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Optimal length of the grass-clover period in crop rotations: results of a 9-year field experiment under organic conditions

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Abstract
The results of a 9-year crop rotation experiment with grass-clover are reported and discussed.

Keywords
Grass-clover, crop rotation, nitrate leaching, N uptake, soil fertility, ley

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Summary

A nine year crop rotation experiment was carried out in the Netherlands from 2002 through 2010 to (1) determine the optimal length of the grass-clover (mixture) and maize period in crop rotations and (2) to determine which crops should receive slurry application with priority in case the availability of cattle slurry is limited. The optimal crop rotation and slurry application strategy were defined in terms of crop yield and N uptake, mineral N in soil in autumn (nitrate leaching potential) and maintenance of soil fertility. A mixture of ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) was grown for two, three or four years (G2, G3 and G4), followed by one or two years of maize (M1 or M2) and one year of triticale. Cattle slurry application strategies were: (S1) no slurry application; (S2) application to the fodder crops maize and triticale; (S3) application to the grass-clover; or (S4) application to all crops. Cattle slurry was applied at a rate of 120 kg N ha^{-1} $year^{-1}$ (≈ 30 Mg ha^{-1} $year^{-1}$). The treatments were replicated three times on three fields on different sandy soils, previously also grown with grass-clover. The experiment was carried out under organic conditions: e.g. no use of synthetic fertilizers, herbicides or pesticides.

The results show that dry matter yield decreased for all treatments over the experimental period, due to a decrease in plant-available N (from N fixation by the clover, applied slurry and mineralization of soil N). For G2M1S1 (used as a reference treatment in the report), yield of the first year of grass-clover decreased from 10.0 Mg DM ha^{-1} in year 1 to 6.6 Mg DM ha^{-1} in year 9. N-fixation by the white clover could not maintain the initial yield level, not for G2M1S1 but also not for G4M1S1 (with higher grass-clover frequency in the rotation). For G4M1S1, grass-clover yield decreased from 10.2 Mg DM ha^{-1} in year 1 to 7.2 Mg DM ha^{-1} in year 9. The negative trend in yield for G2M1S1 could largely be offset by slurry application (S2, S3 or S4), addition of one or two extra years of grass-clover to the rotation and addition of an extra year of maize to the rotation.

The relative yield increase (increase relative to the negative trend) due to slurry application was largest when all crops received slurry application, and the effects of slurry application increased over time. For G2M1S4 instead of G2M1S1, yield of grass-clover decreased from 10.2 Mg DM ha^{-1} in year 1 to 7.9 Mg DM ha^{-1} in year 9. Addition of one or two extra years of grass-clover and/or an extra year of maize to G2M1 increased yields for all crops over time. The highest increase was realized with one or two extra years of grass-clover and one extra year of maize (G3M2). For G3M2S4, yield of grass-clover (third consecutive year) was 9.6 Mg DM ha^{-1} in year 9, 0.9 Mg lower compared to the grass-clover yield in year 1 (first grass-clover year) and 3.1 Mg higher compared with the grass-clover yield for G2M1S1 in year 9.

When grass-clover was grown for four subsequent years, yield peaked in the second year and decreased thereafter. Maize grown for the first year (after grass-clover) had the highest relative contribution to the total dry matter yield of a crop rotation, not only because maize yield was highest in the first year, but also because additional yield was realized with the grass-clover harvested before the maize was sown. Over the entire experimental period, the highest total yield of crop rotations with S4 was realized with G2M2 and the lowest with G4M1. The difference in total dry matter yield between these two rotations was 17 Mg DM ha^{-1} over nine years. The highest total maize yield was realized with G2M2 and the highest total grass-clover yield with G4M1/G4M2. The most important contribution to the relative share of these crops is realized by the frequency of these crops in the crop rotations.

Treatment effects on N uptake were roughly comparable to treatment effects on dry matter yield, though the trend in N uptake was more negative than the trend in yield. This reflects a higher N utilization at lower N availability. Over the entire experimental period, the highest total N uptake was realized with crop rotations with three or four years of grass-clover and slurry application for each crop/each year. With slurry application strategy S4, G2M2 had the highest average N use efficiency over the experimental period (53 kg DM produced per kg N taken up) and G4M1 the lowest (42 kg DM kg^{-1} N).

Mineral N in soil layer 0 to 90 cm at the end of the growing season decreased over the experimental period for all crops, along with the decrease in N uptake. For G2M1S1, mineral N after growth of the first year of grass-clover decreased from 28 kg N ha^{-1} in year 1 to 15 kg N ha^{-1} in year 9. Crop type had a large effect on mineral N in soil in autumn, with the highest level of mineral N after maize, followed by triticale and grass-clover. Crop rotations with a higher frequency of maize will therefore

have a higher risk of nitrate leaching. Application of cattle slurry (S2, S3 and S4) resulted in a relative increase (relative compared to the negative trend) in mineral N in soil in autumn over the experimental period. Slurry application had a much smaller initial effect on mineral N than crop type, but this effect increased over time for all crops. The relative increase in mineral N in soil in autumn suggests that, whereas general level of N uptake decreased over the years, the risk of N leaching relatively increased for rotations with slurry application. This effect can be explained by a higher availability of mineralized N at the end of a growing season, due to an increase in soil organic matter (SOM) and soil organic N (SON) over time for S4 compared to S3, S2 and S1. At the end of the growing season, only part of this mineralized N can be taken up by the crops; the remainder is subject to leaching.

The addition of extra years of grass-clover or an extra year of maize to the rotation did not result in changes in soil mineral N over time for all crops. However, when grass-clover was grown for four subsequent years, mineral N in soil increased up to the third year and decreased thereafter. These results suggest that prolonged growth of grass-clover (for more than three years) will not necessarily result in a higher potential for nitrate leaching. When maize was grown for two years in succession, the level of mineral N at the end of the second season was not different from the level at the end of the first season. The general risk of nitrate leaching was low for grass-clover, with mineral N in layer 0 to 90 cm not exceeding 30 kg ha^{-1} during the experimental period. For maize, the risk was considerably higher, with amounts varying between 40 to 60 kg N ha^{-1}.

There were no statistically significant effects of treatments on the development of soil organic matter (SOM) or soil organic N (SON), probably due to (unavoidable) large sampling errors. For G2M1S1, SOM decreased between year 1 and year 9 from 5.0 to 4.2% in soil layer 0 to 30 cm, from 3.9 to 3.1% in soil layer 30 to 60 cm and remained stable at 1.9 to 2.0% in soil layer 60 to 90 cm. The decrease can be explained by a decreased input of organic matter during the experimental period, as a result of decreasing yields and lower input with slurry, compared to the period before. For G4M1S4 instead of G2M1S1, SOM in layer 0 to 30 cm still decreased over the experimental period, from 5.1% in year 1 to 4.5% in year 9, though the decrease was smaller than for G2M1S1.

Decreases in SON were larger than decreases in SOM. For G2M1S1, SON decreased between year 1 and year 9 from 2.1 to 1.4% in soil layer 0 to 30 cm, from 1.3% to 0.7% in layer 30 to 60 cm, and from 0.7% to 0.4% in layer 60 to 90 cm. For G4M1S4, SON in soil layer 0 to 30 cm decreased from 2.1% in year 1 to 1.5% in year 9. This decrease is only slightly smaller for G4M1S4 than for G2M1S1. When the decreases in SOM and SON had been significant, the conclusion could have been drawn that the observed decline in both soil characteristics appears to be rather difficult to prevent with the input levels used. N fixation by grass-clover was not able to maintain initial yield levels or to maintain soil fertility as measured by SOM and SON.

Of all crop rotations, G2M2 had the highest total yield and maize yield over the experimental period, but also the highest potential for nitrate leaching and the lowest (expected) contribution to the maintenance of soil quality. G4M1 had the highest grass-clover frequency in the rotation (67%) and the lowest maize frequency (17%), so G4M1 will contribute the most to maintenance of soil fertility and has the lowest potential for nitrate leaching. However, G4M1 had also the lowest total yield and the lowest total maize yield. G4M2 seems to be the best compromise, having one of the highest inputs of organic matter by the grass-clover (maintenance of soil fertility), a total yield only slightly lower than for G2M2, and an average nitrate leaching potential. Considering slurry application strategies, slurry application to all crops (S4) resulted in maximal yield and also likely in the highest soil fertility. However, application of S4 also slowly increased mineral N in soil over time, and therefore also the nitrate leaching potential. This increase was relatively small within the timeframe of this experiment, but may become larger over time. There were no significant differences between slurry application strategies S2 and S3 in dry matter yield, N uptake, mineral N in soil and soil fertility. Slurry application to the fodder crops was therefore as effective as slurry application to the grass-clover.

Samenvatting

In Nederland werd tussen 2002 en 2010 een negenjarig vruchtwisselingsexperiment uitgevoerd om (1) de optimale lengte van de gras-klaver- en snijmaïsperiode in vruchtwisseling te bepalen en (2) om te bepalen hoe drijfmest over de gewassen verdeeld moet worden als de beschikbare hoeveelheid drijfmest beperkend is. De optimale vruchtwisseling en drijfmeststrategie werden gedefinieerd in termen van drogestofopbrengst en N-opname van de gewassen, de hoeveelheid minerale stikstof (N) in de bodem in de herfst (indicator voor potentiële nitraatuitspoeling) en het behoud van bodemvruchtbaarheid. Een mengsel van Engels raaigras (*Lolium perenne* L.) en witte klaver (*Trifolium repens* L.) werd verbouwd voor twee, drie of vier jaar (G2, G3 of G4), gevolgd door één of twee jaar snijmaïs (M1 of M2) en tenslotte één jaar triticale voor GPS. De drijfmeststrategiën waren: (S1) geen toediening van drijfmest; (S2) toediening van drijfmest aan de voedergewassen snijmaïs en triticale; (S3) toediening van drijfmest aan gras-klaver; en (S4) toediening van drijfmest aan alle gewassen. Bij drijfmestbemesting werd met runderdrijfmest 120 kg N ha^{-1} $jaar^{-1}$ toegediend (≈ 30 ton ha^{-1}). Alle behandelingen werden drie keer herhaald op drie percelen op verschillende zandgronden. Op deze percelen was voorheen ook gras-klaver verbouwd. Het experiment werd uitgevoerd onder biologische condities: o.a. geen gebruik van kunstmest, herbiciden of pesticiden.

Uit de resultaten blijkt dat de drogestofopbrengst bij alle behandelingen afnam over de duur van het experiment, als gevolg van een afname van de voor het gewas opneembare N (uit N-binding door de klaver, toegediende drijfmest en mineralisatie van bodemorganische stof). Bij G2M1S1 (gebruikt als referentiebehandeling in het rapport) nam de opbrengst van gras-klaver af van 10,0 ton DS ha^{-1} in jaar 1 tot 6,6 ton DS ha^{-1} in jaar 9. De N-binding door de witte klaver in het mengsel met Engels raaigras kon het opbrengstniveau bij start van het experiment niet op peil houden, niet bij G2M1S1 maar ook niet bij G4M1S1 (met hogere gras-klaver frequentie in de vruchtwisseling). Bij G4M1S1 nam de gras-klaver opbrengst af van 10,2 ton DS ha^{-1} in jaar 1 tot 7,2 ton DS ha^{-1} in jaar 9. De negatieve trend in opbrengst bij G2M1S1 kon verminderd worden door drijfmest toe te dienen (S2, S3 of S4), één of twee jaar extra gras-klaver aan de vruchtwisseling toe te voegen en één jaar extra snijmaïs aan de vruchtwisseling toe te voegen.

De vermindering van de opbrengstdaling als gevolg van drijfmesttoediening was het grootst wanneer aan alle gewassen drijfmest werd toegediend. De effecten van drijfmesttoediening namen toe over de tijd. Bij G2M1S4 in plaats van G2M1S1 nam de opbrengst van gras-klaver af van 10,2 ton DS ha^{-1} in jaar 1 tot 7,9 ton DS ha^{-1} in jaar 9 (in plaats van 6,6 ton DS ha^{-1}). Toevoeging van één of twee extra jaren gras-klaver en/of een extra jaar snijmaïs aan G2M1 leidde tot een toename van opbrengst in de tijd. De grootste toename werd gerealiseerd met toevoeging van één of twee extra jaren gras-klaver en één extra jaar snijmaïs aan G2M1. Bij G3M2S4 was de opbrengst van gras-klaver in jaar 9 (derde jaar opeenvolgend gras-klaver) 9,6 ton DS ha^{-1}. Dit was 0,9 ton lager vergeleken met de gras-klaver opbrengst in jaar 1 (eerste jaar gras-klaver) en 3,1 ton hoger vergeleken met de gras-klaver opbrengst bij G2M1S1 in jaar 9.

Als gras-klaver vier jaar achtereen werd verbouwd, piekte de opbrengst in het tweede jaar en nam daarna af. Het eerste jaar maïs na gras-klaver had de hoogste relatieve bijdrage aan de totale drogestofopbrengst van een vruchtwisseling, niet alleen omdat de maïsopbrengst het hoogst was in het eerste jaar (vergeleken met het tweede jaar), maar ook omdat er extra opbrengst werd gerealiseerd door de oogst van een snede gras-klaver voorafgaande aan de maïs. De totale drogestofopbrengst over de hele proefperiode, bij de behandelingen met S4, was het hoogst bij G2M2 en het laagst bij G4M1. Het verschil in totale drogestofopbrengst tussen deze twee behandelingen was 17 ton DS ha^{-1} over negen jaar. De hoogste totale maïsopbrengst werd behaald met G2M2 en de hoogste totale gras-klaver opbrengst met G4M1/G4M2. Voor een belangrijk deel is dit verschil te wijten aan de frequentie van deze gewassen in deze vruchtwisselingen.

De behandelingseffecten op de N-opname van de gewassen waren grofweg vergelijkbaar met de behandelingseffecten op de drogestofopbrengst, hoewel de negatieve trend in N-opname sterker was dan de negatieve trend in drogestofopbrengst. Dit betekent dat er sprake was van een hogere N-benutting bij lagere N-beschikbaarheid. Over de hele proefperiode werd de hoogste totale N-opname gerealiseerd door vruchtwisselingen met drie of vier jaar gras-klaver en drijfmesttoediening aan elk gewas. Bij S4 had G2M2 de hoogste gemiddelde N-efficiëntie over de proefperiode (53 kg DS geproduceerd per kg opgenomen N) en G4M1 de laagste (42 kg DS kg^{-1} N).

De minerale N in bodemlaag 0 tot 90 cm aan het einde van het groeiseizoen nam bij alle gewassen af over de duur van het experiment, tegelijk met de afname in plantopneembare N. Bij G2M1S1 nam de hoeveelheid minerale N aan het einde van het groeiseizoen van gras-klaver (eerste jaar) af van 28 kg N ha^{-1} in jaar 1 tot 15 kg N ha^{-1} in jaar 9. Gewassoort had een groot effect op minerale N in de bodem, met het hoogste niveau na snijmaïs, gevolgd door triticale en gras-klaver. Vruchtwisselingen met een hogere frequentie snijmaïs hebben daardoor een groter risico van nitraatuitspoeling. Toediening van drijfmest (S2, S3 en S4) leidde tot een geringere afname van minerale N over de experimentele periode. Drijfmesttoediening had in het begin van het experiment een veel kleiner effect op minerale N dan gewassoort, maar dit effect nam in de loop van de tijd toe. De kleinere daling van minerale N over de tijd als gevolg van bemesting suggereert dat, terwijl het niveau van N-opname daalde tijdens de proefduur, het risico op nitraatuitspoeling toenam voor vruchtwisselingen met drijfmesttoediening. Dit effect kan verklaard worden door een hogere beschikbaarheid van gemineraliseerde N aan het eind van het groeiseizoen, als gevolg van een toename van het gehalte organische stof (OS) en organische N (ON) in de bodem bij S4, S3 en S2 vergeleken met S1. Aan het eind van het groeiseizoen kan maar een deel van deze beschikbare minerale N opgenomen worden door het gewas; de rest is gevoelig voor uitspoeling.

De toevoeging van extra jaren gras-klaver of een extra jaar snijmaïs aan de vruchtwisseling leidde niet tot een toename van minerale N in de bodem voor alle gewassen in de tijd. Echter, wanneer gras-klaver vier jaar achter elkaar werd verbouwd, nam de minerale N in de bodem toe tot en met het derde jaar, en nam daarna af. Deze resultaten suggereren dat een langere teeltduur van gras-klaver niet hoeft te leiden tot een hoger risico op nitraatuitspoeling. Als snijmaïs twee jaar achter elkaar werd verbouwd was de hoeveelheid minerale N in de bodem aan het einde van het tweede groeiseizoen niet verschillend van het eerste groeiseizoen. Het algemene risico op nitraatuitspoeling was laag voor de gras-klaver, met hoeveelheden minerale N in bodemlaag 0 tot 90 cm onder de 30 kg N ha^{-1} gedurende de proefperiode. Voor snijmaïs was het risico op nitraatuitspoeling aanzienlijk groter, met hoeveelheden minerale N variërend tussen 40 en 60 kg N ha^{-1}.

Er waren geen statistisch significante effecten van behandelingen op de ontwikkeling van OS en ON, waarschijnlijk als gevolg van (onvermijdbare) grote bemonsteringsfouten. Tussen jaar 1 en jaar 9 nam bij G2M1S OS af van 5,0 tot 4,2% in bodemlaag 0 tot 30 cm, van 3,9 tot 3,1% in bodemlaag 30 tot 60 cm en bleef OS stabiel op 1,9 tot 2,0% in bodemlaag 60 tot 90 cm. De afname in OS kan verklaard worden door een afnemende input van organische stof tijdens de proefperiode, als gevolg van dalende gewasopbrengsten en een lagere drijfmesttoediening, vergeleken met de periode daarvoor. Bij G4M1S4 in plaats van G2M1S1 nam OS in bodemlaag 0 tot 30 cm nog steeds af over de proefperiode, van 5,1% in jaar 1 tot 4,5% in jaar 9, hoewel de afname kleiner was dan voor G2M1S1. De afname van ON in de bodem was groter dan van OS. Bij G2M1S1 nam ON tussen jaar 1 en jaar 9 af van 2,1 tot 1,4% in bodemlaag 0 tot 30 cm, van 1,3% tot 0,7% in bodemlaag 30 tot 60 cm, en van 0,7% tot 0,4% in bodemlaag 60 tot 90 cm. Bij G4M1S4 nam ON in bodemlaag 0 tot 30 cm af van 2,1% in jaar 1 tot 1,5% in jaar 9. Deze daling was bij G4M1S4 maar weinig minder dan bij G2M1S1. Als de dalingen in OM en ON significant waren geweest, had de conclusie getrokken kunnen worden dat deze afname moeilijk te stoppen lijkt bij de inputniveaus van OS en ON in de proef. De N-binding door witte klaver was in deze proef niet in staat om het oorspronkelijke opbrengstniveau van de gewassen en de bodemvruchtbaarheid op peil te houden.

G2M2 had van alle vruchtwisselingen de grootste totale opbrengst en snijmaïsopbrengst over de proefperiode, maar ook het grootste risico op nitraatuitspoeling en de kleinste (veronderstelde) bijdrage aan het behoud van bodemkwaliteit. G4M1 had van alle vruchtwisselingen de hoogste frequentie gras-klaver (67%) en de laagste frequentie snijmaïs (17%), waardoor G4M1 de grootste bijdrage aan behoud van bodemkwaliteit levert en het kleinste risico op nitraatuitspoeling. Echter, G4M1 had ook de laagste totale opbrengst en maïsopbrengst van alle vruchtwisselingen. G4M2 lijkt het beste compromis, met een hoge input van organische stof door de gras-klaver (behoud bodemkwaliteit), een totale opbrengst die maar weinig lager is dan bij G2M2, en een gemiddeld risico op nitraatuitspoeling. Drijfmesttoediening aan alle gewassen (S4) gaf de hoogste opbrengsten en de grootste (verwachte) bijdrage aan bodemkwaliteit. S4 gaf echter ook een geleidelijke toename van minerale N in de bodem over de proefperiode, en daardoor ook van het risico op nitraatuitspoeling. Deze toename was tijdens de proefperiode nog relatief klein, maar kan na verloop van tijd verder toenemen. Er waren geen significante verschillen tussen drijfmeststrategiën S2 en S3 in opbrengst, N-opname, minerale N in de bodem en bodemvruchtbaarheid. Drijfmesttoediening aan de voedergewassen was daarmee even effectief als drijfmesttoediening aan de gras-klaver.

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1 Introduction

In dairy farming systems, the use of synthetic fertilizer can be reduced by using grass-clover mixtures in crop rotations. Reduction of the use of synthetic N fertilizer reduces fertiliser costs and the associated emission of greenhouse gasses to the atmosphere during the fertilizer life-cycle. Clover (e.g. white clover, *Trifolium repens* L.) fixes N_2 from the atmosphere. This N is used by the clover, but also by the grass, for the production of biomass.

When grass is grown in rotation with other crops, grassland has to be destroyed before the other crops can be grown. As a result of cultivation, large amounts of N can be mineralized in a relatively short period of time. N utilization by the succeeding crop is often suboptimal, resulting in a surplus of mineral N in soil, which can result in nitrate leaching to groundwater and emission of greenhouse gasses to the atmosphere. The risk for these N losses tends to increase with age of the sward. N losses resulting from grassland cultivation apply to both grassland fertilized with synthetic fertilizer as well as grassland 'fertilized' with N-fixation by clover.

These unwanted N losses can be reduced by reducing the length of the grass-clover period in the crop rotation. However, this reduction increases the costs of grassland production through higher re-sowing costs and higher re-sowing production losses. Reduction in years of grass-clover also reduces the addition of organic matter to the soil, which can have a negative impact on soil fertility and crop productivity in the future. The dilemma is to determine an optimal length of the grass-clover period in rotations. Grass-clover grown in rotation is often succeeded by silage maize, for one or more years. Silage maize produces high yields, but has a rather negative effect on long-term soil quality, because little organic matter is added to the soil. Also, the growing of maize in general increases the risk of nitrate leaching because the N uptake of mineral N from soil by maize is relatively low compared to grass, especially later during the growing season. To reduce N losses, it is therefore also important to determine the optimal length of the maize period in rotation.

The addition of organic matter by grass-clover to the soil is important to maintain soil quality and long-term crop productivity. Organic matter can also be added to the soil by application of organic manure, such as cattle slurry. Cattle slurry usually provides a variety of nutrients and is especially rich in N. In spring, N-fixation by clover and N provided by soil N mineralization is relatively low because of low temperatures. In organic farming systems, the application of cattle slurry is then important to achieve sufficient yield and feed quality. When cattle slurry is applied to crop rotations, and especially when the availability of slurry is limiting, an important question is which crops should receive slurry with priority, to realize the highest N utilization of the rotation.

To investigate these questions, a crop rotation experiment was carried out at the experimental organic dairy farm 'Aver Heino' in the Netherlands. This experiment lasted for 9 years. The experiment was carried out under organic conditions: e.g. there was no use of synthetic fertilizers, herbicides or pesticides. The main questions to be answered were: (1) what is the optimal length of the grass-clover period and maize period in crop rotation in order to realize a maximal yield and N uptake, a minimal risk of nitrate leaching and a maximal maintenance of soil fertility and (2) what is the optimal slurry application strategy for these crop rotations when the availability of cattle slurry is limiting? In this report, the results of this experiment are reported and discussed.

This experiment was funded from 2002 through 2004 by the Dutch Ministry of Agriculture, Nature and Fisheries (LNV) and by the Dutch Dairy Board (Productschap Zuivel, Zoetermeer). From 2005 through 2010, the experiment was funded by the Dutch Ministry of LNV. In 2012, the reporting was funded by the Dutch Ministry of Economic Affairs, Agriculture and Innovation (EL&I).
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2 Materials & methods

2.1 Experimental design

The experiment was laid out as a randomized block trial with three replications, each on a different field with a different sandy soil type. The treatments consisted of all combinations of six different crop rotation schemes and four different slurry application strategies. The experiment was intended to run from 2002 through 2012, totalling eleven experimental years. However, due to budget cuts, the experimental period had to be reduced to nine years and ended in 2010.

In all crop rotations, the growing of grass-clover (G) was succeeded by silage maize (M) and then by silage triticale (T). The mixture of ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) was grown for two, three or four years, maize for one or two years and triticale for one year. All combinations of crop growing periods resulted in six different rotation schemes (Table 1; bold). The shortest crop rotation lasted four years, the longest seven. When a crop rotation had been completed, the rotation was repeated. For instance, for the shortest rotation scheme, with two years of grass-clover followed by one year of maize and one year of triticale (G2M1), with start of the first year of grass-clover in 2002, grass-clover was grown in 2002 and 2003, in 2006 and 2007, and in 2010.

Table 1 Composition and length of all crop rotation schemes

Crop rotation scheme	Year								
	1	2	3	4	5	6	7	8	9
G2M1	**G**	**G**	**M**	**T**	G	G	M	T	G
G2M2	**G**	**G**	**M**	**M**	**T**	G	G	M	M
G3M1	**G**	**G**	**G**	**M**	**T**	G	G	G	M
G3M2	**G**	**G**	**G**	**M**	**M**	**T**	G	G	G
G4M1	**G**	**G**	**G**	**G**	**M**	**T**	G	G	G
G4M2	**G**	**G**	**G**	**G**	**M**	**M**	**T**	G	G

Though the overview in Table 1 suggests that all rotation schemes started at the same time with growing of grass-clover in year 1, this was not the case. To reduce entanglement of year effects (mainly weather effects) with certain crop rotations, and to reduce the effects of the existing grass-clover sod, a different starting year was chosen for each replication. As a result, all crop rotation schemes started in year 4 for the first replication, in year 5 for the second replication and in year 6 for the third replication. Because the longest rotation lasted seven years, and started in the fourth year for the first replication, the whole experiment had to last for at least 11 years to have one complete run of the longest crop rotation. The crop sequence per plot is given in Appendix 1.

There were four different slurry application strategies: no slurry application (S1); application to the fodder crops (S2); application to the grass-clover (S3); or application to all crops (S4) (Table 2). Slurry application consisted of a single application of 120 kg N ha^{-1} $year^{-1}$ with cattle slurry.

Table 2 Description of all slurry application strategies and the amount of N applied (kg total N ha^{-1} $year^{-1}$)

Slurry application strategy	Crop	
	Grass-clover	Maize and triticale
S1	0	0
S2	0	120[1)]
S3	120	0
S4	120	120[1)]

[1)] When maize was grown for the first time after grass-clover, the 120 kg N ha^{-1} were applied before the first cut of the grass-clover, in order to realize a maximal N use efficiency of the slurry N

All treatments were replicated on three different fields on different sandy soils to reduce the effects of a specific sandy soil/location. The fields were located at experimental dairy farm ‘Aver Heino’ (Heino, the Netherlands). The first replication was located on field 1-2, an elevated Plaggic Anthrosol, with an A-horizon of 80 to 100 cm. The second and third replication were located on field 13-15 and field 23-24, both ‘Beekeerd’ loamy sandy soils, common in the eastern part of the Netherlands (a ‘Beekeerd’ soil can be described as a humus-rich, hydromorphic sandy soil). Field 23-24 was located at slightly lower altitude and had a coarser grain of sand compared to field 13-15. The gross dimensions of the experimental units (plots) were 15 (l) x 6 (w) = 90 m^2. There were 24 plots per field/replication and 72 plots in total. The layout of the plots on the three fields is given in Appendix 2.

The grass-clover sods of fields 1-2, 13-15 en 23-24 were sown in April 2001, March 1997 and the spring of 1994, respectively. Field 13-15 was also sod-seeded in September 1997 and August 1998; field 23-24 was sod-seeded in June 1999. At the start of the experiment (autumn of 2001), the grass sods were aged one, five and eight years, respectively. The grass-clover on field 1-2 was preceded by silage maize in 2000 and monoculture grass from 1997 through 1999.

2.2 Methods

2.2.1 Crop sowing

Grass-clover
In March 2002, the existing grass-clover sod was ploughed-down and re-sown on the plots where grass-clover would be grown for more than one year. From 2003 onwards, grass-clover was always sown after the harvest of triticale, usually in July. After triticale harvest, the plots were spaded to a depth of 20 cm and immediately sown with ryegrass, using a combined spading-sowing machine. For sowing, 3 to 4 kg ha^{-1} of white clover seed was mixed with 25 to 30 kg ha^{-1} of ryegrass. The mixture was sown at a depth of 1 to 1.5 cm and rolled afterwards. Before spading and sowing in March 2002, about 80 kg total N ha^{-1} with cattle slurry was applied on plots with S3 and S4. When grass-clover was sown after triticale harvest, no cattle slurry was applied before sowing. Grass and clover seeds were obtained from organic sources and the best performing varieties of the Dutch variety list were selected. For clover, the variety 'Riesling' was used throughout the experiment, except for 2002 (mixture of varieties Choice, Barlett and Alice). For grass, the mixture 'Bar Eko' (Barenbrug, the Netherlands) was used throughout the experiment.

Maize
Maize was sown after grass-clover or a first year of maize. When maize followed grass-clover, the grass-clover was harvested first, usually in May. From 2002 through 2004, 40 kg N ha^{-1} was then applied with cattle slurry on the plots with S2 and S4. The grass-sod was cultivated with a rotavator and ploughed. Maize was sown with a precision sowing machine, with a distance between seeds of 15 cm in the row and of 75 cm between rows. The seeding rate corresponds with nine to ten plants per m^{-2}. Maize seeds were obtained from organic sources and the best performing varieties of the Dutch variety list were selected. Sown varieties were Symphony, Symphony, Rosalie, Rosalie, Rosalie, Rosalie, not recorded, Rosalie, Aastar and Aastar, for 2002 through 2010, respectively.

When maize was grown for two years, a green manure crop was sown after the first harvest. The purpose of this crop was to catch nutrients (mainly N), but also to improve soil quality (soil structure, organic matter, soil life). The green manure crop was sown after loosening the (top) soil with a cultivator. Usually, the green manure was winter rye, but in the first years also mixtures of rye and Italian ryegrass were sown. Plots were rolled after sowing. The green manure crop was not harvested and was destroyed in March. Just before sowing of the second year of maize, the plots received 120 kg N ha^{-1} with cattle slurry (S1 and S4), were cultivated, ploughed or spaded (20 cm), and sown.

Triticale
At start of the field experiment, the triticale plots were sown in the fall of 2001 after cultivation and ploughing of the existing grass-clover sod. From 2002 onwards, triticale was always sown after the harvest of maize. Plots were spaded to a depth of 20 cm and immediately sown with triticale, using a combined spading-sowing machine. Depending on the 1000-grain weight of the triticale seeds, 150 to 200 kg ha^{-1} of seeds was used. Triticale seeds were obtained from organic sources and the best performing varieties of the Dutch variety list were selected. Sown varieties were Binova, Binova, Santop, Santop, Santop, Santop, Talentro, not recorded, and Sequenz, from 2001 through 2009, respectively. In the season of 2002/2003 and 2006/2007, the triticale crop failed during winter. Plots were re-sown in the spring of 2003 or 2007 with summer barley, at a rate of 160 kg ha^{-1} (2003) or 120 kg ha^{-1} (2007). The used variety of the summer barley was not recorded in 2003; the variety in 2007 was 'Class'.

2.2.2 Weed and pest control

During the growth of grass-clover, no specific weed control practices were applied. Most of the weeds were removed with the first harvest. Field 23-24 experienced persistence of dandelion (*Taraxacum officinale* L.) throughout the experimental period, which may have resulted in lower yield of the first harvest (dandelion growth was rather abundant in spring). Maize plots were harrowed once or twice before seedling emergence, depending on the weed pressure. After seedling emergence, the harrowing was repeated (once or twice), until the seedlings became too large. After that moment, plots were hoed until the maize plants outgrew the weeds. Triticale plots were harrowed several times in spring to control weeds.

Plots sown with maize experienced predation from birds (mainly pigeons) on the seeds and seedlings after sowing, especially from 2005 onwards. Little could be done to prevent this predation; removed seeds and seedlings were re-sown as much as possible. Though the surrounding maize plants were able to largely fill open spots, predation is likely to have lowered maize yields.

Maize yields were likely also depressed by feeding damage caused by *Elateridae* larvae, especially on field 23-24. No control practices were available. Plants lost due to feeding damage were re-sown as much as possible.

2.2.3 Slurry and fertilizer applications

Cattle slurry
Cattle slurry was applied on the grass-clover and triticale plots as soon as the soil conditions were right, usually mid-March. From 2002 through 2004, a slurry application on grass-clover plots was split in an application of 80 kg N ha^{-1} for the first growth period and 40 kg N ha^{-1} for the second growth period. From 2005 onwards, the 120 kg N ha^{-1} was applied with one application for the first growth period. On grass-clover plots, slurry was applied with a sod-injector.

From 2002 through 2004, a slurry application on plots with maize grown for the first year was split in an application of 80 kg N ha^{-1} before the first cut of grass-clover and an application of 40 kg N ha^{-1} just before maize sowing. From 2005 onwards, the 120 kg N ha^{-1} was applied before the first cut of the grass-clover. When maize was grown for the second year (maize after maize), cattle slurry was applied at the beginning of May, just before spading and sowing. On maize and triticale plots, slurry was applied with an injector. At each moment of slurry application, slurry was sampled from the tank of the injector, sent to a laboratory (Blgg, Oosterbeek) and analysed for dry matter (DM), organic matter (OM), $N\text{-}NH_3$, total N, K_2O and P_2O_5.

Fertilizer (P and K)
All treatments received P and K fertilizer in surplus of the annual P and K uptake by the crops. P was always applied as rock phosphate (‘Gafsa’; 27 % P_2O_5, 37 % CaO) and potassium as Patentkali© (30 % K_2O, 10 % MgO, 42% SO_3).

Plots with grass-clover as the main crop received 120 kg P_2O_5 ha^{-1} $year^{-1}$ and 730 kg K_2O ha^{-1} $year^{-1}$. P was applied during the first growth period; K distribution over the growth periods was 180, 150, 150, 150 and 100 kg K_2O ha^{-1} for the five growth periods, respectively. For treatments with slurry application, the amounts of P and K applied with slurry were subtracted from the total P and K application rates. P and K application with 30 Mg cattle slurry (fresh weight) was roughly 40 kg P_2O_5 and 180 kg K_2O, respectively.

Plots with maize after maize received 80 kg P_2O_5 ha^{-1} $year^{-1}$ and 240 kg K_2O ha^{-1} $year^{-1}$, applied immediately after sowing. When maize was grown for the first year, and when the preceding grass-clover had received cattle slurry, the maize was fertilized with (80 - 40) = 40 kg P_2O_5 and (240 - 180) = 60 kg K_2O immediately after sowing of the maize. Plots with triticale as the main crop received 60 kg P_2O_5 ha^{-1} $year^{-1}$ and 180 kg K_2O ha^{-1} $year^{-1}$ in week 12 of the calendar year. When cattle slurry was applied, no additional P or K was applied with fertilizer.

Liming
The soil layer 0 to 30 cm of all plots was analysed for pH-KCl in 2004 and 2008. In 2004, average pH-KCl had decreased to a value of 4.7, whereas the target pH-KCl for maize cultivation on this type of sandy soil is between 5.3 and 5.7 (Adviesbasis bemesting, 2002). All plots were then limed with 1400 kg ha^{-1} Dologran 15 (54% CaO) in October 2005 and 1200 kg ha^{-1} in November 2006. In 2008, average pH-KCl was 5.2. All plots were additionally limed with 1400 kg ha^{-1} Dologran 15 in October 2009.

2.2.4 Harvest

Grass-clover was harvested when the dry matter production was estimated to be around 3 Mg DM ha^{-1} (above stubble height, 6 cm) for the first harvest and around 2 Mg DM ha^{-1} for later harvests. Just before harvest, the percentage of clover in the grass-clover mixture was visually assessed as percentage of surface cover. Maize and triticale were harvested for silage when the grains were in the 'dough'-stage (containing 30 to 35% of dry matter). Grass-clover and triticale were harvested with a 'Haldrup' plot harvester at a height of 6 and 15 cm, respectively. Maize was harvested with a converted maize harvesting machine, at a height of 15 cm. Harvests were taken from the centre of each plot. Dimensions of the harvested area were 13 x 1.5 m for grass-clover and triticale and 13 x 3.0 m for maize. The harvested material was weighed and sampled. The sample was oven-dried at 70°C, weighed back to determine moisture content and sent to a laboratory (Blgg, Oosterbeek) for analysis of total N, P and K. All harvested material was removed from the plots.

2.2.5 Soil sampling

Soil fertility
Plots were analysed for soil fertility characteristics in 2002, 2004, 2006, 2008 and 2010. Plots were sampled in February/March of 2002, 2004, 2006 and 2008, and in November of 2010. Sampled soil layers were 0 to 30 cm, 30 to 60 cm and 60 to 90 cm. Samples (at least six subsamples per plot in a representative pattern, at least 1 m from the plot borders) were taken with an auger, sent to a laboratory (Blgg, Oosterbeek) and analysed for organic matter and organic N.

Soil mineral N
After harvest of all the plots grown with maize (usually in October), all 72 plots were sampled for mineral N in soil layers 0 to 30, 30 to 60 and 60 to 90 cm. Samples were taken with an auger (at least six subsamples per plot in a representative pattern, at least 1 m from the plot borders), stored in a refrigerator at max. 5 °C and sent to a laboratory (Blgg, Oosterbeek) within 24 hours for analysis of N-NH_4 en N-NO_3.

2.2.6 Statistical analysis

The collected data were analysed as one set per measured characteristic, using REML (statistical package Genstat, 2012). Data were ln-transformed before REML-analysis. The initial fixed model per characteristic consisted of all main factors and their interactions. During the analysis process, non-significant factors and interactions were removed from the model, except when they were part of a significant/vital higher-order interaction. Significance of model terms was determined using Wald's Chi-squared test ($\alpha = 0.05$). The analysis resulted in a fixed and a random model for each analysed characteristic. The fixed model was used to produce fitted values that are reported as results. Fixed and random models for each analysed characteristic are given in Appendices 3 to 7. The fitted results can be calculated from the fixed models; an example is given in Appendix 3. The LSD's for model terms can be calculated by multiplying average SED's for each model term with factor 1.96 (critical t-value at $\alpha = 0.05$, with more than 60 degrees of freedom). In this report, a difference is considered significant (s) when $P < 0.05$, unless stated otherwise.

3 Results

3.1.1 General

In this report, G2M1S1 is chosen as a reference treatment (shortest crop rotation, no slurry application). All reported results (except clover percentages) are fitted results, using the fixed models from the statistical analysis. Fitted results are largely independent of conditions of specific growing seasons or experimental fields. Trends in results were analysed with the factor vjaar in the fixed model (see Appendices for REML analysis results). The mention of 'a relative increase or decrease' means relative to the trend for a reference treatment. When the trend is negative, a relative increase means a less negative trend.

3.1.2 Dry matter yield

Yield level significantly decreased over the experimental period for all combinations of rotation schemes and slurry application strategies (factor vjaar = -0.05307, Appendix 3). As a result of the In-conversion (see paragraph 2.2.6), the decrease depends on the yield level of a treatment. For G2M1S1, yield of grass-clover, grown for the first year, decreased over the experimental period from 10.0 Mg DM ha^{-1} in year 1 to 6.6 Mg DM ha^{-1} in year 9 (-35%) (Figure 3-1).These results show that, under the conditions of this experiment, N-fixation by white clover could not maintain initial yield level over time. An increase in the grass-clover frequency of a rotation only slightly reduced the negative yield trend for grass-clover. For G4M1S1, yield of grass-clover (third consecutive year) was 7.2 Mg DM ha^{-1} in year 9 compared to 10.2 Mg DM ha^{-1} in year 1 (first year) (-29%). The decrease in yield can not be explained by a decrease in clover percentages (area cover) over the experimental period (Figure 3-2).

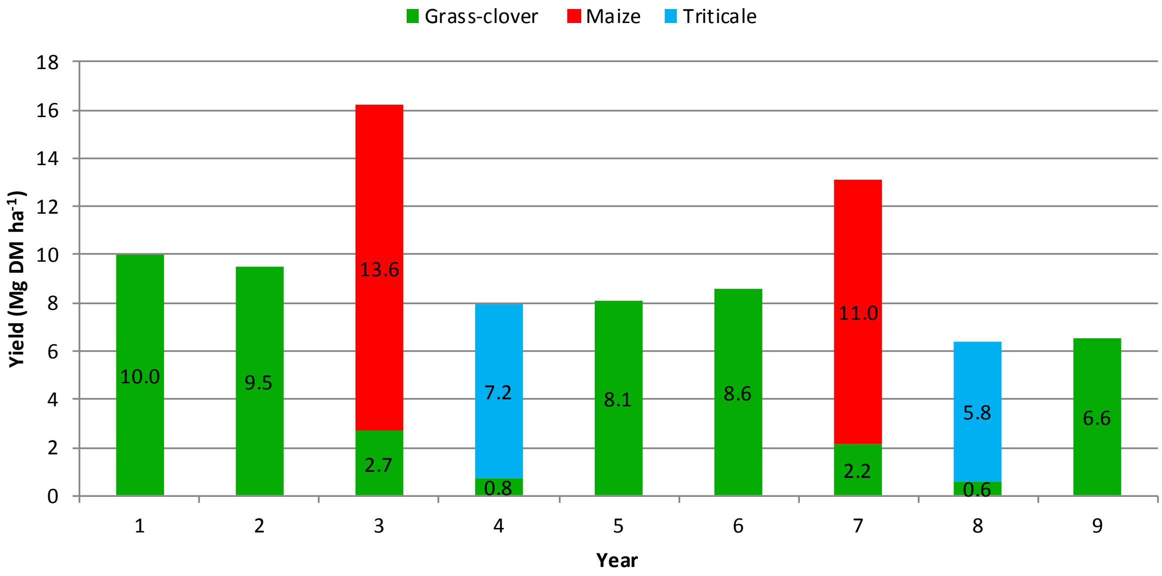

Figure 3-1 Development of (fitted) yield of grass-clover grown in rotation with maize and triticale, for treatment G2M1S1 and with start of the grass-clover period in year 1

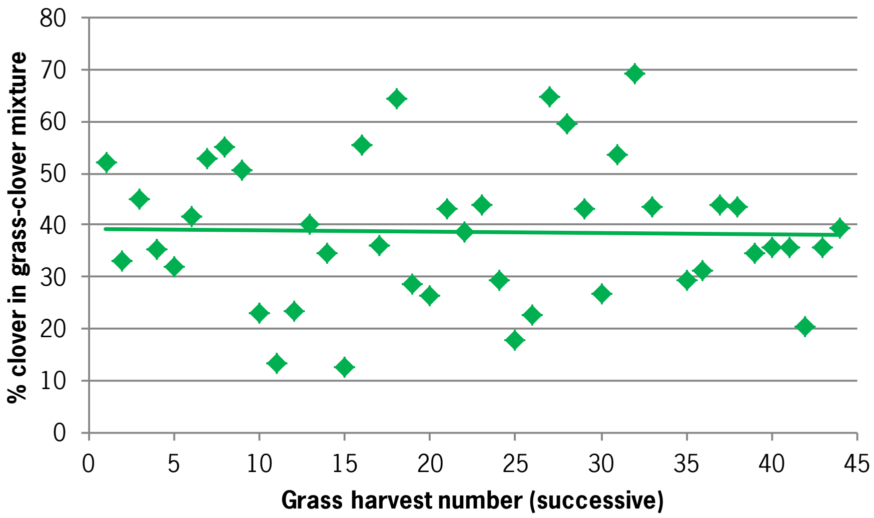

Figure 3-2 Clover percentage (area cover, visually assessed) in the grass-clover mixture throughout the experimental period; mean of all grass-clover plots, hence each data point represents grass-clover swards of a range of ages and slurry application treatments

The negative yield trend could partially be offset by application of cattle slurry, inclusion of more years of grass-clover in the rotation or inclusion of an extra year of maize in the rotation. The increase in yield due to slurry application was significant for all slurry application strategies and increased over time (interaction vjaar.Bemestingsstrategie, Appendix 3). The largest yield increase was realized with S4). For S2 and S3, the extra yield was about half the extra yield realized with S4. This corresponds roughly with the fact that, over time, S2 and S3 received half the amount of slurry compared with S4. There was no significant difference between S2 and S3 in dry matter yield (see factor vjaar.Bemestingsstrategie, Appendix 3). When for G2M1 S4 instead of S1 was applied, the yield of grass-clover decreased from 10.2 Mg DM ha^{-1} $year^{-1}$ in year 1 to 7.9 Mg DM ha^{-1} $year^{-1}$ in year 9 instead of to 6.6 Mg DM ha^{-1} $year^{-1}$.

Inclusion of one or two extra years of grass-clover and/or an extra year of maize in the rotation resulted in an increase of yield for all crops in the rotation over time (interaction vjaar.Mais.Gras, Appendix 3). The largest significant increase was realized with the combined inclusion of one or two extra years of grass-clover and one extra year of maize. Yield increases due to inclusion of an extra year of maize were larger (though not significant) than increases due to inclusion of one or two extra years of grass-clover. Growing four successive years of grass-clover in rotations did not result in additional yield for all crops when compared to three years of grass-clover. For rotation scheme G3M2S4, yield of grass-clover (third consecutive year) was 9.6 Mg DM ha^{-1} in year 9, 0.9 Mg lower compared to the grass-clover yield in year 1 and 3.1 Mg higher compared to the yield of grass-clover in rotation scheme G2M1S1 in year 9 (Figure 3-1, Figure 3-3).

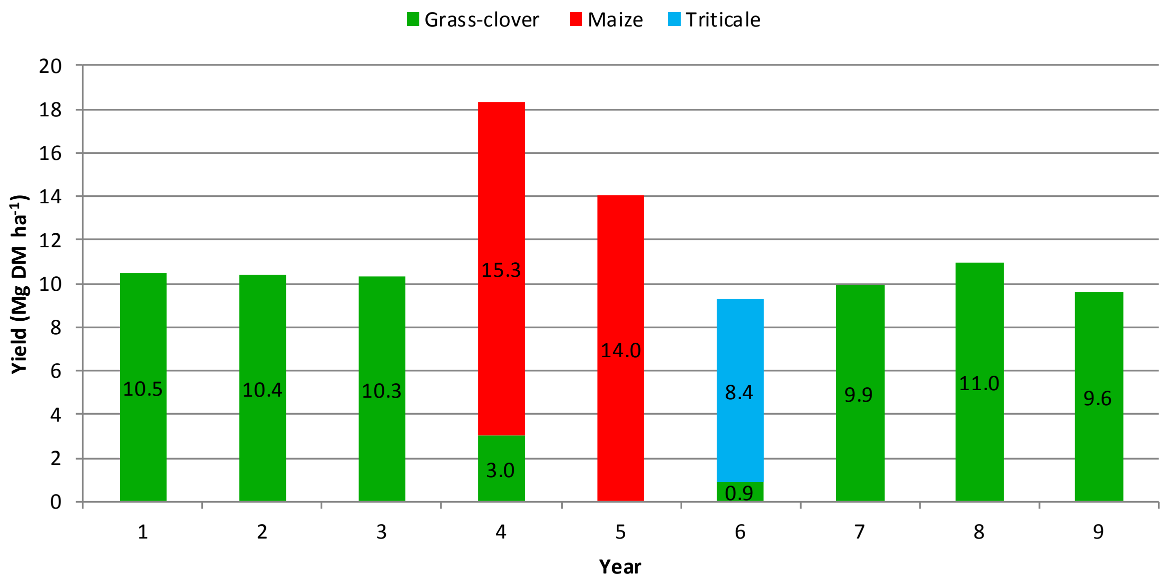

Figure 3-3 Development of (fitted) yield of grass-clover grown in rotation with maize and triticale, for treatment G3M2S4 and with start of the grass-clover period in year 1

When grass-clover was grown for four subsequent years (after at least one year of maize and one year of triticale), yield peaked in the second year and then decreased in the third and fourth year (Figure 3-4). In this example, year 6 was used as the starting year

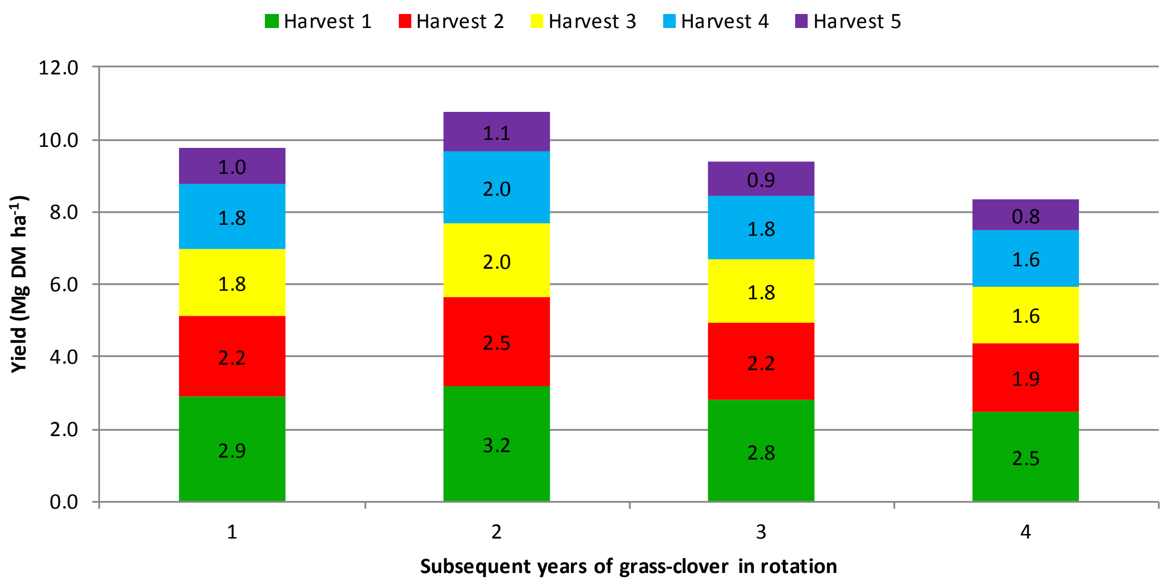

Figure 3-4 (Fitted) yield during four years of grass-clover, per growing season and per successive harvest number with the growing seasons, for treatment G4M2S4 and with start of the first year of grass-clover in year 6 of the experiment

For maize, the highest yield was realized in the first year, when maize was grown after a harvest of grass-clover and cultivation of the existing grass-clover sward. When G3M2S4 started in year 1, maize yielded 15.3 Mg DM ha^{-1} in year 4 (first year of maize) and 14.0 Mg DM ha^{-1} in year 5 (second year of maize). The most obvious explanation for the higher yield in the first year is the availability of extra N for uptake by maize from the cultivated and mineralizing grass-clover sward. Apart from the higher maize yield, additional yield was realized in the first year from the grass-clover harvested before the maize was sown. This yield was 3.0 Mg DM ha^{-1} for G3M2S4 in year 4. With a total annual yield of

18.3 Mg DM ha^{-1}, the first year of maize had the highest relative contribution to the total yield of G3M2S4 (Figure 3-3). The first year of maize also had the highest relative contribution to the total yield of all other treatments. For triticale, the additional yield of grass-clover, sown and harvested after the triticale harvest, was much smaller than for grass-clover harvested before maize was sown. When G3M2S4 started in year 1, yield of the main crop triticale in year 6 was 8.4 Mg DM ha^{-1} and yield of the grass-clover was 0.9 Mg DM ha^{-1}.

When yields are totalled over the entire experimental period, application of S4 (closest to practical conditions) realized the highest yield with crop rotation G2M2 and the lowest with G4M1 (Figure 3-5). The difference in total yield between the highest and lowest yielding crop rotation was 17 Mg DM ha^{-1} over nine years. The highest total maize yield was realized with crop rotation G2M2 and the highest total grass-clover yield with rotation G4M1/G4M2.

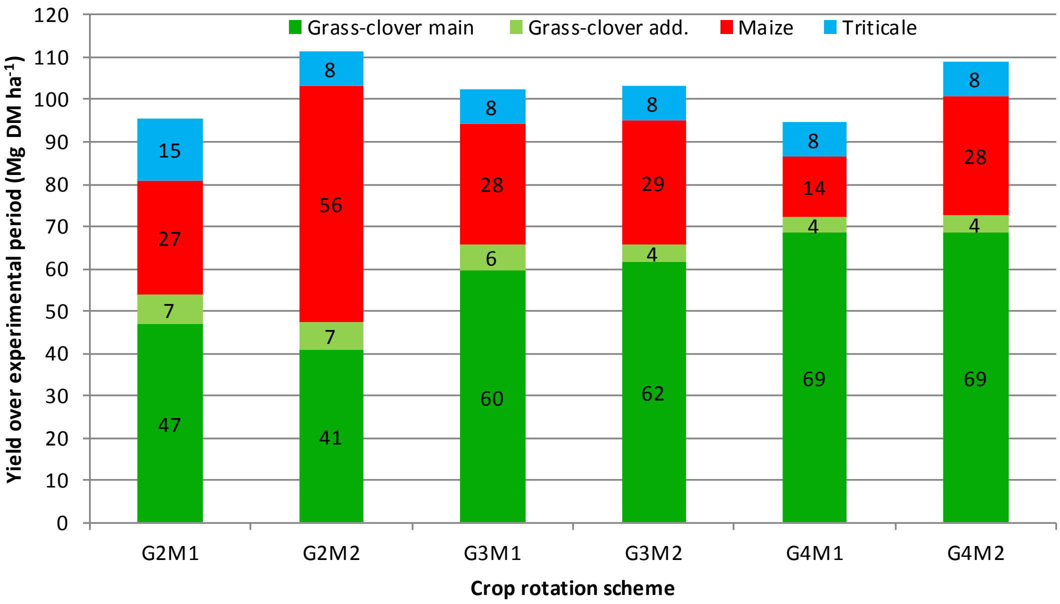

Figure 3-5 (Fitted) total yield (Mg DM ha^{-1}) of all crop rotations over the 9-year experimental period, after start of the first year of grass-clover in year 1 and application of 120 kg N ha^{-1} year^{-1} with cattle slurry to all crops

3.1.3 N uptake

The structure of the fixed model was the same for N uptake as for yield, with the same main factors and interactions of factors. N uptake significantly decreased over the experimental period for all treatments (factor vjaar = -0.07021, Appendix 4). For G2M1S1, N uptake of grass-clover decreased over the experimental period from 290 kg ha^{-1} in year 1 to 165 kg ha^{-1} in year 9 (-43%) (Figure 3-6). The decrease in N uptake was larger than the decrease in dry matter yield (-35%), reflecting a more efficient conversion of N into dry matter at lower levels of N uptake. The negative trend in N uptake could partially be offset by application of cattle slurry, inclusion of more years of grass-clover in the rotation or inclusion of an extra year of maize in the rotation.

The increase in N uptake due to slurry application was significant for all slurry application strategies and significantly increased over time (interaction vjaar.Bemestingsstrategie, Appendix 4). The largest increase was realized with S4. For S2 and S3, the extra uptake compared to S1 was about half the extra uptake realized with S4. This corresponds roughly with the fact that S2 and S3 received half the amount of slurry compared with S4. There was no significant difference in N uptake between S2 and S3 (see factor vjaar.Bemestingsstrategie, Appendix 4). When for G2M1 S4 instead of S1 had been applied, N uptake of grass-clover had decreased from 296 kg N ha^{-1} in year 1 to 202 kg N ha^{-1} in year 9, instead of to 165 kg N ha^{-1} year^{-1}.

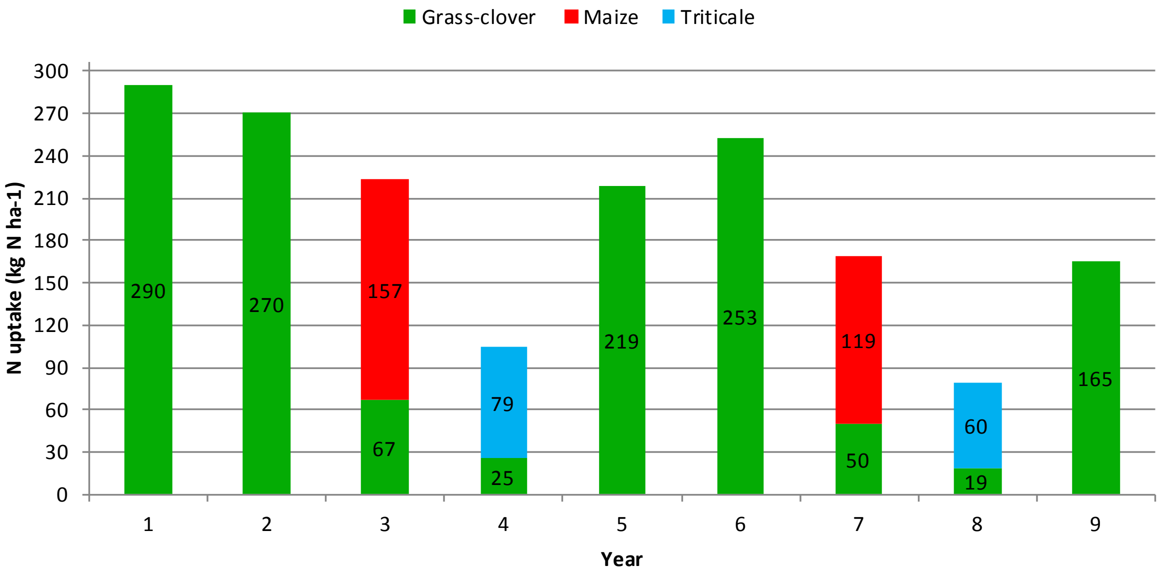

Figure 3-6 Development of (fitted) N uptake of grass-clover grown in rotation with maize and triticale, for treatment G2M1S1 and with start of the grass-clover period in year 1

Compared to G2M1, inclusion of one or two extra years of grass-clover and/or an extra year of maize in the rotation increased N uptake for all crops over the experimental period (interaction vjaar.mais.gras, Appendix 4). The largest significant increase was realized with inclusion of an extra year of maize; addition of extra years of grass-clover did not result in further increases. N uptake increases due to inclusion of an extra year of maize were larger (though not significantly) than increases due to inclusion of one or two extra years of grass-clover. Growing four years of grass-clover in succession in rotations did not result in additional N uptake for all crops when compared to three years of grass-clover. For G3M2S4, N uptake of grass-clover (third year in succession) was 260 kg N ha^{-1} in year 9 (Figure 3-7), 43 kg lower when compared with the N uptake of grass-clover (first year) in year 1 and 95 kg higher compared with the uptake of grass-clover for G2M1S1 in year 9 (Figure 3-6).

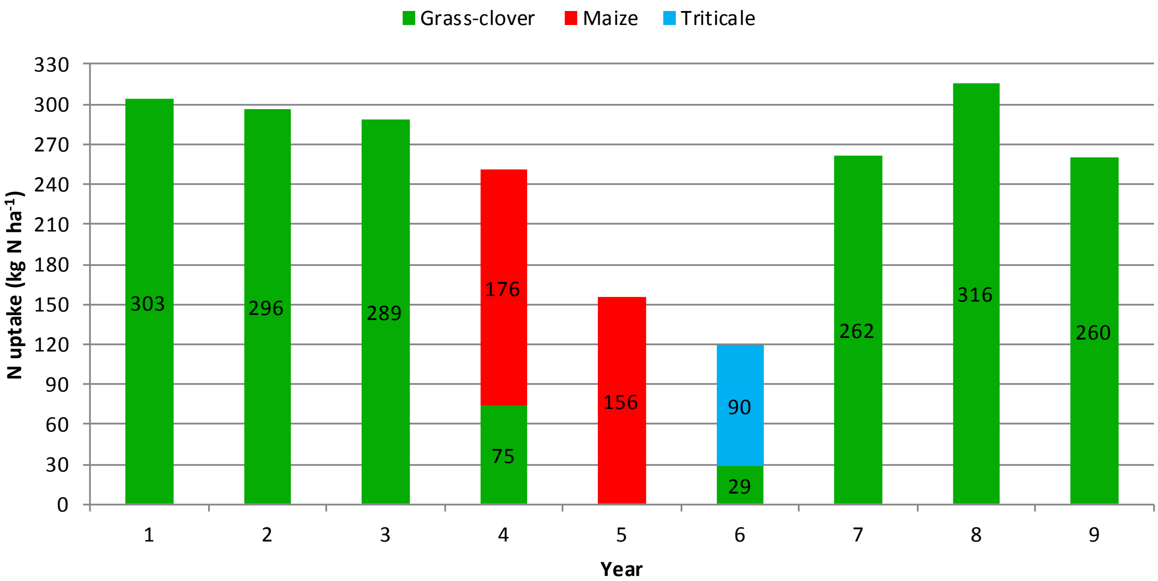

Figure 3-7 Development of (fitted) N uptake of grass-clover grown in rotation with maize and triticale, for treatment G3M2S4 and with start of the grass-clover period in year 1

When grass-clover was grown for four subsequent years (after at least one year of maize and one year of triticale), N uptake peaked in the second year and decreased in the third and fourth year (Figure 3-8). In this example, year 6 of the experimental period was used as the starting year.

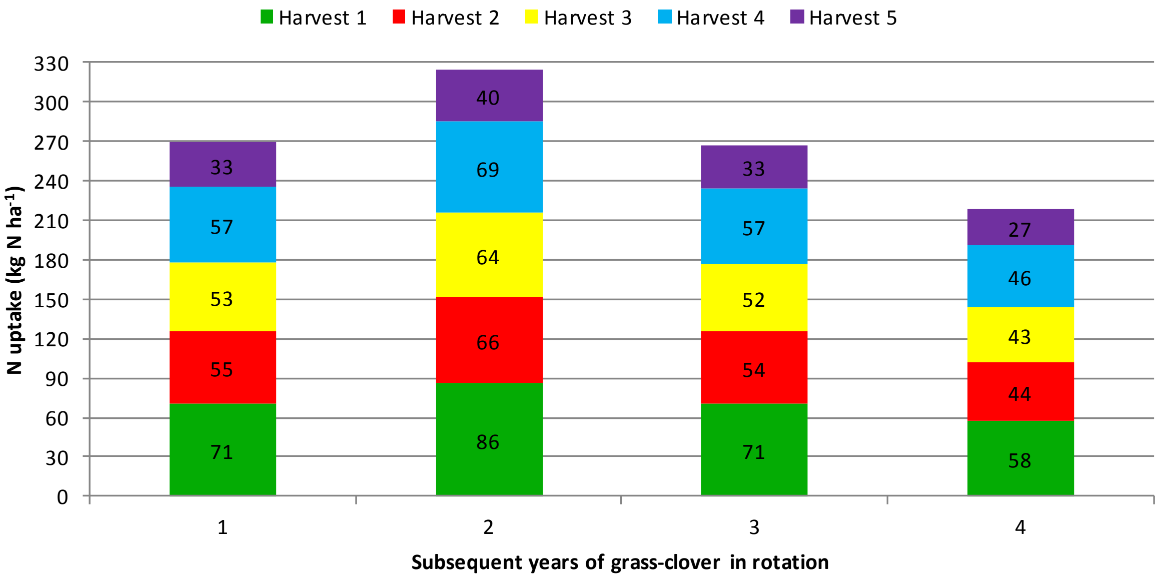

Figure 3-8 (Fitted) N uptake during four years of grass-clover, per growing season and per successive harvest number with the growing seasons, for treatment G4M2S4 and with start of the first year of grass-clover in year 6 of the experiment

For maize, the highest N uptake was realized in the first year, when maize was grown after a harvest of grass-clover and cultivation of the existing grass-clover sward. When G3M2S4 started in year 1, maize N uptake was 176 kg N ha^{-1} in year 4 (first year of maize) and 156 kg N ha^{-1} in year 5 (second year of maize). The most obvious explanation for the higher N uptake in the first year is an extra uptake by maize from the cultivated, N mineralizing grass-clover sward. Apart from the higher N uptake by maize in the first year, additional uptake was realized by the grass-clover harvested before the maize was sown. This uptake was 75 kg N ha^{-1} for G3M2S4 in year 5. With a total annual N uptake of 250 kg N ha^{-1}, the first year of maize had the highest relative contribution to the total N uptake of G3M2S4 (Figure 3-7). The first year of maize had also the highest relative contribution to the total N uptake of all other treatments. For triticale, the additional N uptake by grass-clover, sown and harvested after the triticale harvest, was much smaller than the uptake by the grass-clover harvested before maize was sown. When G3M2S4 started in year 1, N uptake by the main crop triticale in year 6 was 90 kg ha^{-1} and N uptake by the side-crop grass-clover was 29 kg ha^{-1} (Figure 3-7).

When N uptakes are totalled for all crop rotations over the entire experimental period, application of S4 (closest to practical conditions) realized the highest N uptake with crop rotation G3M1 and the lowest with G2M1 (Figure 3-9). The difference in total N uptake between the highest and lowest yielding crop rotation was 320 kg N ha^{-1} over nine years. Differences among crop rotations were smaller for N uptake than for yield. Total N uptake was highest for rotations with three or four years of grass-clover, with little differences among them, and lowest for rotations with two years of grass-clover. Combination of the data for total yield and total N uptake shows that rotation G2M2 had the highest N use efficiency (53 kg of DM produced per kg N taken up) and G4M1 the lowest (42 kg DM kg^{-1} N).

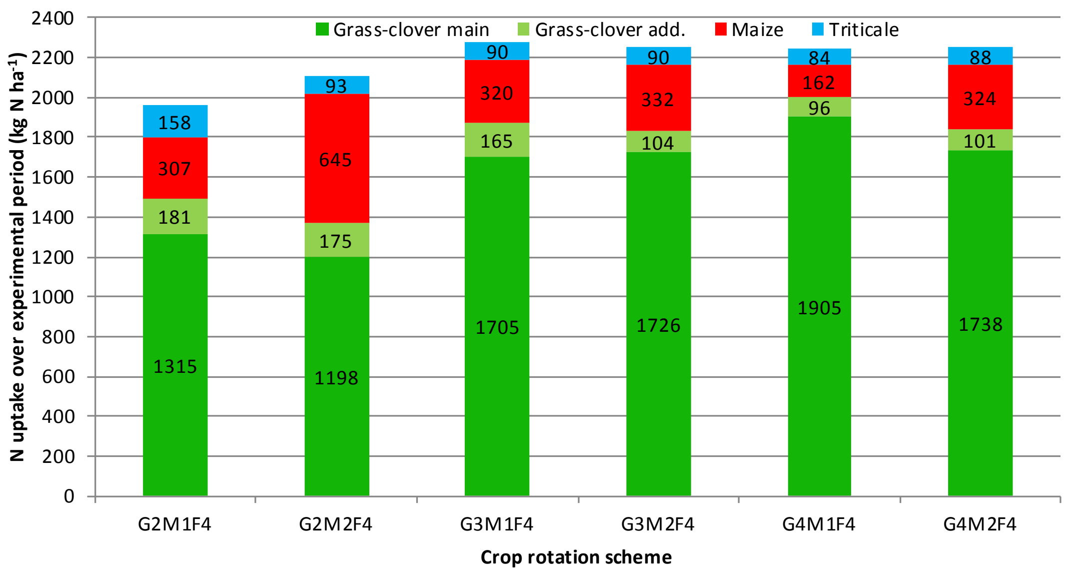

Figure 3-9 (Fitted) total N uptake (kg N ha^{-1}) of all crop rotations over the 9-year experimental period, with start of the first year of grass-clover in year 1 and application of 120 kg N ha^{-1} year^{-1} with cattle slurry to all crops

3.1.4 Mineral N in soil

Mineral N (NH_4-N + NO_3-N) in the soil at the end of the growing season significantly decreased over the experimental period for all treatments (factor vjaar = -0.08381, Appendix 5).

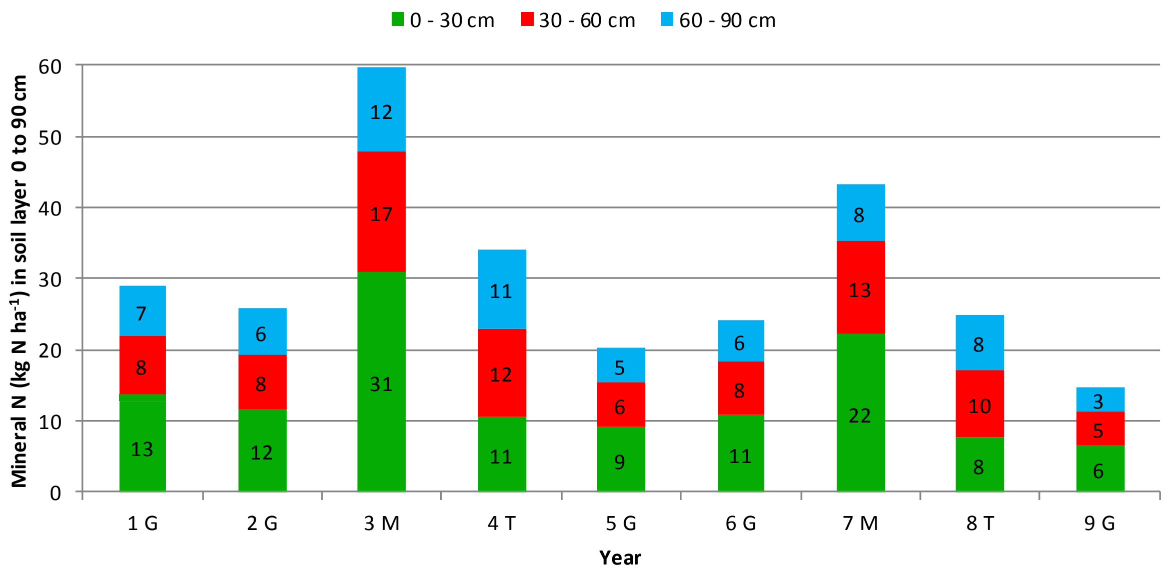

Figure 3-10 Development of (fitted) mineral N (kg NH_4-N + NO_3-N) in soil layer 0 – 90 cm at the end of the growing season, over an experimental period of 9 years, for treatment G2M1S1, with start of the first year of grass-clover in year 1

For G2M1S1, the amount of mineral N in soil after the growing season of grass-clover (first year) decreased over the experimental period from 28 kg N ha^{-1} in year 1 to 15 kg ha^{-1} in year 9 (Figure 3-10). This decrease in mineral N, and therefore in the potential for nitrate leaching, can be explained by a decrease in available N over the experimental period, also resulting in a decrease in N uptake (Figure 3-6) and yield (Figure 3-1).

When maize was grown, the amount of mineral N in soil in autumn was significantly larger than for grass-clover or triticale (factor Type, Appendix 5). The risk of nitrate leaching will therefore be higher after maize than after grass-clover or triticale, and crop rotations with a higher frequency of maize will on average have a larger risk of nitrate leaching.

Slurry application had initially a much smaller impact on mineral N in soil than crop type. Slurry application with S2, S3 and S4 resulted in a significant increase in mineral N in soil over the experimental period, if compared to S1 (Figure 3-11). The absolute levels of increase were initially relatively small but increased in later years. For example, mineral N after growth of the first year of grass-clover increased for G2M1 only with 1 kg N ha^{-1} if S1 was replaced by S4 in year 1, but increased by 3 kg N ha^{-1} in year 9 (Figure 3-11). Mineral N after growth of the first year of maize increased by 4 kg N ha^{-1} when strategy 1 was replaced with strategy 4 in year 3, but with 7 kg ha^{-1} in year 8. These results suggest that, while N availability was decreasing over time, the risk of N leaching increased as a result of slurry application. Generally speaking, the increase in mineral N in soil in autumn due to slurry application can be attributed to increased soil N mineralization resulting from accumulated slurry organic matter. There was no significant difference in mineral N in soil between S2 and S3 (see factor vjaar.Bemestingsstrategie, Appendix 5).

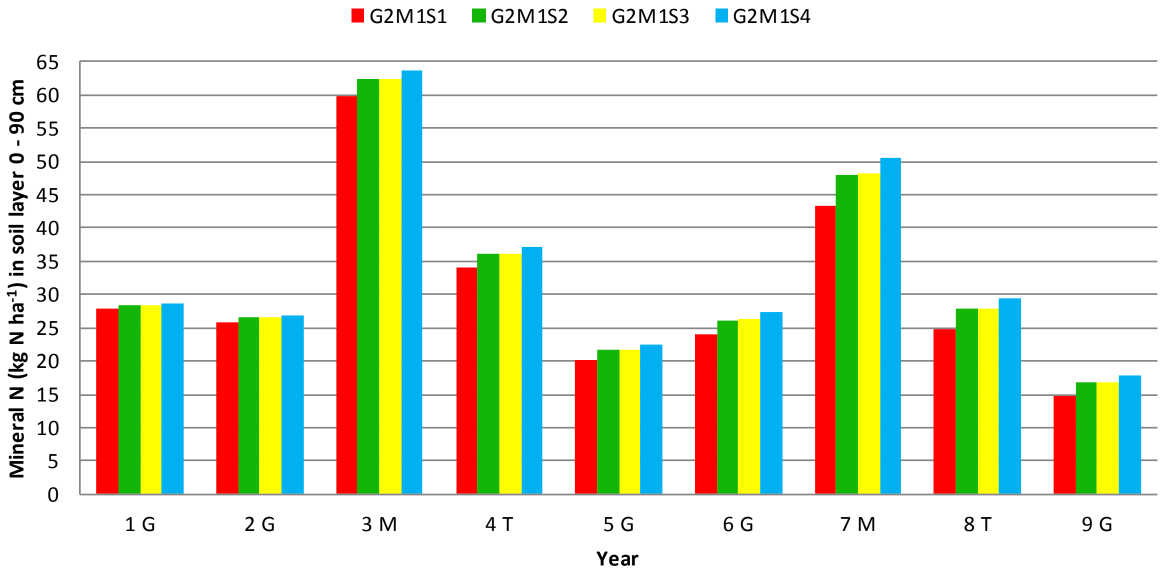

Figure 3-11 Development of (fitted) mineral N (NH_4-N + NO_3-N) in soil layer 0 to 90 cm, at the end of the growing season, over an experimental period of 9 years, for crop rotation scheme G2M1 and four different cattle slurry application strategies, with start of the first year of grass-clover in year 1

Inclusion of extra years of grass-clover or an extra year of maize in the rotation did not result in significant increases of soil mineral N over time for all crops, if compared to G2M1 (interaction vjaar.Gras for grass-clover; interaction vjaar.Maize for maize; Appendix 5). The non-significant changes were a slight increase in mineral N when an extra year of maize was included in the rotation and a slight increase in mineral N when one or two extra years of grass-clover were included.

When grass-clover was grown for several subsequent years, mineral N content significantly changed in these periods (main effects secondjaarGK, thirdjaarGK, fourthjaarGK; Appendix 5). With grass-clover grown for four subsequent years (after at least one year of maize and one year of triticale), mineral N in soil was significantly higher in the second and third year compared to the first year (Figure 3-12). In the fourth year, mineral N was back at the level of the first year. It might be expected that prolonged growth of grass-clover would increase the N leaching potential (as measured by mineral N in soil), but the results do not point in that direction. When maize was grown for two seasons in succession, the amount of mineral N in soil at the end of the second season was not significantly different from the amount at the end of the first season (factor 'msecondjaarMAIS, Appendix 5).

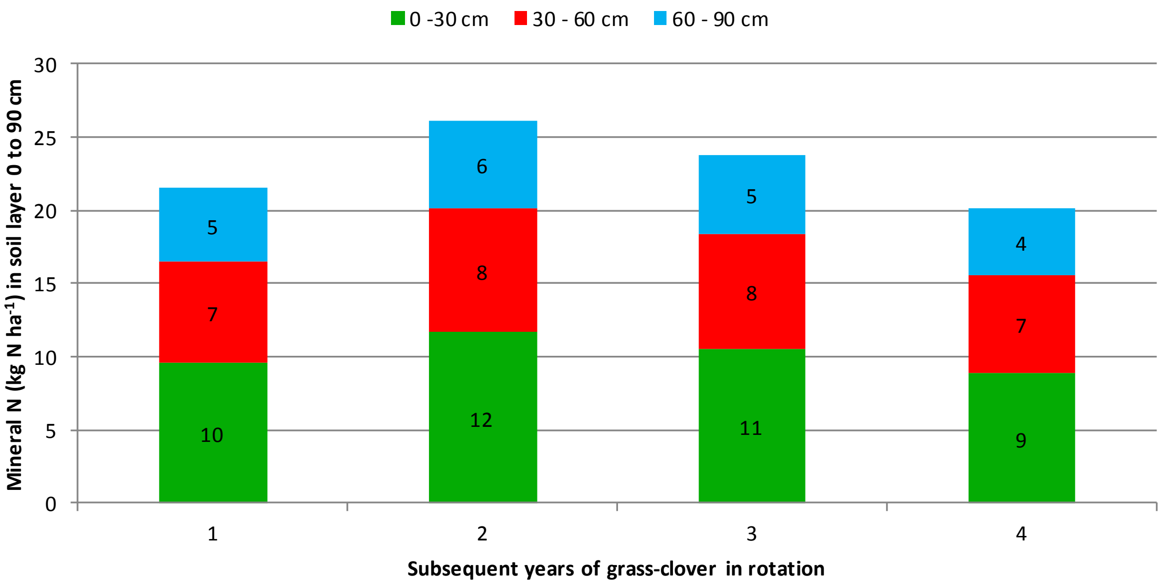

Figure 3-12 Effects of length of the grass-clover period on (fitted) mineral N in soil at the end of the growing season, in soil layer 0 to 90 cm, for G4M2S4 and with start of the first year of grass-clover in year 6 of the experimental period

The general risk of nitrate leaching was in this experiment relatively low for grass-clover, with amounts of mineral N in soil layer 0 to 90 cm not exceeding 30 kg N ha^{-1} (Figure 3-11; Figure 3-12). The situation was different for maize, with amounts of mineral N in soil layer 0 to 90 cm roughly varying between 40 and 60 kg N ha^{-1} (Figure 3-11).

3.1.5 Soil organic matter

None of the main factors or interactions of the full fixed model were significant for soil organic matter (SOM). This lack of significance is probably caused by large fluctuations between years due to (unavoidable) sampling errors. Though none of the treatment effects were significant, the results are shortly discussed to give a general impression. The results presented are fitted using the full fixed model.

For G2M1S1, SOM decreased over the experimental period for soil layers 0 to 30 cm and 30 to 60 cm (Figure 3-13). SOM in layer 0 to 30 cm decreased from 5.0% in year 1 to 4.2% in year 9 (-16%). SOM in layer 30 to 60 cm decreased from 3.9% in year 1 to 3.1% in year 9 (-20%). SOM in layer 60 to 90 cm remained stable at 1.9% to 2.0%. The decrease in SOM is explained by a lower input of organic matter due the decreasing yield level and a lower application of organic matter during the experimental period as compared to the period before.

SOM should benefit from more years of grass-clover in the rotation and application of cattle slurry, because both practices add extra organic matter to the soil. When crop rotation G4M1 was combined with S4, SOM in layer 0 to 30 cm decreased from 5.1% (first year of grass-clover) in year 1 to 4.5% in year 9 (third year of grass-clover) (-11%). Though this decrease is smaller for G4M1S4 than for G2M1S1, it is still considerable. For G4M1S4, SOM in soil layer 30 to 60 cm decreased from 3.9% in year 1 to 3.3% in year 9 (-15%), and SOM in soil layer 60 to 90 cm remained stable at 2.0 to 2.1%. Had these decreases been significant, the conclusion could have been drawn that the observed decline in SOM appears to be rather difficult to prevent under the experimental conditions.

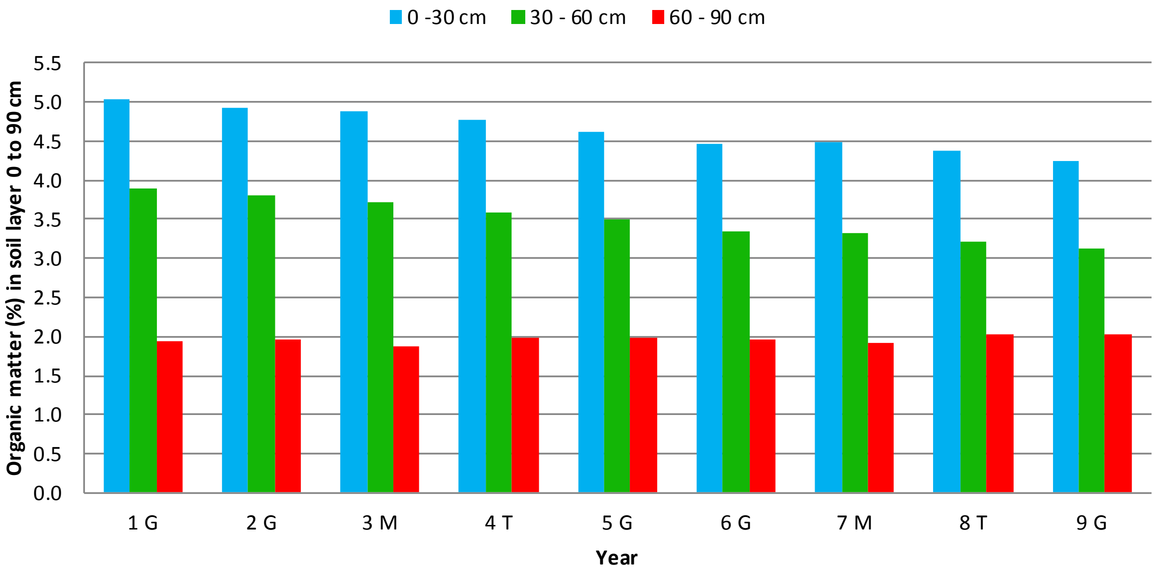

Figure 3-13 Development of (fitted) soil organic matter (%) in soil layer 0 to 30 cm, 30 to 60 cm, and 60 to 90 cm, over an experimental period of 9 years, for treatment G2M1S1, after start of the first year of grass-clover in year 1

3.1.6 Soil organic N

None of the main factors or interactions of the full fixed model were significant for soil organic N (SON). This lack of significance is probably caused by large fluctuations between years due to (unavoidable) sampling errors. Though none of the treatment effects were significant, the results are shortly discussed to give a general impression. The results presented are fitted using the full fixed model.

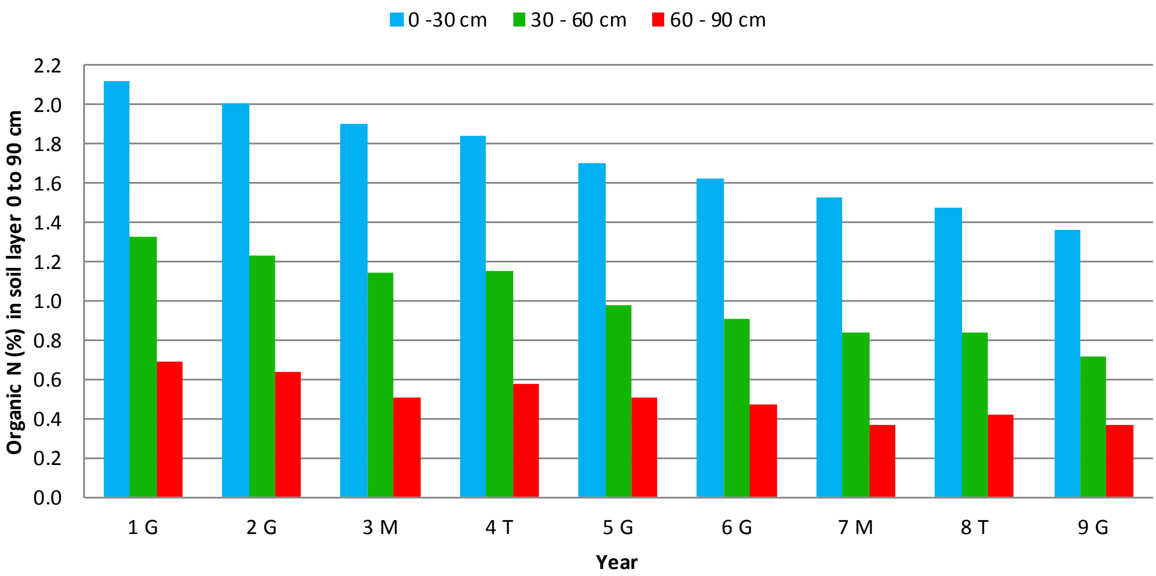

Figure 3-14 Development of (fitted) soil organic N (%) in soil layer 0 to 90 cm over an experimental period of 9 years, for treatment G2M1S1, after start of the first year of grass-clover in year 1

Similar to the development in SOM, SON for G2M1S1 decreased over the experimental period for soil layers 0 to 30 cm and 30 to 60 cm (Figure 3-14; Figure 3-13). However, decreases in SON were much larger than decreases in SOM. Also, whereas SOM was stable in soil layer 60 to 90 cm, SON in this layer decreased in line with the decreases in the other two layers. SON in layer 0 to 30 cm decreased from 2.1% in year 1 to 1.4% in year 9 (-36%). SON in layer 30 to 60 cm decreased from 1.3% in year 1 to 0.7% in year 9 (-46%) and SON in layer 60-90 cm decreased from 0.7% in year 1 to 0.4% in year 9 (-46%). The decrease in SON can be attributed to the lower input level of N during the experimental period as compared to the situation before.

The much larger decrease in SON compared to SOM (and therefore compared to organic C) results in increased C/N-ratio’s. Using an averaged (measured) C concentration in SOM of 53%, the average C/N ratio in soil layer 0 to 30 cm increased for G2M1S1 from 12.6 in year 1 to 16.5 in year 9.

SON should benefit from more years of grass-clover in the rotation and application of cattle slurry, because both practices add extra organic N to the soil. For G4M1S4, SON in layer 0 to 30 cm decreased from 2.1% (first year of grass-clover) in year 1 to 1.5% in year 9 (third year of grass-clover) (-31%). This decrease is only slightly smaller for G4M1S4 than for G2M1S1. For G4M1S4, SON in soil layer 30 to 60 cm decreased from 1.3% in year 1 to 0.8% in year 9 (-42%) and SON in soil layer 60 to 90 cm decreased from 0.7% in year 1 to 0.4% in year 9 (-43%). Had these decreases been significant, the conclusion could have been drawn that the observed decline in SON appears to be rather difficult to prevent given the input levels of the experiment. N fixation by grass-clover was not only not able to maintain initial yield levels (paragraph 3.1.2), but was also not able to maintain soil fertility as measured by SOM and SON.

4 Discussion

4.1 Experimental design

This field experiment lasted nine years, two years less than the intended 11 year period, due to budget cuts from 2010 onwards. An experimental period of nine (or 11) years is relatively short for crop rotation research, bearing in mind that the longest crop rotation scheme was seven years long, and that this scheme was repeated only 1.3 times. The shortest crop rotation was four years long and was repeated 2.3 times. Ideally, all crop rotations should have been repeated several times. The elimination of the last two years has a relatively large influence on the results, because effects of crop rotations and slurry application become more visible over time. The relatively short experimental period therefore has consequences for the conclusions that can be drawn. Whereas the relatively short experimental period can be seen as a weakness of this experiment, the randomisation of the starting year over time and over replications is a strong point. Because of this randomisation, the effects of interactions between specific growing seasons and crop rotation schemes have been limited. This is especially important for the longer lasting crop rotations, which were less repeated.

4.2 Statistical analysis

A strong point of this study is the statistical analysis using REML. This approach takes maximal advantage of all data collected. Splitting the data in a detailed fixed and random model enables to focus more on the systematic effects. All relationships in the fixed model are linear of nature; non-linear relationships were tested but were not significant. A minor drawback of the model, and of the fitted results, is that due to the linearity of relationships there are small inaccuracies in the fitted results, e.g. because the effects of a crop rotation are also fitted for the first years, whereas these effects are mainly realized in later years. For example, the fitted yield for the first year of grass-clover differs a little between treatments, whereas this fitted yield should be the same.

4.3 Decrease in dry matter yield and N uptake

Based on the decrease in dry matter yield and N uptake of G2M1S1, the conclusion can be drawn that the N fixation by the white clover in this experiment was insufficient to maintain original yield levels. However, this conclusion should not be generalized, because in other studies, higher levels of dry matter yield and N uptake are realized for unfertilized mixtures of ryegrass and white clover. In a study by Elgersma and Hassink (1997) on a clay soil, dry matter yield of a mixture of ryegrass and white clover varied between 9.2 and 13.0 Mg ha^{-1}. Apparent N fixation by white clover in the mixture was up to 545 kg N ha^{-1}. Elgersma and Hassink (1997) also recorded a decrease in yield and N uptake in the first three years of the experimental period, followed by an increase in the final year. In a four-year field experiment by Bommele (2007), the average dry matter yield of a mixture of ryegrass and white clover was 11.3 Mg ha^{-1} when grown on former arable land and 10.5 Mg ha^{-1} when grown on ploughed permanent grassland. The average N uptake was 359 and 318 kg N ha^{-1} year^{-1}, respectively. A possible explanation for the decrease in yield in the present study is that, due to prolonged growing of grass-clover (grass-clover was also grown on the three fields before start of the experiment, see paragraph 2.1), an increase in soil-borne pathogens reduced N fixation of the white clover and thus the productivity of the mixture. Lager and Wallenhamar (2003) found increases in white clover yield with more that 200% following soil treatment with fungicides. It is interesting to note that the experiment by Elgersma and Hassink (1997) started on former arable land, meaning that a (relative) absence of soil-borne pathogens may have been responsible for the relatively high N fixation by clover and consequently high yields of the mixture. The experiment by Bommele (2007) also started on fields without previous growing of white clover. An analysis on nematode infestation levels on fields adjacent to the experimental fields in 2009 did not show elevated levels of nematodes or nematode cysts, so it seems less likely that nematodes were responsible for lower grass-clover yields. Another possible explanation for the decrease in yield in this experiment is an insufficient development of white clover in the mixture; a relatively high percentage of clover area cover does not necessarily mean a high percentage of clover in DM. However, though clover development could be lagging behind in the first year after sowing, clover development was usually good in later years (as observed during regular field visits and by clover area cover). A final possible explanation is that N-fixation by the white clover may have been hampered by a lack of certain (micro)-nutrients, such as molybdenum. See for the possible effects of Mo-deficiency and Mo-fertilization on clover yield for instance Vistoso et al. (2012). At start of the experiment in 2002, Mo-concentration in grass-clover DM was determined throughout the year for field 1-2 and averaged 1.2 mg kg^{-1} DM for the treatments without application of cattle slurry and1.6 mg kg^{-1} DM for the treatments with application of cattle slurry. A limited number of data suggests that below a concentration of 4 mg kg^{-1} in clover DM (≈ 2 mg kg^{-1} in grass-clover DM by 50% clover in total DM) the clover yield can decrease due to a decrease in N fixation (pers. comm. Nick van Eekeren). The relatively low Mo-concentration in grass-clover DM in 2002 may have further decreased during the experimental period, since no fertilization of Mo was applied (apart from the Mo that may have been applied with cattle slurry or possibly with the P or K fertilizer).

4.4 Effect of liming on decrease in SOM and SON

Liming in 2005, 2006 and 2009 may have contributed to the decrease in SOM, because liming can stimulate the decomposition of SOM (Whitehead, 1995) by the soil food web. A large application of lime could therefore give a sudden drop in SOM. Measured (not fitted) SOM in soil layer 0 to 30 cm (averaged over all treatments/plots) was 5.1 (2002), 4.8 (2004), 4.9 (2006), 4.4 (2008) and 4.5 (2010), respectively. The decrease between 2006 and 2008 suggests a sudden decrease due to liming, but this seems not plausible. Soil liming was carried out in autumn and soil sampling in spring (except for 2010). Because the liming in October 2005 did not result in a decrease in SOM, measured in February 2006, it seems unlikely that the next liming in November 2006 did result in such a decrease, as measured in March 2008. Furthermore, despite the liming in October 2009, there was no decrease in SOM between soil sampling in March 2008 and November 2010. However, even if liming had accelerated the decrease in SOM and SON, it should have had little to no effect on differences between treatments. The conclusion that a combination of an increase in grass-clover frequency in the crop rotation and application of cattle slurry did little to reduce the negative trend in SOM and SON, therefore remains intact.

In a field experiment by Van Eekeren et al. (2010), located next to a replication of the present experiment on field 13-15, SOM in the soil of a permanent grass-clover mixture decreased on average from 5.2% in 2003 to 4.9% in 2007 in soil layer 0 to 10 cm. This experimental field had been limed in February 2004 and 2006 with 1163 and 1200 kg ha^{-1} of Dolomitic lime, respectively. Van Eekeren et al. (2011) explains this decrease e.g. by a lower input of organic matter (because of a reduced input of manure and/or purely cutting management), a higher decomposition of SOM caused by liming, and a lower accumulation of SOM with a grass-clover mixture as compared to grass in monoculture. A difference between the experimental conditions of Van Eekeren et al. (2010) and the present study is that the crop rotation, and the involved soil cultivation practices, will additionally have stimulated the decomposition of SOM in the present experiment compared to permanent grassland with clover.

4.5 Synthesis

Given the objectives of this experiment, the question remains which combination of crop rotation and slurry application strategy is optimal in terms of yield and N uptake, mineral N in soil at the end of the growing season, and the maintenance of soil fertility. The answer depends partly on the specific requirements of a particular farmer. For example, it makes a difference whether a farmer wants more yield from maize (energy) or more yield from grass-clover (protein) in the ration of his cows. The highest total maize yield was realized with G2M2S4, but the highest total grass-clover yield with G4M1S4 or G4M2S4. From a long-term perspective, crop rotations and slurry application strategies that contribute most to the maintenance of soil fertility (and future soil productivity) are the treatments of choice. However, effects of crop rotation and slurry application on SOM and SON (including the (trend) factor vjaar) were not significant by far. Therefore, it cannot be concluded from the results that a higher frequency of grass-clover in the rotation and extra application of cattle slurry maintains or increases SOM or SON over time. Although significant effects of treatments on soil fertility were not measured in this experiment, it still makes sense to assume that it will be easier to maintain soil fertility with a higher frequency of grass-clover in the rotation, minimal re-sowing events and the addition of extra organic matter with cattle slurry. G4M1S4 (67% grass-clover frequency) would therefore be the treatment of choice, followed by G3M1S4 (60%), G4M2S4 (57%) etc. G4M1S4 had one of the highest N uptakes and the lowest risk of nitrate leaching (due to the lowest frequency of maize growing). However, total yield was second lowest of all crop rotations and the percentage of maize yield in total yield was the lowest of all treatments (Figure 3-5). G4M2, on the other hand, was second best in terms of total yield and the percentage of maize yield in total yield was considerably higher than for G4M1. Re-sowing costs will also be relatively low for G4M1 and G4M2 due to the relatively low maize frequency in rotation. G4M2 therefore seems to be the treatment of choice, both from an environmental/sustainability perspective as well as from an economic perspective. Crop rotation G2M2 had the highest total yield, the highest maize yield and the highest N use efficiency (in terms of kg DM produced per kg N uptake). However, G2M2 also had the highest risk of nitrate leaching and the lowest (assumed) contribution to the maintenance of soil fertility. Though G2M2 is attractive from a (short-term) economic perspective, this rotation scheme appears less attractive considering environmental and sustainability issues. Considering slurry application strategies, application of cattle slurry to all crops (S4) results in maximal yield and also likely in the highest soil fertility. Over time, however, application of S4 slowly increased mineral N in soil in autumn and therefore the nitrate leaching potential. Interestingly, this relative increase was realized despite a general decrease in plant-available N and an increase in N use efficiency. This effect can be explained by a relatively higher N mineralization at the end of the growing season due to a relative increase in SOM and SON for S4 compared to S3, S2 and S1. At the end of the growing season, only part of this mineralized N can be taken up by the crops. There were no significant differences between slurry application strategies S2 and S3 in dry matter yield, N uptake, mineral N in soil and soil fertility. Slurry application to the fodder crops was therefore as effective as slurry application to the grass-clover. It should however be noted that from 2005 onwards, the slurry application to the maize grown after grass-clover was applied to the grass-clover harvested before the maize was sown. This was done to increase the utilization of slurry N (because the decomposing grass-clover sod already supplies a large amount of mineral N to the maize). This practice may have affected the differences between S2 and S3, resulting in an overestimation of yield etc. of S2 compared to S3.

5 Summary of results

Dry matter yield

- Yield level of all treatments decreased over the experimental period due to a decrease in plant-available N, as shown by a decrease of N uptake. For G2M1S1 (chosen as a reference treatment), yield of the first year of grass-clover decreased from 10.0 Mg DM ha^{-1} in year 1 to 6.6 Mg DM ha^{-1} in year 9

- N-fixation by white clover, grown in mixture with ryegrass, could not maintain initial yield levels, not for G2M1S1 but also not for G4M1S1. For G4M1S1, grass-clover yield decreased from 10.2 Mg DM ha^{-1} in year 1 to 7.2 Mg DM ha^{-1} in year 9. The decrease in yield cannot be explained by a decrease in clover percentages (area cover) over the experimental period

- The negative yield trend for G2M1 could largely be offset by a combination of application of cattle slurry to all crops (= each year), addition of one or two more years of grass-clover to the rotation and addition of an extra year of maize to the rotation

- The relative yield increase (yield increase relative to a negative trend) due to slurry application was largest when 120 kg N ha^{-1} with cattle slurry was applied each year. Slurry application effects increased over time. For G2M1S4 instead of G2M1S1, yield of grass-clover decreased from 10.2 Mg DM ha^{-1} in year 1 to 7.9 Mg DM ha^{-1} in year 9 (instead of 6.6 Mg DM ha^{-1})

- The highest relative yield increase due to addition of extra years of grass-clover and an extra year of maize to G2M1 was realized with one or two extra years of grass-clover and one extra year of maize (G3M2)

- For G3M2S4, yield of grass-clover (third successive grass-clover year) was 9.6 Mg DM ha^{-1} in year 9, only 0.9 Mg lower compared to the grass-clover yield in year 1 (first grass-clover year) and 3.1 Mg higher compared with the grass-clover yield for G2M1S1 in year 9

- When grass-clover was grown for four subsequent years (G4), yield peaked in the second year and decreased thereafter. Yield in the fourth year was lower than yield in the preceding years

- Maize grown for the first year had the highest relative contribution to the total yield of a crop rotation, not only because maize yield was highest in the first year, but also because additional yield was realized with harvest of the grass-clover grown before the maize was sown

- Over the entire experimental period, the highest total yield of crop rotations with slurry application each year (S4) was realized with G2M2 and the lowest with G4M1. The difference in total yield between these two rotations was 17 Mg DM ha^{-1} over nine years. The highest total maize yield was realized with G2M2 and the highest total grass-clover yield with G4M1/G4M2

N uptake

- Treatment effects on N uptake were roughly comparable to treatment effects on yield. The trend in N uptake was more negative than the trend in yield, reflecting a higher N use efficiency at lower N availability

- Over the entire experimental period, the highest total N uptake of crop rotations with slurry application each year (S4) was realized with rotations with three or four years of grass-clover. Differences in total N uptake between treatments were smaller than differences in total yield. With S4, G2M2 had highest average N use efficiency over the experimental period (53 kg of DM produced per kg N taken up) and G4M1 the lowest (42 kg DM kg^{-1} N)

Soil mineral N

- Mineral N in soil layer 0 to 90 cm at the end of the growing season decreased over the experimental period for all crops, along with the decrease in N uptake. For G2M1S1, mineral N after growth of the first year of grass-clover decreased from 28 kg N ha^{-1} in year 1 to 15 kg N ha^{-1} in year 9

- Crop type had a large effect on mineral N in soil, with the highest level of mineral N after maize, followed by triticale and grass-clover. Crop rotations with a higher frequency of maize will therefore have a higher risk of nitrate leaching

- Application of cattle slurry had a much smaller effect on mineral N in soil than crop type. Slurry application (strategy 2, 3 and 4) resulted in a relative increase (relative to negative trend) of mineral N for all crops, and this effect also increased over time. The relative increase in mineral N was realized despite the negative trend in N uptake, with N from slurry organic matter becoming available at times when plant N uptake already decreases (autumn)

- The addition of extra years of grass-clover or an extra year of maize to the rotation did not result in changes in mineral N over time for all crops. However, when grass-clover was grown for four subsequent years, mineral N in soil increased up to the third year, but decreased thereafter. These results suggest that prolonged growth of grass-clover (more than three years) will not necessarily result in a higher potential for nitrate leaching

- When maize was grown for two years in succession, mineral N at the end of the second season was not different from the level at the end of the first season

- The general risk of nitrate leaching was low for grass-clover, with mineral N in layer 0 to 90 cm not exceeding 30 kg ha^{-1} during the experimental period. For maize, the risk was considerably higher, with amounts varying between 40 to 60 kg N ha^{-1}

SOM and SON

- There were no significant effects of treatments on the development of SOM or SON, probably due to (unavoidable) large sampling errors

- For G2M1S1, SOM decreased between year 1 and year 9 from 5.0 to 4.2% for soil layer 0 to 30 cm, from 3.9 to 3.1% for soil layer 30 to 60 cm and remained stable at 1.9 to 2.0% for soil layer 60 to 90 cm. These decreases can be explained by a lower input of organic matter during the experimental period compared to the period before, as a result of decreasing yields and a lower application of organic matter.

- For G4M1S4 instead of G2M1S1, SOM in layer 0 to 30 cm still decreased over the experimental period, from 5.1% in year 1 to 4.5% in year 9, though the decrease was smaller than for G2M1S1. These results suggest that the observed decline in SOM may be rather difficult to prevent under the experimental conditions

- Decreases in SON were larger than decreases in SOM. For G2M1S1, SON decreased between year 1 and year 9 from 2.1 to 1.4% for soil layer 0 to 30 cm, from 1.3% to 0.7% for layer 30 to 60 cm, and from 0.7% to 0.4% for layer 60 to 90 cm

- For G4M1S4, SON in soil layer 0 to 30 cm decreased from 2.1% in year 1 to 1.5% in year 9. This decrease is only slightly smaller for G4M1S4 than for G2M1S1

- When the decreases in SOM and SON had been significant, the conclusion could have been drawn that, under the experimental conditions, the observed decline in both soil characteristics appears to be rather difficult to prevent at the experimental input levels. N fixation by grass-clover was not only not able to maintain initial yield levels, but was also not able to maintain initial soil fertility as measured by SOM and SON

Synthesis

- G2M2 had the highest total yield and total maize yield of all crop rotations, but also the highest potential for nitrate leaching and the lowest (assumed) contribution to the maintenance of soil quality

- G4M1 had the highest grass-clover frequency in the rotation (67%) and the lowest maize frequency (17%), so G4M1 will result in maximal maintenance of soil fertility and the lowest potential for nitrate leaching. G4M1 had also the lowest total yield and the lowest total maize yield.

- G4M2 seems to be the best compromise, having one of the highest inputs of organic matter by the grass-clover (maintenance of soil fertility), a total yield only slightly lower than for G2M2 and an average nitrate leaching potential.

- Application of cattle slurry to all crops (S4) resulted in maximal yield and also likely in the highest soil fertility. However, S4 also slowly increased mineral N in soil at the end of the growing season over time, and therefore also increased the nitrate leaching potential. This increase was relatively small within the timeframe of this experiment, but may become larger over time.

- There were no significant differences between slurry application strategies S2 and S3 in dry matter yield, N uptake, mineral N in soil and soil fertility. Slurry application to the fodder crops was therefore as effective as slurry application to the grass-clover.

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Appendices

Appendix 1. Treatment per plot per year

Field/replication 1

Plot	Treatment	Year								
		2002	2003	2004	2005	2006	2007	2008	2009	2010
1	G3M2S3[1)]	M[2)]	M	T	G	G	G	M	M	T
2	G2M2S3	M	T	G	G	M	M	T	G	G
3	G2M1S4	T	G	G	M	T	G	G	M	T
4	G3M2S1	M	M	T	G	G	G	M	M	T
5	G2M1S2	T	G	G	M	T	G	G	M	T
6	G3M1S3	M	T	G	G	G	M	T	G	G
7	G2M1S1	T	G	G	M	T	G	G	M	T
8	G3M1S1	M	T	G	G	G	M	T	G	G
9	G4M2S2	G	M	M	T	G	G	G	G	M
10	G3M1S4	M	T	G	G	G	M	T	G	G
11	G4M2S4	G	M	M	T	G	G	G	G	M
12	G2M1S3	T	G	G	M	T	G	G	M	T
13	G2M2S1	M	T	G	G	M	M	T	G	G
14	G3M2S4	M	M	T	G	G	G	M	M	T
15	G4M2S3	G	M	M	T	G	G	G	G	M
16	G2M2S2	M	T	G	G	M	M	T	G	G
17	G4M1S2	G	M	T	G	G	G	G	M	T
18	G4M1S3	G	M	T	G	G	G	G	M	T
19	G4M1S4	G	M	T	G	G	G	G	M	T
20	G3M1S2	M	T	G	G	G	M	T	G	G
21	G4M2S1	G	M	M	T	G	G	G	G	M
22	G3M2S2	M	M	T	G	G	G	M	M	T
23	G4M1S1	G	M	T	G	G	G	G	M	T
24	G2M2S4	M	T	G	G	M	M	T	G	G

1) Treatment G3M2S3 refers to a crop rotation with 3 consecutive years of grass-clover, followed by 2 years of maize and one year of triticale, with application of slurry application strategy 3
2) G = grass-clover, M = maize, T = triticale

Field/replication 2

Plot	Treatment	Year								
		2002	2003	2004	2005	2006	2007	2008	2009	2010
25	G3M2S1[1)]	M[2)]	T	G	G	G	M	M	T	G
26	G4M1S3	M	T	G	G	G	G	M	T	G
27	G4M2S3	M	M	T	G	G	G	G	M	M
28	G3M1S1	T	G	G	G	M	T	G	G	G
29	G2M2S2	T	G	G	M	M	T	G	G	M
30	G2M2S1	T	G	G	M	M	T	G	G	M
31	G2M1S2	G	G	M	T	G	G	M	T	G
32	G4M1S2	M	T	G	G	G	G	M	T	G
33	G2M1S3	G	G	M	T	G	G	M	T	G
34	G2M1S4	G	G	M	T	G	G	M	T	G
35	G2M2S3	T	G	G	M	M	T	G	G	M
36	G4M1S1	M	T	G	G	G	G	M	T	G
37	G3M1S2	T	G	G	G	M	T	G	G	G
38	G3M1S3	T	G	G	G	M	T	G	G	G
39	G4M1S4	M	T	G	G	G	G	M	T	G
40	G3M2S3	M	T	G	G	G	M	M	T	G
41	G2M2S4	T	G	G	M	M	T	G	G	M
42	G3M1S4	T	G	G	G	M	T	G	G	G
43	G2M1S1	G	G	M	T	G	G	M	T	G

Plot	Treatment	Year								
		2002	2003	2004	2005	2006	2007	2008	2009	2010
44	G3M2S2	M	T	G	G	G	M	M	T	G
45	G4M2S4	M	M	T	G	G	G	G	M	M
46	G4M2S1	M	M	T	G	G	G	G	M	M
47	G4M2S2	M	M	T	G	G	G	G	M	M
48	G3M2S4	M	T	G	G	G	M	M	T	G

[1)] Treatment G3M2S3 refers to a crop rotation with 3 consecutive years of grass-clover, followed by 2 years of maize and one year of triticale, with application of slurry application strategy 3
[2)] G = grass-clover, M = maize, T = triticale

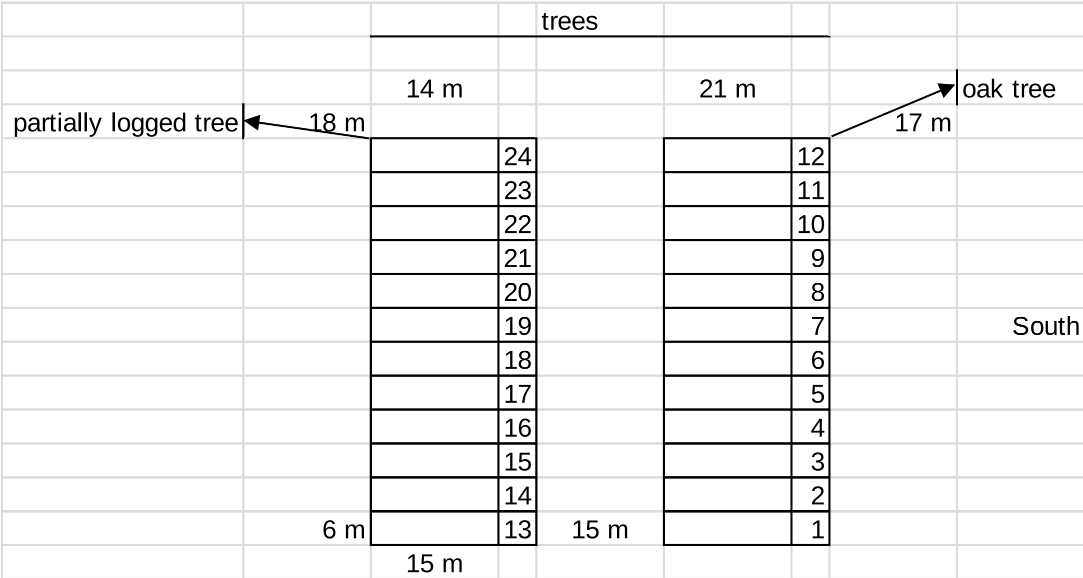
Field/replication 3

Plot	Treatment	Year								
		2002	2003	2004	2005	2006	2007	2008	2009	2010
49	G3M1S4[1)]	G[2)]	G	G	M	T	G	G	G	M
50	G2M1S3	G	M	T	G	G	M	T	G	G
51	G3M2S1	T	G	G	G	M	M	T	G	G
52	G4M1S3	T	G	G	G	G	M	T	G	G
53	G3M1S2	G	G	G	M	T	G	G	G	M
54	G4M2S1	M	T	G	G	G	G	M	M	T
55	G2M2S4	G	G	M	M	T	G	G	M	M
56	G4M1S4	T	G	G	G	G	M	T	G	G
57	G3M1S3	G	G	G	M	T	G	G	G	M
58	G4M2S4	M	T	G	G	G	G	M	M	T
59	G4M2S3	M	T	G	G	G	G	M	M	T
60	G4M1S1	T	G	G	G	G	M	T	G	G
61	G2M2S3	G	G	M	M	T	G	G	M	M
62	G3M1S1	G	G	G	M	T	G	G	G	M
63	G2M2S2	G	G	M	M	T	G	G	M	M
64	G3M2S3	T	G	G	G	M	M	T	G	G
65	G2M1S4	G	M	T	G	G	M	T	G	G
66	G2M1S2	G	M	T	G	G	M	T	G	G
67	G4M2S2	M	T	G	G	G	G	M	M	T
68	G4M1S2	T	G	G	G	G	M	T	G	G
69	G2M2S1	G	G	M	M	T	G	G	M	M
70	G3M2S2	T	G	G	G	M	M	T	G	G
71	G3M2S4	T	G	G	G	M	M	T	G	G
72	G2M1S1	G	M	T	G	G	M	T	G	G

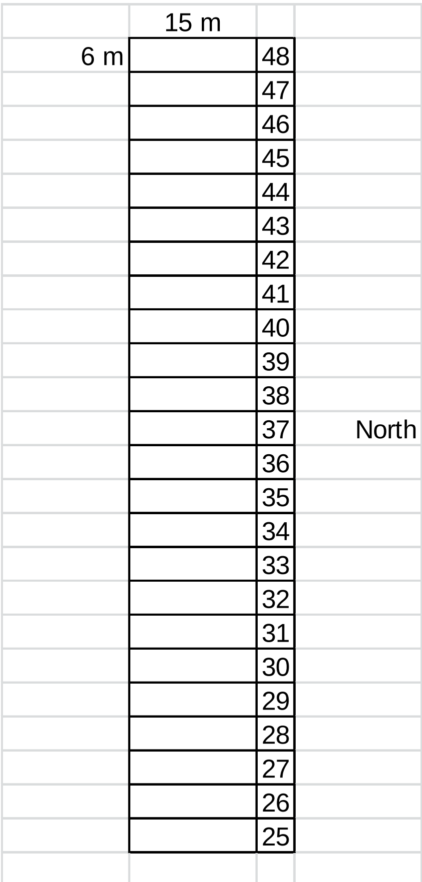
[1)] Treatment G3M2S3 refers to a crop rotation with 3 consecutive years of grass-clover, followed by 2 years of maize and one year of triticale, with application of slurry application strategy 3
[2)] G = grass-clover, M = maize, T = triticale

Appendix 2. Layout of the plots per field

Field 1-2, 'de Es' (first replication)



Field 13-15 (second replication)



Field 23-24 (third replication)

	15 m					
6 m		72	15 m		60	
		71			59	
		70			58	
		69			57	
		68			56	
		67			55	North
		66			54	
		65			53	
		64			52	
		63			51	
		62			50	
		61			49	

Appendix 3. REML analysis yield

Legend of model terms for yield and N uptake:

Oogstnr:	*effect of number of consecutive harvest in a growing season (e.g. for the first year of maize, pre-harvest of grass-clover is harvest nr. 1 and main crop maize is harvest nr. 2*
Vjaar:	*effect of number of years the experiment is running (vjaar is 1 for 2002, 2 for 2003, etc.)*
Type:	*effect of type of main crop grown (grass-clover, maize or triticale)*
Stype:	*effect of type of crop grown (irrespective of being main crop or side-crop)*
Bemestingsstrategie:	*effect of slurry application strategy*
Naoogst GK:	*effect for harvest of grass-clover after triticale*
TnaGK:	*effect for harvest of triticale after grass-clover instead of maize (only applies to the first year of the experiment)*
SecondjaarGK:	*effect of growing grass-clover for the second year*
SecondjaarGK:	*effect of growing grass-clover for the third year*
SecondjaarGK:	*effect of growing grass-clover for the fourth year*
Perceel:	*effect of field*
Jaar:	*effect of year*
Blok:	*effect of replication/block*

Calculation example: yield of the second harvest of the third year of grass-clover in 2009 for treatment G3M1S4 with start of the first year of grass-clover in 2002:

= inverse ln (8.052 (Constant) – 0.2579 (second harvest nr of grass-clover) + (-0.05307 x 8) (8 years effect of vjaar) + 0.02124 x 8 (8 years effect of slurry application strategy 4) + (0.01368 x 8) (8 years effect of interaction between 3 years of grass-clover and 1 year of maize) – 0.0126 (effect of the third year of grass-clover)) = inverse ln (7.6363) = 2072 kg ha^{-1}.

Response variate: LOG(yield)

Fixed model: Constant + Oogstnr.stype + vjaar + vjaar.Bemestingsstrategie + vjaar.Mais.Gras + naoogstGK + TnaGK + secondjaarGK + thirdjaarGK + fourthjaarGK

Random model: Perceel + Jaar + Perceel.Blok + Perceel.Jaar + Perceel.Blok.Veldnr + Perceel.Blok.Jaar + Perceel.Blok.Veldnr.Jaar + Perceel.Blok.Veldnr.Jaar.Oogstnr + Jaar.Type + Jaar.Oogstnr.Type + Jaar.Type.stype + Jaar.Oogstnr.Type.stype

Number of units: 2205 (35 units excluded due to zero weights or missing values)

Perceel.Blok.Veldnr.Jaar.Oogstnr used as residual term
Sparse algorithm with AI optimisation
Covariates not centred

<u>Estimated variance components</u>

Random term	component	s.e.
Perceel	0.00622	0.00770
Jaar	0.00905	0.01099
Perceel.Blok	0.00022	0.00149
Perceel.Jaar	0.00829	0.00424
Perceel.Blok.Veldnr	0.00312	0.00108
Perceel.Blok.Jaar	0.01168	0.00262
Perceel.Blok.Veldnr.Jaar	0.00000	bound
Jaar.Type	0.00141	0.00926
Jaar.Oogstnr.Type	0.00000	bound
Jaar.Type.stype	0.00000	bound
Jaar.Oogstnr.Type.stype	0.05454	0.01117

Residual variance model

Term	Factor	Model(order)	Parameter	Estimate	s.e.
Perceel.Blok.Veldnr.Jaar.Oogstnr					
		Identity	Sigma2	0.0728	0.00234

Tests for fixed effects

Sequentially adding terms to fixed model

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Oogstnr.stype	1108.34	7	156.84	62.4	<0.001
vjaar	4.16	1	4.16	7.1	0.080
vjaar.Bemestingsstrategie	30.14	3	10.05	120.7	<0.001
vjaar.Mais.Gras	9.06	5	1.81	20.1	0.156
naoogstGK	61.24	1	61.24	56.0	<0.001
TnaGK	7.11	1	7.11	29.8	0.012
secondjaarGK	12.43	1	12.43	95.5	<0.001
thirdjaarGK	0.05	1	0.05	87.8	0.818
fourthjaarGK	3.19	1	3.19	82.3	0.078

Dropping individual terms from full fixed model

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Oogstnr.stype	882.62	7	124.89	62.4	<0.001
vjaar.Bemestingsstrategie	30.12	3	10.04	120.7	<0.001
vjaar.Mais.Gras	9.44	5	1.89	20.1	0.142
naoogstGK	60.57	1	60.57	56.0	<0.001
TnaGK	6.56	1	6.56	29.8	0.016
secondjaarGK	8.15	1	8.15	95.5	0.005
thirdjaarGK	0.07	1	0.07	87.8	0.794
fourthjaarGK	3.19	1	3.19	82.3	0.078

Message: denominator degrees of freedom for approximate F-tests are calculated using numerical derivatives ignoring fixed/boundary/singular variance parameters.

3459 vdisp [pr=eff,dev]

Deviance: -2*Log-Likelihood

Deviance	d.f.
-2952.27	2171

Note: deviance omits constants which depend on fixed model fitted.

Table of effects for Constant

8.052 Standard error: 0.1237

Table of effects for Oogstnr.stype

stype Oogstnr	gk	m	t
1	0.0000	1.5423	1.0363
2	-0.2579	1.6226	*
3	-0.4603	*	*
4	-0.4611	*	*
5	-1.0975	*	*

Standard errors of differences

Average:	0.1155
Maximum:	0.1345
Minimum:	0.09659

Average variance of differences: 0.01346

Table of effects for vjaar

-0.05307 Standard error: 0.019183

Table of effects for vjaar.Bemestingsstrategie

Bemestingsstrategie	1	2	3	4
	0.00000	0.00945	0.01290	0.02124

Standard errors of differences

Average:	0.003924
Maximum:	0.003926
Minimum:	0.003923

Table of effects for vjaar.Mais.Gras

Gras Mais	2	3	4
1	0.00000	0.01368	0.01214
2	0.01619	0.02267	0.01848

Standard errors of differences

Average:	0.008140
Maximum:	0.008491
Minimum:	0.007779

Table of effects for naoogstGK

-0.9481 Standard error: 0.12182

<u>Table of effects for TnaGK</u>

0.5521 Standard error: 0.21559

<u>Table of effects for secondjaarGK</u>

0.1099 Standard error: 0.03850

<u>Table of effects for thirdjaarGK</u>

-0.01262 Standard error: 0.048192

<u>Table of effects for fourthjaarGK</u>

-0.1178 Standard error: 0.06602

Appendix 4. REML analysis N uptake

Response variate:	LOG(N uptake)
Fixed model:	Constant + Oogstnr.stype + vjaar + vjaar.Bemestingsstrategie + vjaar.Mais.Gras + naoogstGK + TnaGK + secondjaarGK + thirdjaarGK + fourthjaarGK
Random model:	Perceel + Jaar + Perceel.Blok + Perceel.Jaar + Perceel.Blok.Veldnr + Perceel.Blok.Jaar + Perceel.Blok.Veldnr.Jaar + Perceel.Blok.Veldnr.Jaar.Oogstnr + Jaar.Type + Jaar.Oogstnr.Type + Jaar.Type.stype + Jaar.Oogstnr.Type.stype
Number of units:	2199 (41 units excluded due to zero weights or missing values)

Perceel.Blok.Veldnr.Jaar.Oogstnr used as residual term
Sparse algorithm with AI optimisation
Covariates not centred

Estimated variance components

Random term	component	s.e.
Perceel	0.00715	0.00950
Jaar	0.00813	0.01268
Perceel.Blok	0.00180	0.00312
Perceel.Jaar	0.01040	0.00583
Perceel.Blok.Veldnr	0.00536	0.00184
Perceel.Blok.Jaar	0.02129	0.00444
Perceel.Blok.Veldnr.Jaar	0.00804	0.00214
Jaar.Type	0.00000	bound
Jaar.Oogstnr.Type	0.00000	bound
Jaar.Type.stype	0.01361	0.01378
Jaar.Oogstnr.Type.stype	0.04561	0.01068

Residual variance model

Term	Factor	Model(order)	Parameter	Estimate	s.e.
Perceel.Blok.Veldnr.Jaar.Oogstnr		Identity	Sigma2	0.0795	0.00281

Tests for fixed effects

Sequentially adding terms to fixed model

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Oogstnr.stype	267.69	7	37.73	60.7	<0.001
vjaar	6.53	1	6.53	7.0	0.038
vjaar.Bemestingsstrategie	20.23	3	6.74	122.2	<0.001
vjaar.Mais.Gras	8.31	5	1.66	23.4	0.183
naoogstGK	25.42	1	25.42	54.5	<0.001
TnaGK	5.70	1	5.70	62.6	0.020
secondjaarGK	21.68	1	21.68	106.5	<0.001
thirdjaarGK	1.30	1	1.30	97.9	0.257
fourthjaarGK	2.49	1	2.49	92.2	0.118

Dropping individual terms from full fixed model

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Oogstnr.stype	206.77	7	29.15	60.7	<0.001
vjaar.Bemestingsstrategie	20.25	3	6.75	122.2	<0.001
vjaar.Mais.Gras	7.06	5	1.41	23.4	0.257
naoogstGK	22.88	1	22.88	54.5	<0.001
TnaGK	5.00	1	5.00	62.6	0.029
secondjaarGK	18.05	1	18.05	106.5	<0.001
thirdjaarGK	0.45	1	0.45	97.9	0.503
fourthjaarGK	2.49	1	2.49	92.2	0.118

Message: denominator degrees of freedom for approximate F-tests are calculated using numerical derivatives ignoring fixed/boundary/singular variance parameters.

3468
3469
3470 vdisp [pr=eff,dev]

Deviance: -2*Log-Likelihood

Deviance	d.f.
-2564.44	2165

Note: deviance omits constants which depend on fixed model fitted.

Table of effects for Constant

4.410 Standard error: 0.1326

Table of effects for Oogstnr.stype

stype Oogstnr	gk	m	t
1	0.0000	0.7615	0.2412
2	-0.2657	0.8588	*
3	-0.3028	*	*
4	-0.2153	*	*
5	-0.7564	*	*

Standard errors of differences

Average:	0.1171
Maximum:	0.1445
Minimum:	0.09222

Average variance of differences: 0.01397

Table of effects for vjaar

-0.07021 Standard error: 0.021407

Table of effects for vjaar.Bemestingsstrategie

Bemestingsstrategie	1	2	3	4
	0.00000	0.01244	0.01000	0.02222

Standard errors of differences

Average:	0.004968
Maximum:	0.004971
Minimum:	0.004966

Table of effects for vjaar.Mais.Gras

Gras	2	3	4
Mais			
1	0.00000	0.01781	0.01211
2	0.02451	0.02330	0.02376

Standard errors of differences

Average:	0.01143
Maximum:	0.01183
Minimum:	0.01097

Table of effects for naoogstGK

-0.6296 Standard error: 0.13164

Table of effects for TnaGK

0.5176 Standard error: 0.23146

Table of effects for secondjaarGK

0.2142 Standard error: 0.05041

Table of effects for thirdjaarGK

0.04281 Standard error: 0.063630

Table of effects for fourthjaarGK

-0.1373 Standard error: 0.08709

Appendix 5. REML analysis soil mineral N

Legend of model terms for soil characteristics mineral N, organic matter and organic N:

Laag:	*effect of soil layer (0 to 30 cm, 30 to 60 cm, 60 to 90 cm)*
Type:	*effect of type of main crop grown (grass-clover, maize or triticale)*
Vjaar:	*effect of number of years the experiment is running (vjaar is 1 for 2002, 2 for 2003, etc.)*
Bemestingsstrategie:	*effect of slurry application strategy*
Mais:	*effect of main crop maize*
Gras:	*effect of main crop grass-clover*
msecondjaarMais:	*effect of growing maize after maize in the second year*
TnaGK:	*effect for harvest of triticale after grass-clover instead of maize (only applies to the first year of the experiment)*
SecondjaarGK:	*effect of growing grass-clover for the second year*
SecondjaarGK:	*effect of growing grass-clover for the third year*
SecondjaarGK:	*effect of growing grass-clover for the fourth year*
Perceel:	*effect of field*
Jaar:	*effect of year*
Blok:	*effect of replication/block*

Response variate: LOG(soil mineral N)

Fixed model: Constant + laag + Type + laag.Type + vjaar + laag.vjaar + vjaar.Bemestingsstrategie + vjaar.Mais + vjaar.Gras + msecondjaarMAIS + TnaGK + secondjaarGK + thirdjaarGK + fourthjaarGK
Random model: Perceel + Blok + Veldnr + Perceel.laag + Blok.laag + Veldnr.laag + Jaar + Perceel.Jaar + Blok.Jaar + Veldnr.Jaar + laag.Jaar + laag.Jaar.Type + Blok.laag.Jaar
Number of units: 1944

Separate residual terms for each level of experiment factor: laag

Sparse algorithm with AI optimisation
Covariates not centred

Estimated variance components

Random term	component	s.e.
Perceel	0.0000	bound
Blok	0.0000	bound
Veldnr	0.0070	0.0030
Perceel.laag	0.0028	0.0027
Blok.laag	0.0000	bound
Veldnr.laag	0.0000	bound
Jaar	0.0000	bound
Perceel.Jaar	0.0308	0.0138
Blok.Jaar	0.0496	0.0106
Veldnr.Jaar	0.0382	0.0049
laag.Jaar	0.0143	0.0098
laag.Jaar.Type	0.0257	0.0092
Blok.laag.Jaar	0.0317	0.0050

Residual model for each experiment

Experiment factor: laag

Experiment	Term	Factor	Model(order)	Parameter	Estimate	s.e.
1	Residual		Identity	Variance	0.0627	0.0057
2	Residual		Identity	Variance	0.0893	0.0071
3	Residual		Identity	Variance	0.146	0.011

Tests for fixed effects

Sequentially adding terms to fixed model

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
laag	39.76	2	19.88	15.8	<0.001
Type	114.42	2	57.21	126.8	<0.001
laag.Type	64.40	4	16.09	31.9	<0.001
vjaar	8.15	1	8.15	37.4	0.007
laag.vjaar	1.23	2	0.62	15.6	0.552
vjaar.Bemestingsstrategie	11.90	3	3.97	111.5	0.010
vjaar.Mais	1.49	1	1.49	127.0	0.224
vjaar.Gras	0.92	2	0.46	128.4	0.631
msecondjaarMAIS	1.07	1	1.07	120.8	0.302
TnaGK	2.84	1	2.84	120.4	0.095
secondjaarGK	6.27	1	6.27	139.3	0.013
thirdjaarGK	3.89	1	3.89	130.6	0.051
fourthjaarGK	0.50	1	0.50	126.1	0.481

Dropping individual terms from full fixed model

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
laag.Type	64.40	4	16.09	31.9	<0.001
laag.vjaar	1.23	2	0.62	15.6	0.552
vjaar.Bemestingsstrategie	11.90	3	3.97	111.5	0.010
vjaar.Mais	0.82	1	0.82	127.0	0.368
vjaar.Gras	0.77	2	0.38	128.4	0.682
msecondjaarMAIS	1.01	1	1.01	120.8	0.316
TnaGK	3.95	1	3.95	120.4	0.049
secondjaarGK	9.58	1	9.58	139.3	0.002
thirdjaarGK	4.36	1	4.36	130.6	0.039
fourthjaarGK	0.50	1	0.50	126.1	0.481

Message: denominator degrees of freedom for approximate F-tests are calculated using algebraic derivatives ignoring fixed/boundary/singular variance parameters.

3296 vdisp [pr=eff,dev]

Deviance: -2*Log-Likelihood

Deviance	d.f.
-1308.19	1905

Note: deviance omits constants which depend on fixed model fitted.

Table of effects for Constant

2.625 Standard error: 0.1599

Table of effects for laag

laag	1	2	3
	0.0000	-0.4604	-0.5748

Standard errors of differences

Average:	0.1849
Maximum:	0.1858
Minimum:	0.1837

Table of effects for Type

Type	GK	M	T
	0.0000	1.0620	0.0743

Standard errors of differences

Average:	0.1134
Maximum:	0.1167
Minimum:	0.1079

Average variance of differences: 0.01288

Table of effects for laag.Type

Type	GK	M	T
laag			
1	0.0000	0.0000	0.0000
2	0.0000	-0.2200	0.5325
3	0.0000	-0.3422	0.6613

Standard errors of differences

Average:	0.1289
Maximum:	0.1575
Minimum:	0.1227

Average variance of differences: 0.01668

Table of effects for vjaar

-0.08381 Standard error: 0.027317

Table of effects for laag.vjaar

laag	1	2	3
	0.00000	0.02069	-0.01190

Standard errors of differences

Average:	0.02959
Maximum:	0.02974
Minimum:	0.02939

Table of effects for vjaar.Bemestingsstrategie

Bemestingsstrategie	1	2	3	4
	0.00000	0.01421	0.01494	0.02187

Standard error of differences: 0.006517

Table of effects for vjaar.Mais

Mais	1	2
	0.000000	-0.007933

Standard error of differences: 0.008783

Table of effects for vjaar.Gras

Gras	2	3	4
	0.000000	0.004107	0.009614

Standard errors of differences

Average:	0.01079
Maximum:	0.01099
Minimum:	0.01052

Table of effects for msecondjaarMAIS

-0.1028 Standard error: 0.10213

Table of effects for TnaGK

-0.3876 Standard error: 0.19493

Table of effects for secondjaarGK

0.2519 Standard error: 0.08139

Table of effects for thirdjaarGK

0.2138 Standard error: 0.10238

Table of effects for fourthjaarGK

0.1004 Standard error: 0.14193

Appendix 6. REML analysis soil organic matter

Response variate: LOG(soil organic matter)

Fixed model: Constant + laag + Type + laag.Type + vjaar + laag.vjaar + vjaar.Bemestingsstrategie + vjaar.Mais + vjaar.Gras + TnaGK + secondjaarGK + thirdjaarGK + fourthjaarGK
Random model: Perceel + Blok + Veldnr + Perceel.laag + Blok.laag + Veldnr.laag + Jaar + Perceel.Jaar + Blok.Jaar + Veldnr.Jaar + laag.Jaar + laag.Jaar.Type + Blok.laag.Jaar
Number of units: 1074 (6 units excluded due to zero weights or missing values)

Separate residual terms for each level of experiment factor: laag

Sparse algorithm with AI optimisation
Covariates not centred

Estimated variance components

Random term	component	s.e.
Perceel	0.1589	0.2000
Blok	0.0000	bound
Veldnr	0.0219	0.0055
Perceel.laag	0.1130	0.0809
Blok.laag	0.0000	bound
Veldnr.laag	0.0217	0.0036
Jaar	0.0022	0.0099
Perceel.Jaar	0.0013	0.0008
Blok.Jaar	0.0000	bound
Veldnr.Jaar	0.0000	bound
laag.Jaar	0.0236	0.0142
laag.Jaar.Type	0.0001	0.0002
Blok.laag.Jaar	0.0007	0.0004

Residual model for each experiment

Experiment factor: laag

Experiment	Term	Factor	Model(order)	Parameter	Estimate	s.e.
1	Residual		Identity	Variance	0.00408	0.00040
2	Residual		Identity	Variance	0.0351	0.0032
3	Residual		Identity	Variance	0.108	0.010

Tests for fixed effects

Sequentially adding terms to fixed model

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
laag	9.01	2	4.51	5.0	0.076
Type	0.99	2	0.49	12.7	0.622
laag.Type	1.87	4	0.46	138.3	0.761
vjaar	0.54	1	0.54	3.0	0.516
laag.vjaar	0.95	2	0.47	5.9	0.644
vjaar.Bemestingsstrategie	0.95	3	0.32	318.9	0.814
vjaar.Mais	0.41	1	0.41	81.1	0.523
vjaar.Gras	2.27	2	1.13	81.9	0.327
TnaGK	0.42	1	0.42	9.0	0.533

secondjaarGK	0.83	1	0.83	324.9	0.362
thirdjaarGK	0.74	1	0.74	259.8	0.391
fourthjaarGK	0.00	1	0.00	312.9	0.994

Dropping individual terms from full fixed model

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
laag.Type	1.88	4	0.47	138.3	0.760
laag.vjaar	0.95	2	0.48	5.9	0.644
vjaar.Bemestingsstrategie	0.76	3	0.25	318.9	0.859
vjaar.Mais	0.46	1	0.46	81.1	0.501
vjaar.Gras	2.13	2	1.06	81.9	0.350
TnaGK	0.44	1	0.44	9.0	0.522
secondjaarGK	0.73	1	0.73	324.9	0.393
thirdjaarGK	0.73	1	0.73	259.8	0.394
fourthjaarGK	0.00	1	0.00	312.9	0.994

Message: denominator degrees of freedom for approximate F-tests are calculated using algebraic derivatives ignoring fixed/boundary/singular variance parameters.

8102 vdisp [pr=eff,dev]

Deviance: -2*Log-Likelihood

Deviance	d.f.
-2113.18	1036

Note: deviance omits constants which depend on fixed model fitted.

Table of effects for Constant

1.637 Standard error: 0.3363

Table of effects for laag

laag	1	2	3
	0.0000	-0.2476	-0.9752

Standard errors of differences

Average:	0.3414
Maximum:	0.3424
Minimum:	0.3401

Table of effects for Type

Type	GK	M	T
	0.000000	0.015105	0.009927

Standard errors of differences

Average:	0.01312
Maximum:	0.01409

Minimum: 0.01118

Average variance of differences: 0.0001739

Table of effects for laag.Type

Type laag	GK	M	T
1	0.000000	0.000000	0.000000
2	0.000000	-0.008483	-0.010208
3	0.000000	-0.059832	-0.007047

Standard errors of differences

Average: 0.04182
Maximum: 0.05646
Minimum: 0.02864

Average variance of differences: 0.001855

Table of effects for vjaar

-0.02139 Standard error: 0.025872

Table of effects for laag.vjaar

laag	1	2	3
	0.00000	-0.00623	0.02612

Standard errors of differences

Average: 0.03490
Maximum: 0.03513
Minimum: 0.03460

Table of effects for vjaar.Bemestingsstrategie

Bemestingsstrategie	1	2	3	4
	0.0000000	-0.0006243	0.0016783	0.0014156

Standard errors of differences

Average: 0.003092
Maximum: 0.003123
Minimum: 0.003071

Table of effects for vjaar.Mais

Mais	1	2
	0.0000000	-0.0018130

Standard error of differences: 0.002683

Table of effects for vjaar.Gras

Gras	2	3	4
	0.000000	0.004592	0.003402

Standard errors of differences

Average:	0.003292
Maximum:	0.003317
Minimum:	0.003274

Table of effects for TnaGK

-0.01490 Standard error: 0.022348

Table of effects for secondjaarGK

-0.01378 Standard error: 0.016097

Table of effects for thirdjaarGK

0.01496 Standard error: 0.017534

Table of effects for fourthjaarGK

0.0002199 Standard error: 0.02910873

Appendix 7. REML analysis soil organic N

Response variate: LOG(soil organic N)

Fixed model: Constant + laag + Type + laag.Type + vjaar + laag.vjaar + vjaar.Bemestingsstrategie + vjaar.Mais + vjaar.Gras + TnaGK + secondjaarGK + thirdjaarGK + fourthjaarGK
Random model: Perceel + Blok + Veldnr + Perceel.laag + Blok.laag + Veldnr.laag + Jaar + Perceel.Jaar + Blok.Jaar + Veldnr.Jaar + laag.Jaar + laag.Jaar.Type + Blok.laag.Jaar
Number of units: 1074 (6 units excluded due to zero weights or missing values)

Separate residual terms for each level of experiment factor: laag

Sparse algorithm with AI optimisation
Covariates not centred

Estimated variance components

Random term	component	s.e.
Perceel	0.1388	0.1855
Blok	0.0000	bound
Veldnr	0.0000	bound
Perceel.laag	0.1303	0.0933
Blok.laag	0.0000	bound
Veldnr.laag	0.0020	0.0010
Jaar	0.0845	0.0830
Perceel.Jaar	0.0008	0.0009
Blok.Jaar	0.0000	bound
Veldnr.Jaar	0.0136	0.0025
laag.Jaar	0.0463	0.0282
laag.Jaar.Type	0.0000	bound
Blok.laag.Jaar	0.0012	0.0009

Residual model for each experiment

Experiment factor: laag

Experiment	Term Factor	Model(order)	Parameter	Estimate	s.e.
1	Residual	Identity	Variance	0.00242	0.00232
2	Residual	Identity	Variance	0.119	0.010
3	Residual	Identity	Variance	0.327	0.026

Tests for fixed effects

Sequentially adding terms to fixed model

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
laag	14.62	2	7.31	5.7	0.027
Type	0.34	2	0.17	320.9	0.843
laag.Type	8.06	4	2.01	501.3	0.092
vjaar	1.64	1	1.64	3.0	0.290
laag.vjaar	0.29	2	0.14	5.9	0.869
vjaar.Bemestingsstrategie	1.14	3	0.38	139.0	0.767
vjaar.Mais	0.01	1	0.01	96.7	0.922
vjaar.Gras	1.61	2	0.80	93.5	0.451
TnaGK	4.74	1	4.74	319.0	0.030
secondjaarGK	0.21	1	0.21	321.1	0.646

thirdjaarGK	0.63	1	0.63	321.7	0.429
fourthjaarGK	1.24	1	1.24	320.0	0.267

Dropping individual terms from full fixed model

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
laag.Type	7.84	4	1.96	501.3	0.100
laag.vjaar	0.29	2	0.14	5.9	0.869
vjaar.Bemestingsstrategie	0.79	3	0.26	139.0	0.853
vjaar.Mais	0.10	1	0.10	96.7	0.755
vjaar.Gras	1.26	2	0.63	93.5	0.536
TnaGK	5.13	1	5.13	319.0	0.024
secondjaarGK	0.12	1	0.12	321.1	0.730
thirdjaarGK	0.33	1	0.33	321.7	0.566
fourthjaarGK	1.24	1	1.24	320.0	0.267

Message: denominator degrees of freedom for approximate F-tests are calculated using algebraic derivatives ignoring fixed/boundary/singular variance parameters.

8092 vdisp [pr=eff,dev]

Deviance: -2*Log-Likelihood

Deviance	d.f.
-1249.87	1036

Note: deviance omits constants which depend on fixed model fitted.

Table of effects for Constant

7.715 Standard error: 0.4452

Table of effects for laag

laag	1	2	3
	0.0000	-0.4445	-1.0930

Standard errors of differences

Average:	0.4098
Maximum:	0.4124
Minimum:	0.4066

Table of effects for Type

Type	GK	M	T
	0.000000	0.000762	0.026281

Standard errors of differences

Average:	0.02131
Maximum:	0.02311
Minimum:	0.01823

Average variance of differences: 0.0004591

Table of effects for laag.Type

Type laag	GK	M	T
1	0.00000	0.00000	0.00000
2	0.00000	0.00195	0.06158
3	0.00000	-0.16185	0.02742

Standard errors of differences

Average:	0.06632
Maximum:	0.09055
Minimum:	0.04542

Average variance of differences: 0.004686

Table of effects for vjaar

-0.05519 Standard error: 0.057421

Table of effects for laag.vjaar

laag	1	2	3
	0.00000	-0.02243	-0.02288

Standard errors of differences

Average:	0.04925
Maximum:	0.04977
Minimum:	0.04861

Table of effects for vjaar.Bemestingsstrategie

Bemestingsstrategie	1	2	3	4
	0.000000	0.002737	0.000599	0.002643

Standard errors of differences

Average:	0.003890
Maximum:	0.003938
Minimum:	0.003856

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Table of effects for vjaar.Mais

Mais	1	2
	0.0000000	-0.0009390

Standard error of differences: 0.003002

Table of effects for vjaar.Gras

Gras	2	3	4
	0.000000	-0.000188	0.003431

Standard errors of differences

Average:	0.003631
Maximum:	0.003635
Minimum:	0.003626

Table of effects for TnaGK

-0.09060 Standard error: 0.039996

Table of effects for secondjaarGK

0.01054 Standard error: 0.030532

Table of effects for thirdjaarGK

0.01923 Standard error: 0.033505

Table of effects for fourthjaarGK

0.06231 Standard error: 0.056051