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# Nitrification in Dutch heathland soils

## *II. Characteristics of nitrate production*

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**Abstract**

Some characteristics of nitrification in 41 humus samples of Dutch heathlands were studied. Most of the acid humus samples (30) showed accumulation of nitrate during a 4-week incubation of field-moist material. In these samples net nitrate production was completely blocked by 0.06% acetylene indicating that nitrification was probably of a chemolithotrophic nature. From a comparison of the net production of nitrate in humus suspensions at pH 4 and pH 6 a differentiation into four patterns could be made:

I. No nitrate production at either pH value studied (12 samples)
II. Acid-sensitive nitrate production (3 samples)
III. Acid-tolerant, pH-dependent nitrate production (10 samples)
IV. Acid-tolerant, pH-independent nitrate production (16 samples)

The results show that acid-tolerant, chemolithotrophic nitrification is widespread among Dutch heathland soils. Absence of potential net nitrate production in humus samples is most likely caused by limitations in the supply of ammonium or oxygen.

## Introduction

The annual acid load in Dutch heathland and forest soils located in areas with high values of ammonium deposition, is mainly due to nitrogen transformations, including nitrification (Mulder, 1988). In heathland soils the process of nitrification is mainly restricted to the organic horizons (De Boer *et al.*, 1989a). It was shown that at least two types of chemolithotrophic nitrification exist in the humus of two heathland soils that were studied whereas no indications were found for the activity of heterotrophic nitrifiers (De Boer *et al.*, 1989a). These two types differed mainly with respect to the pH-dependency of the process: an acid-sensitive type of nitrification predominated in slow nitrate producing humus whereas an acid-tolerant type predominated in fast nitrate-producing humus.

In an earlier paper dealing with this subject, it was indicated that nitrate is produced in many of these acid soils (Troelstra *et al.*, 1990). In this paper attention is focused on the distribution of different nitrification types in the heathland soils studied.

## Material and methods

Samples of the FH horizon were taken in early spring 1988 from 17 heathland locations described by Troelstra *et al.* (1990). Per location all vegetation-types, *i.e.* dominance of *Calluna vulgaris*, *Deschampsia flexuosa*, *Erica tetralix* or *Molinia caerulea*, were sampled. Sampling was done by taking randomly at least 40 cores ($\phi$ = 2.4 cm). The cores were stored at 4°C. Most experiments were started within two weeks after

sampling. Before the start of the experiments the cores were mixed and sieved (<4 mm).

*Characterization of nitrification*

The nature of nitrification, autotrophic or heterotrophic, was examined by determing the effect of low concentrations of acetylene (0.06%) on the production of nitrate in field-moist humus. At this concentration acetylene, is believed to be a specific inhibitor of autotrophic nitrification (Hynes and Knowles, 1982). Of every humus sample two field-moist portions, equivalent to 5 grams of dry humus each, were weighed and put into screw-cap bottles (315 mL). Water-saturated samples were dried at 20°C to 65% of the water holding capacity. Acetylene (0.2 mL) was added through the septum to one of the portions, whereas the other portion served as a control. The flasks were incubated during 4 weeks at 20°C. At the start as well as at the end of the incubation period all humus samples were analysed for ammonium and nitrate. The net production of ammonium and nitrate was calculated by subtracting the initial concentrations of mineral N from the final concentrations.

The sensitivity of the nitrifying micro-organisms towards acidity was determined by comparing the production of nitrate in 5% suspensions that were maintained at pH 4 and pH 6, respectively. The pH was controlled by dthe addition of 1% ammonia. The suspensions were incubated in Erlenmeyer flasks on a rotary shaker (100 rpm) at 20°C. Net production of nitrate was determined over a 3-week incubation period.

To study the presence of urea-stimulated ammonium-oxidizing bacteria, production of nitrate in 1% humus suspensions was compared in the presence and absence of urea at pH 5 (De Boer *et al.*, 1989b).

*Analytical procedures*

Humus was analysed for pH, moisture, total N, total C and mineral N. Analytical procedures were the same as described by Troelstra *et al.* (1990).

**Results**

*Properties of humus samples*

Results of the humus samples analyses are listed in Table 1 for each vegetation-type. All humus samples were acid (range of pH: 3.5–4.6). Mean C/N ratios of humus from Deschampsia- and Molinia-sites were significantly lower (small sample t-test, $p < 0.05$) than those of humus from sites dominated by dwarf-shrubs. Mineral N concentrations ranged from 10 to 270 ppm. Mean concentrations were not significantly different between the humus samples of the locations dominated by the different plant species. All samples contained more ammonium-N than nitrate-N but the ammonium-N/nitrate-N ratios of the samples differed greatly (2–1600). The lowest values were observed in some of the samples from Molinia-sites, whereas the highest were found in samples from extremely wet locations (*e.g.* Kampshei).

*Effect of acetylene on nitrification in field-moist humus*

Data of net mineral N production in field-moist humus, in the absence of acetylene, are listed in Table 2. Net mineral N production ranged from 0.3 to 77 ppm per week. The mean value of net N mineralization in humus of Deschampsia-sites was significantly higher ($p < 0.1$) than that of humus from other sites. Net mineral N production was not affected by the addition of acetylene (not shown).

Humus samples from 11 sites did not show net nitrate productions with or without addition of acetylene. These samples originated from 1 Molinia-site, 4 Calluna-sites and 6 Erica-sites. In some of these samples (*e.g.* Dwingeloo-Calluna) nitrate concentrations decreased during the incubation period which may indicate that nitrate reduction occurred. In the other humus samples (30) net nitrate-N production ranged from 0.1 to 16 ppm per week. Relative nitrification, *i.e.* the proportion of total mineral N that is produced as nitrate-N, ranged from 0–86%. Nitrate producing humus samples were not restricted to sites dominated by certain plant species. In all humus

*Table 1.* Properties of humus samples from Dutch heathlands (April '88)

| Location | Depth of FH (cm) | pH | Moisture (%)[a] | Total N (%)[b] | C/N | $NH_4^+$–N (ppm)[b] | $NO_3^-$–N (ppm)[b] |
|---|---|---|---|---|---|---|---|
| *Calluna locations* | | | | | | | |
| Asset | 2.8 | 3.8 | 67 | 1.45 | 21.9 | 46.4 | 1.9 |
| Dwingeloo | 2.4 | 4.4 | 65 | 1.17 | 21.0 | 211.8 | 7.0 |
| Ede | 2.8 | 3.9 | 60 | 1.14 | 23.9 | 97.8 | 6.7 |
| Ginkel | 2.5 | 3.8 | 66 | 1.40 | 21.6 | 86.5 | 9.6 |
| Hoorneboeg | 2.4 | 3.9 | 64 | 1.28 | 22.6 | 56.1 | 0.7 |
| Kampina 1 | 2.7 | 3.8 | 67 | 0.98 | 26.3 | 9.3 | 0.2 |
| Kampina 2 | 1.5 | 3.8 | 53 | 1.15 | 21.7 | 80.0 | 12.4 |
| Loon/Drunen | 0.6 | 4.3 | 50 | 0.35 | 23.7 | 17.8 | 0.0 |
| Molenveld | 3.3 | 3.8 | 65 | 1.23 | 23.5 | 10.0 | 0.4 |
| Reemsterveld | 3.3 | 4.4 | 66 | 1.12 | 22.4 | 158.6 | 0.9 |
| Terlet | 2.6 | 4.1 | 52 | 0.78 | 18.7 | 83.9 | 5.9 |
| Wolfheze | 3.0 | 4.1 | 44 | 0.48 | n.d. | 44.7 | 5.3 |
| *Erica locations* | | | | | | | |
| Assel | 3.1 | 3.8 | 73* | 1.26 | 25.2 | 40.2 | 5.7 |
| Balloo | 4.4 | 4.0 | 65 | 1.20 | 20.8 | 76.1 | 0.1 |
| Deelen | 3.9 | 3.7 | 84* | 1.41 | 26.0 | 133.4 | 0.2 |
| Dwingeloo | 2.5 | 4.3 | 70 | 0.98 | 25.1 | 264.4 | 2.2 |
| Hoenderloo | 3.5 | 3.8 | 69 | 1.45 | 23.5 | 64.3 | 2.1 |
| Kampina | 3.5 | 3.8 | 65 | 1.13 | 24.6 | 125.0 | 12.1 |
| Kampshei | 3.4 | 3.9 | 80* | 1.80 | 19.3 | 160.3 | 0.1 |
| Kootwijk | 3.0 | 3.8 | 64 | 1.14 | 24.6 | 47.4 | 2.1 |
| Molenveld | 3.4 | 3.8 | 69 | 1.25 | 23.4 | 14.4 | 1.7 |
| Uddel | 3.6 | 3.7 | 80* | 1.71 | 21.3 | 66.5 | 0.3 |
| *Deschampsia locations* | | | | | | | |
| Ede | 4.7 | 4.0 | 59 | 1.20 | 17.4 | 144.1 | 20.4 |
| Ginkel | 3.8 | 4.1 | 56 | 1.04 | 20.9 | 114.1 | 9.2 |
| Hoornboeg | 3.7 | 3.9 | 63 | 1.02 | 24.7 | 75.7 | 0.1 |
| Kootwijk | 3.8 | 4.4 | 66 | 1.63 | 16.1 | 23.9 | 8.3 |
| Molenveld | 3.2 | 3.8 | 58 | 1.20 | 18.8 | 92.5 | 2.3 |
| Reemsterveld | 4.7 | 4.0 | 54 | 0.85 | 22.9 | 69.9 | 5.6 |
| Terlet | 4.5 | 4.5 | 57 | 1.02 | 15.9 | 15.6 | 3.2 |
| Wolfheze 1 | 3.9 | 4.2 | 63 | 1.56 | 17.6 | 125.1 | 5.1 |
| Wolfheze 2 | 2.9 | 4.2 | 52 | 1.12 | 16.9 | 112.9 | 6.0 |
| *Molinia locations* | | | | | | | |
| Assel | 3.8 | 3.5 | 64 | 1.30 | 22.1 | 48.4 | 22.7 |
| Balloo | 4.2 | 3.9 | 68 | 1.16 | 81.4 | 81.4 | 10.0 |
| Dwingeloo | 1.3 | 4.1 | 64 | 1.16 | 22.1 | 145.3 | 7.5 |
| Ede | 2.6 | 3.6 | 50 | 1.00 | 20.7 | 37.6 | 23.5 |
| Kampina | 3.2 | 3.7 | 58 | 0.91 | 21.1 | 53.6 | 29.0 |
| Kampshei | 4.3 | 3.9 | 79* | 1.44 | 21.8 | 109.2 | 0.1 |
| Kootwijk | 3.6 | 3.6 | 59 | 1.22 | 20.8 | 61.9 | 34.1 |
| Reemsterveld | 2.9 | 3.6 | 61 | 1.34 | 20.1 | 49.3 | 23.8 |
| Terlet | 2.1 | 4.6 | 53 | 0.91 | 21.8 | 49.3 | 8.3 |
| Uddel | 3.5 | 3.8 | 85* | 1.98 | 17.3 | 142.6 | 0.5 |

[a](wet weight – dry weight)/wet weight × 100.
[b]On basis of dry humus.
n.d. not determined.
*Water-saturated samples.

*Table 2.* Some data on N mineralization and nitrate production in heathland humus samples

| Locations | Accumulation of mineral-N and nitrate-N (mg N/kg humus/wk) | | | | Ratio pH6/pH4 | Urea stimulation | Pattern |
|---|---|---|---|---|---|---|---|
| | mineral-N in humus* | $NO_3^-$-N in humus* | $NO_3^-$-N in suspensions | | | | |
| | | | pH4 | pH6 | | | |
| *Calluna locations* | | | | | | | |
| Assel | 20.3 | 7.4 | 70.0 | 76.0 | 1.1 | | IV |
| Dwingeloo | 15.5 | −0.7 | 18.7 | 483.1 | 25.8 | + | III |
| Ede | 19.3 | 8.2 | 59.7 | 99.9 | 1.7 | | III |
| Ginkel | 42.5 | 13.7 | 154.9 | 119.9 | 0.8 | | IV |
| Hoorneboeg | 18.1 | 0.3 | 0.0 | 119.8 | ∞ | + | II |
| Kampina 1 | 0.3 | 0.0 | 0.0 | 0.0 | – | | I |
| Kampina 2 | 27.5 | 8.3 | 107.3 | 144.7 | 1.3 | | IV |
| Loon/Drunen | 3.2 | 0.0 | 0.0 | 0.0 | – | | I |
| Molenveld | 0.8 | 0.0 | 0.0 | 0.0 | – | | I |
| Reemsterveld | 14.6 | 0.6 | 18.7 | 193.7 | 10.4 | + | III |
| Terlet | 24.0 | 8.0 | 26.1 | 24.2 | 0.9 | | IV |
| Wolfheze | 12.1 | 8.3 | 37.3 | 47.3 | 1.3 | | IV |
| *Erica locations* | | | | | | | |
| Assel | 2.1 | −0.8 | 0.0 | 0.0 | – | | I |
| Balloo | 17.9 | 0.0 | 0.0 | 0.0 | – | | I |
| Deelen | 4.8 | 0.0 | 0.0 | 0.0 | – | | I |
| Dwingeloo | 8.4 | 2.7 | 39.2 | 45.2 | 1.1 | | IV |
| Hoenderloo | 26.1 | 1.1 | 10.3 | 20.5 | 2.0 | | III |
| Kampina | 18.1 | 9.1 | 71.9 | 149.3 | 2.1 | + | III |
| Kampshei | 13.2 | 0.0 | 0.0 | 0.0 | – | | I |
| Kootwijk | 16.6 | −0.1 | 0.0 | 0.0 | – | | I |
| Molenveld | 5.0 | −0.2 | 0.0 | 0.0 | – | | I |
| Uddel | 10.0 | 0.1 | 0.0 | 0.0 | – | | I |
| *Deschampsia locations* | | | | | | | |
| Ede | 32.3 | 14.9 | 107.3 | 162.9 | 1.5 | | III |
| Ginkel | 36.1 | 13.7 | 99.9 | 127.6 | 1.3 | | IV |
| Hoorneboeg | 24.3 | 0.2 | 0.0 | 0.0 | – | | I |
| Kootwijk | 46.8 | 7.7 | 42.0 | 152.1 | 3.6 | | III |
| Molenveld | 27.3 | 1.5 | 0.0 | 35.8 | ∞ | n.d. | II |
| Reemsterveld | 19.0 | 7.6 | 45.7 | 77.0 | 1.7 | | III |
| Terlet | 77.3 | 15.9 | 60.7 | 121.5 | 2.0 | + | III |
| Wolfheze 1 | 34.1 | 7.2 | 40.1 | 44.8 | 1.1 | | IV |
| Wolfheze 2 | 38.0 | 11.9 | 36.4 | 36.4 | 1.0 | | IV |
| *Molinia locations* | | | | | | | |
| Assel | 14.4 | 12.4 | 224.9 | 224.5 | 1.0 | | IV |
| Balloo | 21.9 | 11.1 | 82.1 | 153.1 | 1.9 | | III |
| Dwingeloo | 24.2 | 5.3 | 55.1 | 64.4 | 1.2 | | IV |
| Ede | 22.3 | 10.8 | 196.9 | 235.5 | 1.2 | | IV |
| Kampina | 20.0 | 11.5 | 223.1 | 251.1 | 1.1 | | IV |
| Kampshei | 9.9 | 0.0 | 0.0 | 0.0 | – | | I |
| Kootwijk | 20.9 | 10.6 | 165.2 | 177.8 | 1.1 | | IV |
| Reemsterveld | 21.2 | 11.3 | 99.9 | 137.6 | 1.4 | | IV |
| Terlet | 72.4 | 7.8 | 27.1 | 102.9 | 3.8 | + | III |
| Uddel | 4.9 | 0.1 | 0.0 | 12.1 | ∞ | n.d. | II |

*Incubation of sieved field-moist humus at 20°C.

+ Accumulation of nitrate at pH 5 was at least 1.5 times higher in suspensions with urea-addition than in suspensions with ammonium-addition; n.d. not determined.

samples nitrate production was completely blocked by 0.06% acetylene indicating that nitrifiction was probably of a chemolithotropic nature (not shown).

Using data from all humus samples (n = 41) regression-analysis revealed that nitrate production was significantly ($p < 0.005$) and positively correlated with N mineralization (r = 0.59) and significantly, but negatively with the (initial) moisture content (r = −0.54) and the C/N ratio (r = −0.51). Regression of nitrate production against pH and initial mineral N content did not reveal significant correlations.

*Effects of pH and urea on nitrate production in humus suspensions*

From a comparison of the net nitrate production in suspensions of the humus samples at pH 4 and at pH 6 a differentiation into four patterns could be made:

*I. No nitrate production* at both pH values studied (n = 12)

*II. Acid-sensitive nitrate production*: nitrate production at pH 6 but not at pH 4 (n = 3)

*III. Acid-tolerant, pH-dependent nitrate production*: nitrate production at both pH 6 and pH 4 with the production at pH 6 being at least 1.5 times faster than at pH 4 (n = 10)

*IV. Acid-tolerant, pH-independent nitrate production*: nitrate production at both pH 6 and pH 4 with the production at both pH values being almost equal (n = 16)

Most of the humus samples, that did not show accumulation of nitrate in suspensions at either pH value studied, originated from Erica-dominated sites. Acid-tolerant nitrification, that is nitrate production at pH 4 (patterns III and IV) is wide-spread among Dutch heathland soils; it was not found to be restricted to humus of certain vegetation-types. The acid-sensitive type of nitrification (pattern II) was found to be uncommon.

All humus samples that accumulated nitrate during the incubation of field-moist material also did so in suspensions, with the exception of Dwingeloo-Calluna.

The effect of urea on nitrification in suspensions at pH 5 was said to be stimulating when the accumulation of nitrate in 3 weeks was at least 1.5 times higher in suspensions with urea-addition than in those with ammonium-addition. All humus samples that showed a stimulation of nitrate production by urea were also found to produce more nitrate at pH 6 than at pH 4 (patterns II and III). However, there were also some humus samples with pH-dependent nitrate production (pattern III) that did not show urea-stimulated nitrate production.

## Discussion

In all humus samples acetylene completely blocked the nitrate production indicating that nitrification in heathland humus is probably due to the activities of chemolithotropic bacteria (De Boer *et al.*, 1989a). Therefore, nitrate production by fungi does not seem to be of quantitative importance in these soils. Using the same acetylene-block technique combined with antibiotic-treatments, it was indicated that nitrate production in some forest soils is mainly caused by fungi (Adams, 1986; Killham, 1986). At the moment, it is not obvious which factors determine whether nitrification in acid soils is mainly autotrophic or heterotrophic.

In this study acid-tolerant chemolithotrophic nitrifying bacteria were shown to be widespread in Dutch heathland soils. Although the isolation of acid-tolerant nitrite-oxidizing bacteria has been reported (De Boer and Laanbroek, 1989; Hankinson and Schmidt, 1988), thusfar no acid-tolerant ammonium-oxidizing bacteria have been isolated. Therefore, there is only indirect evidence for the existence of acid-tolerant, chemolithotrophic ammonium-oxidizing bacteria and all information about their physiology has to be deduced from suspension experiments.

The pH-dependency of nitrate production in humus suspensions varied greatly between the humus samples studied. One group of humus samples (pattern IV) produced nitrate at almost equal rates at both pH 4 and pH 6. Another group of humus samples (pattern III) produced considerably more nitrate at pH 6 than at pH 4. This difference in response to a pH-rise may be due to the presence of predominantly acid-tolerant ammonium-oxidizing strains with growth rates that are more (pattern III) or less (pattern

IV) affected by an increase in pH from 4 to 6. However, another possibility may be the presence of both acid-tolerant and acid-sensitive strains in humus samples that show pH-dependent (pattern III) nitrate production. In that latter case, it can be expected that nitrate production at pH 6 is the result of a combined activity of acid-tolerant and acid-sensitive strains whereas at pH 4 only the acid-tolerant strains are active. The presence of both acid-sensitive and acid-tolerant strains in one humus sample may be indicated by those humus samples in which nitrate production in suspensions was both pH-dependent and stimulated by urea. Previously, it was shown that a stimulation of the nitrate production in suspensions may indicate the presence of acid-sensitive, ureolytic ammonium-oxidizing bacteria (De Boer *et al.*, 1989b). Urea-stimulated nitrate production was not detected in any of the humus samples with pH-independent nitrate production (pattern IV) indicating that the acid-tolerant ammonium-oxidizing bacteria may not be ureolytic.

It should be noted that there were humus samples with nitrification characteristics that deviated from those discussed above. These are samples with a pH-dependent, acid-tolerant nitrate production (pattern III) that is not stimulated by urea (*e.g.* Kootwijk-Deschampsia). This type of nitrification may be the result of combined activities of acid-tolerant – and non-ureolytic, acid-sensitive ammonium-oxidizing strains.

Nitrite was not detected in any of the suspension experiments indicating that under all conditions studied, nitrite-oxidation was not the limiting step with respect to nitrate production. Therefore, the suspension experiments do not give information about the effect of a rise in pH on the process of nitrite-oxidation. It is obvious that acid-tolerant nitrite-oxidizing bacteria must be present in humus samples showing nitrate production at pH 4 but it is unknown whether acid-sensitive, nitrite-oxidizing bacteria do contribute to nitrate production at pH 6. Hankinson and Schmidt (1988) showed that acid-tolerant – and acid-sensitive nitrite-oxidizing bacteria do coexist in an acid forest soil.

It seems that acid-sensitive nitrate production, as opposed to acid-tolerant nitrate production, requires special conditions (suitable pH or the presence of urea). Therefore, it can be expected that the former type will be quantitatively less important than the latter with respect to nitrate production in heathland humus. Indeed, it was observed that humus samples with little or no nitrate formation in suspensions at pH 4 but with much nitrate formation at pH 6 produced only little nitrate during incubation of field-moist material.

Nitrate production in heathland soils is correlated with some soil factors which may give information about the regulation of nitrification in these soils (Troelstra *et al.*, *submitted*). The first prerequisite for nitrification is that a population of nitrifying bacteria can develop. Therefore, the properties of humus in which nitrifying bacteria seem to be absent may give information on the factors that are required to allow nitrifying bacteria to grow. It is supposed that the absence of nitrate production in suspensions either at pH 4 and at pH 6 indicates that low numbers of nitrifying bacteria were present in the humus at the time of sampling. This was the case with 12 humus samples. These samples also showed no or little accumulation of nitrate during incubation of field-moist material.

One factor that may determine whether nitrifying bacteria can grow is the supply of ammonium. It is known that in a vegetation dominated by young dwarf-shrubs immobilization of nitrogen predominates with, subsequently, low amounts of available ammonium both for plants and micro-organisms (Berendse, 1988). Low numbers of nitrifying bacteria in the humus of the dwarf-shrub sites in Kampina-1, Loon/Drunen and Molenveld may be due to low amounts of available ammonium as both initial ammonium concentrations and N-mineralization were low in these humus samples. It was observed that also in many forest soils ammonium availability appears to control nitrification (Robertson, 1982; Vitousek *et al.*, 1982).

Not only ammonium but also oxygen is needed in nitrate production. Soils in wet heathlands have been reported to be anaerobic during part of the year (Lache, 1976). Therefore, it can be imagined that the oxygen supply in such locations may not be sufficient for nitrification. In this study this may have been the case for the

sites dominated by Erica or Molinia in Asselt, Deelen, Kampshei and Uddel. Hence, it is concluded that low numbers of nitrifying bacteria in 9 out of 12 humus samples may be due to lack of ammonium or oxygen.

The three remaining sites (Kootwijk-Erica, Balloo-Erica and Hoorneboeg-Deschampsia) are very interesting because low numbers of nitrifying bacteria seem to be present in the humus although ammonium supply and moisture conditions are apparently suitable. The low numbers of nitrifying bacteria in both Erica-sites is surprising as these sites are surrounded by a Molinia-vegetation with a strongly nitrate-producing humus. The possibility was studied whether Erica-humus on these sites contained toxic compounds because allelochemical inhibition has been reported to be a factor controlling nitrification (Rice, 1984). Furthermore, it was observed that the activity of pure cultures of nitrite-oxidizing bacteria was retarded by extracts of Erica-leaves (Bertru and Goma Tchimbakala, 1985). It appeared that nitrate production in suspensions containing both nitrifying Molinia-humus (Kootwijk) and non-nitrifying Erica-humus (Kootwijk) was not different from nitrate production in suspensions containing only Molinia-humus (undescribed experiment). Thus allelochemical inhibition does not seem to be the cause of low numbers of nitrifying bacteria in the Erica-humus. Still, it can be imagined that even in these two sites without extreme moisture conditions, oxygen supply may not have been sufficient for the nitrifying bacteria to proliferate. The Erica-humus in these sites, especially in the Kootwijk-site, is very compact as opposed to the surrounding Molinia-humus. Oxygen supply in the Erica-humus of these sites may be limited by diffusion. Clays-Josserand *et al.* (1988) suggested that the occurrence of nitrification in different organic layers of forest soils is dependent on the diffusion capacity for oxygen.

Also the absence of nitrate production in Hoorneboeg-Deschampsia remains puzzling. All conditions for nitrification seem to be suitable. It may be possible that the apparently favourable conditions did not exist until recently and therefore nitrifying bacteria may not have had enough time to build up a population of sufficient size.

From this study it has become obvious that pH is not a factor that determines whether or not chemolithotrophic nitrifying bacteria can exist in heathland humus. Previously, it has been shown that acid-tolerant nitrifying bacteria were able to grow exponentially even at pH values as low as 3.5 (De Boer *et al.*, 1989a). In addition, the production of nitrate in suspensions at pH 4 of humus samples with acid-tolerant nitrification (pattern III and IV, $n = 26$) is negatively correlated ($r = -0.726$) with humus-pH indicating that at the time of sampling the highest numbers of acid-tolerant, ammonium-oxidizing bacteria were present in the most acid humus. This may imply that the activities of acid-tolerant ammonium-oxidizing bacteria have resulted in a drop of the humus-pH, although a low *in situ* pH might also be explained by other processes.

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