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DENITRIFICATION IN A CULTIVATED SOIL: OPTIMAL GLUCOSE AND NITRATE CONCENTRATIONS

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Summary—An experiment was conducted in the laboratory on a cultivated soil incubated in serum bottles with a range of C-to-nitrate concentrations. C was added in form of glucose and nitrate in form of $Ca(NO_3)_2$. It was shown that an C–N concentration of respectively 500 μg C (glucose-equivalent, Glc-Eq.) and 36 μg N g^{-1} dry soil was optimal for denitrification. Results obtained either in the laboratory, in soil columns or in the field were in good agreement with one another. In particular, the root zone was shown to be favorable for denitrifying activity because the water-soluble C (Glc-Eq.) and N concentrations were more favorable than in bare soil. Furthermore, the water-soluble extractable Glc-Eq. appeared to be closely related to the denitrification rate and is thus likely to represent the energetic C pool supporting denitrification.

This was related to an inhibiting effect of increasing NO_3^- and NO_2^- concentrations on NO_3^- loss and N_2O production. Such inhibition can affect short-term measurements of denitrification in the field.

INTRODUCTION

The influence of nitrate and C on denitrification was studied by Bowman and Focht (1974) although they determined only the NO_3^- loss. A few laboratory studies have followed nitrate disappearance (Kohl *et al.*, 1976; Ryzhova, 1979) or N_2O production in the presence of acetylene (Klemedtsson *et al.*, 1977; Yoshinari *et al.*, 1977) in saturated soil. Actually there is no clear evidence in the field of the influence of changing C and NO_3^- concentrations on the N_2O flux, although the NO_3^- concentration was often considered to have no effect on the N_2O flux (Bremner and Shaw, 1958; Patrick, 1960). Furthermore, there are very few observations of the influence of the variation of the C level in a given soil. It is generally accepted that the easily-decomposable, readily-oxidizable organic substances serve as electron donors (Burford and Bremner, 1975; Standford *et al.*, 1975; Beauchamp *et al.*, 1980) and these substances are often considered to correspond to the soluble organic C in cold-water extracts of soils (Burford and Bremner, 1975; Katz *et al.*, 1985).

We have related the denitrification rate to a range of C–nitrate concentrations and to defined optimal concentrations. We then checked for their occurrence in field conditions and compared rhizospheric and bare soil.

MATERIALS AND METHODS

We used a sandy clay loam either sieved (< 2 mm) at a water content of 125 mg g^{-1} dry soil, or in undisturbed soil columns, or directly in the field. Some physical characteristics of the soil were as follows: 34.8% sand, 46.4% silt, 16.9% clay, 1.27% organic matter, pH 6.6.

In the laboratory

The soil was divided into 5 parts and amended with $Ca(NO_3)_2$ solutions of 5 different concentrations. After a 48-h incubation at 21°C the quantities of NO_3^--N in the 5 different samples were: 15, 36, 113, 169 and 330 μg N g^{-1} dry soil. Each soil sample was then divided into seven 55 g portions, placed in separate 150 ml serum bottles, and glucose added at 30, 80, 100, 120, 250 or 500 μg C g^{-1}. The final average water content was 380 mg g^{-1} dry soil or about 180% of the water holding capacity. Each flask received 10% acetylene (v/v). The 35 flasks were incubated for 44 h at 21.5°C and analyzed for N_2O and mineral N (no NH_4^+ was detected). This is referred to as Experiment I.

Experiment II was carried out for one C input, 130 μg C g^{-1}, and 6 different NO_3^--N concentrations (three replicates): 8.5, 17.4, 27.1, 43.1, 79.1, 259.6 μg N g^{-1} dry soil. At the end of the incubation N_2O-N, NO_3^--N and NO_2^--N were measured. The final water content was 630 mg g^{-1} dry soil.

Twenty-three undisturbed soil columns, taken from bare soil, were amended with 100 mg of NO_3-N in the form of $Ca(NO_3)_2$, (equivalent to 120 kg N ha^{-1}) and incubated for 1 month at 21°C. One week later, 15 columns were sown to ryegrass so that a root zone could develop in the top of the column. Incubation was continued for a further 3 wk after which, 1 mg of glucose-C dissolved in 5 ml of water was added to certain columns. Water content was maintained at 230 mg d^{-1} dry soil by regular readjustments to the initial weight with distilled water. After different incubation times (before and after the addition of nitrate and 1, 2, or 3 days after the addition of glucose) denitrification was measured.

In the field

The field experiment was carried out on a plot of 2-month old maize plants. The plot had received 120 kg N ha^{-1} of $Ca(NO_3)_2$ 1.5 months before the experiment. The N_2O production in presence of C_2H_2 was measured three times during the day, either directly above the plants' root zone (3 measurements), or on the bare soil between the maize stalks (3 measurements). Totally rigid plexiglass enclosures made of 2 cylinders, 10 cm high and of different diameters fitted into one another were chosen to enclose the stems without damaging the plants or the roots. The enclosures were pushed down into the soil to a depth of 5 cm, a few minutes before the beginning of the experiment.

Denitrification was assayed by injecting 10% (v/v) acetylene into the gas chambers. N_2O concentration was measured in 5 ml samples of air removed from the chamber after 60 min and N_2O flux calculated. Preliminary experiments showed that diffusion was sufficient to reach 0.1% C_2H_2 at 10 cm depth, and that N_2O was often the major product.

A gas chromatograph Girdel 30 equipped with an ECD (^{63}Ni source) operating at 280°C with a 2-m long Porapack Q column set at 80°C was used. We also used a TCD operating at 200°C and 200 mA with a 2-m long silicagel column set at 60°C.

The first 5 cm of soil within the enclosure were removed and homogenized before measuring the water content. A fraction of the soil was frozen for later C and N analyses.

The soil samples (55g) were shaken in the presence of 100 ml of distilled water for 1 h and then centrifuged at 6000 rev min^{-1}. The supernatant was filtered and frozen. NO_3^--N and NO_2^--N together were measured by the steam distillation method and the NO_2^--N by the Griess–Illevay method. The Glc.Eq. was determined on another aliquot by the anthrone method (based on a standard glucose curve) (Brink *et al.*, 1960). Due to reaction interference with NO_3^- (Katz *et al.*, 1983), we eliminated the NO_3^- with a H^+ and OH^- ion exchange resin beforehand.

RESULTS

In the laboratory

Experiment I. Increasing C concentration from 30 to 120 μg C g^{-1} sharply stimulated N_2O production; thereafter there was minor increase (Fig. 1a). At each level of carbon it was observed a slowdown of N_2O production (Fig. 1b) for NO_3^--N concentrations higher than 36 μg N g^{-1}. The maximum activity was obtained for concentrations of 36 μg NO_3^--N g^{-1} and 500 μg C(Glc.Eq.) g^{-1}. In spite of the fact that NO_3^--N was limiting for inputs of 10 and 36 μg N g^{-1}, the same inhibition was observed on the pool of N that was not in the form of either NO_3^- or NO_2^- at the end of the incubation (Fig. 2).

Experiment II showed the same tendencies, in spite of a higher water content (Fig. 3). The accumulation of nitrite was measured and was found to be important, up to 28 μg NO_2^--N g^{-1}, for inputs of 80 and 260 μg NO_3^--N g^{-1} corresponding to a less important N_2O production than expected. NO_3^--N disappearance behaved in a fashion similar to that of N_2O produced. The data are internally consistent as all N added was approximately accounted for by NO_3^-, NO_2^- and N_2O at the end of the incubation. Each point is the mean of 3 incubations.

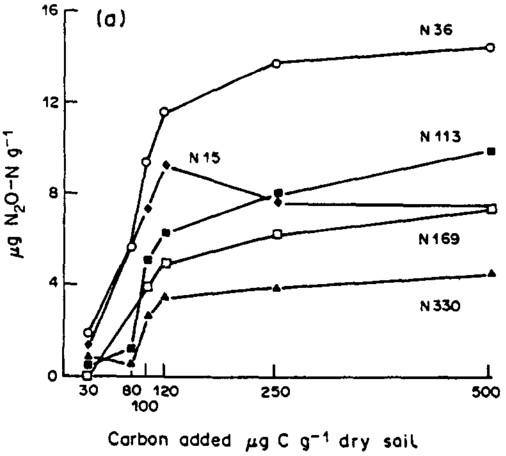

Fig. 1. (a) Experiment I: N_2O accumulation at the end of the 44 h incubation as a function of the C (Glc) added, for different nitrate levels (μg N g^{-1} dry soil).

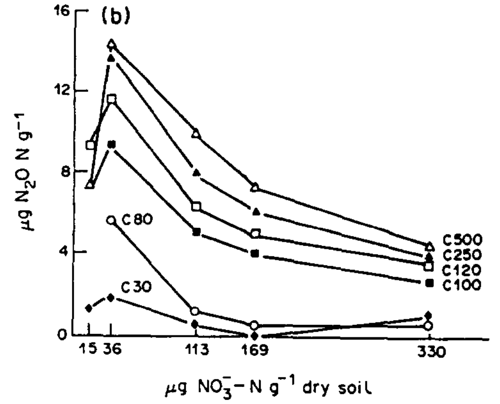

Fig. 1. (b) Experiment I: N_2O accumulation at the end of the 44 h incubation as a function of the NO_3^- concentration, for different C levels (μg C g^{-1} dry soil).

The results of the soil columns (Fig. 4a and b) enabled us to simply show the trend of the relationship between denitrification and its substrates. For better understanding, the data were classified into arbitrary ranges of glucose and mineral N, corresponding to limiting, low, medium and high concentrations.

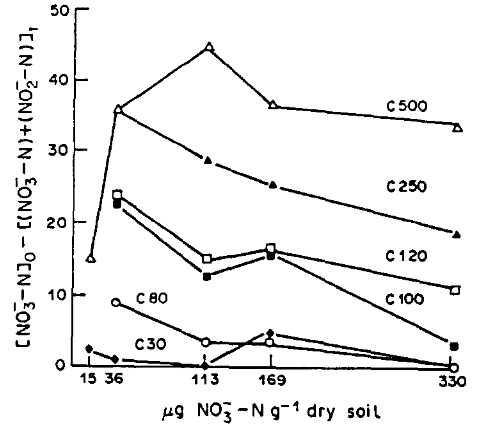

Fig. 2. Experiment I: quantity of N not recovered in the form of NO_3^- or NO_2^- at the end of the incubation as a function of the nitrate added, for different C levels (μg C g^{-1} dry soil). *(NO_3^--N added)—(mineral N measured at the end of the incubation)(in μg N g^{-1} dry soil).

Denitrification seemed to be favoured by simulta-

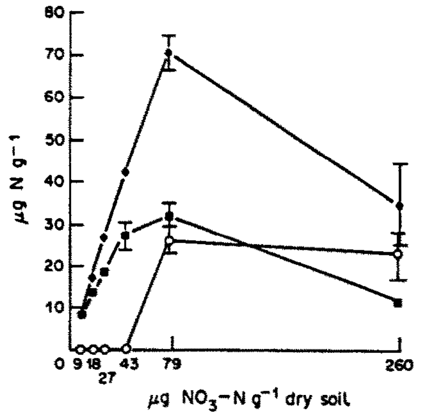

Fig. 3. Experiment II: N_2O-N and NO_2^--N concentrations at the end of the incubation and NO_3^--N disappeared, for 6 different NO_3^--N inputs. The glucose added was equivalent to 130 µg glucose-C g^{-1} dry soil. Bars indicate SD. ◆, Nitrate disappeared; ○, nitrite; ■, nitrous oxide.

neous Glc.Eq. and mineral N concentrations between 6 and 260 µg C g^{-1} and 15 and 130 µg N g^{-1}, respectively. Larger concentrations of mineral N led to a slowdown of the gas production, and at lower concentrations, denitrification remained constant but at a low level. The maximum activity was obtained for 30 µg N g^{-1} and 260 µg Glc.Eq.C g^{-1}.

In the field

Although NO_3^- contents were lower in planted than in bare soil (Fig. 5) the presence of higher

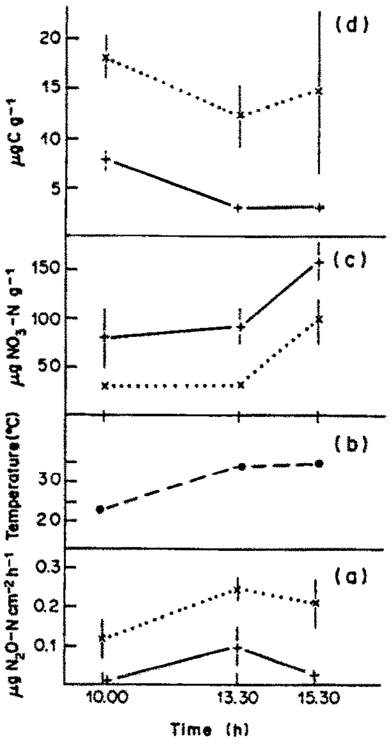

Fig. 5. (a) N_2O production in the field during one day in bare soil—, under maize ---. Each point represents an average of 3 measurements, bars indicate the SD; (b) soil temperature; (c) nitrate concentration (d) Glc.Eq. concentration.

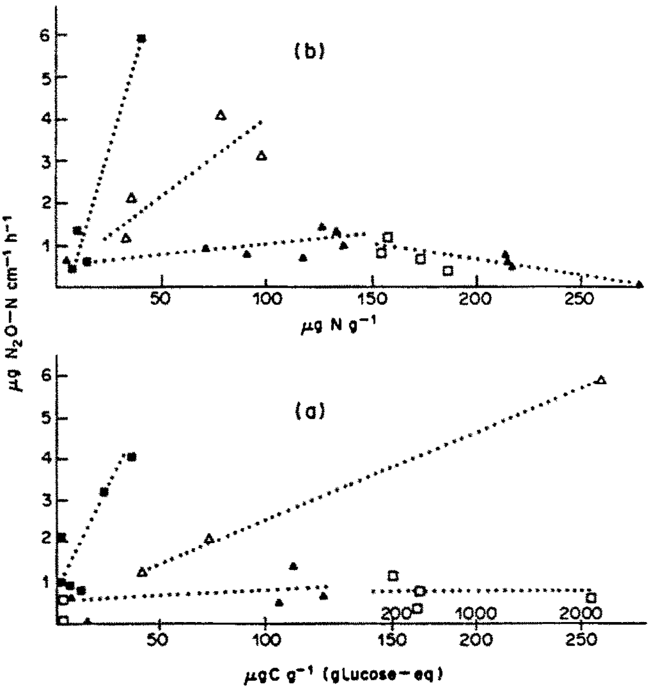

Fig. 4. N_2O production rate in soil columns as a function of Glc.Eq. Concentration (a); mineral nitrogen concentration. ■, 71 < N < 129; △, 34 < N < 41; □, 200 < N < 2000; ▲, S < N < 15. (b) Data unit is µg g^{-1} dry soil. ■, 106 < C < 260; △, 22 < C < 74; □, 204 < C < 2116; ▲, 1 < C < 6. Dashed lines represent only the tendency of the relationship between the activity and the substrate.

Glc.Eq. concentrations under plants would explain the development of a greater denitrifying activity. We obtained an average C content of 15 μg C g^{-1} under plants, despite a considerable dilution of the C exuded into the soil. The N_2O production was shown to be 3 to 5 times higher, with values as high as 500 g N ha^{-1} day^{-1} at 24°C and at 220 mg g^{-1} dry soil water content.

DISCUSSION

The results obtained in the laboratory in serum bottles demonstrate the importance of following simultaneously the production of N_2O in the presence of C_2H_2 and the disappearance of NO_3^-. Indeed, the inhibiting effect observed on N_2O production could be due either to a true inhibition, or to an incomplete blockage of the N_2O-reductase by C_2H_2. The fact that the same inhibiting effect was observed on the disappearance of $NO_3^- + NO_2^-$ (Experiment I) and NO_3^- (Experiment II) confirms the validity of the first hypothesis.

Experiment I shows a less important N_2O production seemingly related to increasing initial NO_3^- concentration. Because of a lower water content in Experiment I than in Experiment II, the rates of production of N_2O and of mineral N disappearance were lower (Lalisse-Grundmann *et al.*, 1983). Besides, this lower water content and the addition of C suggest the likely occurrence of an assimilative reduction of NO_3^-, thus accounting for a part of the N not recovered (no NH_4^+ was detected). The other possibilities are:

- NO accumulation or other intermediate gaseous compounds (McKenney *et al.*, 1982).
- Nitroxyl intermediate (Renner and Becker, 1970).

The influence of mineral N concentrations and soluble C concentration on denitrification in the field will be discussed later.

Likewise, Experiment I showed on the one hand a simultaneity between nitrite accumulation and slowdown of the N_2O production and NO_3^- reduction, and on the other hand different accumulation of intermediates depending on the concentration of nitrate added.

These results obtained on soil are in good agreement with other observations reported in the literature for various conditions. The pattern of intermediaries accumulation during denitrification is indeed still controversial. A few studies have reported such an inhibition, where nitrate or nitrite were supposed to play an important role. Even if a few hypotheses have been proposed, it is not clear yet whether nitrite or nitrate is the effector and further explanation will be needed for a better understanding of the mechanisms involved.

High concentrations of nitrate are supposed to inhibit not only N_2O-reductase activity (Firestone *et al.*, 1979; Gaskell *et al.*, 1981; Terry and Tate, 1980) but also NO-reductase activity (Payne and Riley, 1969) and nitrate reductase (Renner and Becker, 1970). Some authors have shown that nitrate *per se* had an inhibiting effect on nitrite reduction (Gaskell *et al.*, 1981) or NO-reductase (Payne and Riley, 1969), others suggested that nitrite accumulation during nitrate anaerobic reduction (Betlach and Tiedje, 1981; McKenney *et al.*, 1982, Shimuzu *et al.*, 1978) is responsible for the inhibiting effect noted on N_2O reduction (Firestone *et al.*, 1979), on NO reduction (Renner and Becker, 1970) or on NO_3^- reduction (Bailey and Beauchamp, 1973). On the other hand, Payne and Riley (1969) suggested that the inhibition of the NO-reductase by nitrate resulted in nitrite accumulation.

We observed an inhibiting effect of NO_3^- in a low range of concentrations between 36 and 113 μg N g^{-1}. They are lower than most of the reported ones, but higher than those that Nakajima *et al.* (1984) observed. The nitrite concentration obtained in our study is higher than observed by Firestone *et al.* (1979) and Nakajima *et al.*, (1984) as being inhibiting. Care must be taken nevertheless, that most studies were conducted on pure culture, except for a few: Firestone *et al.*, (1979) and Bailey and Beauchamp (1973) used soil, Nakajima *et al.* (1984) used lake sediments. In fact, understanding of the mechanisms involved is unlikely to be solved in the soil directly.

The total denitrification rate was shown to be inhibited under high nitrate concentration conditions (Nakajima *et al.*, 1984; Renner and Becker 1970; Payne and Riley, 1969; Avnimelech, 1971) and by high nitrite (Nakajima *et al.*, 1984; Renner and Becker, 1970).

The higher denitrification rates related to higher C concentrations under plants are an evidence of the rhizospheric effect (Woldendorp, 1963; Stefanson, 1972; Smith and Tiedje, 1979). This can be related also to photosynthesis (Scaglia *et al.*, 1985) and to O_2 depletion within the root zone (Garcia, 1975). In addition, the NO_3^--N concentrations ranged from 80 to 150 μg N g^{-1} in the bare soil, compared to 30–80 μg N g^{-1} in the rhizosphere, and thus fell within the inhibition range observed in the laboratory. So the C–N values were likely to be favorable to denitrification in the root zone but less favorable in bare soil. However, concentrations of C in planted soil were lower than expected. This might be because: (a) the exuded C is diluted in a large amount of soil in the soil sample and the C was underestimated; or (b) the C turnover is very fast in the root zone and no C can accumulate (De Catanzano and Beauchamp, 1985).

These results show that a slight variation of the Glc.Eq. concentration (from 5 to 20 μg C g^{-1} for example) can lead to a noticeable variation in N_2O production. Thus, we assume that the C pool extracted with cold water and analyzed for the Glc.Eq. is likely to represent the available C to the denitrifying microorganisms, as confirmed by Katz *et al.* (1985).

This C pool might often be limiting in the field, explaining the important role played by the C mineralization on denitrification (Focht and Verstraete, 1977; Reddy *et al.*, 1982). Furthermore, in the field, the occurrence of a substrate-inhibiting effect would explain some unexpected low denitrification rates.

Our results determined ranges of favorable soluble C and nitrate concentrations for denitrification comparatively in the laboratory and in the field.

In undisturbed soil columns we could only point out trends of the relationship between C–N and denitrification. A stimulating effect by plants was confirmed by favorable C–N concentrations in the rhizosphere. It was also shown that the Glc.Eq. of the cold-water soil extract was related to the available C pool used as an energy source for denitrification. We thus propose it as a C pool reference for denitrification, as suggested by Chalamet (1985). Furthermore, there was a good agreement between the laboratory and the field results.

In the laboratory we obtained a maximum denitrifying activity for 500 μg C g^{-1} and 36 μg NO_3^--N g^{-1}. These concentrations are likely to occur in the field, a concentration of 60 μg NO_3^--N g^{-1} is roughly equivalent to 120 kg N ha^{-1} in the top 20 cm. Even if the observed pattern of accumulation corresponds to N-reductive steps that occur at different kinetic rates as suggested by Betlach and Teidje (1981) and not to an inhibiting effect, it remains that the rates of denitrification noted for a given length of time are lower than those expected in the presence of high concentrations of nitrate. That is why these results have a particular meaning having been obtained on soil, in as much as these denitrification rates actually correspond to short-term measurements in the field.

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