beaucoup de N_2O au cours du processus de dénitrification étudié au laboratoire. Nous proposons donc un indicateur empirique, mesuré au laboratoire, des potentialités des sols à émettre N_2O au champ. L’apport de carbone à l’un des Gleyic Luvisols a favorisé la réduction du N_2O et limité l’accumulation transitoire de N_2O au cours de la dénitrification étudiée au laboratoire.

N_2O / sols / dénitrification / indicateur empirique / carbone

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1. INTRODUCTION

Nitrous oxide, N_2O , is one of the six greenhouse gases of which industrial countries, on the basis of the Kyoto protocol, have to reduce their levels of emission [26]. Agriculture is largely involved in anthropogenic N_2O emission due to losses during the synthesis and use of N-fertilizer and the storage and use of livestock manure [22]. One assumption in the IPCC method for estimating N_2O emission on the country scale is that the main N_2O emissions are proportional to the level of N input from mineral and organic fertilizers, symbiotic fixation and culture residues [27]. For obvious economic and demographic reasons, the global reduction of inputs of N-fertilizer to limit N_2O emission as suggested by the IPCC method is difficult to implement. It is thus important to improve our understanding of the mechanisms of N_2O emission by agricultural soils so that methods can be proposed for reducing N_2O emission which do not conflict with agricultural production.

N_2O is produced by soils mainly through two microbiological processes: (1) nitrification, the successive aerobic oxidation of ammonia to nitrite and nitrate, and (2) denitrification, the successive anaerobic reduction of nitrate to nitrite, nitric oxide, nitrous oxide and dinitrogen [29]. N_2O can also be reduced in soils during the last step of denitrification [16]. The proportion of N_2O emitted during denitrification, i.e. the $N_2O:N_2$ ratio during denitrification varies considerably from soil to soil and also for a given soil when the environmental factors are altered by field management [28]. This ratio is regulated at different levels which include (i) the cellular level through enzyme inhibition and the relative availabilities of electron donors and electron acceptors, ... and (ii) gaseous diffusion into soils which is affected by soil texture and soil moisture... [9].

Different methods are used to determine the influence of agricultural practices on the levels of N_2O emitted by soils. These have been experimentally tested "in situ" by different authors [e.g. 1, 14, 18] but the general trends are difficult to determine partly due to uncontrolled heterogeneities between the experimental sites. Some operational models are available for predicting N_2O emission on the field scale and testing the effect of different agricultural practices on N_2O emissions [17, 21]. Nevertheless their performances vary with the conditions studied and estimations of N_2O emission by soils with these models should be used with caution [23]. Moreover, N_2O emissions are highly dependent on local climatic conditions, rainfall and temperature, that remain unpredictable. Chaussod [5] underlined the necessity of defining the relevant indicators of soil biological quality which includes the environmental impact of the soil sys-

tem on the surrounding atmosphere. With this end point in mind, we report here some observed similarities between laboratory incubations and high levels of "in situ" N_2O emission. We then propose a rapid laboratory test which can be useful for discriminating soils with potentially high levels of "in situ" N_2O emission. This test is based on a study of the kinetics of production and reduction of N_2O by anaerobic denitrification during the incubation of soil slurries.

2. MATERIALS AND METHODS

2.1. Soils

This study involved 12 soils which were classified into three systems. The first system, called the Champagne-Burgundy system, was mainly investigated in 1994–1995. It included 3 cultivated soils from the Champagne-Burgundy area, (1) an Eutric Leptosol (47°46 N, 4°95 E), (2) a Gleyic Luvisol (47°16 N, 5°18 E) and a Rendzic Leptosol (48°57 N, 2°25 E). These soils were seeded with rapeseed in autumn 1994 and received equivalent levels of N-fertilizer during spring 1995 based on the balance sheet method [14]. The second system, called the Beauce system, was investigated in 1999. This also included 3 cultivated soils from the Beauce-Faux-Perche area, (1) a Haplic Calcisol (47°98 N, 1°34 E), (2) a Gleyic Luvisol (48°08 N, 1°06 E) and (3) a Haplic Luvisol (48°24 N, 1°34 E). These soils were seeded with winter wheat in autumn 1998 and received comparable rates of fertilizer, with regard to the balance sheet method, during spring 1999. The third system, or grassland/cultivated system, contained 3 pairs of soils. Each pair consisted of cultivated and adjacent grassland soils, both from the same pedological unit. This system included (1) a pair of soils from a Calcaric Fluvisol (47°32 N, 4°45 E), and (2) 2 pairs from two Gleyic Cambisols (47°15 N, 4°37 E - Cambisol A and 47°22 N, 4°28 E - Cambisol B). These soils were investigated in 1997. Their main characteristics are shown in Table I.

2.2. Measurements of N_2O emission on the field scale

Different methods were used to determine N_2O emissions on the field scale. In the two systems which compared N_2O emissions in different cultivated fields (i.e. (1) the Champagne-Burgundy system and (2) the Beauce system), N_2O emission was measured about twice a month from February to June 1995 in the Champagne-Burgundy system and from February to June 1999 in the Beauce system, using the chamber method. 8 chambers (0.5 m in diameter, height varying in accordance with

Table I. Main characteristics of the studied soils (ND: not determined).

	Land use	Clay content g·kg ⁻¹	Sand content g·kg ⁻¹	Organic C g·kg ⁻¹	Organic N g·kg ⁻¹	CEC cmol·kg ⁻¹	pH water	Bulk density
Burgundy-Champagne system								
Eutric Leptosol	Rapeseed	502	115	30.9	3.34	28.9	7.9	1.0
Gleyic Luvisol	Rapeseed	210	346	8.6	0.77	9.1	6	1.4
Rendzic Leptosol	Rapeseed	100	305	16.7	1.49	7.6	8.1	1.2
Beauce system								
Haplic Calcisol	Wheat	334	26	14.2	1.69	22.8	7.9	1.4
Gleyic Luvisol	Wheat	136	60	10.8	1.18	7.8	6.3	1.3
Haplic Luvisol	Wheat	242	57	11.8	1.21	16.5	7.6	1.3
Cultivated/grassland system								
Calcaric Fluvisol	Wheat	287	15	33.4	3.62	21.1	8	ND
	Grassland	311	15	51.8	5.24	25.8	8	ND
Gleyic Cambisol (A)	Rapeseed	362	63	15.2	1.66	14.2	6.9	ND
	Grassland	358	60	23.8	2.70	17.1	6.7	ND
Gleyic Cambisol (B)	Wheat	374	48	19.9	2.24	20.6	7.4	ND
	Grassland	370	26	30.3	3.23	23	7.1	ND

plant height) were inserted into each soil. These chambers were closed for 2 hours during the measurement of N₂O emission. The chamber atmosphere was sampled 4 times during this period in 3 ml Terumo vacutainer tubes that we had previously purged. These samples were analyzed on a Varian 3400 Cx GC equipped with an electron capture detector coupled to an automatic sampler (HSS 86-20 SRA instruments).

In the system which compared N₂O emission on cultivated and grassland soils, nitrous oxide emission was measured on undisturbed soil cores (10 cm diameter and 20 cm height) placed in controlled conditions of temperature, water and nitrate contents. Sixteen cores per treatment were sampled during autumn 1997 by manually driving steel cylinders into the soil and removing them with a spade [15]. Half of the cores from cultivated soils were sampled within the row and contained plants while the other half were sampled between the rows. Controlled conditions of nitrate and water contents were obtained by (1) determining the soil water-filled pore space and nitrate contents under sampling conditions and (2) adding a nitrate solution drop by drop to the soil cores. The volumes and concentrations of the nitrate solution were adjusted so that the cores in each pair of grassland and cultivated soils were at equivalent water-filled pore space (Tab. II) and nitrate concentrations of approximately 50 mg N·kg⁻¹ soil. N₂O emission by the soil cores was measured 24 h after addition of the solution at 20 °C in the laboratory. The soil cores were kept airtight during the measurements. Each closed core contained about 1.5 dm³ of soil and 1.5 dm³ of gas. This gas atmosphere was sampled 4 times over a period of

Table II. Nitrous oxide fluxes and conditions of water-filled pore space during measurement in the grassland/cultivated system. Numbers in parentheses are standard deviations.

Soil	Land Use	WFPS %	N ₂ O emission g N·ha ⁻¹ ·d ⁻¹
Fluvisol	Cultivated	0.70 (0.16)	3 (2)
	Grassland	0.68 (0.02)	90 (97)
Neoluvisol A	Cultivated	1.00 (0.13)	173 (197)
	Grassland	1.00 (0.12)	3300 (1000)
Neoluvisol B	Cultivated	0.68 (0.05)	19 (14)
	Grassland	0.67 (0.04)	152 (76)

3 hours in 3 ml Terumo vacutainer tubes. These samples were also analyzed on the Varian GC described above.

In this paper, the comparisons of N₂O emissions were made between soils in a given system, that is between the three Champagne-Burgundy soils and the 3 Beauce soils. The comparisons in the third system were only made of paired soils (pasture and cultivated) from the same site. A comparative study which included all these soils would have required identical “in situ” systems of measurement and homogenization, or at least consideration, of numerous parameters such as climatic conditions. This was beyond the scope of the present study.

2.3. Soils' capacity to produce and to reduce N₂O in the laboratory without carbon addition

The capacities for production and reduction of N₂O during denitrification were investigated in the laboratory

under anaerobic conditions (N_2) using fresh soil samples sieved through 5-mm mesh. Four sets of incubation conditions were established in order to determine the activities of the different stages of denitrification. Each set included 3 replicates equivalent to 50 g of dry soil placed in 565-ml flasks. Two series of incubations were carried out with the addition of NO_3^- (50 ml KNO_3 solution with 100 mg $N \cdot l^{-1}$) as electron acceptor (1) in the presence of acetylene (2.5% of the gas atmosphere) in order to measure the potential activity for total denitrification, and (2) without the addition of acetylene in order to measure the production of N_2O during denitrification. N_2O emission was assumed to be negligible during nitrification in these experimental conditions. Two other series of incubations were carried out with the addition of water (50 ml) and N_2O (5 ml) as electron acceptor to test the capacity of the soil to reduce N_2O (1) in the absence of acetylene and (2) in the presence of acetylene (2.5% of the gas atmosphere). Before 1996, the acetylene (stabilized with acetone) was washed in water before use. After 1996, we used acetone-free acetylene (quality AAS27). The difference between these two treatments was used to calculate the soil potential for N_2O reduction. Anaerobic conditions in the flasks were established before gas addition by three successive cycles of evacuation and refilling with N_2 . The kinetics at 20 °C were determined over a period of one week with the flasks under constant agitation on a rotating shaker. The flask atmosphere was periodically sampled in 3 ml Terumo vacutainer tubes. The samples with a N_2O concentration higher than 500 ppm were analyzed on a Girdel 3000 when sampled before 1998 and on a MTI microGC when sampled after this date, both instruments being equipped with a TCD detector. Samples with lower N_2O concentrations were analyzed with the Varian GC described above. We consider this methodology derived from Blackmer and Bremner [4] and Tiedje [25] to efficiently reveal the capacities of the soil denitrifying enzymes to utilize the soil carbon available under field conditions.

2.4. Characterization of soil capacities to produce and to reduce N_2O with carbon addition

The Gleyic Luvisol of the Burgundy-Champagne system was further sampled during February 2000 and sieved through 5-mm mesh. Carbon was added to the soil samples in 565 ml flasks during a pre-incubation period of 48 hours. 12 soil samples equivalent to 50 g of dry soil were left untreated, 12 soil samples equivalent to 50 g of dry soil received 25 ml of a carbon solution (202.5 mg of sodium succinate and 123 mg of sodium acetate per litre) and 12 soil samples equivalent to 50 g of dry soil received 25 ml of diluted (1/25) liquid pig

manure (20 g·l⁻¹ of dissolved organic carbon). After this preincubation period, (1) 6 flasks of untreated soil received a nitrate solution (50 ml of KNO_3 equivalent to 100 mg $N \cdot l^{-1}$) and the 6 other flasks of untreated soil received 50 ml of water, (2) 6 flasks with soil treated with the carbon solution were then amended with 25 ml of a carbon-nitrate solution (202.5 mg of sodium succinate, 123 mg of sodium acetate and 1.44 g of KNO_3 per litre) and the 6 other flasks with the soil treated with the carbon solution were then amended with 25 ml of the previous carbon solution (202.5 mg of sodium succinate and 123 mg of sodium acetate per litre) and (3) 6 flasks with the soil and the diluted pig manure were then amended with 25 ml of a nitrate solution (200 mg $N \cdot l^{-1}$) and the 6 other flasks with the soil and the diluted pig manure received 25 ml of water. When anaerobic conditions had been produced, 5 ml of N_2O were added to the soil samples without nitrate addition. Half of the samples to which nitrate had been added and half of the samples to which N_2O had been added were incubated in the presence of acetylene (2.5% of the gas atmosphere).

3. RESULTS

3.1. N_2O emission on the field scale

The mean “in situ” N_2O emissions measured from February to June 1995 in the Champagne-Burgundy system were 1 g $N_2O \cdot N \cdot ha^{-1} \cdot d^{-1}$ on the Rendzic Leptosol, 24 g $N_2O \cdot N \cdot ha^{-1} \cdot d^{-1}$ on the Gleyic Luvisol and 6 g $N_2O \cdot N \cdot ha^{-1} \cdot d^{-1}$ on the Eutric Leptosol (Fig. 1). These results have been reported in detail in Hénault et al. [14]. The different rates of N_2O emission observed between these sites could not be explained by differences between climatic conditions or agricultural practices which were very similar on all the sites during this experiment.

The mean “in situ” N_2O emissions measured from February to June 1999 in the Beauce system were 5 g $N_2O \cdot N \cdot ha^{-1} \cdot d^{-1}$ on the Haplic Calcisol, 29 g $N_2O \cdot N \cdot ha^{-1} \cdot d^{-1}$ on the Gleyic Luvisol and 4 g $N_2O \cdot N \cdot ha^{-1} \cdot d^{-1}$ on the Haplic Luvisol (Fig. 2). The N_2O fluxes were higher on the Gleyic Luvisol than on the two other soils at each date. During this experiment, the rainfall was slightly higher on the Gleyic Luvisol compared to the other sites. Nevertheless, the water-filled pore space in the 0–20 cm layer of this soil was generally lower than in the Haplic Calcisol and the Haplic Luvisol due to lower bulk density. Thus, the soil hydric conditions in the Beauce system study could not explain the higher emissions observed on the Gleyic Luvisol.

N_2O emissions measured at equivalent WFPS in soil cores from pairs of grassland and cultivated soils

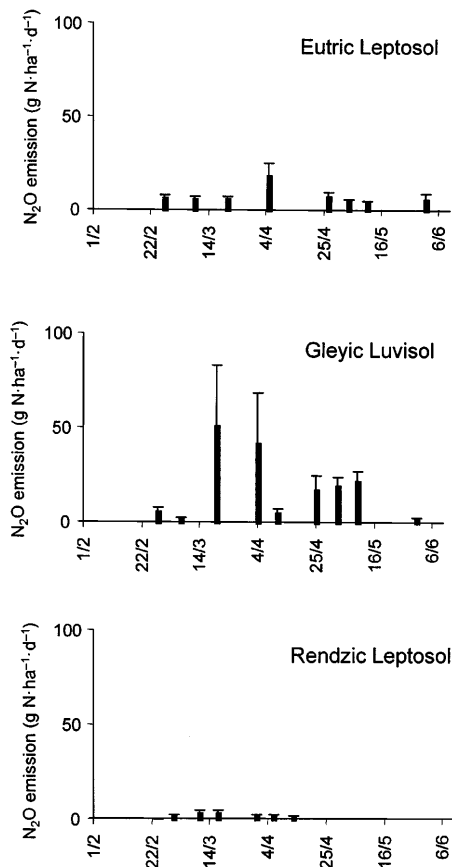


Figure 1. In situ N₂O fluxes measured during 1995 in the Champagne-Burgundy system. Bars represent the standard errors.

exhibited considerable differences. The rates ranged between 3 and 100 g N·ha⁻¹·d⁻¹ on the cultivated soils and between 90 and 3300 g N·ha⁻¹·d⁻¹ on the grassland soils (Tab. II).

3.2. N₂O production and consumption by soil slurries without carbon addition

The kinetics of both NO₃⁻-denitrification and N₂O-reduction varied considerably from soil to soil in the Champagne-Burgundy system (Fig. 3). All the added nitrate was rapidly denitrified on the Eutric Leptosol and the Rendzic Leptosol. The maximum denitrification rate assessed during the first 96 hours of incubation on the Eutric Leptosol was 0.91 μg N·g⁻¹ soil·h⁻¹. The maxi-

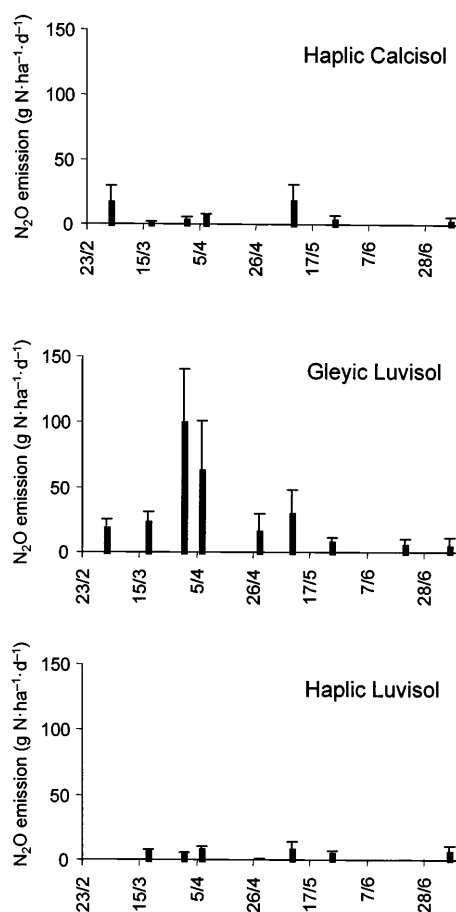


Figure 2. In situ N₂O fluxes measured during 1999 on the Beauce system. Bars represent the standard errors.

imum denitrification rate, estimated from the first 48 hours of incubation was 1.8 μg N·g⁻¹ soil·h⁻¹ on the Rendzic Leptosol. Both soils also reduced N₂O very efficiently at similar rates, of roughly more than 2.3 μg N·g⁻¹ soil·h⁻¹. Both accumulated very low levels of N₂O during denitrification. In contrast, the Gleyic Luvisol in this system denitrified the added nitrate very slowly, with constant rates of 0.14 μg N·g⁻¹ soil·h⁻¹ during the incubation period. This soil also reduced N₂O very slowly. A latency period of 48 hours was observed before N₂O reduction occurred. The N₂O reduction rate was thus 0.30 μg N·g⁻¹ soil·h⁻¹. This soil accumulated a large proportion of N₂O (> 70%) during denitrification.

The kinetics of NO₃⁻-denitrification and of N₂O-reduction in the Beauce system varied little between the three soils under study (Fig. 3). The NO₃⁻-denitrification

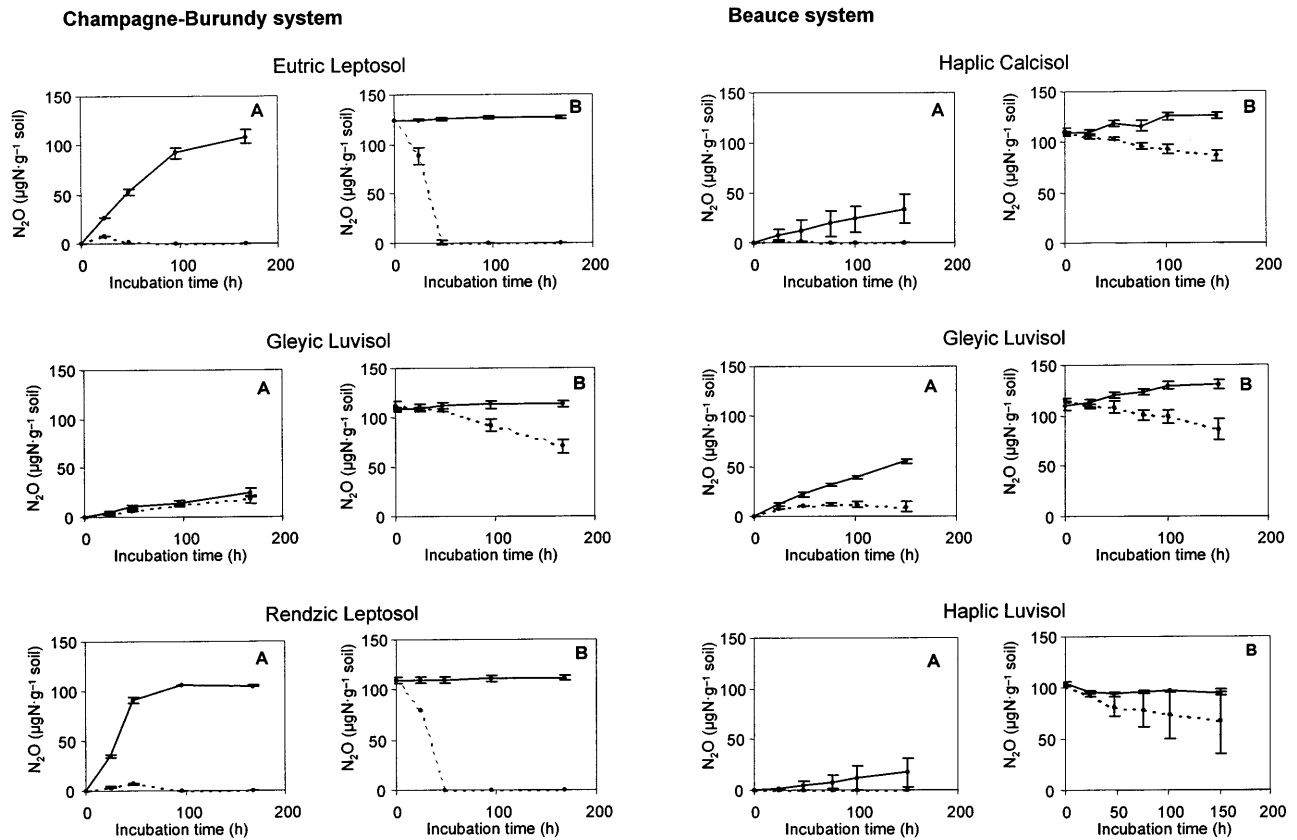


Figure 3. Anaerobic kinetics of N_2O production (A) and consumption (B) by soil slurries from the Champagne-Burgundy and Beauce systems. Soil slurries were incubated under anaerobiosis with nitrate addition (A) or nitrous oxide addition (B) in the presence of C_2H_2 (—) or without C_2H_2 (---). Symbols represent the means of the 3 replicates and error bars are the standard errors or are smaller than the symbol.

rates remained constant during the incubation period at 0.21, 0.33 and 0.11 $\mu\text{g N}\cdot\text{g}^{-1}\text{ soil}\cdot\text{h}^{-1}$ for the Haplic Calcisol, Gleyic Luvisol and Haplic Luvisol respectively. These three soils also reduced N_2O at similar rates of 0.28, 0.33 and 0.22 $\mu\text{g N}\cdot\text{g}^{-1}\text{ soil}\cdot\text{h}^{-1}$, respectively. The Haplic Calcisol and Haplic Luvisol in this system accumulated very low levels of N_2O during denitrification. The Gleyic Luvisol, in contrast, although its N_2O reduction rate was as high as its NO_3^- -denitrification rate, accumulated N_2O during denitrification, during the first 100 hours of incubation.

The NO_3^- -denitrification rates in the cultivated/grassland system were always higher on the grassland than on the paired cultivated soils (Fig. 4). This difference was particularly large on the two Cambisols. The cultivated Fluvisol denitrified nitrate at higher rates than the other cultivated soils. This was also the soil with the shorter history of cultivation (around 10 years). The N_2O -reduc-

tion rates were also always higher on the grassland than on the paired cultivated soils. This rate tended to fluctuate on the two cultivated Cambisols. The grassland soils could be clearly distinguished from the cultivated ones on the basis of the rate of N_2O accumulation during denitrification. Despite the high immediate N_2O reduction rates observed on the grassland soils, these also accumulated high levels of N_2O during denitrification. The cultivated soils in this system accumulated very low levels of N_2O during denitrification.

3.3. Relation between the capacities of the soil to produce and to reduce N_2O without carbon addition and the level of soil N_2O emission on the field scale

Both the Gleyic Luvisol of the Champagne-Burgundy system and the Gleyic Luvisol of the Beauce system

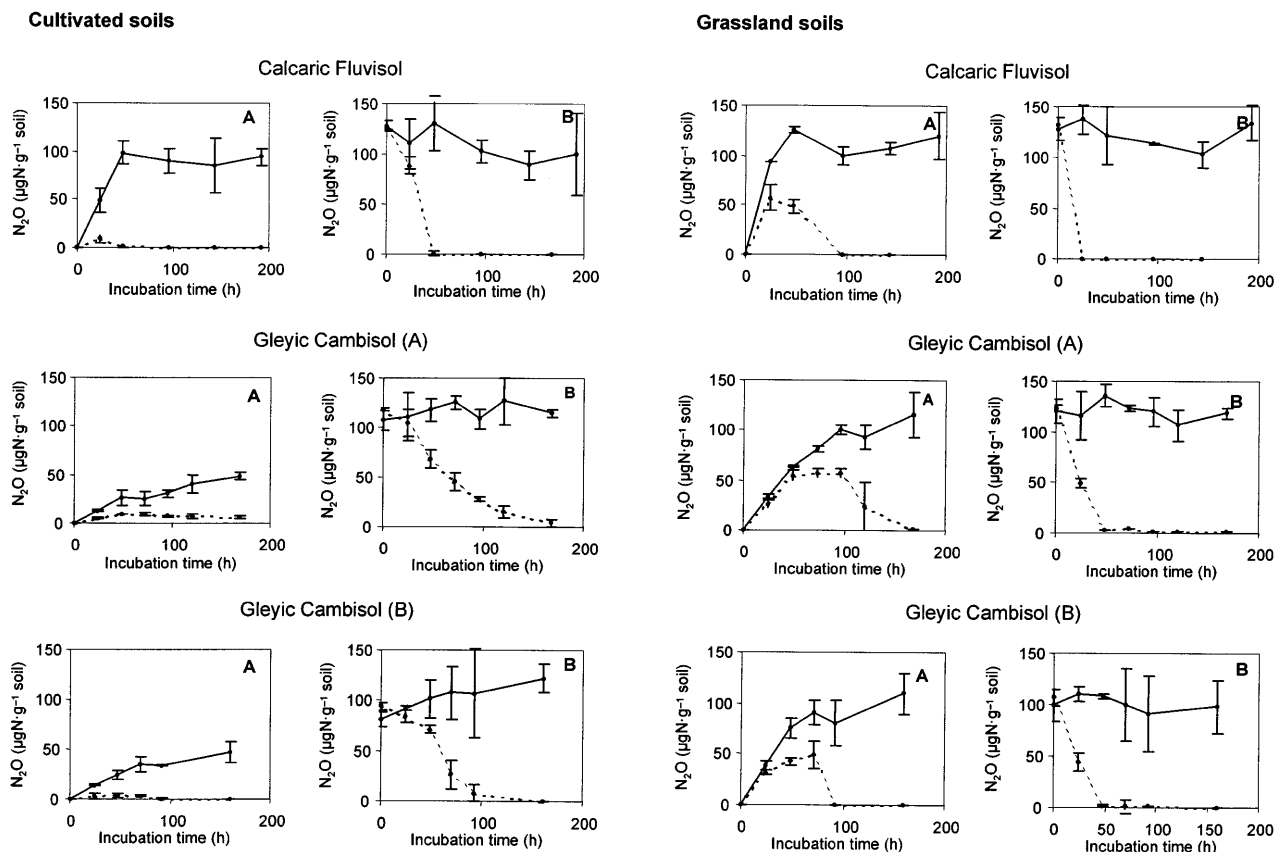


Figure 4. Anaerobic kinetics of N₂O production (A) and consumption (B) by soil slurries from paired cultivated and grassland soils from Burgundy. Soil slurries were incubated in anaerobiosis with nitrate addition (A) or nitrous oxide addition (B) in the presence of C₂H₂ (—) or without C₂H₂ (- - -). Symbols represent the means of 3 replicates and error bars are the standard errors or are smaller than the symbol.

emitted high levels of N₂O on the field scale. Grassland soils appeared to emit potentially higher levels of N₂O than the paired cultivated soils when maintained under comparable conditions of water-filled pore space and nitrate contents.

The high level of N₂O emission on the field scale could not be correlated with the soils' capacity to denitrify nitrate in the laboratory under total anaerobiosis. The Gleyic Luvisol in the Champagne-Burgundy system emitted high levels of N₂O in the field whereas it exhibited the lowest denitrification capacity in the laboratory.

The low N₂O reduction capacity of the Gleyic Luvisol in the Champagne-Burgundy system seemed to partially explain "in situ" emission. This low capacity to reduce N₂O during anaerobiosis greatly suggests that almost all denitrified nitrate is emitted as N₂O by this soil. Not all the soils which emitted high levels of N₂O on the field

scale exhibited very low capacities of N₂O reduction under total anaerobiosis in the laboratory. In contrast, all the grassland soils exhibited higher N₂O reduction rates than the paired cultivated soils. Nevertheless a common point was apparent among all the soils which emitted high levels of N₂O on the field scale, i.e. the two Gleyic Luvisols of the Champagne-Burgundy and Beauce systems and the grassland soils. All accumulated high levels of N₂O during NO₃⁻ denitrification by soil slurries even though they had a high potential to reduce N₂O.

The "in situ" levels of N₂O emission were apparently influenced both by the proportion of N₂O emitted during NO₃⁻ denitrification and by the time for which N₂O continued to accumulate in the flasks during denitrification. This latter parameter reflects the time required for the efficient reduction of the N₂O formed during denitrification. We therefore calculated an empirical index

(Tab. III) which was the product of (1) the maximum ratio of the accumulated N_2O to the denitrified nitrate observed during the laboratory incubation and (2) the time for which N_2O continued to accumulate in the flasks during denitrification. In both the Burgundy-Champagne and Beauce systems, the levels of “in situ” N_2O emission are classed in the same order as in this empirical index. This index clearly discriminates the two Gleyic Luvisols which exhibited high levels of N_2O emission in the field. In the cultivated/grassland system, this index also clearly distinguishes the cultivated soil, with lower N_2O emission, from its paired grassland soil with higher N_2O emission.

3.4. Effect of carbon addition on soil capacities to produce and to reduce N_2O

After carbon addition, NO_3^- -denitrification in the Gleyic Luvisol of the Champagne-Burgundy system was increased to around $0.40 \mu\text{g N}\cdot\text{g}^{-1}\text{soil}\cdot\text{h}^{-1}$ with the added acetate and succinate. The N_2O -reduction rate was also significantly increased by the carbon addition. A latency time for N_2O reduction was not observed and the subsequent N_2O reduction rate was higher than $0.60 \mu\text{g N}\cdot\text{g}^{-1}\text{soil}\cdot\text{h}^{-1}$. The transient accumulation of N_2O during denitrification was reduced after the addition of carbon (Fig. 5). The empirical index assessed on the reference samples was still higher than 150. It decreased to values

of 30 and 25 in the soils with added pig manure and added acetate and succinate, respectively.

4. DISCUSSION

The proportion of N_2O emitted during denitrification is known to vary considerably for a given soil in relation to different physical and agronomic parameters such as the soil water-filled pore space, soil temperature and soil nitrate content [2]. In this study, this proportion appeared to vary greatly with the duration of incubation under anaerobic conditions. We were nevertheless able to verify for the Gleyic Luvisol and the Rendzic Leptosol of the Champagne-Burgundy system that the kinetics obtained were reproducible with time over a period of several years without the introduction of exogenous carbon. For example, similar kinetics were observed on the Gleyic Luvisol sampled in 1994–1995 (Fig. 3) and in 2000 (Fig. 5), (data not shown for the Rendzic Leptosol).

These experiments which include both measurements of N_2O emission in the field and of soil kinetics in the laboratory show that the “in situ” levels of soil N_2O emission are linked to the transient accumulation of N_2O during denitrification in controlled conditions.

Some relationships between “in situ” denitrification rates and soil biological factors have already been investigated. Parsons et al. [20] found that the denitrifying

Table III. Relation between an empirical index calculated from the laboratory kinetics of soil denitrification and “in situ” N_2O emission into each system and in the grassland system between each pair of soils.

Soils	Land use	Mean of measured in situ N_2O emission $\text{g N}\cdot\text{ha}^{-1}\cdot\text{d}^{-1}$	Maximum ratio of accumulated N_2O (measured without C_2H_2) to denitrified nitrate (measured with acetylene)	Time of N_2O accumulation during denitrification (h)	Empirical index = Max ratio \times Time of accumulation
Burgundy Champagne					
– Eutric Leptosol	Rapeseed	6	0.32	24	8
– Gleyic Luvisol	Rapeseed	23	0.88	>168	>147
– Rendzic Leptosol	Rapeseed	1	0.09	48	4
Beauce					
– Haplic Calcisol	Wheat	5	0.22	48	11
– Gleyic Luvisol	Wheat	29	0.64	101	64
– Haplic Luvisol	Wheat	4	0.22	48	11
Grassland/Cultivated					
– Calcaric Fluvisol	Cultivated	4	0.19	24	4
	Grassland	80	0.62	24	15
– Gleyic Cambisol (A)	Cultivated	173	0.38	48	18
	Grassland	3300	0.86	72	62
– Gleyic Cambisol (B)	Cultivated	19	0.20	48	10
	Grassland	152	0.82	70	57

Gleyic Luvisol of the Burgundy-Champagne system

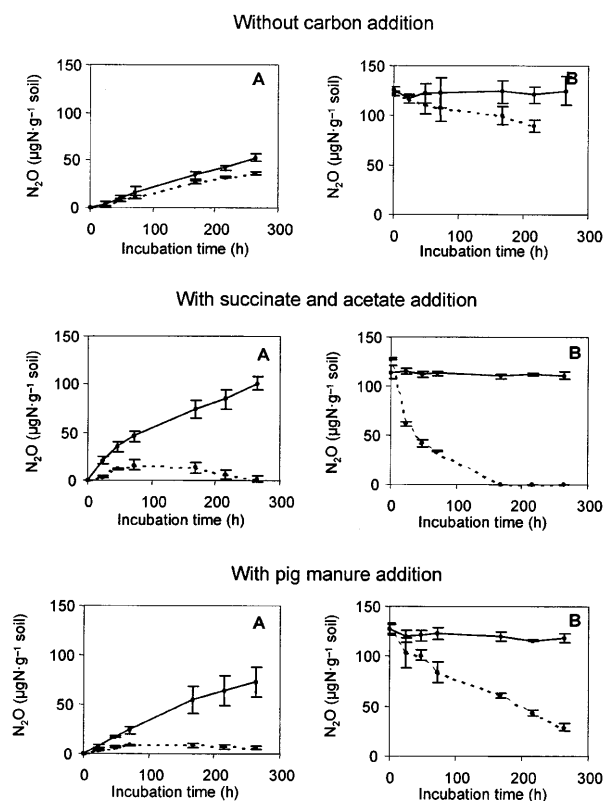


Figure 5. Anaerobic kinetics of N₂O production (A) and consumption (B) by soil slurries from the Gleyic Luvisol of the Champagne-Burgundy system amended with different forms of carbon. Soil slurries were incubated in anaerobiosis with nitrate addition (A) or nitrous oxide addition (B) in the presence of C₂H₂ (—) or without C₂H₂ (- - -). Symbols represent the means of 3 replicates and error bars are the standard errors or are smaller than the symbol.

enzymes' activities (DEA) were not good predictors of denitrification rates because N-gas loss was not always tightly coupled to the synthesis of denitrifying enzymes. In contrast Groffman and Tiedje [13] observed that the annual loss of N due to denitrification in nine Northern temperate forest soils could be related to soil DEA, [25], and to the DEA-to-biomass C ratio. The present study does not deal with total denitrification but with N₂O emission. The laboratory kinetics examined did not strictly pertain to DEA as no carbon or chloramphenicol was added. The level of "in situ" N₂O emission was observed to be related to the soil transient N₂O accumu-

lation during anaerobic denitrification, simply estimated during laboratory kinetic analyses over a 1-week period.

In the present study, the transient accumulation of N₂O during denitrification appeared to be a relevant indicator of soil "in situ" N₂O emission. In fact, we think that if a soil is unable to efficiently reduce the N₂O produced through denitrification in anaerobic conditions, the N₂O produced through denitrification under field conditions (i.e. high water-filled pore space, temperature and nitrate contents) will escape from the soil before being reduced.

A large transient accumulation of N₂O during denitrification corresponds to a high N₂O:N₂ ratio during the first days of incubation. The N₂O:N₂ ratio during denitrification is known to be regulated by numerous factors including the relative activities of the enzymes involved (e.g., nitrate reductase and nitrous oxide reductase, ...) and the gaseous diffusion in soils [9]. Environmental factors such as soil pH and soil water content also clearly influence the N₂O:N₂ ratio [11]. Moreover the existence of bacterial populations with different affinities for N₂O cannot be excluded [8]. Dendooven and Anderson [10] proposed that the emission of N₂O during the first hours of denitrification could be due to the different latency times of the enzymes involved in the production and consumption of N₂O in anaerobic conditions after nitrate addition. They had observed on a pasture soil, a lower persistence and a retarded de-repression time of N₂O reductase than of the nitrate and nitrite reductases.

During our experiments, the soil carbon content was apparently an important driving factor for the level of transient accumulation of N₂O during denitrification. The soil's capacity to reduce N₂O was restored in the Gleyic Luvisol from the Champagne-Burgundy system by the addition of carbon and the accumulation of N₂O during denitrification decreased in consequence. Firestone and Davidson [12] proposed that the relative availability of reductant vs. oxidant controls the end product of denitrification (N₂O or N₂). If the availability of oxidant (N-oxide) greatly exceeds the availability of reductant (most commonly organic carbon), then the oxidant may be incompletely utilized, i.e. N₂O will be produced. In the case of the Gleyic Luvisol of the Burgundy system without carbon addition, we can hypothesize that the availability of NO₃⁻ was high compared to the available carbon, so that N₂O was the major product of denitrification. With added carbon, the ratio of available carbon to available N increased, leading to lower N₂O accumulation. However the effect of organic carbon on the level of transient accumulation of N₂O during denitrification appears to be complex. The organic contents of the grassland soils were higher than those of the paired cultivated soils. In accordance both NO₃⁻-denitrification

rates and N_2O -reduction rates were higher on the grassland soils than on the cultivated ones. Nevertheless the observed transient accumulation of N_2O was higher on grassland soils, despite the absence of latency time for N_2O reduction for incubations with N_2O addition. As N_2O reduction apparently started more slowly for incubations with NO_3^- addition, we can hypothesize that either the high soil nitrate content limits the N_2O reductase activity or that an apparent minimal level of N_2O , around $50 \mu\text{g } N_2O\text{-N}\cdot\text{g}^{-1}$ soil, is required for the N_2O reductase to become active in grassland soils. This transient accumulation of N_2O during denitrification is in accordance with the high level of N_2O emission observed on soil cores placed in controlled conditions of water and nitrate content. This observation is not sufficient to conclude that N_2O emissions are higher on grassland soils in field conditions than on cultivated ones. Soil porosity was previously observed to be higher on natural grassland clods than on cultivated ones [19], which suggests that grassland soils could less frequently be under conditions favorable to denitrification than cultivated soils.

Chèneby et al. [6, 7] had studied the possible relationship between the structure of the denitrifying community in soils, the capacities of isolated strains to produce and to consume N_2O and soil abilities to emit N_2O on the Rendzic Leptosol of this study and a Gleyic Luvisol in the Burgundy area, which also poorly reduces N_2O during denitrification. The structure of the denitrifying communities differed from site to site. Nevertheless the capacities of the isolated strains to produce and consume N_2O could not be correlated to the soils' denitrifying characteristics. Most of the denitrifying strains isolated from the Gleyic Luvisol possess the *nosZ* gene, which encodes N_2O reductase synthesis, and reduce N_2O during cultivation on synthetic media. From these different studies, the control of the end product of denitrification in this soil appears to depend more on the relative availability of nitrates and organic carbon than on the specific characteristics of its microbial community.

Although Blackmer and Bremner [3] had underlined the lack of data on soil capacities to act as sinks for N_2O , few specific studies have been carried out on N_2O consumption processes [8]. Blackmer and Bremner [3] published N_2O reduction rates on 9 cultivated Iowa soils, globally constant over a 14-day period, and between 0.57 and $1.11 \mu\text{g } N\cdot\text{g}^{-1}$ soil $\cdot\text{d}^{-1}$, i.e. 0.024 and $0.045 \mu\text{g } N\cdot\text{g}^{-1}$ soil $\cdot\text{h}^{-1}$. These values are much lower than the ones we observed. These differences could be due both to the presence of oxygen and to the small amount of N_2O present during the incubations performed by Blackmer and Bremner [3]. Both our study and that of Blackmer and Bremner [3] demonstrate that the capacity of a soil to

take up N_2O is, in certain soils, higher than its capacity to produce N_2O . Nevertheless in field conditions soils generally act as a source and not as a sink of N_2O [24].

The empirical index defined in this paper could be introduced into a Geographical Information System (GIS) as one of the criteria for assessing a soil's potential for emitting N_2O on a regional scale. Although this index cannot be used to predict absolute levels of soil N_2O emissions, it could be useful for classifying soils on the regional scale. Other soil characteristics would be required to classify soils as a function of their potential to emit N_2O , particularly those revealing soil water behavior, such as hydric conductivity and bulk density. These parameters reveal the occurrence in soils of the anaerobic conditions favorable to denitrification. The empirical index of this study provides information on the end product of denitrification. Other parameters such as the soil pH and the soil nitrate content are known to determine the end product of denitrification [4]. We do not know at present if and how these parameters interact with each other.

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REFERENCES

- [1] Arah J.R.M., Smith K.A., Crichton I.J., Li H.S., Nitrous oxide production and denitrification in Scottish arable soils, *J. Soil Sci.* 42 (1991) 351–367.
- [2] Aulakh M.S., Doran J.W., Mosier A.R., Soil denitrification: significance, measurement, and effects of management, *Adv. Soil Sci.* 18 (1992) 1–57.
- [3] Blackmer A.M., Bremner J.M., Potential of soils as a sink for atmospheric nitrous oxide, *Geophys. Res. Lett.* 3 (1976) 739–742.
- [4] Blackmer A.M., Bremner J.M., Inhibitory effect of nitrate on reduction of N_2O to N_2 by soil microorganisms, *Soil Biol. Biochem.* 10 (1978) 187–191.
- [5] Chaussod R., La qualité biologique des sols. Évaluation et implications, *Étude et gestion des sols* 3 (1996) 261–278.

- [6] Chèneby D., Hartmann A., Hénault C., Topp E., Germon J.C., Diversity of denitrifying microflora and ability to reduce N₂O in two soils, *Biol. Fertil. Soils* 28 (1998) 19–26.
- [7] Chèneby D., Philippot L., Hartmann A., Hénault C., Germon J.C., 16SrDNA analysis for characterization of denitrifying bacteria isolated from three agricultural soils, *FEMS Microbiol. Ecol.* 34 (2000) 121–128.
- [8] Conrad R., Soil microbial processes involved in production and consumption of atmospheric trace gases, *Adv. Microbiol. Ecol.* 14 (1995) 207–250.
- [9] Davidson E.A., Fluxes of nitrous oxide and nitric oxide from terrestrial ecosystems, in: Rogers J.E., Whitman W.B. (Eds.), *Microbial production and consumption of greenhouse gases: methane, nitrogen oxides, and halomethanes*. American Society for Microbiology, Washington, DC, 1991, pp. 219–235.
- [10] Deendooven L., Anderson J.M., Dynamics of reduction enzymes involved in the denitrification process in pasture soil, *Soil Biol. Biochem.* 26 (1994) 1501–1506.
- [11] Firestone M.K., Firestone R.B., Tiedje J.M., Nitrous oxide from soil denitrification: factors controlling its biological production, *Science* 208 (1980) 749–751.
- [12] Firestone M.K., Davidson E.A., Microbiological basis of NO and N₂O production and consumption in soil, in: Andreae M.O., Schimel D.S. (Eds.), *Exchange of trace gases between terrestrial ecosystems and the atmosphere*, John Wiley & Sons Ltd, 1989, pp. 7–21.
- [13] Groffman P.M., Tiedje J.M., Denitrification in north temperate forest soils: relationships between denitrification and environmental factors at the landscape scale, *Soil Biol. Biochem.* 21 (1989) 621–626.
- [14] Hénault C., Devis X., Page S., Justes E., Reau R., Germon J.C., Nitrous oxide emissions under different soil and land management conditions, *Biol. Fertil. Soils* 26 (1998) 199–207.
- [15] Hénault C., Germon J.C., NEMIS, a predictive model of denitrification on the field scale, *Eur. J. Soil Sci.* 51 (2000) 257–270.
- [16] Hopkins D.W., Dundee L., Webster E.A., Simek M., Biological production and consumption of nitrous oxide in soils, *Eur. J. Soil Biol.* 33 (1997) 145–151.
- [17] Li C., Narayanan V., Harriss R.C., Model estimates of nitrous oxide emissions from agricultural lands in the United States, *Global Biogeochem. Cycles* 10 (1996) 297–306.
- [18] Mosier A.R., Hutchinson G.L., Sabey B.R., Baxter J., Nitrous oxide emissions from barley plots treated with ammonium nitrate or sewage sludge, *J. Environ. Qual.* 11 (1982) 78–81.
- [19] Parry S., Renault P., Chenu C., Lensi R., Denitrification in pasture and cropped soil clods as affected by pore space structure, *Soil Biol. Biochem.* 31 (1999) 493–501.
- [20] Parsons L.L., Murray R.E., Smith M.S., Soil denitrification dynamics: spatial and temporal variations of enzyme activity, populations, and nitrogen gas loss, *Soil Sci. Soc. Am. J.* 55 (1991) 90–95.
- [21] Parton W.J., Mosier A.R., Ojima D.S., Valentine D.W., Schimel D.S., Weier K., Kulmala A.E., Generalized model for N₂ and N₂O production from nitrification and denitrification, *Global Biogeochem. Cycles* 10 (1996) 401–412.
- [22] Prather M., Derwent R., Ehhalt D., Fraser P., Sanhueza E., Zhou X., Other trace gases and atmospheric chemistry, in: Houghton J.T., Meira Filho L.G., Bruce J., Lee Hoesung, Callander B.A., Haites E., Harris N., Maskell K. (Eds.), *Climate change: radiative forcing of climate change and evaluation of the IPCC IS92 emission scenarios*, Cambridge University Press, 1994, pp. 72–126.
- [23] Renault P., Les modèles opérationnels d'émissions de N₂O par les sols aux échelles régionales, *C. R. Acad. Agric. Fr.* 85 (1999) 163–176.
- [24] Seiler W., Conrad R., Field measurements of natural and fertilizer-induced N₂O release rates from soils, *J. Air Poll. Contr. Assoc.* 31 (1981) 767–772.
- [25] Tiedje J.M. Denitrification, in: Page A.L. (Ed.), *Methods of soil analysis part 2*, 2nd ed., Madison, 1982, pp. 1011–1026.
- [26] UNFCCC, Le protocole de Kyoto, 1997, [online] <http://www.unfccc.de> (page viewed on 2 October 2001).
- [27] Van Moortel E., Boeckx P., Van Cleemput O., Inventory of nitrous oxide emissions from agriculture in Belgium – calculations according to the revised Intergovernmental Panel on Climate Change guidelines, *Biol. Fertil. Soils* 30 (2000) 500–509.
- [28] Weier K.L., Doran J.W., Power J.F., Walters D.T., Denitrification and the dinitrogen/nitrous oxide ratio as affected by soil water, available carbon, and nitrate, *Soil Sci. Soc. Am. J.* 57 (1993) 66–72.
- [29] Williams E.J., Hutchinson G.L., Fehsenfeld F.C., NO_x and N₂O emissions from soil, *Global Biogeochem. Cycles* 6 (1992) 351–388.