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Effect of cellulose and straw incorporation in soil on total denitrification and nitrogen immobilization at initially aerobic and permanent anaerobic conditions

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Summary. Laboratory experiments were used to examine the influence of cellulose and straw on denitrification and N immobilization in a sandy loam soil. The soil was mixed with 300 µg nitrate-N/g and incubated in a special vessel under conditions that changed from aerobic to anaerobic or in the permanent absence of O_2. Gases (O_2, CO_2, N_2, N_2O, NO and CH_4) were analysed by gas chromatography at regular intervals and the soil was examined for nitrate, nitrite, ammonium and cellulose. Compared with controls, the application of straw and cellulose (0.5% and 1.0%, respectively) enhanced nitrate immobilization and decreased denitrification, under both anaerobic and originally aerobic ($PO_2 = 20$ vol%) conditions. However, a comparison of results from the aerobic and the anaerobic incubations shows that an increase in denitrification and N immobilization was apparent at an original O_2 concentration of 20 vol%. N_2 was the major product of denitrification in all experiments. Free methane was apparent as soon as nitrate was respired. The stimulating effect of O_2 on total denitrification in the presence of relatively high amounts of easily decomposable cellulose is ascribed to a higher turnover and an intensified mineralization rate (CO_2 production), which increased the total demand for electron acceptors.

Key words: Denitrification – Cellulose hydrolysis – Straw – Cellulolytic-denitrifying bacteria – N immobilization – Methane – Decreasing aerobiosis – Permanent anaerobiosis

In soil, O_2 need not necessarily inhibit or decrease denitrification if the supply of easily decomposable organic matter is relatively high (Abou Seada and Ottow 1985; Ottow et al. 1985). Under fully aerobic conditions (permanent O_2 introduction at maximum saturation) denitrification may even be intensified, because a bacterial population growing rapidly under favourable conditions requires a supply of both O_2 and nitrate (Ottow and Fabig 1984, 1985; Robertson and Kuenen 1984). These observations from experiments using pure cultures under controlled conditions may have far-reaching consequences for practical soil management. A recommendation made during the last decade, that straw be incorporated into arable soils, together with an additional amount of mineral N, has been increasingly put into practice in several European countries (Debruck and von Boguslawski 1979; Lynch et al. 1980; Mengel and Schmeer 1985; Christensen 1986). In general, proper incorporation of straw increases N immobilization (Scherer and Mengel 1981, 1983; Asmus and Hübner 1985; Kwong et al. 1986) and seems to reduce gaseous N losses by denitrification (Scherer and Mengel 1983; Mengel and Schmeer 1985). Under certain soil conditions, however, ploughing-in of straw may stimulate denitrification (Ganry et al. 1978; Schmeer 1983). In the present paper, the effect of cellulose or straw on nitrate immobilization and denitrification was examined under both initially aerobic and permanently anaerobic conditions in controlled laboratory experiments.

Materials and methods

Soil used. An air-dried and sieved (<2 mm) sandy loam soil (Table 1), as described previously (El Demerdash and Ottow 1983), was used throughout the experiments.

Laboratory experiments. For each sample, 400 g air-dried soil was mixed carefully in an Erlenmeyer flask (500 ml) with 300 µg N-

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Table 1. Physico-chemical properties of the sandy loam soil used in the laboratory experiments. Data refer to air-dried soil

pH (H_2O/KCl)	7.3/7.1	$N\text{-}NO_3^-$	22 mg/kg
Total C	4.4%	$N\text{-}NO_2^-$	0.2 mg/kg
Total N	0.27%	$N\text{-}NH_4^+$	7 mg/kg
C:N	16.3	$CaCO_3$	20.3%
$C_{(H_2O)}$[a]	0.55% of total C		
Cellulose[b]	1.4%		

[a] Water-soluble extractable C as a measure of the potential denitrification capacity (El Demerdash and Ottow 1983)
[b] As reducing sugars

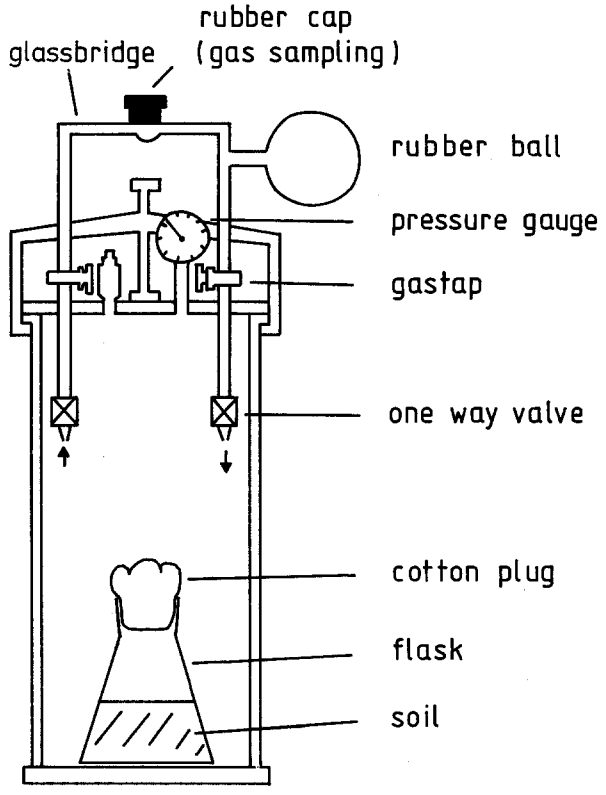

Fig. 1. Modified anaerobic jar designed for experiments with soil under controlled conditions. The flask was loosely closed with a cotton plug. Before gas sampling, the gas taps were opened and the atmosphere was mixed by pressing the rubber ball several times

NO_3^-/g (as KNO_3) and cellulose ("reinst" = "purest", Serva) or oatstraw powder (milled and sieved <1 mm) at 0.5 and 1.0%, respectively. The soil was brought to 80% of its water-holding capacity with distilled water. Each flask was enclosed in a 3.6-litre anaerobic plastic jar (Gössner, Hamburg, FRG) specially equiped (Fig. 1) with a glass bridge, serum cap (for gas sampling), two gas-tight one-way valves and a rubber ball for mixing the jar atmosphere (just before sampling). Details of this technique have been described elsewhere (El Demerdash and Ottow 1983; Ottow et al. 1985; Sommer and Ottow 1985). The jar was evacuated, flushed six times with helium gas (99.996%; Messer Griesheim, Frankfurt, FRG) or with a He/O_2 mixture (20 vol% O_2) using a "Labomix" gas mixer (Drägerwerk, Lübeck, FRG). All jars were incubated in the dark for 45 days (30°C), and soils without cellulose or straw were induced as controls.

All treatments were prepared in sufficient replicates to allow gas measurements of at least three, and soil analyses of two, jars at a time.

Oatstraw and cellulose "reinst". Both substrates were applied on the same weight basis, since the oatstraw and the cellulose HBS reinst (Serva no. 45421, ca. 87% cellulose) had a total C content of 42.2% and 42.4%, respectively. The oatstraw had a cellulose content of 41.6% and a total N content of 0.45% giving a C:N ratio of 94.

Gas chromatography. Gas samples were taken through the rubber sampling cap at the top of the apparatus (0.5 ml, Hamilton syringe) at regular intervals to measure O_2, CO_2, N_2, NO, N_2O and CH_4 production. Two Perkin-Elmer F22 gas chromatographs equipped with thermal conductivity detectors were used, one to determine NO, N_2O, CO_2 and CH_4 with columns of porapak Q and R (Wilhite and Hollis 1968), and the other with a molecular sieve (5 Å) to separate N_2 and O_2 (Moretti et al. 1974). Data were collected on a Perkin-Elmer recorder 56 and evaluated by Perkin-Elmer GC data system SIP-1. Details of the gas chromatography methods have been described previously (Fabig et al. 1978).

Chemical analyses. From each flask, 10 g homogenized soil was shaken (30 min) with 50 ml 1% $KAl(SO_4)_2$ solution and filtered (<0.45 µm, Sartorius Co.). The extract was used to determine nitrate, nitrite and ammonia by colorimetric methods (Abou Seada and Ottow 1985), using a Zeiss Spectrophotometer PM 2DL. Total N was determined by the Kjeldahl method (Bremner 1965). For the determination of water-extractable organic C, 30 g soil was shaken with 60 ml distilled water, centrifuged (15 min at 6000 rpm), filtered by suction (<0.45 µm, Sartorius Co.), and analysed as suggested by Halliwell (1960). For the determination of cellulose, 1 g fresh soil was extracted with 10 ml acetic-nitric acid (80% acetic acid with concentrated nitric acid at 10:1, v/v) for 3 min. After incubation in boiling water for 30 min, the sample was centrifuged (15 min at 6000 rpm) and the supernatant discharged. The residue was subsequently extracted with 10 ml 67% H_2SO_4, and the extract allowed to stand for 1 h at room temperature. In the supernatant, cellulose was determined colorimetrically as reducing sugars at 620 nm with anthrone reagent according to Updegraff (1969). For the calibration curve, Serva-cellulose "reinst" (="purest") was used.

Results

Denitrification under anaerobic conditions

Figure 2 shows the effects of increasing amounts of cellulose (0, 0.5% and 1.0%) on denitrification and mineral N turnover under anaerobic conditions for a 45-day period. The supply of cellulose apparently increased CO_2 production and ammonium immobilization. Denitrification (N_2) occurred only at a minor level and changed non-significantly with increasing cellulose content. In all samples, denitrification was completed within 5 days (Fig. 2). Figure 3 shows the effects of oatstraw on denitrification and changes in nitrate, nitrite and ammonium under anaerobic conditions (He atmosphere). The effects of oatstraw on both denitrification and N turnover were quite similar to those of cellulose.

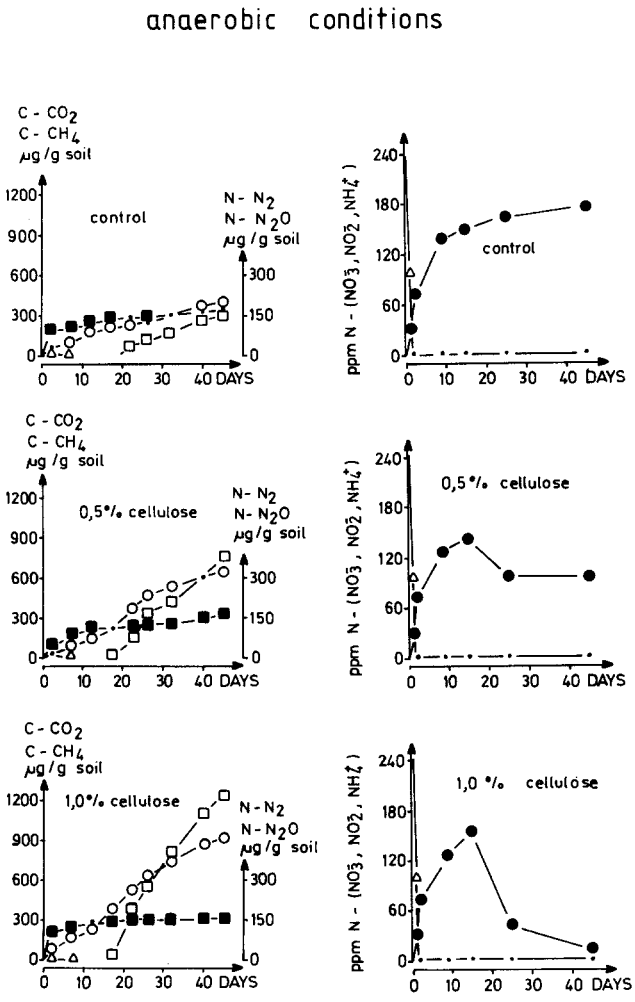

Fig. 2. Effect of increasing amounts of cellulose (0.5% and 1.0%) on CO_2 production and denitrification (*left;* ○, C-CO_2; ■, N-N_2; △, N-N_2O; □, C-CH_4) and changes in nitrate, nitrite and ammonium (*right;* △, N-NO_3^-; ●, N-NH_4^+) in a sandy loam soil under permanently anaerobic (He atmosphere) conditions during an incubation period of 45 days (80% water-holding capacity, 30 °C)

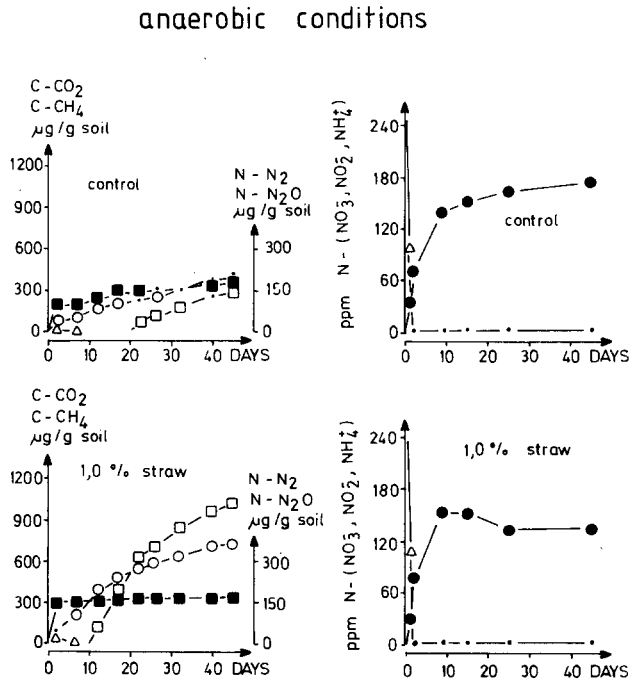

Fig. 3. Effect of straw (1.0%) on CO_2 formation and denitrification (*left*) and on changes in nitrate, nitrite and ammonium (*right*) in a sandy loam soil, under permanently anaerobic (He atmosphere) conditions during an incubation period of 34 days (80% water-holding capacity, 30 °C). Symbols as for Fig. 2

Denitrification under originally aerobic conditions

Figure 4 shows the influence of increasing amounts of cellulose on CO_2 production, denitrification and N turnover under aerobic conditions (original pO_2 = 20 vol%). Mineralization (CO_2 production) increased considerably, although the rate of O_2 consumption remained constant. Denitrification was suppressed with increasing cellulose content, although nitrate disappeared more rapidly. In controls, the nitrate concentration increased temporarily after 9–10 days, probably as a result of nitrification; this was reflected by a decrease in ammonium concentration. Finally, ammonium formation diminished with increasing amount of cellulose. The decrease in both denitrification and ammonium production suggests that N was increasingly immobilized in the biomass by the increasing cellulose supply.

A comparison with Fig. 2 (anaerobic conditions) shows that denitrification caused by a supply of cellulose was more intensive at an initial pO_2 of 20 vol% than under fully anaerobic conditions. Under aerobic conditions, cellulose hydrolysis and mineralization were clearly more intensive than in the absence of O_2 (Table 2), and consequently more N must have been turned over. Apparently, the enhanced O_2 demand, caused by increased mineralization of more available substrates (hydroxylated aliphatic and aromatic compounds), forced the microorganisms to use nitrate as an electron acceptor. The simultaneous and nearly parallel decrease in O_2 and nitrate (Fig. 4) suggests that both electron acceptors are used at the same time, presumably in different microsites and/or organisms.

As expected, methane formation was more rapid and intensive under permanently anaerobic than under initially aerobic conditions (cf. Figs. 2, 4). Since methane formation is known to be inhibited by nitrate, its release may indicate that the supply of nitrate has been exhausted. Since methane was released considerably earlier under permanently anaerobic conditions (Fig. 2) than under initially aerobic conditions

Table 2. Effect of increasing cellulose and straw concentrations on CO_2 production, cellulose hydrolysis, N turnover and release of N gases from a sandy loam soil (total C 4.4%, pH 7.3), under aerobic (20 vol% O_2 initial concentration) and anaerobic (He atmosphere) conditions, after 45 days of incubation at 30 °C

Starting pO_2 (v/v %)	Cellulose or straw added (%)	C-CO_2 (mg/kg)	Loss in cellulose (g/100 g soil)	At end of experiments			
				N-(N_2 + N_2O) (mg/kg)	N-NO_3^- (mg/kg)	N-NO_2^- (mg/kg)	N-NH_4^+ (mg/kg)
	Cellulose						
0	0.5	645 (1.4)[a]	0.34 (17.9)[b]	170 (5.6)[c]	2.0	0.1	100
0	1.0	912 (1.9)	0.35 (14.6)	163 (5.4)	2.0	0.1	16
	Straw						
0	1.0	733 (1.5)	0.30 (16.5)	168 (5.5)	5.0	1.0	157
0	Control	407 (0.9)	0.17 (12.1)	182 (6.1)	3.0	0.1	174
	Cellulose						
20	0.5	1016 (2.2)	0.58 (30.5)	240 (7.9)	4.0	0.6	68
20	1.0	1162 (2.4)	0.78 (32.5)	189 (6.2)	3.0	0.5	37
	Straw						
20	0.5	937 (2.0)	0.43 (26.7)	266 (8.7)	6.0	1.1	86
20	1.0	1029 (2.1)	0.33 (18.1)	257 (8.5)	5.0	0.7	83
20	Control	736 (1.7)	0.27 (19.3)	511 (16.9)	4.0	0.9	101

[a] % of original total C
[b] % of original cellulose
[c] Gaseous N-losses (N_2 + N_2O-N) in % of original total N. Values are averages of at least three parallel experimental set up with three replicate determinations each

(Fig. 4), nitrate must have been exhausted more rapidly under the permanently anaerobic conditions.

Figure 5 shows the effect of a supply of oatstraw on denitrification and other parameters of N turnover. The results were essentially the same as those obtained with cellulose (Fig. 4). This result suggests that the effects of the oatstraw on N turnover and denitrification were dominated by cellulose rather than by other components of the straw.

Table 2 summarizes the effects of aerobic or anaerobic incubation on cellulose losses, CO_2 production and N turnover by the end of the experiments. These results clearly show that cellulose mineralization, CO_2 liberation and denitrification were more intensive under initially aerobic than under permanently anaerobic conditions, both with cellulose and with straw.

Discussion

The results obtained support the view that straw manuring is useful for the immobilization of N in soil biomass, both under temperate (Debruck and von Boguslawski 1979; Mengel and Schmeer 1985) and tropical climates (Ganry et al. 1978; Kwong et al. 1986). With respect to the effect of O_2, however, some important observations were made in the present study.

First, nitrate reduction and ammonia formation appear to be considerably more intensive under permanently anaerobic than under initially aerobic conditions, at least with the present study conditions (30 °, 80% water-holding capacity). This may be explained by nitrate ammonification (respiration) rather than by true denitrification. The rapid decrease in ammonium after approximately 15 days under anaerobic and after 30 days under initially aerobic conditions is likely to be due to immobilization within the biomass of methane-forming bacteria; it was significantly greater in the presence of pure cellulose than with straw and may therefore be caused by lignin. This complex compound is apparently less suitable as a substrate for methanogenic bacteria than cellulose, at least under permanent anaerobiosis with a preceding period of nitrate respiration. The rapid release of methane from cellulose products left by nitrate respiration or from cellulose remains under permanent anaerobiosis is important because it reflects unfavourable conditions for seed germination and root growth. The incorporation of cellulose-rich compounds into a well-structured soil will be generally less harmful than into compacte material.

Secondly, denitrification losses from soil after straw and cellulose incorporation appeared to be increased under initially aerobic conditions. This type of initially aerobic situation with a progressively restricted supply of O_2 is quite common in the field, particularly after periods of rain during a relatively warm autumn in temperate regions or under generally tropical conditions. Under these ecological conditions, aerobic microorganisms use the trapped O_2 for cel-

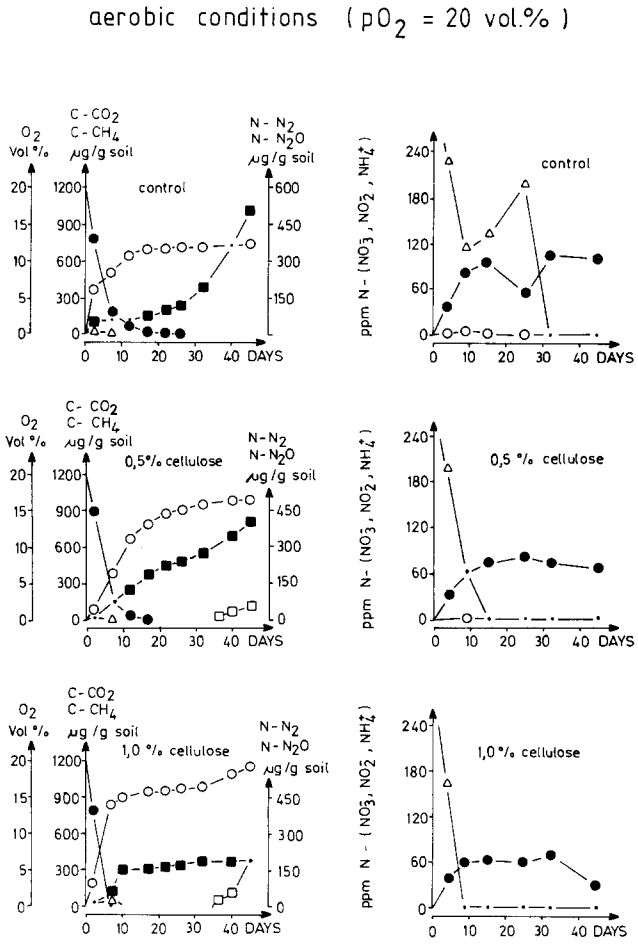

Fig. 4. Effect of increasing amounts of cellulose (0.5% and 1.0%) on CO_2 production and denitrification (*left*) as well as on changes in nitrate, nitrite and ammonium (*right*) in a sandy loam soil, under initially aerobic conditions (pO_2 = 20 vol%), during an incubation period of 45 days (80% water-holding capacity, 30 °C). ●, O_2 (*left*); ○, $N\text{-}NO_2^-$ (*right*); other symbols as for Fig. 2

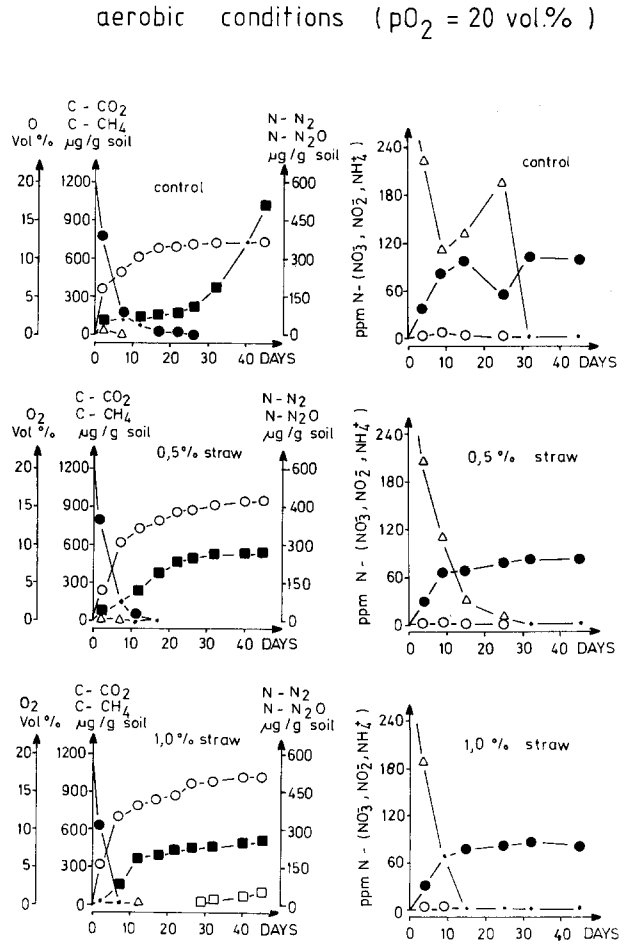

Fig. 5. Effect of increasing amounts of straw (0.5% and 1.0%) on CO_2 production and denitrification (*left*) and on changes in nitrate, nitrite and ammonium (*right*) in a sandy loam soil under initially aerobic conditions (pO_2 = 20 vol%), during an incubation period of 45 days (80% water-holding capacity, 30 °C). Symbols as for Fig. 4

lulose hydrolysis and respiration, and change to nitrate respiration (denitrification) in order to continue the energy-conserving reactions (ATP synthesis). In a well aerated, microbiologically active soil, the rapid mineralization of straw or other energy-rich compounds in the presence of fertilizer N may cause intensive O_2 consumption through respiration and nitrification; this could create locally and periodically anaerobic microsites with early, high denitrification rates. This ecological situation is quite likely to occur in soils, the more so since common cellulolytic-denitrifying bacteria (such as *Cellulomonas* spp., *Bacillus* spp., *Acinetobacter-Moraxella* spp. and *Pseudomonas* spp.) are able to hydrolyse cellulose under aerobic conditions, using nitrate as an electron acceptor (Sommer and Ottow 1985). The higher the microbial activity, the more intensive will be the demand for electron acceptors, and the earlier may be the onset of denitrification at appropriate microsites. These conclusions are consistent with the hypothesis that the demand for electron acceptors by an intensively metabolizing aerobic microflora is more decisive for the beginning and the intensity of denitrification than the establishment of anaerobiosis per se (Ottow and Fabig 1985). In the light of this background, it is likely that total denitrification losses from a microbiologically active, organically manured and "fertile" soil may be even more intensive than those from wet, physico-chemically poorly structured and compact sites. This, however, must await final proof in field measurements.

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