Prediction of the non-fertilizer N supply of mineral grassland soils

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Abstract

Different methods for estimating the non-fertilizer N supply (NFNS) of mineral grassland soils were compared. NFNS was defined as the N uptake on unfertilized plots. The potential mineralization rate (0-12 weeks), macroorganic matter and active microbial biomass (determined by the substrate-induced respiration method; SIR) were correlated positively with NFNS. The difference between the actual soil organic N or microbial N content (determined by the fumigation incubation method) and their contents under equilibrium conditions (Δ org. N and Δ MB-N), however, gave the best estimations of NFNS. For field conditions the best estimation for NFNS was: NFNS (kg N ha⁻¹ yr⁻¹) = 132.3 + 42.1 \times Δ org. N (g kg⁻¹ soil; r = 0.80). This method is based on the observation that, under old grassland swards, close relationships exist between soil texture and the amounts of soil organic N and microbial N. These relationships are assumed to represent equilibrium conditions as under old swards under constant management, the gain in soil organic N and microbial N equals the losses. Soils under young grassland and recently reclaimed soils contained less soil organic N and microbial N. In such soils the amounts of organic N and microbial N increase with time, which is reflected in a lower NFNS. The annual accumulation of organic and microbial N gradually becomes smaller until organic N, microbial N and NFNS reach equilibrium. The main advantage of the "difference method" in comparison with the other methods is its speed and simplicity.

Introduction

On unfertilized fields, the non-fertilizer N supply (NFNS) consists of soil organic N that is mineralized during the growing season, mineral N that is present in the soil profile in spring, N in dry and wet deposition, and N fixation by free and symbiotic living microbes in the soil. Soils differ greatly in the amounts of nitrogen made available for uptake by plants during the growing season; the contribution to NFNS by deposition and N fixation, however, is in the absence of clover quite constant or small, respectively. So, the amount of N taken up from plots receiving no fertilizer N provides a good indication of the NFNS (Warren and Whitehead, 1988). Annual N uptake rates on unfertilized plots can range between 10 and 900 kg N per ha in grassland soils (Brockman, 1969; Richards and Hobson, 1977). A reliable prediction of NFNS would be very helpful to advise farmers on the optimum fertilizer application rate. Different methods have been proposed to predict NFNS, such as quantification of actual mineralization rates in the field (Raison et al., 1987) and potential mineralization rates in the laboratory (Nordmeyer and Richter, 1985; Stanford and Smith, 1972). The determination of potential mineralization rates in the laboratory has the advantage that soils from different locations can be compared relatively simply under the same conditions. Hassink (1994) incubated fieldmoist, homogenized samples from different grassland sites and determined the potential mineralization rate as the increase in inorganic N after 2 and 12 weeks incubation. When field-moist samples are incubated, N mineralization rates remain relatively constant during incubation (Addiscott, 1983; Hassink, 1994), so that a short incubation period might be sufficient to give a good estimation of NFNS.

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The active organic matter fractions (such as macroorganic matter and the microbial biomass) are assumed to play a dominant role in the availability of nutrients (Janzen et al., 1992). Warren and Whitehead (1988) observed that the N in macroorganic matter contributes substantially to available N. It has been found that, for soils differing in texture, the light fraction of macroorganic matter ($> 150 \mu m$; Meijboom et al., 1995) and the active microbial biomass (determined by the substrate-induced respiration method; Anderson and Domsch 1978) correlate particularly well with their potential mineralization rates (Hassink, 1995). The amounts of active organic matter and microbial biomass could therefore be expected to give a good measure of the amount of available soil N. A drawback of the present methods for estimating NFNS is that they are time-consuming and consequently not very attractive for general application.

A new approach to estimate NFNS is based on the observation that in old grasslands a close relationship exists between the organic N content and the size fraction < 50 μ m (clay and silt fraction; Hassink, 1994). The increase in organic N content with increasing clay and silt content is because organic matter is better protected in fine-textured soils than in coarse-textured soils (Hassink et al., 1995; Tisdall and Oades, 1982). In addition to organic N, microbial N generally also correlates positively with the clay and silt content of a soil (Amato and Ladd, 1992; Gregorich et al., 1991; Hassink et al., 1995; Van Veen et al., 1985). It may be assumed that in old grassland soils, where the amount and quality of inputs of organic residues to the soil is relatively constant, the amount of both organic N and microbial N reach an equilibrium that is mainly controlled by soil texture (Hassink, 1994).When the organic N content is in equilibrium, the annual amount of N that is incorporated into the organic matter pool balances the amount of N that mineralizes in the same year. Soils that have an organic N content that is below the equilibrium level will mineralize less N than is incorporated into the organic N pool and will consequently increase their organic N content (Cuttle and Bourne, 1992; Hassink et al., 1990; Ryden, 1984). In young swards and in recently reclaimed polder soils in the Netherlands, the amount of soil organic N in grasslands increased by more than 100 kg N per ha annually (Hassink and Neeteson, 1991; Hassink, 1994). During the first years after arable soil is sown to grass, the difference between the actual soil organic N content and the equilibrium value is at its maximum and the accumulation of organic N is high. Accumulation of organic N is finite, however, and the annual increases become smaller with time, and the organic N content reaches equilibrium asymptotically (Jenkinson, 1988; Ryden, 1984). In line with this the difference between the actual amount of organic and microbial N, and the amounts under equilibrium conditions might give a good estimation of the NFNS. The great advantage of this new approach to estimate NFNS is its speed and simplicity.

The objective of this paper is to see which approach gives the best estimation of the nonfertilizer N supply of grassland soils under field conditions. To do this, estimates of N availability obtained by the different approaches were correlated with the amounts of N taken up by unfertilized perennial ryegrass growing under uniform environmental conditions in the greenhouse and under field conditions.

Materials and methods

Fields sampled for incubation and determination of potential N mineralization rates and soil organic N

In March 1989 and in March 1991 samples were collected from mineral grassland soils located in different areas in the Netherlands. The land was grazed by dairy cattle and received 400-500 kg fertilizer-N per ha per year. Three mixed samples, each consisting of 20 bulked cores, were taken from the $0-10$ and $10-25$ cm soil layer at each location: For details of the soils see Hassink (1994).

Soil samples were sieved through a 0.008-m mesh screen; roots and stubble were removed. N mineralization was determined by measuring the increase in mineral N after 2 and 12 weeks of incubation of soil samples in glass jars at 20° C: details are given by Hassink (1994).

Mineral N was measured colorimetrically after extraction with $1 N$ KCl solution for $1 h$ using a soil:water ratio of 1:2.5. Total soil N, including mineral N, was determined according to Deys (1961), after destruction with sulfuric acid and salycylic acid.

Determination of the microbial biomass and the active microbial biomass

The amount of N in the microbial biomass was determined in field-moist samples by the chloroform fumigation-incubation (FI) technique (Jenkinson and Powlson, 1976). A k value of 0.4 was used to calculate the biomass from the flush. The exact procedure has been described by Hassink et al. (1991).

The active microbial biomass was determined by a modification of the substrate-induced respiration (SIR) method (Anderson and Domsch, 1978): for details see Hassink (1993). The active microbial biomass was only determined in the grassland soils sampled in 1991.

Determination of the amount of macroorganic matter and its light fraction

Rewetted soil samples (250 g) were washed on two sieves (top sieve: mesh size $250 \mu m$; bottom sieve: 150 μ m) till the washings became clear. By pushing the soil through the top sieve all the macroaggregates ($> 250 \mu m$) that can be destroyed by agricultural practices (Tisdall and Oades, 1982) were dispersed. Clogging occurred if the sieve had a mesh size < 150 μ m, making the washing procedure much more time-consuming. The mineral fraction was discarded by decantation. The organic fraction $> 150 \mu m$ was called macroorganic matter. The combined organic fraction from both sieves was fractionated in silica suspensions with a density of 1.13 g $cm⁻³$. The light fraction is the fraction with a density < 1.13 g cm⁻³. The exact procedure used for density fractionation has been described by Meijboom et al. (1995). Macroorganic matter was only determined in a selection of the soils sampled in 1989 and 1991, The characteristics of these soils are given by Hassink (1995).

Assessment of the non-fertilizer N supply (NFNS) of soils

NFNS is defined as the amount of N taken up by ryegrass from unfertilized plots during a growing season. Information on NFNS was obtained in different ways:

(i) In March 1989 and in March 1991 soil samples were taken for the determination of potential N mineralization. At the same time, undisturbed soil columns with a diameter of 20 cm and a height of 30 cm were pushed into the soils. From each location 5 undisturbed columns were taken to determine the N uptake by the herbage. They were installed in a greenhouse with a sliding roof that was closed automatically when it started raining. The temperature in the greenhouse was the same as outside. The columns were placed in distilled water, so that the water table was constant at

Table 1. Some characteristics of the top 20 cm of mineral grassland soils where NFNS was determined under field conditions (the NMI and PR experiments)

	Experimental Total N period		Particle size distribution $(g \text{ kg}^{-1})$ % particles <		
			$2 \mu m$	$50 \ \mu m$	
Sandy soils					
Gortel	1973-83	1.4	$\overline{4}$	12	
Finsterwolde	1977-78	1.0	10	23	
Hemrik	1978-81	2.1	3	15	
Den Ham	1979-83	4.2	8	35	
Ruurlo	1980-84	2.0	4	30	
Achterberg	1988	1.6	$\overline{4}$	9	
Peest	1988	2.7	5	17	
Cranendonck	1989-92	1.1	3	15	
Tynaarlo	1989-93	2.3	3	25	
Dalfsen	1991	1.9	\overline{c}	10	
Slootdorp	1992	1.6	5	45	
Wageningen	1993	1.0	4	10	
Loams and clays					
Finsterwolde	1977-78	1.4	59	85	
Ten Boer	1977-78	4.0	40	63	
Friens	$1981 - 83$	3.8	25	70	
Burum	1982-91	4.1	29	72	
Aduard	1987-88	3.4	30	70	
Marknesse	1988	1.8	23	67	
Swifterbant	1988	1.8	21	72	
Zaltbommel	1991	4.0	51	91	
Lelystad	1991	5.0	22	60	
Haskerdijk	1991	4.4	54	89	

30 cm below the soil surface. Every week some distilled water was applied to the top of the columns to prevent salt accumulation at the surface. All columns were fertilized with P and K to prevent these nutrients becoming limiting. The grass was harvested by cutting 2 cm above the soil surface at five successive occasions. As mineral N was determined at the time of sampling, NFNS could be corrected for the initial amount of mineral N.

At some of the sites where columns were taken in 1991, N uptake by the herbage in the field was measured on unfertilized plots. At the selected sites unfertilized plots were laid out in triplicate, and these were cut on five successive occasions.

(ii) Information on NFNS under field conditions on other mineral grassland soils was obtained from fer-

Fig. 1. Relationship between soil texture (% soil particles < 50 μ m) and the organic N content (g N kg⁻¹) in the top 10 cm of the grassland soils sampled in 1989 and 1991. Old grassland soils have been under grass for more than 10 years, young grassland soils for less than 10 years. Polder soils were reclaimed from the sea less than 50 years ago. Significant relationship: $N = 1.4$ (0.2) + 0.03564 $(0.005) \times$ % particles < 50 μ m. () = standard error of difference.

tilization experiments on mown grassland fields, performed by the Nutrient Management Institute (NMI, Wageningen) and the Research Station for Cattle, Sheep and Horse Husbandry (PR, Lelystad) at different locations in the Netherlands. At all locations, plots that received no fertilizer were laid out in triplicate. At these locations only the organic N and clay and silt content of the top 20 cm of the soil were determined. When N uptake on unfertilized plots was determined in more than one year, the average value was taken. Some characteristics of these soils are given in Table 1.

Statistical analysis

The relationships between NFNS and soil characteristics and between soil texture and soil organic N and N in microbial biomass were analyzed by correlation and stepwise multiple regression techniques (Genstat, 1987). The fraction < 50 μ m (i.e. the clay + silt content) was taken as an index of soil texture (Hassink, 1994).

Results

Relationship between soil texture and soil organic N content and N in microbial biomass

There were highly significant ($p < 0.05$) positive correlations between (i) the organic N content and (ii) the Microbial biomass (g N kg-1 soil; 0-10 cm)

Fig. 2. Relationship between soil texture (% soil particles < 50) μ m) and the amount of microbial biomass (g N kg⁻¹) in the top 10 em of the grassland soils sampled in 1989 and 1991. Significant relationship: microbial biomass $N = 0.0465 (0.0168) + 0.00285$ $(0.00034) \times %$ particles < 50 μ m. () = standard error of difference.

amount of N in the microbial biomass and the fraction $<$ 50 μ m (clay + silt content) for both the top 10 cm and the top 25 cm of old (under grass for more than 20 years) grassland soils sampled in 1989 and 1991 (Table 2; Figs. 1, 2). Recently reclaimed polder soils and arable soils that had recently been sown to grass (less than 8 years ago) had lower organic N contents and less microbial biomass than other grassland soils with the same texture. Although it is recognized that it may take 100 years before equilibrium is reached when arable land is laid down to grass (Jenkinson, 1988), it is assumed that the relationship between soil texture and organic N and microbial N of the grassland soils more than 20 years old represents equilibrium conditions. This allows the calculation of the difference between the actual amounts of microbial and organic N and the amounts under equilibrium conditions for each soil.

In an earlier paper, the relationship between the organic N content in the top 20 cm and the fraction < 50 μ m of old grasland soils with a deep groundwater table was given as organic N $(\%)=0.147(0.014) +$ 0.0026 (0.00039) \times % < 50 μ m (r² = 0.76; Hassink, 1994). This relationship was assumed to represent the equilibrium conditions for the soils that were sampled by NMI and PR (the soils of Table 1).

Non fertilizer N supply (NFNS)

The uptake of N by unfertilized ryegrass, grown in soil columns in the greenhouse, ranged from 58 to 208 kg N per ha per growing season in the mineral soils sampled in 1989 and 1991. For the sandy and loamy

Table 2. Correlations and relationships between soil organic N (g kg⁻¹) microbial biomass-N (g kg^{-1}) and the fraction < 50 μ m (clay and silt content) for the top 10 cm and the 0-25 cm layer of old grassland soils sampled in 1989 and 1991 for the determination of potential N mineralization

$0 - 10 cm$
Soil organic N (g kg ⁻¹) = 1.4 (0.2) + 0.03564 (0.005) × % < 50 μ m r = 0.87
Microbial biomass-N (g kg ⁻¹) = 0.0465 (0.0168) + 0.00285 (0.00034) \times % < 50 μ m; r = 0.90
$0 - 25$ cm.
Soil organic N (g kg ⁻¹) = 1.2 (0.2) + 0.02833 (0.0037) \times % < 50 μ m; r = 0.88
Microbial biomass-N (g kg ⁻¹) = 0.0404 (0.0139) + 0.001775 (0.000285) \times % < 50 μ m; r = 0.83

() = standard error of difference; $r =$ coefficient of correlation.

Table 3. Comparison of N uptake by the herbage growing on unfertilized columns of soil in the greenhouse and on unfertilized plots in the field (kg N ha⁻¹ yr⁻¹)

	Greenhouse	Field		
Sand				
Achterberg	145	108		
Cranendonck	127	126		
Tynaarlo	134	166		
Dalfsen	208	175		
Average	154	144		
Loam				
Burum	181	181		
Swifterbant	134	91		
Slootdorp	58	67		
Lelystad	180	166		
Average	138	126		
Clay				
Zalthommel	134	151		
Haskerdijk	163	208		
Average	149	180		

soils the field N uptake on unfertilized plots was not significantly different from the amounts taken up in the greenhouse (Table 3). The N uptake on the clay soils, however, was significantly higher (average 30 kg N ha^{-1}) under field conditions than in the greenhouse (Table 3). It is assumed that the lower N uptake in the greenhouse was due to the wet conditions in the greenhouse experiment which caused denitrification (see discussion). It was therefore assumed that, for clay soils, NFNS was 30 kg higher than measured in the greenhouse.

For the soils from the NMI and PR experiments: Table 1, NFNS ranged from 45 to 233 kg N ha⁻¹.

Correlation between herbage N uptake in soil columns incubated in the greenhouse under non-limiting water conditions and estimates of NFNS by different approaches

When all soils were pooled, N mineralized during an incubation period of 12 weeks correlated best with total N uptake by the herbage (NFNS), but the coefficient of correlation was still only 0.60 for the top 10 cm and 0.63 for the top 25 cm (Table 4). As NFNS is affected by the amount of mineral N in the soil at the time of sampling, NFNS was also expressed as total N uptake minus mineral N in the top 30 cm. However, when NFNS was defined in such a way, correlation coefficients between NFNS and soil characteristics actually decreased (Table 4). Higher correlation coefficients were obtained when NFNS was defined as total N uptake minus N uptake in the first cut (Table 4). The highest correlation coefficients with NFNS were obtained with the difference between the amount of N in the microbial biomass and the amount under equilibrium conditions (\triangle MB-N; r = 0.75), for N mineralized during an incubation period of 12 weeks ($r = 0.74$). for the amount of macroorganic N ($r = 0.69$), for the difference between the amount of organic N and the amount of organic N at equilibrium ($r = 0.65$) and for the amount of active microbial biomass $(r = 0.65;$ Table 4). The correlations of NFNS with the light fraction of the macro-organic matter and the amount of N mineralized during an incubation period of two weeks were considerably lower. Correlations between NFNS and

Soil characteristic	NFNS calculated according to:								
and soil depth	All soils			Sandy soils			Loams and clays		
	a^a	$\mathbf b$	c	a	b	$\mathbf c$	$\mathbf a$	b	c
A. Potential mineralization									
$0-2$ weeks $0-10$ cm	0.18	0.48	0.22	0.28	0.51	0.28	0.27	0.61	0.11
0-2 weeks 0-25 cm	0.30	0.51	0.43	0.52	0.60	0.55	0.31	0.55	0.34
$0-12$ weeks $0-10$ cm	0.60	0.74	0.49	0.62	0.71	0.51	0.58	0.78	0.48
$0-12$ weeks $0-25$ cm	0.63	0.62	0.61	0.65	0.67	0.56	0.70	0.58	0.68
B. Active microbial biomass (SIR)									
$0-10$ cm	0.34	0.65	$\mathbf{0}$	0.55	0.65	0.39	0.13	0.67	-0.36
$0-25$ cm	0.26	0.60	$\bf{0}$	0.46	0.65	0.29	0.19	0.68	-0.29
C. N in L fraction of macroorganic matter									
$0 - 10$ cm	0.24	0.43	0.25	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
$0 - 25$ cm	0.19	0.35	0.21	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
D. N in total macroorganrc matter									
$0 - 10$ cm	0.49	0.69	0.17	0.54	0.79	0.51	0.60	0.79	0.22
$0 - 25$ cm	0.44	0.66	0.17	0.51	0.74	0.50	0.58	0.75	0.22
$E. \Delta$ soil organic N									
$0-10$ cm	0.40	0.65	0.34	0.50	0.56	0.41	0.48	0.89	0.30
$0-25$ cm	0.27	0.56	0.24	0.51	0.54	0.44	0.21	0.70	0.06
$F \Delta MB-N$									
$0 - 10$ cm	0.57	0.75	0.51	0.71	0.90	0.67	0.63	0.83	0.51
$0-25$ cm	0.39	0.68	0.39	0.53	0.77	0.53	0.35	0.65	0.26

Table 4. Correlations between soil characteristics and NFNS calculated as the amount of N harvested in herbage (total amount or corrected) in unfertilized columns incubated in the greenhouse. The highest correlation coefficients are in italics

 a = total N harvested; b = total N harvested minus N harvested in the first cut; c = total N harvested min the initial amount of inorganic N present.

n.d. = not determined.

soil characteristics were stronger for the top 10 cm than for the top 25 cm (Table 4).

The correlation with NFNS increased when the sandy soils and loams and clays were analyzed as two separate groups (Table 4). N in the light fraction of macroorganic matter was not analyzed, as the correlation with NFNS was low when all soils were pooled. For the sandy soils, the highest correlation with NFNS was obtained with the difference between the amount of N in the microbial biomass in the top 10 cm of the soil and the amount of N in the microbial biomass under equilibrium conditions $(\Delta \text{ MB-N})$. The correlation was even higher when NFNS was expressed as total uptake minus N in the first cut $(r = 0.90)$ than when expressed as total N uptake $(r = 0.71)$. The relationship giving the best estimation of NFNS (N uptake

minus N in first cut) was: NFNS (kg N ha⁻¹) = 115 (4) + 740 (100) $\times \Delta$ MB-N (g N kg⁻¹ soil).

For the loams and clays, correlations of soil characteristics with NFNS were higher again when NFNS was defined as N uptake minus N in the first cut, than as total N uptake or total N uptake minus the initial amount of mineral N (Table 4). The difference between the soil organic N content of the top 10 cm and its organic N content under equilibrium conditions (Δ org. N) showed a better correlation ($r = 0.89$) with NFNS (N uptake minus N in the first cut) than any other soil characteristic. The best estimation of NFNS was: NFNS (kg N ha⁻¹) = 114 (3) + 12.0 (3.1 \times Δ org. **N** (g N kg⁻¹ soil) + 130 (50) \times Δ MB-N (g N kg⁻¹ soil).

Fig. 3. Relationship between the N uptake by the herbage (NFNS in kg N ha⁻¹ yr⁻¹) on unfertilized field plots located on different mineral soils (soils of Table 1) and the difference between the actual organic N content in the top 20 cm and the organic N content at equilibrium (g N kg⁻¹ soil). Significant relationship: NFNS = 132.3 $(7.3) + 42.1$ $(7.1) \times \Delta$ org. N. () = standard error of difference

Correlation between NFNS and A org. N under field conditions for the soils of Table 1

As we only had data on the organic N content of the top 20 cm of the soils and total N uptake on unfertilized plots, we correlated the N uptake on unfertilized plots (including the first cut; NFNS) with the Δ org. N and total org. N values of the soils. When all soils were pooled, the correlation between NFNS and Δ organic N was high $(r = 0.80)$. Correlation coefficients were higher for the loams and clays (0.84) than for the sandy soils (0.72). The correlation between NFNS and total organic N was not significant. The relationship giving the best estimation of NFNS was: NFNS (kg N ha^{-1}) yr^{-1}) = 132.3 (7.3) + 42.1 (7.1) $\times \Delta$ org. N (g N kg⁻¹) soil; Fig. 3).

Discussion

Estimate of the non-fertilizer N supply (NFNS) in mineral soils

The objective of this study was to see which approach gives the best estimate of the non-fertilizer N supply of grassland soils under field conditions. Although the amount of N mineralized in 7-to 14-d incubations is generally considered to be the most accurate method currently available for assessing the N availability of soils (Keeney, 1982), the potential N mineralization rate determined by incubating samples for 14 days did

not give a good estimate of NFNS: apparently the time of incubation was too short. The correlation with NFNS was better when an incubation period of 12 weeks was used. In previous experiments both close (Warren and Whitehead, 1988) and very poor (Fox and Piekielek, 1984) correlations were observed between N mineralized during a short incubation and NFNS. The goodness of the correlations found is expected to depend on the range of soils that are used, the variation in mineral N in spring (Machet, 1991) and the amount of mineral N present below the sampling layer but accessible to roots. Although it has been found that the light fraction of macroorganic matter correlates better with the potential mineralization rate (12 weeks incubation) than the total amount of macroorganic matter (Hassink, 1995) the opposite was found for the correlation with NFNS. The explanation might be that the light fraction changes considerably during the growing season (Table 5), whereas the total amount of macroorganic matter changes little.

The difference between the actual amount of soil organic N and microbial biomass and their amounts under equilibrium conditions correlated better with NFNS than the commonly determined potential mineralization rate and the amounts of macroorganic matter and active microbial biomass. For all mineral soils, the difference between the actual soil organic N content and the content under equilibrium conditions correlated well with NFNS. The results suggest that for sandy soils the difference between the actual amount of microbial N and microbial N under equilibrium conditions might even improve the correlation with NFNS. The advantage of this new method is that the difference between the actual organic N and microbial N contents and their equilibrium values is easy to determine and

Table 5. Amount of N (kg ha^{-1}) in stubble, roots and the light fraction of macroorganic matter (light M.O.M.) in the top 25 cm of a sandy (Tynaarlo) and loamy (Lelystad) grassland soil in spring and autumn of 1992

	Tynaarlo		Lelystad		
	Spring	Autumn	Spring	Autumn	
Stubble	17	19	15	20	
Roots	121	55	112	41	
Light M.O.M.	49	117	50	83	
Total	187	191	177	144	

that it is less time consuming than determination of the potential N mineralization rate or the amount of macroorganic N.

The mineral soils that were sampled all had a deep groundwater table (average highest water table > 40 cm below the surface). Soils with a higher water table have a higher organic N content under equilibrium conditions (Hassink, 1994). This means that for soils with a different water table level another equation for equilibrium contents should be used (Hassink, 1994).

Variation in NFNS in individual grassland soils

To exclude differences in soil temperature and water status between sites, soil columns were incubated under uniform environmental conditions in the greenhouse. At the beginning of the incubation, the amount of mineral N differed considerably between columns of different sites. Mineral N concentrations were highest in the clays. For the clays, N uptake in the first cut was considerably lower than the initial amount of mineral N in the soil. The water table was kept at only 30 cm below the surface, leading to partially anaerobic conditions in the clay soils. This suggests that at least a part of the mineral N initially present was denitrified in the fine-textured soils. This is in line with the observation that for clays, N uptake under field conditions was higher than N uptake in the greenhouse, while this was not found for loams and sandy soils. Partial denitrification of mineral N in the fine-textured soils at the beginning of the incubation might also explain why the correlation of soil parameters with NFNS increased, especially for the fine-textured soils when the first cut was excluded.

It was expected that the NFNS in the field would vary between years as temperature and moisture conditions are different every year. At four locations NFNS was determined over a period of four or five years. In individual years NFNS could differ 40% from the average value. The variation was higher in the soils with a low moisture supplying capacity (Den Ham and Tynaarlo) than in soils with a high moisture supplying capacity (Hemrik and Ruurlo). Variations in nitrogen supply between years were caused by differences in available N in spring and differences in N uptake during the summer period (June-September). As an example the cumulative NFNS in five successive years in Den Ham is presented in Figure 4. Variations in NFNS during the summer period were related to the amount of rainfall between June and September; 1982 and 1983 were dry years. Variations in NFNS in spring could not

Fig. 4. Cumulative N uptake by the herbage on unfertilized plots in Den Ham (kg N ha⁻¹) in 1979, 1980, 1981, 1982 and 1983.

be explained by differences in temperature or moisture conditions.

Another aspect to take into account is that mineralized N not only accumulates in aboveground plant parts that are harvested, but also in stubble, roots and plant residues (light fraction of the macroorganic matter pool; Meijboom et al., 1995). When the sum of the amount of N in roots, stubble and plant residues at the end of the growing season differs from the amount at the beginning, NFNS is under- or overestimated. For a sandy soil and a loam, the amounts of N in roots, stubble and light fraction were determined, and they were not significantly different between spring and autumn (Table 5).

Conclusion

It may be concluded that the "difference method" gives a good estimate of NFNS $(r = 0.80)$ on mineral grassland soils. The method has advantages over other methods because it is less time consuming and simpler.

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