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Total denitrification and the ratio between N_2O and N_2 during the growth of spring barley

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Summary Total denitrification ($N_2O + N_2$) and nitrous oxide emission were measured on intact soil cores using the acetylene inhibition technique.

Total denitrification from the depth 0–8 cm during the growth period from April to August was 7 kg N/ha from plots supplied with 30 kg N/ha and 19 kg N/ha from plots supplied with 120 kg N/ha. The amounts of precipitation, plant growth, and N application were found to affect the denitrification rate. These factors also affected the ratio $(N_2O + N_2)/N_2O$, which varied from 1.0 to 7.2. Plant growth and precipitation increased the proportion of N_2 produced, whereas a high nitrate content increased the proportion of N_2O.

Introduction

From an agricultural point of view denitrification is a very important process since considerable amounts of plant-available N may be lost by this process.

Estimates of denitrification losses have been made in several cases and by various methods[1,4,8,11,12]; and losses varying from a few to about 70 per cent of the applied N have been reported. One source of variation could be, that when total denitrification is estimated only from measurements of N_2O, a fixed overall ratio between N_2O and N_2 production is used, and since this ratio is known to vary depending upon factors such as carbon supply[12], soil treatment[9], plant growth[14], and nitrate content in soil[3], the estimate may be erroneous.

In the present investigation the acetylene inhibition technique[17] and measurements of N_2O emission have been combined to estimate the total denitrification, and to calculate the proportion of N_2O and N_2 produced during the growth period of spring barley. Effects of N application, plant growth, and precipitation were also studied.

Materials and methods

Experimental area

Samples were taken from a field plot experiment with continuous barley cultivation started in 1973. The plots are located on a sandy loam soil at Roskilde State Research Station on Plant Science, Sealand. Physical and chemical properties of the soil are described by Vinther[15].

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Two levels of N fertilization were selected for these studies, one below (30 kg N/ha/year) and one above (120 kg N/ha/year) the normal application to barley in this area (90 kg N/ha/year). The N was applied as calcium-ammonium-nitrate (50% ammonium and 50% nitrate) on April 21, and at the same time the barley (cultivar Zita) was sown. Phosphorus and potassium (400 kg/ha of a PK-fertilizer containing 19 kg P, 49 kg K, and 10 kg Mg) were applied in the autumn the year before. Total precipitation during the growth season was 331 mm and the mean temperatures in the months April, May, June, July, and August were 5.7, 12.5, 13.6, 15.4, and 15.4°C, respectively.

Sampling and treatment of samples

Samples for determination of N_2O production were taken to a depth of 8 cm as intact soil cores in steel tubes 35 mm in diameter. Cores were taken both with and without plants. Samples without plants were taken between two barley rows spaced approximately 10 cm apart. These samples therefore were not free from roots, but the density of roots in samples with plants was considerably higher than in samples without. The size of the incubation vessel and the sampling procedure made it necessary to decapitate the plants about 5 cm above the soil surface before sampling.

Samples for determination of nitrate in the soil by extraction with 2*M* KCl for 1 h were taken several times during the growth season.

Measurements of denitrification

All samples were taken as pairs with four replicates. From each pair one sample was incubated with acetylene and the other without acetylene, in order to calculate the ratio

$$N_2O \text{ produced with } C_2H_2/\text{without } C_2H_2.$$

If acetylene completely inhibits N_2O reduction, the calculated ratio is equal to

$$(N_2O + N_2)/N_2O.$$

Results from samples incubated with acetylene correspond to total denitrification. The loss of N in the form of NO was considered to be negligible.

Immediately after arriving at the laboratory the samples were incubated in glass containers (volume 390 ml) fitted with a rubber septum. Samples to be incubated with acetylene received 10% acetylene after removing 10% of the air with a gastight syringe. To obtain activities as near as possible to field activities the incubated samples were placed under outdoor conditions.

Measurements of N_2O, both in samples with and without acetylene, were performed the day after sampling and again 24 h later and the N_2O production was calculated by difference.

Analytical procedure

N_2O was measured by a gas-chromatograph with an electron capture detector as described by Vinther *et al.*[16].

Results

Fluctuations of denitrification ($N_2O + N_2$), NO_3-N in soil and precipitation during the period from April to August are shown in Fig. 1. Before fertilization denitrification was low, probably due to low temperatures and to lack of substrate (nitrate). After application of N denitrification increased only slightly until mid-May while the soil was very dry due to low precipitation. In the last week of May the highest activity occured, coinciding with heavy rainfall, and thereafter the denitrification rate decreased to a constant low level not

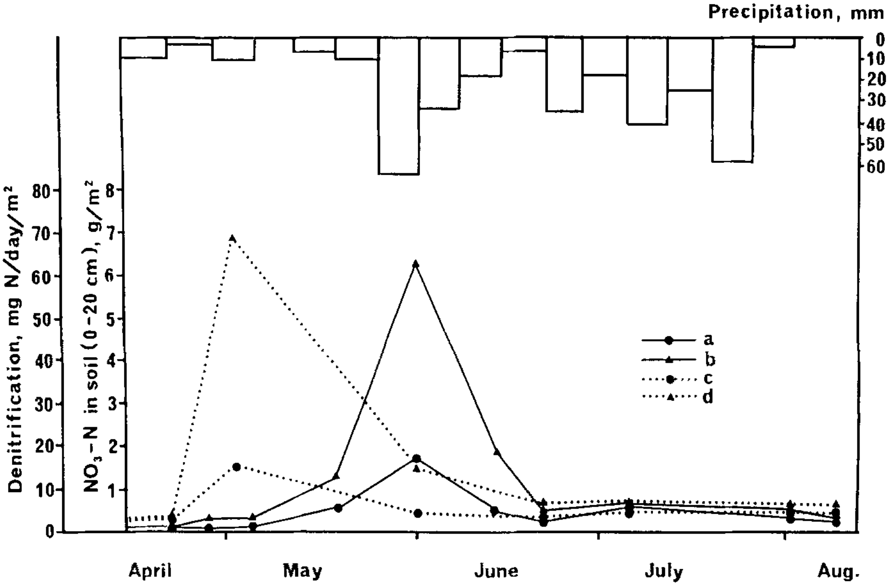

Fig. 1. Denitrification, NO_3-N content in soil and precipitation during the growth period of barley.
a = Denitrification from plots applied with 30 kg N/ha
b = Denitrification from plots applied with 120 kg N/ha
c = NO_3-N content in plots applied with 30 kg N/ha
d = NO_3-N content in plots applied with 120 kg N/ha.

affected by fluctuations of precipitation. In this six week period precipitation and therefore also soil moisture were relatively high but most of the nitrate was used by the plants and low activities of denitrification were measured.

The denitrification losses shown in Fig. 1 amount to 7 kg N/ha from plots supplied with 30 kg N/ha and to 19 kg N/ha from plots supplied with 120 kg N/ha. These are mean values of samples with and without plants.

In a previous investigation[2] no significant difference was found between the denitrifying activity in the depth 0–5 cm and 5–20 cm, and activities in deeper layers were found to be insignificant. In the present investigation denitrification was measured in the depth 0–8 cm, and therefore the total denitrification from plots supplied with 30 kg N/ha and 120 kg N/ha can be estimated to be approximately 15 kg N/ha and 50 kg N/ha respectively.

In Fig. 2 the effect of plant growth on the denitrification shows that when supplied with 30 kg N/ha the presence of plants on an average increases the activity by 2.5 times, whereas when supplied with 120 kg N/ha the increase is 5 times. In the samples without plants there was no difference between the denitrification from the two N levels, but if

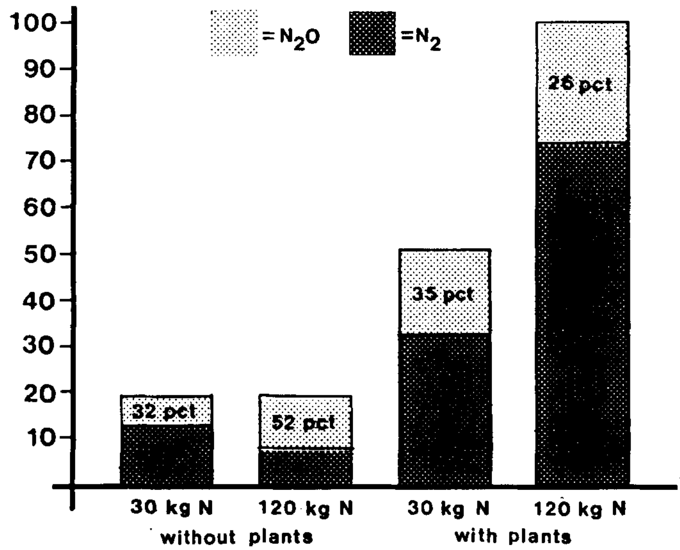

Fig. 2. Relative denitrification and ratio between N and N_2O per cent.

plants were present the denitrification was about twice as high in plots supplied with 120 kg N/ha as in plots with 30 kg N/ha. This indicates that supply of nitrate does not increase the denitrification rate unless other factors like oxygen tension and carbon supply are optimized also.

Fig. 2 also shows the mean ratios between N_2O and N_2 in the four treatments; the ratios at each sampling during the growth period are shown in Table 1. In the absence of plants, increasing the amount of fertilizer decreases the ratio $(N_2O + N_2)/N_2O$, which means that the proportion of N_2O is increased. In plots supplied with 30 kg N/ha, 32% of the N loss was N_2O, whereas the corresponding percentage in plots supplied with 120 kg N/ha was 52% (Fig. 2). In the presence of plants the opposite effect was observed.

Within each of the four treatments the ratio between N_2O and N_2 was relatively constant throughout the growth period, except 31 days after germination (Table 1), when a drastic increase in the amount of N_2 occurred. This increase coincided with a period of heavy rainfall (Fig. 1).

Both plant growth and precipitation (soil moisture) have a positive effect on denitrification by consuming oxygen and restricting oxygen diffusion; but also the ratio between N_2O and N_2 seems to be affected by the availability of oxygen. At low oxygen tension the proportion of N_2 is relatively larger than at higher oxygen tensions. Application of N fertilizer has an opposite effect on the ratio between N_2O and N_2.

Table 1. Effect of plant growth and nitrogen application on the ratio $(N_2O + N_2)/N_2O$ during the growth cycle of barley

Days after germination	Without plants		With plants	
	30 kg N/ha	120 kg N/ha	30 kg N/ha	120 kg N/ha
4	1.2	1.1	3.3	1.9
18	3.0	1.3	3.0	3.9
31	6.5	4.9	4.2	7.2
45	4.0	2.0	2.4	3.4
52	2.3	1.4	2.5	4.3
67	2.0	1.0	–	2.8
94	2.8	1.4	2.1	3.0
Mean	3.1	1.9	2.9	3.8

Discussion

From the available literature it is evident that losses of N due to denitrification vary considerably depending upon various factors such as soil type, irrigation or rainfall, cropping pattern, and fertilization management. Burford and Stefanson[6] found a maximum N_2O flux corresponding to 0.28 kg N/ha/day from a sandy loam soil fertilized with 100 kg N/ha. Since they could not measure the N_2 produced from the denitrification they could not account for all gaseous losses from the soil. A calculation of the maximum N_2O flux in the present investigation gives 0.25 kg N/ha/day, which is in good agreement with the findings of Burford and Stefanson.

Total denitrification has been estimated in a number of investigations[1,5,11,12,13] and losses of gaseous N corresponding to values from 0 to 70% of the fertilizer N applied have been reported. In the present investigation the total loss of gaseous N corresponded to 50% and 42% from plots supplied with 30 kg N/ha and 120 kg N/ha respectively.

In this investigation the factors which chiefly influence denitrification and cause variations in the rate of denitrification appear to be: (1) soil moisture, which at high levels stimulates denitrification by restricting O_2 diffusion, (2) plant growth, which may stimulate denitrification by providing carbon substrates and consuming soil O_2, and (3) soil nitrate level. These factors have also been shown to affect the proportions of N_2O and N_2 produced. Both increasing soil moisture and plant growth reduce oxygen availability in the soil and increase the proportion of N_2 produced, whereas high levels of nitrate increases the proportion of N_2O produced during the denitrification. Similar trends have been reported by Dowdell[7], Stefanson[14] and Blackmer and Bremner[3].

The mean ratios between N_2O and N_2 found in the present investigation are similar in magnitude to those stated by Lensi and Chalamet[10], but the variation from 1.0 to 7.2 indicates that it is unrealistic to use overall ratios for calculating total denitrification from N_2O measurements or to estimate N_2O losses from estimates of total denitrification.

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