Effect of soil moisture on the ability of Italian rye grass (Lolium multiflorum Lam.) to reduce an injurious content of nitrate nitrogen in soil.

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Abstract. The ability of Italian rye grass (*Lolium multiflorum*) to reduce a high content of mineral nitrogen in soil was studied in a pot experiment in greenhouse. The experimental soil consisted of a silty clay rich in humus and a fine sand. The soils were kept at three moisture levels corresponding approximately to pF values 2, 3 and 4. Two levels of nitrogen were applied, 175 and 350 mg/kg, as ammonium nitrate. The clay and fine sand soils initially contained 370 and 780 mg/kg of nitrate nitrogen, respectively. The grass was harvested five times during 205 days.

The excess of the nitrate in the clay soil prouducing high nitrate contents in the grass was exhausted after two cuttings when the soil moisture was kept at pF 2, whereas it was not reduced at all at pF 4. At pF 3, the excess nitrate was exhausted after the 3rd and 4th cutting at lower and higher nitrogen application levels, respectively.

The nitrate content of the grass grown on the fine sand soil was reduced to an acceptable level only at pF 2 and after the lower application of nitrogen, not before the last cutting, however.

Inorganic, mostly ammonium or nitrate nitrogen can be readily taken up by plants. The plant tolerates in its tissue only very low contents of ammonium nitrogen. In practice there is not usually so much ammonium in soil that the uptake would exceed the ability of the plant to utilize it. A dangerously high content of nitrate nitrogen in soil is more common. At a high soil nitrate level the crop may contain so much nitrate that it cannot be used as human food or animal fodder. The plants themselves tolerate relatively high contents of nitrate in their tissues, but only a fraction of it may prevent the utilization of the crop products. Moreover, nitrate nitrogen in soil is readily leached causing a pollution risk to ground water in addition to the direct loss of a part of the nutrient.

The aim of this study was to investigate how fast the injuriously high content of nitrate nitrogen in soil is reduced by growing Italian rye grass *(Lolium multiflorum)* in pots, and how it is affected by different levels of soil moisture and mineral nitrogen content.

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Material and methods

The two experimental soils had the following properties:

	Soil 1	Soil 2
pHCaCl ₂	5.5	6.0
org. C %	10.5	4.1
clay content (<2 µm) %	46	12
NH4-N mg/kg	27	3
NO3-N *	370	780
air dry moisture g/kg	48	26

Both soils had a very high content of nitrate. The largest part of it originated, no doubt, from the calcium nitrate applied to the soils earlier when they were used in pot experiments.

800 g of soil was weighed in plastic pots, 1.3 l by volume. The experimental design was as follows:

Factor M: soil	moisture				
	Soi	11	Soil 2		
	water	pF	water	pF	
	added		added		
	ml/kg		ml/kg		
M ₁	156	4.1	94	3.8	
M ₂	312	3.1	188	3.0	
M ₃	625	1.8	375	2.3	
Factor N: nitre	ogen applica	tion as amn	nonium nitrate		
N ₁		175	mg/kg		
N ₂		350			

There were four replicates. The basal dressing (K, P, S, Ca, Mg, Cu, Mn, Zn and B) as well as the nitrogen application were added in water solution and mixed thoroughly with the soil. The pots were planted on May 10th 1972 allotting 20 seed of Italian rye grass *(Lolium multiflorum)* for every pot. The pots were watered after periods of one or two days to the moisture level fixed in the experimental design. The grass was cut five times:

Cutting	Date	Time from last cutting, days	Time from planting, days	
1st	June 16th	37	37	
2nd	July 8th	22	59	
3rd	August 15th	38	97	
4th	October 5th	56	153	
5th	November 29th	52	205	

The experiment was carried out in greenhouse. An attempt was made to keep the temperature at 20° C, but it appeared impossible to prevent a rise in temperature on warm days. The highest temperatures measured were not less than 30° C. Such rises took place between the first and third cuttings, though only occasionally. The light was obviously deficient at the end of the experiment. The plant was cut the first three times at an earlier stage of development than in the last two cuttings when the ear had just emerged.

The yields were dried at 80° C. They were ground and the total and the nitrate nitrogen were determined. The nitrate nitrogen was determined in a water extract of the plant material by means of a nitrate ion selective electrode. The content of total nitrogen was determined by the common Kjeldahl method modified to include also nitrate nitrogen in the result. After the last cutting the ammonium and nitrate nitrogen in the soil were determined in 0.5 N K₂SO₄ extracts (10 g of soil per 100 ml of extractant, time 1 h) by means of ammonia and nitrate selective electrodes, respectively. The soil pH was measured in 0.01 M CaCl₂ (ratio 1: 2.5).

Results and discussion

Grass yields

Table 1. Yields of rye grass in g of D.M. per kg of soil.

		Cutting					
		1st	2nd	3rd	4th	5th	Total
Soil 1, M ₁ N ₁		0.11ª	0.33ª	0.90ª	0.65 ^a	0.56 ^a	2.55ª
N ₂		0.15 ^a	0.36ª	0.99 ^a	0.84 ^a	0.58 ^a	2.92 ^a
M ₂ N ₁		1.19 ^{ab}	2.93 ^b	4.71 ^b	3.58°	1.29bc	13.70 ^b
N ₂		1.56 ^{ab}	2.91 ^b	5.00 ^b	4.37 ^d	1.89 ^d	15.73 ^{bc}
M ₂ N ₂		2.54 ^b	4.85°	4.16 ^b	2.61 ^b	1.13abc	15.29bc
N ₂	••••••	2.20 ^b	5.22°	5.37 ^b	3.06 ^{bc}	1.66 ^{cd}	17.51°
Soil 2, M ₁ N ₁		0.01 ^a	0.03ª	0.11ª	0.10 ^a	0.10 ^a	0.35ª
N ₂		0.01 ^a	0.00 ^a	0.05 ^a	0.09 ^a	0.13 ^a	0.28ª
M ₂ N ₁		0.32ª	0.93ª	2.47 ^b	3.58 ^b	2.42 ^b	9.72°
N ₂	••••••	0.11ª	0.34ª	0.57ª	0.76 ^a	0.42 ^a	2.20 ^b
M ₃ N ₁		0.87 ^b	2.98 ^b	7.09 ^d	6.07°	2.14 ^b	19.15 ^d
N ₂	••••••	0.71 ^b	2.49 ^b	5.72°	6.38°	2.90°	18.20 ^d
LSD _{0.05} betwe	een soils	1.10	1.00	1.51	1.33	0.65	2.22

Yields of the same soil and the same cutting not marked by a common letter differ significantly (P = 0.05).

In the dry (M_1) soils the growth of grass was poor (Table 1), this was so especially in soil 2. This is to a certain extent surprising, because the water at the moisture level the soil was watered to was not as firmly bound in the soil (pF 3.8) as in the other soil (pF 4.1). Obviously the greater content of clay and humus in the latter one, leads to a greater store of available water.

In soil 1 there was enough water at level M_2 to produce as large an total yield as at the highest moisture level (M_a) . However, the rhythm of growth was different at these moisture levels. At the second cutting the yield in the moister soil was bigger, while later, at the fourth cutting, the situation was reversed. Apparently the plant needed at the beginning of its growth more water than later. This may depend on the stage of development as well as on external factors, such as the rising temperature which again increased transpiration. The smaller yield in the moister soil at the fourth cutting may be caused by the exhaustion of nutrients. Although it cannot be proved in the light of the results that the main reason was a reduction in the nitrogen content, the change in the total nitrogen content of the plant (Table 2) can be considered as an indication. In fact, the difference in the uptake of nitrogen up to the third cutting (Table 4) does not explain the expected difference in the nitrogen status of the soil. On the other hand it is apparent that the nitrogen losses, probably caused by denitrification, were at moisture level M_3 bigger than at level M_2 (cf. Table 5).

The growth of rye grass in soil 1 was not usually significantly influenced by raising the nitrogen application from level N_1 to level N_2 . Only at moisture level M_2 was there an increase in yield at the last two cuttings. Apparently the soil contained at the beginning of the experiment so much nitrogen available to the plant that additional nitrogen made no difference. Later, as available nitrogen in the soil decreased, the plant benefited from the additional nitrogen. The significant increase in growth, particularly at moisture level M_2 may have been a mere chance, but it can be assumed that at a higher moisture level the ability of the plant to take up nitrogen was so much better that the additional nitrogen was exhausted approximately at the same time as the initial plant-available nitrogen in the soil.

In soil 2 the plant grew best at the highest moisture level (M_3) , though at the last cutting, at nitrogen level N_1 , the harvested yield was at level M_2 as large as at level M_3 . At this time of the year the light may have been a growth limiting factor, but it is also possible that a state of nitrogen deficiency had developed (cf. Table 5). At moisture level M_3 , the rye grass grew especially well between the second and the third cutting. The growth was very clearly superior to that taking place in soil 1 at the same time. Apparently the mineral nitrogen content of soil 2 at the second cutting was reduced by the uptake of the plant to a level no longer detrimental to it, while the plant in soil 1 may have suffered from a nitrogen deficiency.

Raising the nitrogen application from level N_1 to level N_2 produced in soil 2 at moisture level M_2 a clear decrease in growth. This was obviously caused by the high mineral nitrogen content of the soil which showed no decrease during the experiment (Table 5). Increasing the soil moisture reduced the injury caused by too high a content of mineral nitrogen in the soil. There was the same tendency at moisture level M_3 at the beginning, but no longer after the third cutting. The last yield benefited by the additional nitrogen.

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Total nitrogen contents

PLANTINE ALL MARCHINE	Cutting					
	1st	2nd	3rd	4th	5th	
Soil 1, M ₁ N ₁	_	66.8 ^d	53.2 ^{bc}	56.6 ^d	55.7°	
N ₂	-	65.7 ^d	62.2°	55.0 ^d	53.7°	
M.N.	64.1 ^b	57.1°	43.8 ^b	22.7 ^b	21.0 ^a	
N ₂	66.7 ^b	61.6 ^{cd}	51.4 ^b	32.8°	25.9 ^b	
M ₃ N ₁	58.7ª	39.3ª	19.1 ^a	14.8 ^a	26.2 ^b	
N ₂	58.0 ^a	48.6 ^b	19.4 ^a	16.1 ^{ab}	23.7 ^{ab}	
Soil 2, M ₁ N ₁	-	_	_	-	_	
N ₂	-	-	-	-	-	
M ₂ N ₁	68.7 ^b	58.2 ^a	56.8 ^a	49.2 ^{bc}	59.3 ^b	
N ₂	79.0°	67.5 ^b	67.1 ^b	56.3°	64.5 ^b	
M ₃ N ₁	66.2 ^a	58.5ª	50.5 ^a	30.7 ^a	27.5ª	
N ₂	69.7 ^b	59.5 ^a	51.6 ^a	42.8 ^b	37.3ª	
LSD _{0.05} between soils	3.2	5.2	8.1	9.7	8.9	

Table 2. Total nitrogen contents of rye grass in mg per g of D.M.

Nitrogen contents of the same soil and the same cutting not marked with a common letter differ significantly (P = 0.05).

The total nitrogen content of rye grass in soil 1 seems clearly to decrease at each cutting (Table 2, statistical testing was not carried out in this direction), except at moisture level M1. At moisture level M2 the decrease in the total nitrogen of the plant after the third cutting was clear. At the highest moisture level (M3) the first decrease took place already after the first cutting, but a clear decrease only after the following cutting. The increasing effect of additional nitrogen on the total nitrogen content of the plant could be detected when the nitrogen content was reduced from its initial level. Apparently the total nitrogen content of the plant was close to the highest level that was possible in the conditions of the experiment. The availability of nitrogen in soil 1 at the highest moisture level (M_a) was obviously reduced in the N_2 treatment to the same level as in the N1 treatment making the soil nitrogen-deficient. A proof of the deficiency is the inability of this soil to produce as high a growth as soil 2 at the same time and at a corresponding level of soil moisture. Apparently the plant took up after the second cutting from soil 1 at the highest moisture level only the nitrogen that had been mobilized from the reserves of organic nitrogen of the soil.

In soil 2 the total nitrogen content of the plant decreased to the lowest level observed in soil 1 only in treatment M_3N_1 and at the last harvest. On the ground of the mineral nitrogen contents determined in the soil after the

last cutting (Table 5) it is obvious that only in this case had the initial reserves of mineral nitrogen come to an end.

The total nitrogen content of the fourth yield harvested in soil 2 at the highest moisture level responded to additional nitrogen, which can be taken as evidence that the mineral nitrogen content of the soil had dropped to a moderate level. Additional nitrogen no longer reduced the growth. In this soil at moisture M_2 there was a tendency of additional nitrogen to increase the total nitrogen content of the plant. This was the case at no other time when there was much mineral nitrogen left in the soil. Thus, increasing the nitrogen application has hardly caused directly the increase in the nitrogen content of the plant. This may be regarded as evidence of poor growth caused by excessive nitrogen in soil. As stated earlier, an additional application of nitrogen was followed by reduction in growth (Table 1).

Nitrate nitrogen contents

	Cutting				
	1st	2nd	3rd	4th	5th
Soil 1, M ₁ N ₁	 _	_	17.5	_	_
N ₂	 -	-	17.9	15.0	12.4
M ₂ N ₁	 15.0	15.2	7.5	1.5	0.6
N ₂	 15.1	16.8	13.9	4.5	2.0
M ₃ N ₁	 12.4	5.5	0.4	0.3	0.6
N_2	 12.2	9.0	0.5	0.5	0.4
Soil 2, M ₁ N ₁	 2	_	_	-	-
N ₂	 —	-	-	-	-
M ₂ N ₁	 _	14.4	18.7	14.8	17.6
N ₂	 .—	-	22.7	-	-
M ₃ N ₁	 _	16.1	18.1	6.8	1.1
N ₂	 -	16.4	18.6	12.6	7.8

Table 3. Nitrate nitrogen contents of rye grass in mg per g of D.M.

Table 3 is not complete, because some experimental yields were so small that no material could be taken for a determination of nitrate nitrogen, consequently statistical testing could not be carried out. The important differences are so clear, however, that the risk of an error is small, although it can not be determined precisely.

The nitrate nitrogen and the total nitrogen contents of the plant correlated rather closely, calculated from values in Tables 2 and 3, $r = 0.94^{***}$. This is natural since an increase of the nitrogen uptake over the utilizability of the plant leads to an accumulation of nitrate in the plant material (cf. LAWRENCE et al. 1968). Although a plant tolerates rather high contents of nitrate, relatively

low contents are detrimental to animals using the plant as fodder. Investigators hold different opinions over the highest tolerable nitrate nitrogen content of animal fodder. For the following discussion it is assumed to be 2 mg/g, a value adopted by LAWRENCE et al. (1968).

In soil 1, the nitrate nitrogen content of the grass had dropped below the expected limit of toxicity clearly only at the highest moisture level. The drop seems to have taken place after the second cutting. At moisture level M_2 the same soil produced toxic fodder for a much longer time; at level N_1 to the third, at level N_2 to the fourth cutting. The nitrate content of rye grass grown in soil 2 was reduced only in the treatment M_3N_1 , and not until the last yield, below the toxic level. The deficiency of light at the end of the experiment may have caused a slight increase in the nitrate nitrogen content of the last yield (cf. CANTLIFFE 1973).

Nitrogen uptake

Table 4. Cumulative uptake of total nitrogen by rye grass in different cuttings in mg of nitrogen per kg of soil.

		Cutting				
		1st	2nd	3rd	4th	5th
Soil 1, M ₁ N ₁		6ª	28ª	72 ^a	108 ^a	139 ^a
N ₂		8ª	31 ^a	93 a	139 ^a	170 ^a
M ₂ N ₁		74 ^{ab}	241 ^b	447 ^b	528 ^b	556 ^b
N ₂		101 ^{ab}	278 ^b	532 ^b	674 ^b	722 ^c
M ₃ N ₁		146 ^b	329 ^b	410 ^b	449 ^b	478 ^b
N ₂		128 ^b	376 ^b	484 ^b	533 ^b	573 ^b
oil 2, M ₁ N ₁		0 ^a	2 ^a	10 ^a	16 ^a	21ª
N ₂		0 ^a	0 ^a	4 ^e	10 ^a	18 ^a
M ₂ N ₁		21ª	75 ^a	215 ^b	390°	533°
N ₂		9 ^a	32 ^a	69 a	112 ^b	139 ^b
M ₃ N ₁		57 ^b	231 ^b	586°	788 ^d	851 ^d
N ₂		49 ^b	196 ^b	484°	776 ^d	894 ^d
LSD. of betw	een soils	79	117	117	93	114

Nitrogen uptakes of the same soil and the same cutting not marked with a common letter differ significantly (P = 0.05).

The uptake of total nitrogen from soil 1 (Table 4) was apparently until the second cut somewhat higher at moisture level M_3 than at M_2 , but the difference was not statistically significant. After this time the uptake from the moistest soil became clearly slower. The reason for this may have been the exhaustion of the initial abundant supply of mineral nitrogen, which was assumed on the ground of reduced yields and their total nitrogen contents. At moisture M_2 the nitrogen uptake continued to be rather abundant even after the second cut. The total uptake of nitrogen until the end of the experiment seems to have been even more abundant than at the higher moisture level, but the difference was significant only at level N_2 . The small addition of nitrogen from level N_1 to level N_2 as compared with the abundant initial mineral nitrogen supply of the soil increased significantly the nitrogen uptake of rye grass only at moisture level M_2 , but not until the third cut, and the tendency was similar in the other cases.

At the lowest moisture level (M_1) soil 2 gave to the plant very little nitrogen indeed, due to the extremely poor growth of the grass (cf. Table 1). The moisture level, M_2 , was not yet sufficient to enable the plant to take up nitrogen effectively. At moisture level M_3 the uptake was more effective in all stages studied. The addition of mineral nitrogen to the soil caused a clear decrease in the nitrogen uptake at moisture M_2 . These differences depend on the differences in growth (Table 1), which are relatively big compared with the differences in the nitrogen contents (Table 2). As stated above, the mineral nitrogen content of the soil at moisture M_2 was too high for sufficient growth. As an increase of moisture diminished this disadvantace, one may assume that the injury was caused by too high a concentration of mineral and nitrate nitrogen in the soil solution.

Nitrogen status in soil

At the end of the experiment both soils that had been kept dry (M_1) contained still a lot of mineral nitrogen (Table 5). In fact no significant reduction in the nitrogen content had taken place. The nitrification seems to have been rather low, too. The content of ammonium nitrogen had not changed during the experiment. In soil 1, increasing the moisture to the level M_2 caused a clear decrease of mineral nitrogen in the soil. It is apparent that by increasing the moisture further to level M_3 the reduction in the mineral nitrogen content was made even greater. However, the difference was not statistically singificant in the applied test, the accuracy of which was much reduced by the great variation observed in treatment M_1 . Increasing the moisture in soil 2 from level M_1 to M_2 caused a significant decrease of mineral nitrogen at level N_1 but not at level N_2 . Increasing the moisture further to level M_3 , almost exhausted the mineral nitrogen in soil. There was no difference between levels N_1 and N_2 .

At the higher moisture levels (M_2 and M_3) both experimental soils contained considerably less ammonium nitrogen at the end of the experiment than at the lowest moisture (M_1). The decrease of ammonium may have been caused both by nitrification and uptake by plant. In soil 2, in treatment M_2N_2 , where the nitrate content of the soil was clearly too high for sufficient growth, the decrease of ammonium does not seem to have slowed down. Obviously the nitrification took place effectively, because the nitrate content of the soil Table 5. Nitrogen status and pH in soils at the end of the experiment.

		Content of			Calculated nitrogen mobilization (+) or immobiliza-	
		NH ₄ -N mg/kg	NO ₃ -N mg/kg	Total mg/kg	tion (-) mg/kg	$pHCaCl_2$
Soil 1, M ₁ N ₁		96 ^b	404 ^b	500 ^b	58°	5.4 ^a
N ₂		151°	451 ^b	602°	17 ^c	5.4 ^a
M ₂ N ₁		11 ^a	8ª	19 ^a	-4 ^{bc}	5.5 ^b
N_2		10 ^a	56 ^a	66 ^a	34°	5.5 ^b
M ₃ N ₁		7 ^a	1 ^a	8 ^a	-93 ^{ab} *	5.5 ^b
N ₂		8 ^a	1 ^a	9 ^a	-173 ^a *	5.5 ^b
Soil 2, M ₁ N ₁		113 ^b	761°	874°	-67 ^{ab}	5.9ª
N ₂		176°	839°	1015°	-106 ^a	5.9 ^a
M.N.		44 ^a	206 ^b	250 ^b	-176 ^a *	6.3 ^b
N ₂		54 ^a	1031 ^d	1085°	91 ^b	5.8 ^a
MaN,		20 ^a	1 ^a	21ª	-88ª	6.6 ^b
N ₂		21 ^a	14 ^a	35 ^a	-208 ^a *	6.4 ^b
LSD0.05 betw	een soils	42	137	145	134	0.2

Values of the same soil and the same cutting not marked with a common letter differ significantly (P=0.05).

* Value deviates significantly (P=0.01) from zero.

even increased during the experiment in spite of the nitrate uptake by the plant.

The amounts of mobilized and immobilized nitrogen (Table 5) have been calculated by subtraction of mineral nitrogen at the beginning from the sum of soil mineral nitrogen and total nitrogen taken up by plant at the end of the experiment. The nitrogen contained in the roots was not determined as it was considered to be immobilized. The temperature in the greenhouse during the experiment ($\geq 20^{\circ}$ C) was rather beneficial for nitrogen mineralization (STANFORD et al. 1973).

In soil 1, near wilting point (M_1) , a slight mobilization of nitrogen seems to have taken place, but it was not significant. By increasing the moisture the mobilization apperently became more vigorous agreeing well with the results reported by STANFORD and EPSTEIN (1974). At the highest moisture level (M_3) , approximating the field capacity, some losses of nitrogen did occur. The significant difference observed between moisture levels M_2 and M_3 at nitrogen level N_2 can hardly be explained by the different amounts of nitrogen contained by the plant roots, since it would assume strong negative correlation between the amounts of nitrogen contained in the roots and those in the tops, which is not likely. Denitrification is more probable. According to STANFORD and EPSTEIN this really is possible at moistures near field capasity (M_3) . The differences observed in soil 2 may well be explained by the different amounts of nitrogen contained in the roots. There is no reason to assume that mobilization had not taken place in this soil, but because of the lower content of mineralizable organic matter the amounts of mobilized nitrogen must have been smaller than in soil 1, and so the possible differences between the treatments have probably been hidden behind the experimental error.

The soil pH and the nitrate nitrogen content of the soil at the end of the experiment are rather closely correlated, $r = -0.97^{***}$ for soil 1 and $r = -0.98^{***}$ for soil 2, as calculated from the treatment means presented in Table 5. As the uptake of nitrate was abundant, indicated here by the low nitrate content of the soil at the end of the experiment, the uptake of anions was to such an extent dominating compared with the cation uptake that the acidity of the soil decreased.

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SELOSTUS

Maan kosteuden vaikutus italialaisen raiheinän (Lolium multiflorum Lam.) kykyyn vähentää maan haitallisen suurta nitraattitypen pitoisuutta.

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Italialaisen raiheinän (Lolium multiflorum) suorittaman typenoton vaikutusta maan haitallisen suuren mineraali- ja varsinkin nitraattitypen pitoisuuden vähentäjänä tutkittiin astiakokeessa kasvihuoneessa. Raiheinästä korjattiin 205 päivän aikana 5 satoa, joiden kokonaistypen ja nitraattitypen pitoisuudet määritettiin. Koemaina oli hieta ja erittäin runsasmultainen hiesusavi. Niitä pidettiin kokeen ajan kolmesta eri kosteustilassa, jotka vastasivat suunnilleen pF-arvoja 2, 3 ja 4. Koe suoritettiin kahdella typpitasolla, jotka saatiin aikaan lisäämällä maihin kokeen alussa 175 ja 350 mg/kg typpeä ammoniumnitraattina.

Savimaassa, joka sisälsi alunperin nitraattityppeä 370 mg/kg, jo kaksi raiheinäsatoa riitti vähentämään maan käyttökelpoista typpeä niin paljon että kasviaineksen nitraattipitoisuus, joka alunperin oli hyvin korkea, laski alle 2 mg/g, silloin kun maata pidettiin kosteudeltaan kenttäkapasiteetissa. Pidettäessä maata lähellä lakastumisrajaa ei sen mineraalityppivarasto juuri vähentynyt. Maan kosteuden ollessa näiden kosteustilojen puolessa välissä, kasviaineksen nitraatinpitoisuus laski alle mainitun rajan ensi kerran kolmannessa sadossa alemmalla typpitasolla ja neljännessä sadossa korkeammalla typpitasolla.

Hietamaassa, joka sisälsi alunperin 780 mg/kg nitraattityppeä, raiheinän nitraattitypen pitoisuus laski alle mainitun rajan vasta viimeisessä sadossa, silloin kun maa oli kosteudeltaan lähellä kenttäkapasiteettia, ja vain alemmalla typpitasolla. Muissa tapauksissa kasviaineksen nitraatinpitoisuus pysyi korkeana kokeen loppuun saakka.

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