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Denitrification under different cultivated plants: effects of soil moisture tension, nitrate concentration, and photosynthetic activity

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Summary. Plant effects on the denitrification rate were investigated in pot experiments at different soil moisture tensions and nitrate concentrations. Nitrate concentrations and the soil moisture tension were regulated immediately before each measurement. The effects of the plants on denitrification rates were dependent on the soil moisture tension. At a low soil moisture tension (−7 cm H_2O), there was a 10-fold increase in the denitrification rate (planted versus unplanted soil). At a medium moisture tension (−30 cm H_2O) the plants had practically no effect, and at the highest tension (−60 cm H_2O) the effect was slightly negative. Large differences in denitrification rates under different plant species were observed. At a low soil moisture tension, the average denitrification rate (μg N kg^{-1} soil h^{-1}) was 39–42 under small grains (barley, wheat, and oats), 47–82 under the grasses (cocksfoot, meadow grass, meadow fescue, and timothy) and 18 under red clover. The differences between the monocots were attributable to differences in plant growth rates, rather than to any specific difference in stimulation or inhibition of denitrification, since the variations in photosynthetic activity fairly well predicted the differences in denitrification rates under different monocots. Clover, however, gave much lower denitrification rates than those predicted by the photosynthetic activity.

Key words: Denitrification – Soil moisture – Roots – Photosynthesis – Acetylene inhibition method

It has often been observed that growing roots have a stimulating effect on denitrification (Stefanson 1972; Von Rheinbaben and Trolldenier 1984; Haller and Stolp 1985; Scaglia et al. 1985; Svensson et al. 1985; Klemedtsson et al. 1987a, b), and this has been attributed to the stimulation of bacterial respiration by exudation as well as to the reduction of pO_2 by root respiration. Other studies have shown that plant roots have neutral or even negative effects on denitrification (Smith and Tiedje 1979; Aulakh et al. 1983; Haider et al. 1985). In most cases, this may have been due to nitrate depletion by root uptake and reduction of soil moisture content by transpiration. However, Haider et al. (1987) observed that young maize roots has a slightly negative effect on denitrification, although they tried to eliminate the effects of moisture and nitrate depletion in the root zone. Thus, there are still some unexplained conflicts between different investigations regarding the effect of plant roots on denitrification. Interpretation of these studies is difficult because the conflicting data were obtained from experiments with different plant species under widely different conditions, especially with respect to soil water status.

The present pot experiment was designed to trace systematic differences between plant species regarding their effects on denitrification at different soil moisture tensions. To ensure a high nitrate concentration and a standardized soil moisture tension, the pots were flooded with nitrate solutions and drained to specific soil moisture tensions, immediately before incubation for measurement of photosynthetic activity and denitrification rate by the acetylene inhibition method.

Materials and methods

Soil and growth conditions

The soil was a clay loam, with 26% clay, 42% silt, 32% sand, pH 5.7 (H_2O), 3% organic C, and 0.3% organic N (Bakken et al. 1987). It was taken during September 1983 from a field which had grown small grains without organic manuring for 4 years. Soil samples were sieved through a 5-mm steel mesh and stored in

polyethylene bags at outdoor temperature (−10 to +5 °C) until the experiment began (November–February).

The pots were made of black polyethylene, 12 cm high × 7 cm wide, with five 1-cm diameter holes in the bottom, and were filled with 500 g fresh weight soil (396 g dry weight). The soil was limed and fertilized with 12.0 g $CaCO_3$ and 1.2 g composite fertilizer kg^{-1} soil dry weight. The composite fertilizer contained 7.6% NO_3^--N, 5.4% NH_4^+-N, 19% K, 4% P, and 1.5% Mg plus microelements (Mo, Bo, Cu, Zn, Mn, and Fe).

The pots were sown with different plants and placed on capillary mats (Fig. 1A) under normal daylight plus artificial light (15 h day^{-1}) from high-pressure sodium lamps (Osram Vialux, NAV-T 400 W, light intensity 220 µE m^{-2} s^{-1} (400 – 700 nm) at the soil surface and 420 mE m^{-2} s^{-1} 30 cm above the soil surface. The unplanted pots were covered with tinfoil to avoid algal growth. The air temperature in the growth chamber was 12 °–15 °C. At intervals, some of the pots were used for denitrification measurements, followed by determination of root and shoot dry weights or extraction of soil samples (2 *M* KCl) for NH_4^+ and NO_3^- determination (Henriksen and Selmer-Olsen 1970; Selmer-Olsen 1971).

Regulation of nitrate concentration and soil moisture tension

Before incubation, the nitrate concentration and the soil moisture tension were regulated by flooding the pots with nitrate solutions followed by draining (Fig. 1B, C) to different moisture tensions on columns of coarse silt (10- to 100-µm diameter particles) in which the level of free water was 7, 30, or 60 cm below the silt surface. To ensure capillary contact between the silt and the soil in the pots, the pots were pressed gently into the surface of the silt columns. To minimize evapotranspiration during draining, the temperature was low (10 – –12 °C) and the air was moistened to about 90% relative humidity. After draining, the soil moisture content of each pot was measured by weight (total weight minus the empty pot weight, the soil dry weight and the plant fresh weight) immediately before incubation (Fig. 1D).

The efficiency of the draining system was checked in initial experiments with plant-free pots equipped with tensiometers. The moisture content as measured by weight fell rapidly during the first 0.5–1 h of draining. After 2 h, the moisture tension reached the desired levels and the moisture content stabilized at different levels depending on the applied tension, 460–490, 360–380 and 340–360 ml H_2O kg^{-1} soil dry weight with water tensions of −7, −30 and −60 cm H_2O, respectively.

Photosynthesis and denitrification measurement

The incubation system (Fig. 1D) consisted of polyacryl cylinders (inner diameter 17 cm, 75 cm high) receiving daylight plus light from six horizontal neon-light tubes which were placed 20 cm from the cylinders (minimum light intensity 230 µE m^{-2} s^{-1} (400–700 nm). The temperature in the cylinders was 14 °–18 °C. The cylinders could be coupled (top and bottom) to a gas circulation system consisting of a membrane pump (P, flow of 1000 ml/min), a T-tube with rubber septum, and an alternative loop (controlled by valves) through a gas-washing bottle (1200 ml). CO_2 was injected through the rubber septum. Acetylene was mixed into the cylinder atmosphere by coupling the gas-washing bottle, filled with pure acetylene, into the circuit. The pump was run for 5 min to ensure homogenous distribution of the introduced gases within the cylinder. Samples of the cylinder atmosphere for gas chromatographic analysis were taken through the rubber septum in the circulation system after running the pump for 5 min.

The photosynthetic activity (CO_2 fixation) and the denitrification rates were measured in sequence. Initially, 50 ml CO_2 was injected (giving 3000 ppm CO_2), and the CO_2 depletion during the following 2 h of incubation (three gas samples) was used to estimate the photosynthetic activity of the plants. Acetylene was then introduced (1200 ml), giving a final concentration of 6.5%), and the measured N_2O accumulation during the following 6–8 h (three gas samples) was used to estimate the denitrification rate. The N_2O and CO_2 concentrations were measured on a gas chromatograph, equip-

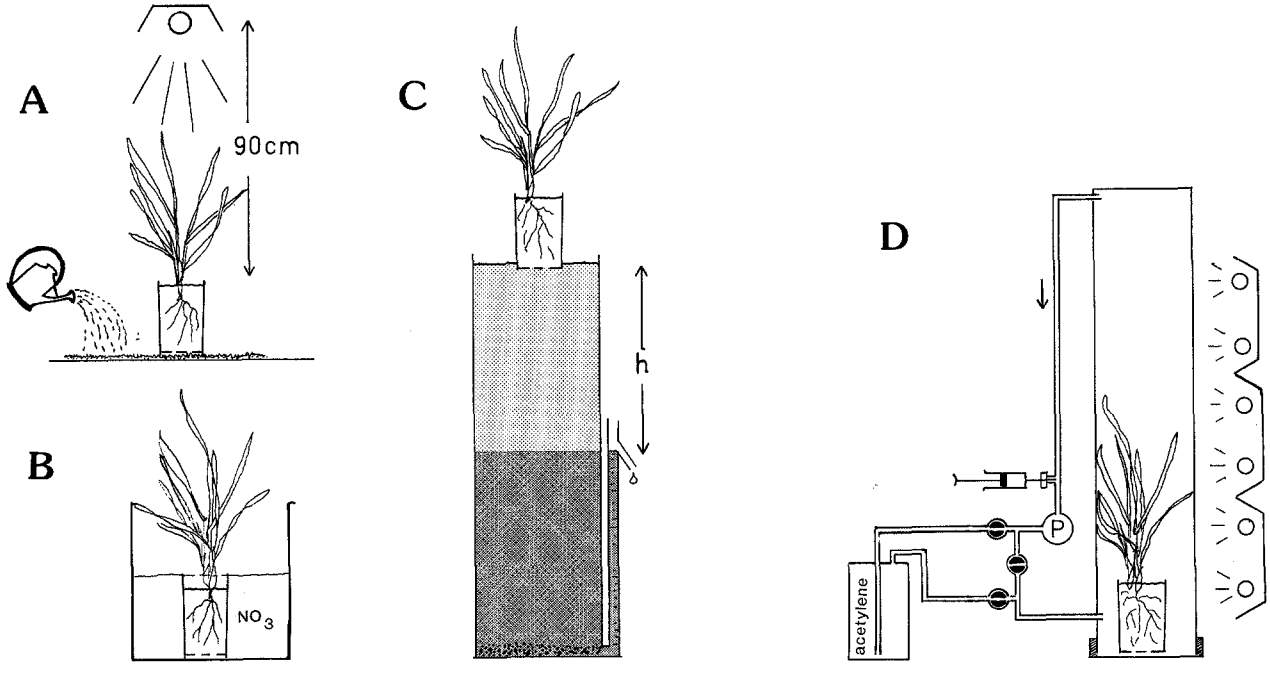

Fig. 1A–D. Plant growth conditions (**A**), method for regulating nitrate concentration by flooding in nitrate solutions (**B**), and drainage on silt column (**C**) prior to incubation for measurement of photosynthesis and denitrification rate (**D**). See text for detailed explanation

ped with a thermal conductivity detector and an electron capture detector. The method has been described in detail by Bakken et al. (1987).

Plant types and experimental design

In the first pot series, 105 pots were sown with barley (*Hordeum vulgare* L. var. Møyar, eight plants per pot), and denitrification measurements were conducted between 29 and 45 days after germination. During this period, plant dry weight (roots plus shoots) increased from 0.74 to 1.94 g per pot while the shoot/root ratio fell from 2.1 to 1.7. Two separate experiments were conducted, with different purposes. One was to test the effect of varying the nitrate concentration and the soil moisture tension: In a series of six incubations, each including nine pots with barley and three unplanted pots, three different nitrate concentrations in the flooding water (10, 100, and 200 ppm) and three different soil moisture tension levels (-7, -30, and -60 cm H_2O) were applied; flooding lasted 2 h and draining 8–12 h. The pots without plants received the highest nitrate concentration (200 ppm), but were drained, like those with plants, to three different soil moisture tensions. The purpose of the second experiment was to determine the effect of the length of the draining period before the denitrification measurements were made. Sixty pots (45 with barley and 15 without plants) were flooded in 100 ppm NO_3 solution for 2 h, and denitrification was measured after draining for 2, 8, 14, 40, and 60 h on the three different draining tensions (nine pots with barley and three without plants in each incubation).

In the second pot series, 120 pots were prepared and sown with the following plants (13 pots each plus 16 unplanted): Barley (*Hordeum vulgare* L. var. Agneta), wheat (*Triticum aestivum* L. var. Runar), oats (*Avena sativa* L. var. Mustang), cocksfoot (*Dactylis glomerata* L. var. Leikerud), meadow grass (*Poa pratensis* L. var. Holt), meadow fescue (*Festuca pratensis* Huds. var. Salten), timothy (*Phleum pratense* L. var. Forus), and red clover (*Trifolium pratense* L. var Molstad). The herbage grasses and clover were sown 12 days earlier than the cereals because of the germination lag. The number of seeds per pot was 50 for the herbage grasses and clover, and 10 for the cereals. Measurements of photosynthesis and denitrification were made between 18 and 60 days after seeding, each measurement covering a group of 9 pots (one of each plant species and one unplanted) that had been given the same pretreatment (flooding and draining). All the groups were flooded for 15–25 min with 100 ppm NO_3^- solution, but two different draining tensions were applied: Six groups were drained at -7 cm H_2O for 2 h, and four groups were drained at -30 cm H_2O for 10–12 h. Each part of the experiment (low and high soil moisture tension) was subjected to a separate two-way analysis of variance. Covariance between denitrification and photosynthetic activity was analysed in a separate analysis of variance, leaving out the data for the pots without plants. A comparison between the regression model estimates, based on all plant species, and the data for each plant species was used to express the possible inherent differences between plant species with respect to stimulation of denitrification.

Results

The intended regulation of the nitrate concentration in the soil, to be obtained by varying the nitrate concentration of the flooding solution, was only partly achieved. The average nitrate concentration, calculated as ppm in the soil water, $n = 12$ for each treatment, after flooding with 10, 100, and 200 ppm solution was 17 (±5), 32 (±19), and 66 (±10) ppm, respectively. Thus the method established systematic differences between the nitrate concentrations, but the differences were smaller than intended.

The results from varying draining periods are shown in Fig. 2. At the low soil moisture tension, the denitrification rate increased during the first 14 h of draining, and then declined gradually to approach the levels of the other treatments. At the high soil

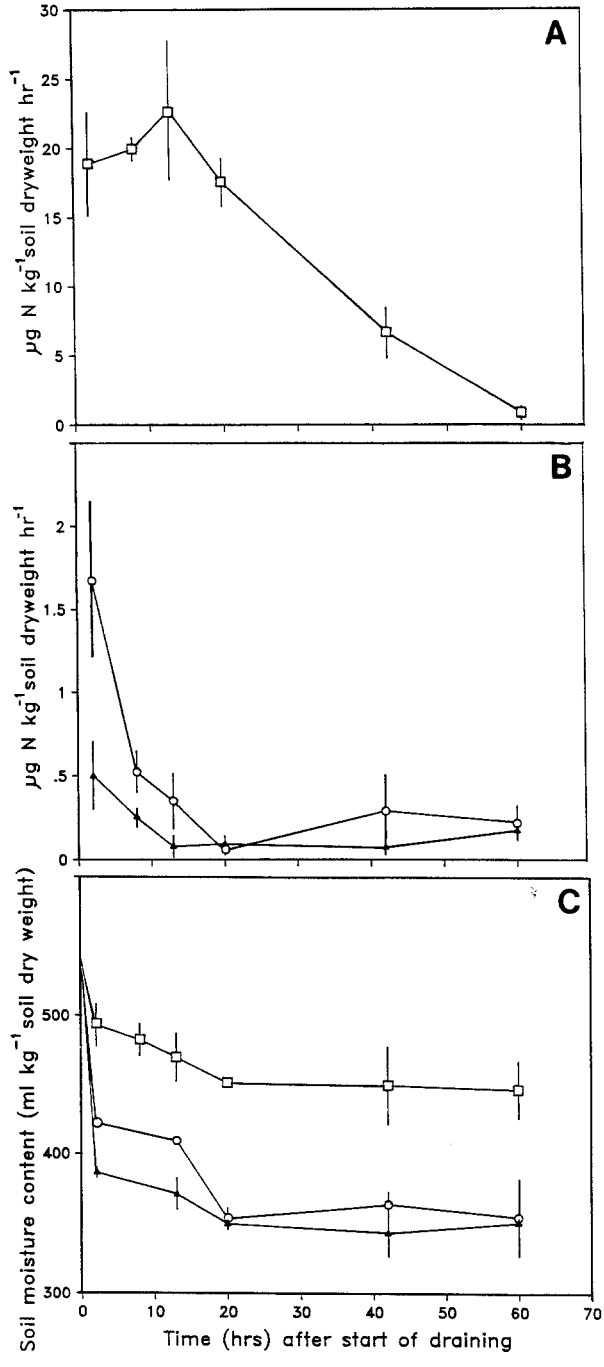

Fig. 2. Denitrification rates (**A, B**) and soil moisture contents (**C**) at three different moisture tension levels during a 60-h draining period after flooding. Note different scales in **A** and **B**. Moisture tension: □, -7 cm H_2O; ○, -30 cm H_2O; ▲, -60 cm H_2O

Table 1. Effect of soil moisture tension and nitrate concentrations in flooding solution on denitrification rates in pots with and without barley plants: Average activity values (μg N kg^{-1} soil h^{-1}) for each treatment

Moisture tension (cm H_2O)	Soil with plants[a]			Soil without plants[a]
	NO_3^--concentration (ppm)			
	10	100	200	200
−7	13.6 (6.3)	25.5 (12.2)	27.0 (7.1)	2.10 (0.13)
−30	0.90 (0.43)	0.73 (0.43)	0.98 (0.40)	0.86 (0.10)
−60	−0.15[b] (0.20)	0.28 (0.13)	0.35 (0.10)	0.78 (0.10)

[a] Standard deviation of mean given in parentheses ($n = 5$ for planted soil and 15 for unplanted)
[b] A slight reduction in N_2O concentration observed, resulting in negative value

moisture tension, the denitrification rate fell gradually during the first 20 h of draining. The pots without plants (not shown), which were all drained at −30 cm H_2O moisture tension, showed similar denitrification rates to those of the planted pots that had been drained at the same tension. The measured soil moisture content (ml kg^{-1} soil) fell rapidly during the first 1−2 h of draining, but continued to decrease at a slow rate during the next 18 h.

Table 1 summarizes the results of varying nitrate concentrations and moisture tensions. There were large and significant ($P = 0.05$) differences in the denitrification rates between the lowest moisture tension and the others (analysis of variance of planted pots only). The effect of increasing nitrate concentrations was apparently positive, but statistically insignificant. The effect of plants (analysis of variance of unplanted versus planted pots at highest nitrate level) was significant ($P < 0.05$), and there was a significant interaction ($P < 0.05$) between the effect of plants and the soil moisture tension: At the low soil moisture tension (−7 cm H_2O), the presence of plants gave a 10-fold increase in the denitrification rate. This plant effect was practically eliminated at −30 cm H_2O moisture tension and slightly negative at the highest soil moisture tension.

The plant dry weights and the shoot/root ratios for the different plant species are shown in Fig. 3; there were large differences in growth rates and in the standing crop between the plant species. Photosynthetic activity (Fig. 4) increased throughout the experiment, but there were some fluctuations due to variations in the light intensity (daylight). In order to determine how far the measured photosynthetic activity predicted the difference in net plant-growth rates, accumulated values of the measured photosynthesis were compared with the measured plant dry weights. The accumulated photosynthesis was calculated according to the following formula:

$$C_n = C_{n-1} + (P_{n-1} + P_n) \cdot (D_n - D_{n-1}) \cdot 15/24$$

where C_n is the accumulated photosynthesis (μg C per pot) at the time of the nth measurement, P is the measured photosynthetic activity (μg CO_2-C pot^{-1} h^{-1}), D_n is the time (days) from start of the measurement. Thus the estimates (C_n) do not account for plant respiration in the dark period, and are based on an assumed photosynthesis period of 15 h day^{-1} (the programmed light period). The values for C_n, and the measured amount of plant C after 39, 48, and 74 days are shown in Fig. 5. Not unexpectedly, the C_n values exceeded measured plant C (factor of 2−5): thus the measured photosynthetic activity grossly overestimated net plant growth. However, C_n was correlated with plant C, showing that the measured photosynthesis data predicted with some accuracy the differences between the plant species with respect to the net plant-growth rate. The correlation coefficient (r) between C_n and plant C was 0.72, 0.53, and 0.32 for the three dates on which plant dry weight was determined, 39, 48, and 74 days after the start of the experiment.

Although denitrification rates at the low soil moisture tension showed large variations between plant species (Fig. 6), the patterns of denitrification and photosynthesis were similar (Figs. 4 and 6): A peak in activity was observed on day 30, and a second rise occurred late in the experiment. Analysis of variance of the low-moisture-tension data (Table 2) demonstrated significantly lower ($P < 0.01$) values for unplanted and clover pots, compared to the others. The relatively large differences between the different monocots were not significant. In a second analysis of variance the measured photosynthetic activity was included as a covariate (excluding the unplanted pots), to see whether this could explain some of the observed differences between the plant species. There was a significant ($P < 0.01$) correlation between the denitrification rate and the photosynthetic activity, and a lower probability for the difference between clover and the other plant species ($P = 0.053$). The relationship between the denitrification rate and measured photosynthetic activity is shown in Fig. 7 together with the regression function ($r = 0.565$) for the denitrification rate in response to the photosynthetic activity: $D = -0.56 + 2.23 \cdot F$, where D is the denitrification rate in μg N kg^{-1} soil h^{-1} and F is the photosynthetic activity in mg CO_2-C kg^{-1} soil h^{-1} (based on data for all plant species). While the regression function explains only some of the variation in the denitrification rate, the observed differences between the monocots are fairly well predicted by the model: Table 3 shows the average measured values, model estimates, and residuals for each plant species. For clover, the measured values were much lower than the model estimate.

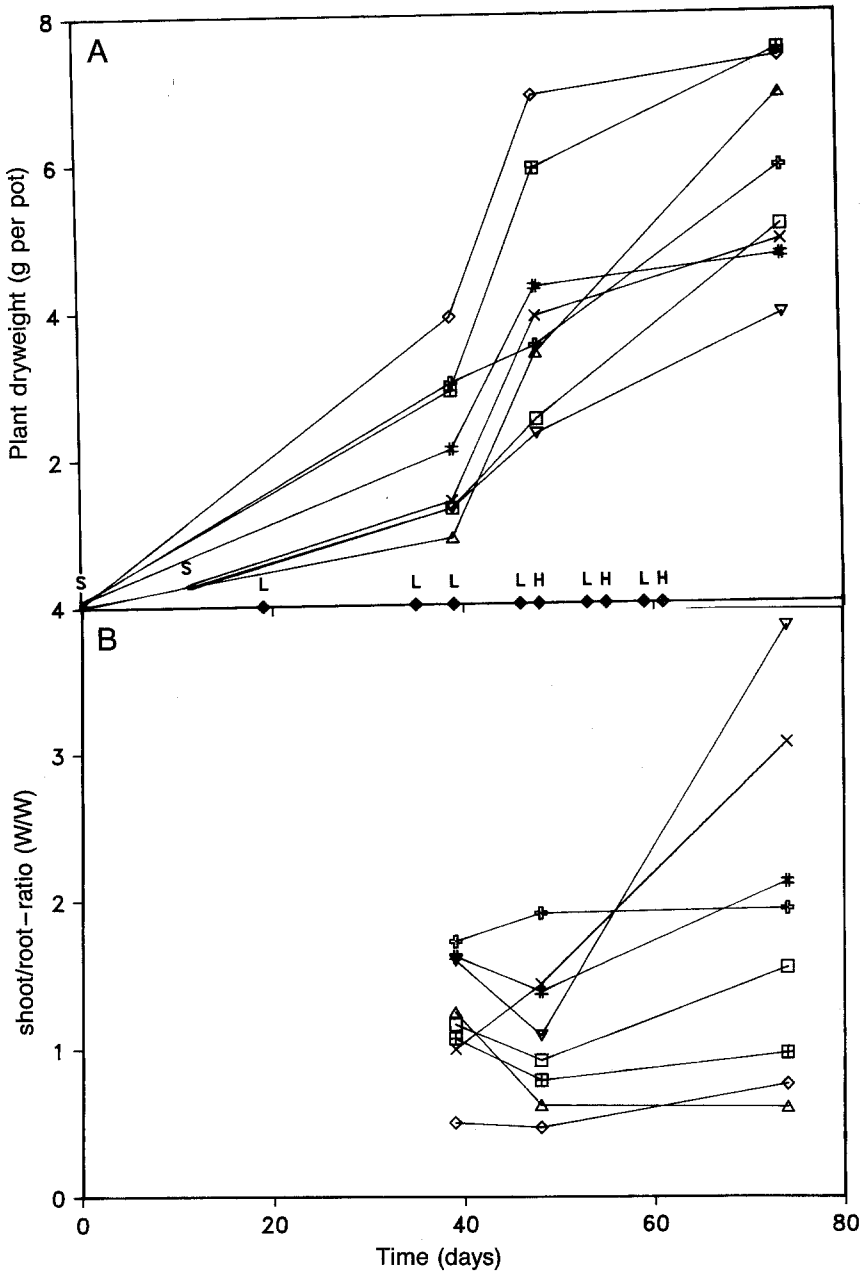

Fig. 3. Plant dry weight (**A**) and shoot/root ratio (**B**) of different plant species. Weight of seeds is also shown (*S*). Time of photosynthesis+ denitrification measurements at high (*H*) and low (*L*) soil moisture tensions are marked on the bottom line of (a). Plants: □, barley; ×, wheat; ▽, oats; #, clover; ◇, timothy; ✤, cocksfoot; △, meadow grass; ⊞, meadow fescue

At a high soil moisture tension, the denitrification rates showed large variations (Table 2), and there were no significant differences between the unplanted and planted pots and between the different plant species. There was a significant ($P = 0.01$) covariance with soil moisture content, as measured by the weight of each pot, but corrections for variations in this parameter did not bring the difference between planted and unplanted pots to significance ($P = 0.298$). However, the high average for planted pots was caused by one wheat measurement. Excluding this value brought the average denitrification rate of the planted pots down to 3 μg N kg^{-1} soil dry weight h^{-1}.

Discussion

Drainage on silt columns established systematic differences in soil moisture contents between treatments within 1–2 h. However, equilibrium was not reached, since the soil moisture continued to decrease at a slow rate (Fig. 2). This was probably due to plant transpiration which, sooner or later, would create a higher moisture tension in the pots than in the silt underneath. Weighing of the pots was not able to reveal small differences in soil moisture, since the correction for water in plant tissue introduced some uncertainty in the estimates. The planted pots may

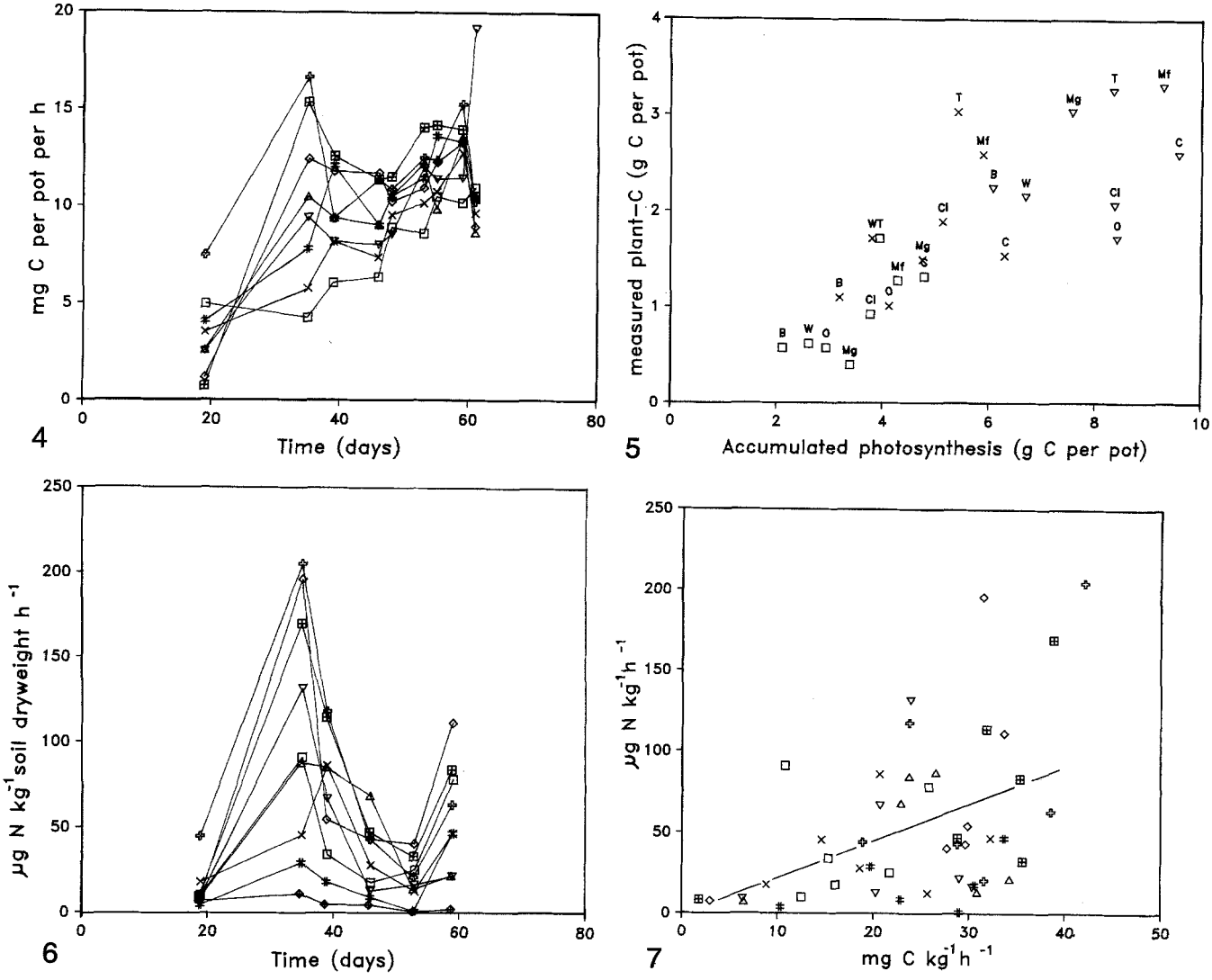

Fig. 4. Measured photosynthetic activity (CO_2 fixation) in different plant species during the experiment (measurements from both high and low soil moisture tension treatments). For explanation of symbols, see Fig. 3

Fig. 5. Relationship between accumulated values for photosynthesis (CO_2 fixation) and measurement plant C (calculated from plant dry weight). Time of incubation: □, 0–39 days; ×, 0–48 days; ▽, 0–74 days. Plants used: B, barley; W, wheat; O, oats; Cl, clover; T, timothy; C, cocksfoot; Mg, meadow grass; Mf, meadow fescue

Fig. 6. Denitrification rates in different plant species at low soil moisture tension treatment. ⊞, unplanted; for explanation of other symbols, see Fig. 3

Fig. 7. Relationship between measured photosynthetic activity (CO_2 fixation) and denitrification rates, and the regression line based on all observations. For explanation of symbols, see Fig. 3

therefore have had slightly lower moisture contents than the unplanted ones, and this would probably have most seriously affected the results at the highest moisture tension treatment. This may explain the slight negative effect of plants on denitrification at −60 cm H_2O (Table 1). The results of Haider et al. (1987) may have been similarly influenced, since these authors used a rather high soil moisture tension.

Measurements after flooding with different nitrate concentrations (Table 1) indicated that the denitrification rate was nitrate-limited at the lowest concentration. The average nitrate concentration in this treatment was 17 ppm (µg NO_3^--N ml^{-1} soil water), which is in the lower range for zero-order kinetics of nitrate reduction (Firestone 1982; Limner and Steele 1982). The lower limit for zero-order kinetics has been

Table 2. Denitrification rate (μg H kg^{-1} soil dry weight h^{-1}) under different plant species, at high ($n = 3$) and low ($n = 6$) soil moisture tension

Plants used	Soil moisture tension (cm H_2O)[a]	
	Low (−7 cm)	High (−30 cm)
Cocksfoot	82.1 (27.9)	5.1 (4.0)
Meadow fescue	75.6 (24.1)	0.8 (0.5)
Timothy	75.3 (27.7)	4.9 (5.5)
Meadow grass	47.0 (15.0)	6.3 (6.2)
Oats	42.9 (19.6)	3.6 (3.6)
Barley	42.7 (13.8)	1.4 (0.3)
Wheat	39.4 (10.9)	19.8 (19.7)
Clover	17.8 (7.1)	0.3 (0.3)
Average of planted pots	52.9 (7.1)	5.3 (2.6)
Unplanted	4.3 (1.6)	2.1 (1.1)

[a] Standard deviation of mean in parentheses

Table 3. Denitrification as a function of photosynthetic activity for each plant species: Measured denitrification rate (μg N kg^{-1} dry weight h^{-1}), model estimates (linear regression), and residuals (Average values at low soil moisture tension)

Plants used	Measured	Estimated	Residual
Cocksfoot	82.1	71.2	10.9
Meadow fescue	75.6	65.5	10.1
Timothy	75.3	57.7	17.6
Meadow grass	47.0	53.7	−6.7
Oats	42.9	47.7	−4.8
Barley	42.7	35.0	7.7
Wheat	39.4	39.1	0.3
Clover	17.8	53.0	−35.2

debated in relation to plant effects, since nitrate depletion around the roots may counteract their potential stimulation of denitrification (Smith and Tiedje 1979; Ryden 1983; Haider et al. 1985). The present experimental technique has circumvented the uncertainty about the effect of nitrate uptake by plants, since the flooding with nitrate solutions shortly before the incubation ensured a high and uniform nitrate concentration in planted as well as unplanted soil. Thus, plant uptake of nitrate cannot be the reason for the plant negative effects on denitrification at the highest moisture tension levels (Table 1). A slight reduction in soil moisture content by plant transpiration is a more likely reason.

The results of the present study of soil moisture tension show that it is a critical parameter, and can reconcile apparently conflicting data on denitrification in the root zone. In their phytotrone experiments with maize plants, Haider et al. (1987) obtained very little stimulation of denitrification from plant roots despite a high nitrate concentration in both planted and unplanted pots. However, they controlled the soil moisture tension at levels similar to (−5 kPa = −50 cm H_2O), or much higher (−330 cm H_2O) than, the highest level applied in the present investigation. In the present study, stimulation of denitrification by roots was practically eliminated at −30 cm H_2O moisture tension (Tables 1 and 2), and at −60 cm H_2O the plant roots even had a slightly negative effect. Thus, there is no conflict between the results of Haider et al. (1987) and the present investigation: Both studies show only a slightly positive, or even a negative root effect at a moderate soil moisture tension. Other experiments which have shown a positive effect of plant roots were conducted at rather high soil moisture contents (Smith and Tiedje 1979; Von Rheinbaben and Trolldenier 1984; Klemedtsson et al. 1987a, b). A detailed comparison is difficult owing to the use of ambiguous units for the water status such as water-holding capacity and field capacity. Even the amount of moisture per kilogram of soil dry weight is an insufficient report of the water status, unless the porosity of the soil is given. Von Rheinbaben and Trolldenier (1984) measured the denitrification rate in pots of sandy soil planted with spring wheat at 60%−100% of "water-holding capacity" which appears to mean the amount of water retained in the pots after free draining. Thus, 100% water-holding capacity is similar to the low soil-moisture-tension treatment in the present investigation. Despite this uncertainty, the results of Von Rheinbaben and Trolldenier (1984) may be considered in general agreement with the present investigation: The denitrification rate in unplanted soil was 3−6 μ*M* N_2O-N pot^{-1} 24 h^{-1}, which is equivalent to 1−2 μg N_2O-N kg soil h^{-1} (2.1 kg soil pot^{-1}). In planted pots at 100% water-holding capacity, the denitrification rate was 5−90 μ*M* N_2O-N pot^{-1} 24 h^{-1}, or 1.5−20 μg N_2O-N kg soil h^{-1}. Von Rheinbaben and Trolldenier (1984) also observed that denitrification was considerably stimulated by an increasing moisture content and preincubation at a high moisture content, but only in planted pots.

The stimulating effect of plant roots on denitrification can be ascribed partly to exudation of organic C (Haller and Stolp 1985), and partly to the O_2 demand by root respiration (Erich et al. 1984; Klemedtsson et al. 1987a, b) The fact that plant species was less important than the measured photosynthetic activity (except for clover) may possibly strengthen the view that root respiration is more important than root exudation, since it seems likely that there is a larger species variation in the amounts and quality of exudates than in the root respiration rate.

There were rather large differences in denitrification rates under different plant species (Table 2). How-

ever, the correlation between the denitrification rate and photosynthetic activity (Fig. 7) and the ability of the regression function to predict the observed differences between monocots (Table 3) indicate that most of the differences between these plant species are attributable to differences in the plant growth rate (net photosynthesis) only, rather than more specific stimulation or inhibition of denitrification. It may be concluded that a good estimator of the plant growth rate or root activity at the time of measurement is important in studies comparing different plant species or varieties. The root density may not be sufficient as a covariate, since it may not necessarily be a good indicator of root activity. The present technique for photosynthesis measurement is rapid and may easily be included in investigations of denitrification under plant cover. It does not give true estimates of net photosynthesis (plant growth rate), but it may predict the difference between plant species (Fig. 5) with respect to growth rate and root activity (respiration and exudation) at the time of the denitrification measurement.

The reason for the low denitrification rate under clover is unknown. The data of Erich et al. (1984) showed that the rhizosphere of another legume, alfalfa, differed from other tested plants (grass and deciduous forest) in having a much lower N_2O-reduction capacity, and a much lower N_2O-generation capacity (anaerobic incubation of freshly sampled soil). Other studies have reported larger denitrification rates under legumes than other plants (Scaglia et al. 1985; B. H. Svensson pers. commun. 1986). It has been hypothesized that this may be due to denitrifying *Rhizobium* spp. (L. Klemedtsson PhD Thesis, Ultuna, Sweden, 1986). The clover roots in the present investigation were heavily nodulated (data not shown), and therefore the low denitrification rate was not due to lack of rhizobia.

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