Reconciling scientific approaches for organic farming research

Part II:
Effects of manure types and white clover *(Trifolium repens)* cultivars on the productivity of grass-clover mixtures grown on a humid sandy soil

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Abstract Part 2

This Part describes the agronomic results of the multidisciplinary grassland study. This project concerned the effects of clover varieties and spring applications of animal manure on the yield of grass-white clover mixtures on a moist sandy soil (1993-1996). To be aware of the context of the findings in a multidisciplinary approach, attention was paid to: chemical soil fertility, damage to clover by slugs and soil borne nematodes. To increase the understanding of soil fertility, earthworm dynamics were also measured. At the end of the period the botanical composition of all plots was assessed. Factors measured besides total yield and clover yield were N, P and K yield. It was found that these 'context'- measurements were important for the overall explanation of the scientific results. Data were used for modelling several relationships between yield parameters. The overall findings of this project led to an understanding and description of the main aspects of manure with regard to grass-white clover growth on a moist sandy soil.

It was concluded that on a moist sandy soil the amount of inorganic and organic N, the N release and the K input were the main manure factors relating to fluctuations in total yields on white clover development and on N yields in the first six years after sward establishment. The inorganic N component in manure can be used strategically to improve the growth of the herbage in spring. Maintenance of soil fertility in terms of P, K and Ca levels is an important key factor for a successful organic grass-clover sward.
Carbon rich FYM derived from a deep litter stable and composted before application increased the earthworm population, reduced the number of nematodes and maintained the highest level of soil pH, all factors which might positively affect white clover growth in the long term. FYM applied in spring resulted in the typical extended growth season in the second part of the growing season. On a sandy soil the high concentration of K in the FYM positively affects the potential white clover growth.
The choice of a persistent white clover cultivar is another important factor affecting herbage and N yields of an organic grass-clover sward. However, winter losses were not found to be the main cause of white clover reductions over the years. Losses in the growing season were related to slugs which reduced white clover leaf area. The literature shows that the cyanide concentration in DM herbage affects the susceptibility of white clover to pests.

Key words: organic agriculture, grass-clover, white clover cultivars, animal manure, potassium, nematodes, earthworms

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1. Introduction

1.1. Legumes and manure in organic farming

Since the first biodynamic farm, Loverendale, was converted in the Netherlands in 1926, organic farms have been mainly located on the more fertile peat and old clay soils in the coastal areas. These soils have a high soil fertility. In the coastal areas, specialised organic dairy farms have been based on permanent pastures with a low content (10-15%) of white clover, mainly a wild type of white clover (Ennik *et al*., 1982; Baars *et al*., 1983; Kramer, 1994). For instance, in 1995 most organic dairy farms in the province of Friesland were extensive (1.5 LU ha^{-1}) and based on a traditional and natural way of farming with a low production per hectare (5.900 kg milk ha^{-1} $year^{-1}$). Mixed farming systems consisting of cattle, arable crops, fodder crops and short term leys were and are rare and mainly located on young clay soils in the central polder area and on clay soils in the coastal areas. Short term leys, based on red and white clover, were extremely suitable for clover growth and therefore had legume contents of over 70% (Van der Meer and Baan Hofman, 1989; Baars and Veltman, 2000).
Conversion to organic dairy farming increased rapidly in the last decade. The production levels increased as well and fodder production relied much more on newly established grass-white clover leys often combined with a second fodder crop to supply energy to animals (Van Eekeren, 2000). There was a shift to other soil types, when dairy farms also started converting to organic farming on sandy soils on the old Pleistocene areas in the east, middle and south of the country. In each European country, organic farming systems show some similarity with the practices of conventional systems. Therefore organic farming in The Netherlands developed as highly specialised and highly productive systems. Nowadays Dutch organic farming consists of stockless arable systems, specialised vegetable or fruit growers and dairy farming mainly based on permanent pastures or resown grass-clover, fodder maize or whole plant silage. The choice for rotation is very much soil related, in coastal clay and peat areas only grassland is found. Because of the specialisation, feed concentrates of (partly) organic origin were imported into dairy farms from outside the farm (Baars and Van Ham, 1996). On the other hand, organic arable or horticulture farms are mainly dependent on animal manure from conventional systems. In arable systems legumes are used as intercropping or green manures. Within these specialised systems there is only a small area of legumes (Nauta and Elberse, 1999). In other countries, however, stockless organic arable systems were based on legumes (Stopes *et al*., 1996; Cormack, 1996).

The regulations on the use of manure have been adapted to this dependency on conventional animal manure. Manure level, manure quality and manure origin have been restricted since 1991, when the organic standards were formulated by the European Commission. Animal manure was restricted for all forms of farming to a level of 170 kg total N ha^{-1}, which is related to the manure production of 2 LU ha^{-1} (EEC Nº 2092/91). However, in the near future, organic arable systems must use manure produced by organically kept and fed animals. A co-operation between specialised farms is allowed as a solution for the cycling of organic manure, straw and fodder between farms. In future, organic cattle farmers should therefore reduce the amount of manure N applied to grass-clover pastures, because this animal manure should partly be exported to arable parts on their own farm or to specialised arable and horticulture farms. This change in regulation means, that specialised animal farms should not only produce meat and/or milk but also organic manure (Nauta *et al*., 1999). The reduction of external N sources in the totality of organic farming systems will lead to a more central role of legumes in crop rotations and in leys and pastures (Lantinga and Oomen, 1996; Baars, 1998, 2001).

In the first place the type and quality of animal manure used on organic farms depends on the housing system. Two main housing systems are distinguished in modern organic farming: the deep litter stable and cubicle housing. Tied barns will no longer be allowed after 2010, because of welfare standards. In the deep litter stable, large amounts of straw are used to keep the animals clean. Once a day 9 kg (range: 7-14 kg) straw per cow is spread for bedding (Krutzinna *et al.*, 1996). In most of the deep litter houses slurry is also produced as the exercise area usually has a slatted floor. In cubicle houses often only low amounts of fibre rich products are used for bedding, like chopped straw or sawdust. Therefore, in cubicle systems only slurry is generally available.
N losses during storage and application of slurry are very low (Buchgraber, 1983; 1984). Since 1990, slurry has to be injected into grassland by law and ammonia losses are strongly reduced compared to above ground application (Van der Meer *et al.*, 1987, Bussink and Bruins, 1992). FYM from deep litter systems has to be collected in the barn and is often composted in a large heap. During storage ammonia losses cannot be avoided. During the heating phase of the compost process considerable amounts of carbon and ammonia are lost by respiration (Bockemühl, 1978). The C/N ratio declines from 21 to 9, if the heap is re-mixed or 11 if not re-mixed (Gottschall, 1985). In the first six months of the transformation, the N percentage increases from 1.8 to 2.8 (Kirchmann, 1985) and is stabilised after this period. Kirchman (1985) found total N losses of 20-35% after composting, but the respiration losses of C and N depend on the method and time of storage, heap density and moisture, amount of straw and maximum temperature during the process (Bockemühl, 1978; Gottschall, 1984). Some N, but mainly K can

be lost by leaching, if composting is done in the open air without storage of effluents (Kolenbrander and De la Lande Cremer, 1967; Buchgraber, 1983; MacNaiedhe, 1994). If FYM is spread on the top of the soil, losses of ammonia can be high if the weather conditions after application are windy and hot (De la Lande Cremer, 1953).

1.2. Prerequisites of legume based grasslands

Legumes commonly used in organic farming systems are white clover (*Trifolium repens*) for permanent pastures (grazing and cutting), red clover (*Trifolium pratense*) for ley pastures (mainly cutting) and lucerne (*Medicago sativa*) in arable crop rotations (cutting only).
With regard to nutrients, much information is available about the effects of single fertilisers on white clover development. With regard to soil fertility and availability of minerals, positive and site specific effects on the development of legumes in general and specifically for white clover, were found from the application of P, K, Ca and S (Andrew, 1960; Fothergill *et al.*, 1996). Vigorous clover growth requires appropriate use of P, K and Na fertiliser, otherwise severe nutrient deficiencies can develop (Jones and Sinclair, 1991). For instance in the UK, in long term experiments on grassland at Rothamsted research station the supply of K-fertilisers was of vital importance for clover growth, whereas at Cockle Park the importance of P fertilisers for legume growth was stressed (Cooke, 1976). In a mixture of grass and clover Simpson *et al.* (1988) found a significant response of white clover to increased levels of K. The grass component did not respond to increased K application, because the grass yield was N-limited. Newton (1995) investigated the yields of organic swards on commercial farms in relation to the production-site classes in the UK. The production of leys and permanent pastures was site specific. There was a highly significant correlation between herbage yield and the level of soil P. Soil K and the percentage of clay or sand in the soil did not affect yields. Bowdler and Pigott (1990) also found significant increase from applied S to total yields of a two-year-old irrigated grass-white clover mixture on a heavy clay soil, whereas P and K fertilisers did not affect yield. Effects of mineral application (P, K, Ca, S and even Na) therefore might be positive, neutral or negative, depending on soil type and availability of nutrients (Frame and Newbould, 1986). Effects also can be linear, quadratic or exponential depending on the level of soil fertility and fertiliser application (see for instance Hernán Acuña, 1995). Fertiliser N, on the other hand, in general negatively affects white clover growth, especially at higher rates of application and after repetitive applications (Frame and Boyd, 1987). Fertiliser N, but in general the level of soluble N in the root zone, negatively affects N fixation by the legume (Nesheim *et al.*, 1990). Nevertheless, N is used in spring as a strategic fertiliser to improve spring herbage growth and to improve the content of protein in the 1st cut. In conventionally managed grass-clover swards, an amount of 50-60 kg N ha^{-1} is often used in spring (Laidlaw, 1980; Frame, 1987), but only if there is an adequate initial

white clover level in spring (Frame, 1989). White clover growth will be slightly depressed by the N application, but might recover later in the season (Frame, 1987). This was confirmed by a New Zealand study by Pinxterhuis (2000), who showed that in N fertilised pastures (100 kg N ha^{-1}) grazed by cattle the clover content of the swards was moderately reduced directly after application. Effects, however, were not permanent.

1.3. Long term FYM effects on grassland

Manure on grassland is used to replace minerals removed in cut herbage, to compensate for leaching (N and K) and aerial losses (N), to build up soil fertility (C, N, P and K) and for N-fixation (P). The N component of manure is used strategically to improve the grass growth rate of the mixture in spring. Animal manure and especially FYM from cows is used for building up the soil quality as well, in terms of water holding capacity, soil structure, release of minerals and to feed the soil fauna. Although the humus content (C) of the grassland soil will always increase in time, the increase in soil C content was greatest after FYM application (Kolenbrander and De la Lande Cremer, 1967).

There are often positive effects of FYM in arable crop rotations (Raupp, 1999). In comparison with soils receiving no fertiliser or only artificial fertilisers, the organic matter content in soils receiving FYM is higher. The increase of soil organic matter positively affects soil structure, water holding capacity, ease of ploughing, number of bacteria and number of soil invertebrates. Negative effects of FYM include, among others, the possibility of importing weed seeds, especially when the temperature during storage is kept low (Ferwerda, 1951a).

Effects of FYM on grassland productivity were summarised by Kolenbrander and De la Lande Cremer (1967). In several parallel trials, Frankena (1936, 1937 and 1938) investigated the effect of fresh FYM levels (20 and 40 tonnes ha^{-1}), application time (December, January and early March) and soil type (sand, clay and peat) on the yield of the 1st cut in permanent pastures. His results showed that the effects of FYM (both level and timing) were most sensitive on a sandy soil, whereas other soil types had a better buffer capacity for FYM application. Results, however, were based on permanent pastures with a low clover content and a traditional management system with a high 1st hay cut yield in June .

Losses of N from FYM can occur, because of early leaching or by ammonia volatilisation. Timing of application in spring is therefore an important factor in controlling N losses. Brünner (1954) evaluated his experiments with FYM on grassland production in the Bavarian Algau area. Precipitation in spring increased spring herbage yield, because of the leaching of nutrients out of FYM into the soil and the better breakdown of the manure. An early winter application (November) was the best solution in relatively dry areas with low winter precipitation. In areas with a high winter precipitation spring application was possible.

An application of FYM late in spring should be avoided, because of aerial losses of N. On arable land N losses were measured over a 4-day period, in relation to the time of ploughing. N losses in April can be up to 25% of the total N in the FYM, if weather conditions are dry and windy with sunshine, but only 2% in February when the weather is damp with rainfall (Iversen and Jensen in: Gerretsen, 1928).
In an experiment on pastures with a high clover content, diluted urine was applied once at five different times between the end of November and the end of March (Drysdale, 1963). The highest yield response was found to urine applied at the end of March. Earlier application showed a stepwise reduction of the N efficiency and there was a strong reduction if urine was applied in November and December. Additionally the N concentrations in DM herbage were highest if urine was applied in March. In grass-only leys the response to November and December application was much higher.

Coleman *et al.* (1987) analysed long term effects of plots with and without application of FYM at Cockle Park. Data from the 1st cut hay yield were recorded over a period of 80 years. Plots receiving FYM varied less in yield from year to year, showing a more independent reaction to the soil moisture balance from year to year. However, there was not a higher level of available water holding capacity on the FYM treated plots. The different species diversity of the plots as a result of treatments and differences in rooting pattern and drought tolerance might explain the variations in response to the moisture balance. Shiel (1995) suggested that herbage growth was more buffered in the manure- treated plots at Cockle Park. FYM plots were less affected by delay or absence of manuring, or by drought, compared with the variation in plots receiving fertilisers. The manure-treated plots contained more N, which was presumably the reason why yield levels were maintained in years without manure application. Manure produced a large herbage yield and good quality with a small annual variation. There was a large botanical diversity and the aftermath was intensively grazed. Only the P-treated plots were comparable in terms of diversity and reliability of yield, but yields were smaller because of an inadequate N supply. Plots receiving FYM and basic slag alone were less dense (bulk density) than the other plots. This was combined with a relatively high pH (pH (H_2O) about 5.6-5.9). Arnold *et al.* (1976) showed that in the Cockle Park soils K could be mineralised from illitic clay and even after 80 years without additional K applications the DM yields were unaffected.
In a 65 years long term comparison between fertiliser NPK and FYM on permanent pastures on a sandy soil, a steady, but significant yearly increase of production was found after yearly applied FYM (De la Lande Cremer, 1976). In this period the yield ratio for FYM compared to that for NPK increased from about 80% to 120%. This increase was accompanied by a higher organic matter content under FYM (7.4% versus 6.2%).

Over a period of 40 years Cooke (1976), however, did not find much difference in effect from animal manure on 1st cut hay yields at Rothamsted (UK) in comparison with NPK fertiliser. FYM was only used every four years.

1.4. Goals and challenges

The input of N into organic systems relies on animal manure, biological N fixation by free living soil micro-organisms, atmospheric deposition and biological fixation by legumes (Watson and Stockdale, 1999). Of these N sources, manure and legume N are the most important on mineral soils, whereas mineralised soil N and manure N are the most important sources on organic soils (peat). As a matter of principle the N input to organic systems in general should be mainly based on legumes. Therefore a basic understanding is necessary of the best ways to introduce and to maintain legumes in the organic system, to develop suitable, site adapted grass-clover mixtures (with a well balanced amount of clover and grass) and to reduce losses of minerals in all parts of the cycle. If organic dairy farmers are pushed to reduce the level of manure applied to grass-white clover pastures, questions will be raised about acceptable minimum levels of soil fertility for maintaining white clover in the sward.
Besides the maintenance of the long term soil fertility in terms of Ca, P and K, also the choice of the clover type plays a key role for a balanced grass-white clover mixture (Evans and Williams, 1987). New cultivars of white clover bred for a better winter survival of stolons (Collins *et al*., 1991; Rhodes and Fothergill, 1993; Fothergill and Collins, 1993), are positively correlated with clover yields in spring and with total annual clover yields (Collins *et al*., 1991). Newly bred cultivars combine a better cold-tolerance with a higher spring yield. In comparison with the often-used New Zealand cv. Huia, the cultivar Aberherald bred by IGER (UK) has been shown to give a significantly higher survival of stolons during the winter period (Rhodes and Ortega, 1996).

Data are lacking about the productivity of newly established organic grass-clover swards on sandy soils, and the interactions between manure quality, soil fertility factors and productivity of grass-white clover mixtures on sandy soils are unknown. Baars and Van Dongen (1993) found that the productivity of the sward and the persistence of white clover in resown grass-clover mixtures were poor on a sandy soil in comparison with those on old marine clay, river clay, or peat soils. It was also clear from farmers experiences, that the development of grassland systems on sandy soils needed much more attention to maintain long term soil fertility, and that there were also specific problems of drought in summer. The low buffer capacity of sandy soils forces organic farmers to develop systems to reduce losses of nutrients.
Early herbage production is an important issue in dairy farming. The start of the growing season

on organic farms is delayed by a period of about two weeks compared to conventional farming. To improve spring growth for early grazing two possibilities are suggested, improved white clover cultivars and optimising the timing of manure application as part of the management of manure handling.
Three parallel experiments were carried out on effects of animal manure on grass-white clover mixtures. The first (overall) aim of these experiments was to understand the effects of animal manure on the productivity of grass-white clover swards and to understand the interaction between positive effects of manure on the development of white clover in terms of P and K and negative aspects of the manure in terms of total and mineral N, also affecting white clover. Specific attention was paid to effects on spring yield and protein yield of the 1st cut silage.
The second aim was to understand the essential preconditions with regard to soil fertility for the growth of grass-white clover on sandy soils, if in the future animal manure would be unavailable due to its use in stockless arable systems or arable crops in a mixed system. Therefore, there is a need to understand the factors necessary to maintain legumes in grassland systems.
Effects of manure application on herbage intake by the grazing animal are not known. Therefore, the third aim was to look at effects of manure type on herbage residues after a grazing period.

The work on the clover cultivars and manure (1993-1995) started as part of a network experiment at three sites in Europe (Wales, Ireland and the Netherlands) (see also Humphreys *et al.* 1997; MacNaiedhe *et al*., 1997), which was funded by the EU (Organic livestock farming, nutritional, environmental and economic implications of conversion (AIR-3C 92-0776)). The continuation of the work was financed by the Dutch Ministry of Agriculture, Nature Conservation and Fisheries (1996-1999).

Results of the trials have been described in Baars *et al.* (1996), Baars (2000), Van der Burgt and Baars (2001), Baars (2001 a and b) and Baars (2002).

2. Methodology, treatment and layout of experiments

Three different trials are described, which will be referred to as: trial 'cutting', trial 'grazing' and trial 'levels'. The first two trials were based on a split plot design of manure types and clover varieties. The last trial set was based on a split plot design of manure types and manure levels. The white clover cultivar Alice was part of all trials and these grass-clover plots were used to compare effects between the three trials.

In this chapter only the methodology, which is relevant for all trials, is described. Specific methods used for analysing the soil fauna and botanical composition are described in those chapters, where results of these analysis are presented.

2.1. Layout of the experiments

All three trials were located in the same pasture of the experimental farm 'Aver Heino' at Heino (52°25' north and 6°15' east), in the eastern part of the Netherlands. The humid sandy soil has been classified as a gleyey sand with a semi-permeable loam horizon at 70 to 80 cm. The history of the field was as cloverless permanent pasture.

2.1.1. Trials 'cutting' and 'grazing'

Experimental site and establishment

The two trials had a comparable plot lay-out. The trials were established in the first week of September 1992 as part of a six-year study on effects of manure types and white clover cultivars on spring yield and total yield. The initial level of soil fertility was: pH (KCl) 5.4, organic matter: 3.0%, phosphorus as P-Al 44.0 P_2O_5 100 g^{-1} air-dry soil; potassium as K-HCl 13.0 K_2O 100 g^{-1} air-dry soil, indicating a slightly acid soil of good P and adequate K status.
Three cultivars of white clover were sown in a mixture with perennial ryegrass (*Lolium perenne*) (cv. Magella): Aberherald, Alice and Retor. Cv. Retor was used as a Dutch medium-leafed standard. Grass seed was drilled with a row width of 7 cm at a rate of 30 kg ha^{-1}. White clover was sown afterwards by hand at 3.5 kg ha^{-1} (Figure 2.1). Only in the trial 'cutting', after the 1st cut in 1993 pure grass plots were created by chemically killing the clover. Only one out of the four grass plots was used as a pure grass stand. The other three plots were used in trial 'levels' (Figure 2.2).

Trials 'cutting' and 'grazing' had the same experimental layout: a split-plot design with randomised blocks replicated four times, with manure type as the main treatment and white clover cultivars as subplots. Trial 'cutting' was trimmed to a height of 4-5 cm five times annually. Trial 'grazing' had a mixed management of cutting (twice) and rotational grazing (three times) by dairy cows or young stock. Cutting and grazing were alternated. Plot size was 10 m x 3 m ('cutting') or 10 m x 6 m ('grazing'). In the trial 'grazing', all treatments were grazed by one group of animals that had access to all treatments at once. After each grazing cycle the herbage residue was measured.

Two different animal manure types were compared, with a fertiliser application without N as a control. Composted farmyard manure from deep litter barns (FYM) and slurry which was shallow injected at a depth of 4-5 cm (SLU), were compared with superphosphate[1] and K salt as 'Kali-60' (PK). Manure and fertiliser were generally applied in the middle of March, except in the first year (see Table 2.1). The objective was to apply FYM and SLU at a rate which supplied approximately 100 kg total N ha^{-1} (1993-1995) or 150 kg N ha^{-1} (1996-1998). Dressings of P and K were applied at an intermediate level between FYM and SLU. The manure was analysed by the Laboratory for Soil and Crop Testing at Oosterbeek (Blgg) for total N, inorganic N, total P and total K.

FYM	Retor	Barfiola*	Aberherald	Alice
SLU	Aberherald	Retor	Barfiola	Alice
PK	Aberherald	Barfiola	Alice	Retor

*** Plots with Barfiola were no longer sampled after 1993 and therefore left out of these results**

Figure 2.1. Lay-out of the trial 'grazing' (one of four replications). All plots on the same row received the same type of manure, as indicated on the left side. Plot size: 10 x 6 m.

[1] Neither superphosphate nor potassium salt as 'Kali-60' were allowed under organic conditions

PK	Alice, level-2	Grass	Alice, level-3	Alice, level-1
PK	Retor	Aberherald	Alice	Barfiola*
SLU	Alice, 30m^3	Grass	Alice, 40m^3	Alice, 50m^3
SLU	Alice	Retor	Barfiola	Aberherald
FYM	Alice, 40t	Grass	Alice, 50t	Alice, 30t
FYM	Aberherald	Alice	Barfiola	Retor

*** Plots with Barfiola were not sampled after 1993 and therefore results were discarded**

Figure 2.2. Lay-out of the trials 'cutting' (grey) and 'levels' (clear) (one of four replications). All plots on the same row received the same type of manure, as recorded on the left side. In the trial 'cutting' all cultivars received the lowest level of manure (level-1, 30m^3 or 30t). Plot size: 10 x 3 m.

2.1.2. Trial 'levels'

Establishment

This trial was established in the same field as the trial 'cutting' (Figure 2.2). Three grass plots created after killing the clover in 1993 were oversown with white clover using the so called 'Pedestrian strip seeder'. Plots were oversown at three different dates between May and August 1994 with white clover cv. *Alice* in a split plot design with manure type as the main treatment and date of sowing as subplots. White clover was sown at a rate of 8 kg ha^{-1}. From 1993-1995 the grass plots were treated as in trial 'cutting'. Plot size was 10 m x 3 m. The cutting interval was the same as in trial 'cutting'.

The average level of soil fertility in the top layer at the start of trial 2 was (average 1994-1995) pH (KCl) 4.9, organic matter: 3.7%, phosphorus as P-Al 39.0 P_2O_5 100 g^{-1} air-dry soil; K as K-HCl 12.5 K_2O 100 g^{-1} air-dry soil, indicating a slightly acid soil of good P and adequate K status. After the establishment of white cloves in 1995, the plots were reshuffled for another experiment on effects of three increasing manure levels. Randomisation of the oversown plots was undertaken in November 1995. The lay-out of trial 'levels' was a split plot design with manure type as the main level and level of application as a subplot. Three levels of the manures used in trials 'cutting' and 'grazing' were investigated during a three-year period (1996-1998).

2.2. Manure application, timing and nutrient concentrations

2.2.1. Trials 'cutting' and 'grazing'

In the 1st year (1993) only the manure was applied after the 1st cut (Table 2.1) to prevent damage of the young sward. On average, FYM was applied about two weeks before SLU and PK. Due to the heavy rainfall in autumn and winter in 1993 (Figure 2.3), the soil conditions were

Table 2.1. Temperature sum (T-value) of 200 ºC, rainfall in the 1st two weeks after manure application, application dates of manure and level of nutrient application and dates of cutting (trials 'cutting' and 'grazing' 1993-1998)

Year	1993 (1)	1994	1995 (2)	1996	1997	1998 (3)	Mean
Application date							
PK	26 Mar	30 Mar	24 Mar	03 Apr	19 Mar	25 Mar	26 Mar (4)
SLU	14 May	21 April	23 Mar	03 Apr	14 Mar	23 Mar	29 Mar
FYM	12 May	30 Mar	16 Mar	07 Mar	04 Mar	28 Feb	14 Mar
T-value (date)	18 Feb	28 Feb	15 Feb	05 Apr	27 Feb	12 Feb	01 Mar (4)
Rainfall (mm)							
SLU		6.9	41.0	8.2	25.1	17.3	19.7
FYM		67.8	52.7	0.0	8.9	75.4	41.0
Cutting dates (5)							
1st	05 May	13/17 May	11/17 May	29 May	22 May	19 May	23 May (6)
2nd	08 Jun	14 Jun	27 Jun	18/24 Jun	17/19 Jun	23/25 Jun	22 Jun
3rd	29Jun/5Jul	7/11 Jul	20/26 Jul	08 Aug	23 Jul	05 Aug	01 Aug
4th	16 Aug	30 Aug	10 Oct	02/06 Sep	18/20 Aug	21/23 Sep	06 Sep
5th	3 Oct	10 Oct	-	30 Oct	30 Sep	-	15 Oct
Total N kg ha-1							
SLU	101	124	94	141	154	141	126
FYM	129	106	103	174	150	177	140
Inorganic N kg ha-1							
SLU	47	59	46	72	76	72	62
FYM	7	12	14	30	30	39	22
Kg P ha-1							
PK	22	24	24	24	24	24	23
SLU	15	17	15	21	18	21	18
FYM	26	32	25	30	38	45	33
Kg K ha-1							
PK	87	133	266	130	130	130	146
SLU	137	150	250	208	198	348	188
FYM	177	128	235	158	260	188	218

(1). In the year of establishment animal manure applied only after the first cut.
(2). In 1995 all plots received an extra K application after the second cut: 133 kg K ha-1.
(3). In 1998 Aberherald plots received an extra K application for experimental reasons.
(4). Averages of 1994-1998.
(5). In the first year the trials were cut mainly to reduce annual weeds. In 1994 and 1995 the 1st cut started as a grazing cycle (2.0-2.5 t DM.ha-1). In 1996-1998 the 1st cut started as a silage cut (3.5-4.0 t DM.ha-1). After each silage cut the following cut was a grazing cycle. If two dates are written, this means the difference between the date of cutting for the 'grazing' trial (1st date) and for the 'cutting' trial (2nd date). At this 2nd date the grazing aftermath was measured in the grazing trial and the whole field was topped.
(6). Averages of 1996-1998 for the date of the 'cutting' trial.

such that slurry application in spring 1994 was delayed. In 1996 there was also a delay, due to the long and severe winter (1995/96) (Figure 2.4). After the dry and mild winter of 1997/98, FYM was applied very early in spring. In the Netherlands it is advised to start manure application when the T-value reaches a value of 200 (T-value = the sum of average daily temperatures above 0 ^{o}C after the 1st of January). This T-value reflects the earlyness of the growing season (Table 2.1).

At the end of the first three year period the amount of applied manure was increased from 20 to 30 tonnes (FYM) or m^3 (SLU) ha^{-1}. The application of the PK fertilisers was not increased. Therefore the average K level was lowest for PK. The slurry composition was constant over the years for all nutrients, but the FYM showed significant fluctuations for P (1.0-1.6 kg t^{-1} DM) and mainly K (5.1-11.6 kg t^{-1} DM). The nutrient concentration of the manure was measured only after its application. The levels of K in FYM were much higher in the last two years. On average FYM plots received most P and K. The amount of directly available N was always highest for SLU. Extra K was applied after the 2nd cut in 1995. Excessive rainfall (Figure 2.3) in the autumn and winter period of 1994/1995, combined with the low levels of soil K, led to a depletion of K in the soil. The older leaves of the clover plants showed typical symptoms of K deficiency and died. After an application of 133 kg K ha^{-1} all these symptoms immediately disappeared.

2.2.2. Trial 'levels'

FYM was applied immediately before the first cut for all levels: 30, 40 or 50 t ha^{-1}. PK and SLU were divided between the first three cuts as follows (per ha): SLU30 as 30m^3 before the first cut, SLU40 was as SLU30 plus an extra 10 m^3 before the second cut; SLU50 was as SLU40 plus an extra 10 m^3 before the third cut. PK-1 received a full dose before the first cut; PK-2 was as PK-1 plus an extra half a dose before the second cut; PK-3 was as PK-2 plus an extra half a dose before the third cut (Table 2.2). The application of manure in spring was at the same date as in trials 'cutting' and 'grazing' (Table 2.1).

On average FYM was applied three weeks before PK and SLU. PK plots received the lowest amount of K and an intermediate level of P. Due to differences in composition of FYM over the experimental period, the average K application in FYM was 75% higher than in PK and 44% higher than in SLU. The application of P in FYM was 75% higher than in SLU and in PK 32% than in SLU. The SLU treatment received the highest amount of directly available N: 73 kg ha^{-1} before the first cut (243% of FYM). For FYM the total N amount was equal to SLU.

Table 2.2. Average date of manure application and total input of nutrients ha^{-1} (1996 – 1998), amounts of SLU (m^3 ha^{-1}) and of FYM (t ha^{-1}) (trial 'levels').

	----- Date of application ------			Amount	Total N	Inorg. N	P	K
Manure Treatment:	**1st cut**	**2nd cut**	**3rd cut**	**t ha^{-1} or m^3 ha^{-1}**		-------- in kg ha^{-1} ---------		
PK-1	26 Mar				0	0	24	130
PK-2	26 Mar	1 June			0	0	35	195
PK-3	26 Mar	1 June	26 Jun		0	0	47	260
SLU30	24 Mar			29.3	145	73	20	198
SLU40	24 Mar	28 May		39.3	198	98	27	268
SLU50	24 Mar	28 May	26 Jun	49.3	246	122	33	336
FYM30 (1)	4 Mar			26.7	148	30	34	238
FYM40 (2)	4 Mar			36.7	203	41	47	323
FYM50 (3)	4 Mar			46.7	259	52	59	408
Mean:								
PK					0	0	35	195
SLU				39.3	196	98	26	267
FYM				36.7	203	41	47	323
Low level					98	34	26	188
Medium level					134	46	36	262
High level					168	58	46	335

(1, 2, 3). In 1996 FYM was applied at 20, 30 and 40 t ha^{-1} instead of 30, 40 and 50 t ha^{-1}.

2.3. Meteorological data

The daily meteorological data (Figures 2.3 and 2.4) were obtained from the automatic meteorological station present at the experimental site. The winters of 1992/1993, 1994/95 and 1997/98 were mild and those of 1995/96 and 1996/97 were severe. The average temperatures were low from December 1995 until February 1996 and the absolute minimum temperatures were between -10 and -15 ^oC. The growing season in 1996 was delayed because of the long duration of the winter. The T-value of 200 ^oC was only reached by the 5th of April (Table 2.1). Additionally, the winter of 1996/97 had absolute minimum temperatures ranging from -10 to -15 ^oC between November and January. The temperature increased rapidly in early spring with high maximum values in February and March. Therefore, the growing season had an early start. In almost all years average daily temperatures in spring and summer were above the long term average. Rainfall was very different between the years. For instance in March 1994 heavy rainfall delayed the time of manure application. A dry period occurred between August 1995 and September 1996, but in 1997 the amount of rainfall was also well below average.

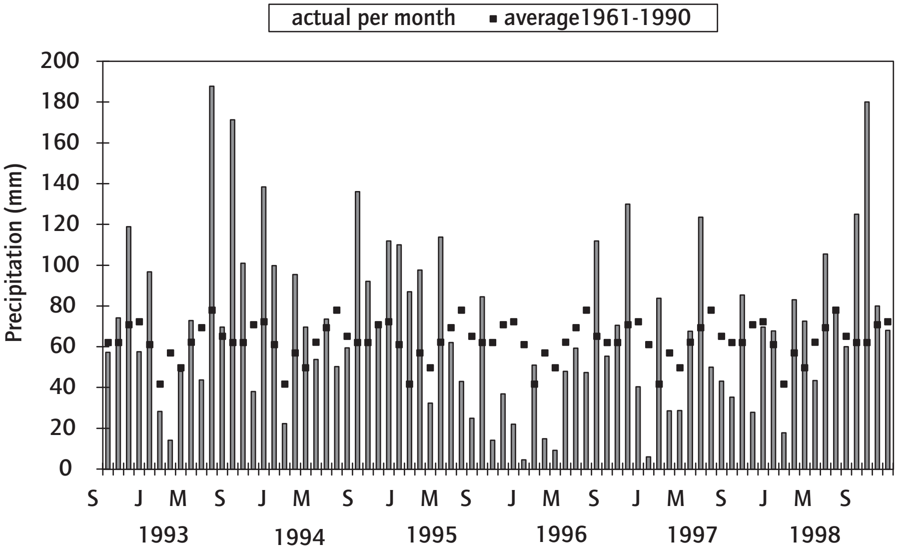

Figure 2.3. Rainfall per month (mm); actual rainfall versus long term average

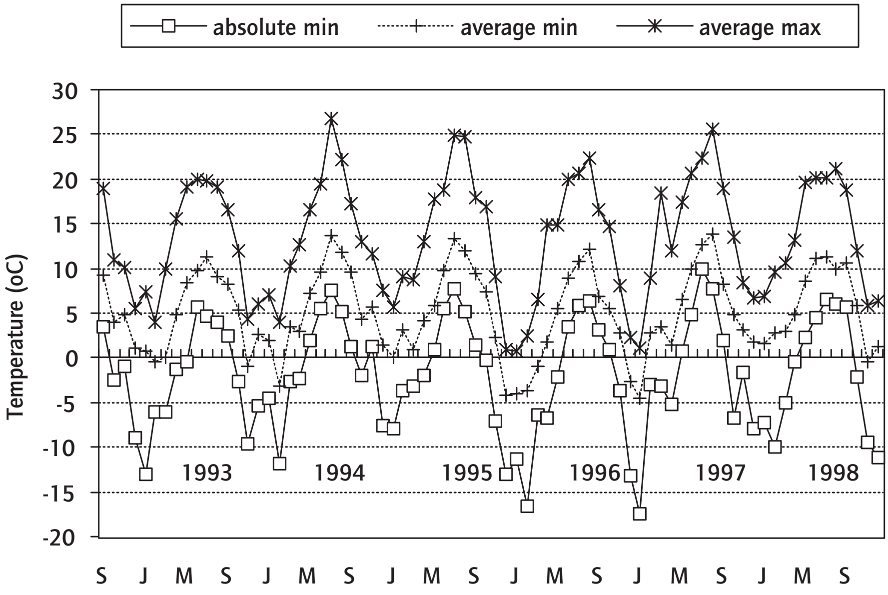

Figure 2.4. Monthly minimum and average temperatures (ºC); actual daily temperature versus long term daily average

2.4. Soil sampling

For each manure treatment in February of each year, a single soil sample was taken at a depth of 0-5 cm. In trials 'cutting' and 'grazing', the 40 subsamples were bulked for the three clover cultivars (1993-1996) or only of cvs. Alice and Aberherald (1997-1999). The samples were analysed by the Laboratory for Soil and Crop Testing at Oosterbeek (Blgg) for organic matter, pH, available P (as P-Al) and K (as K-HCl), and Mg. The soil fertility values in trials 'cutting' and 'grazing' were averaged per year to study effects of manure type on soil fertility development or were averaged per trial to compare effects of management regime.

2.5. Herbage yield measurements

Plots were harvested with a Haldrup forage harvester. The cutting height was approximately 4 to 5 cm above ground level. After harvest a 'core sample' of herbage (300-400 g) was taken from each plot and dried at 70 ºC for 24 hours. These herbage samples were used for analysis of dry matter and total N, P and K. From each plot a second 'grab sample', based on 20-25 subsamples was taken for hand-separation into grass, white clover and other species. The separated samples were dried at 100 ºC for 16 hours.

Grass-clover plots with cv. Alice and pure grass plots were analysed for N, P and K in herbage.

2.6. Statistical analyses

Data were analysed by analysis of variance (ANOVA) using GENSTAT for Windows (Lane and Payne, 1998; Oude Voshaar, 1995). The experiment was analysed as a split plot design (manure type x manure level) or as a one way ANOVA with randomised blocks (manure type).

Regression analyses were conducted in GENSTAT 5.22. Linear as well as polynomial models were used.

3. Effects of white clover cultivars on yields of grass-clover mixtures

3.1. Additional methodology

In this chapter only data of the trials ‘cutting’ and ‘grazing’ are used to analyse effects of the white clover cultivars on herbage yield and early spring growth during 1994-1996 (cvs. Aberherald and Alice compared with cv. Retor as the local standard). Clover cultivars were evaluated using an average of the three manure types (FYM, SLU and PK). Results of the year of establishment (1993) are excluded from this evaluation.
From 1993-1996 SLU and FYM plots received the equivalent of about 100 kg N ha^{-1} and in 1996 the level increased to 150 kg N ha^{-1}.

In 1994 and 1995 slug damage was measured as the area of leaves eaten by slugs. Three cuts were analysed for slug damage (May, June 1994 and May 1995) by the method described in Clements and Murray (1992).

Stolons were measured twice a year, in November and in spring after the 1st cut. Per plot, three core samples were taken of 56.4 cm^2 each at a depth of 10 cm. Losses during winter were calculated as the difference in stolon length and weight between November and the following measurement in spring. Relative losses were calculated as (November values – Spring values)/November values.

3.2. Herbage yields, white clover yields and nitrogen yields

The effects of white clover cultivars on total herbage yield, white clover yield and N yield are shown in Table 3.1, 3.2 and 3.3 respectively. The development of white clover in DM herbage per cut is shown in Figure 3.1.

In both trials mixtures with cv. Retor had the lowest total herbage yield in 1995 and 1996 (Table 3.1). Compared with the trial ‘cutting’, effects were somewhat delayed under ‘grazing’. Cv. Alice out-yielded cv. Aberherald in 1995 (‘cutting’) and 1996 (‘grazing’). In 1995 and 1996 there was an interaction between clover cultivar and manure type for both trials. The interaction was caused by a larger yield reduction in mixtures with cv. Retor for PK than for SLU and FYM compared to the reduction in cvs. Alice and Aberherald in 1995. In 1996 in the trial ‘cutting’ the interaction was caused by the reduced yield from the mixture with cv. Retor for PK and FYM compared to cvs. Alice and Aberherald. In the trial 'grazing' the mixture with cv. Retor was reduced in the SLU plots.

In all years white clover yields were lowest for mixtures with cv. Retor under 'cutting' (P < 0.001), (Table 3.2). Cv Retor had almost disappeared from the sward in 1996. There were no interactions

Table 3.1. Total herbage yields (t DM ha^{-1} $year^{-1}$): effect of white clover cultivars averaged over three manure types of trials 'cutting' (left) and 'grazing' (right) (1994-1996).

Year	1994	1995	1996	1994	1995	1996
Trial		**------ 'cutting' -------**			**------ 'grazing' ------**	
Mean	10.46	9.79	8.88	9.83	9.63	8.81
Clover cultivar						
Retor	10.21	8.97 a	7.77 a	9.85	9.30 a	7.94 a
Aberherald	10.56	9.89 b	9.21 b	9.62	9.63 ab	8.83 b
Alice	10.61	10.40 c	9.45 b	10.01	9.96 b	9.66 c
P value						
Clover cultivar	0.220	< 0.001	0.001	0.488	0.053	< 0.001
Clover * manure	0.722	0.021	0.042	0.664	0.252	0.042
LSD (5%)						
Clover cultivar	0.50	0.47	0.74	0.67	0.53	0.43

Within a column, data followed by the same letter are not significantly different at P < 0.05.

Table 3.2. White clover yields (t DM ha^{-1} $year^{-1}$): effect of white clover cultivars averaged over three manure types of the trials 'cutting' (left) and 'grazing' (right) (1994-1996).

Year	1994	1995	1996	1994	1995	1996
Trial		**------ 'cutting' -------**			**------ 'grazing' ------**	
Mean	4.50	2.48	2.37	3.85	3.30	2.61
Clover cultivar						
Retor	3.28 a	0.80 a	0.61 a	3.62	2.05 a	1.07 a
Aberherald	5.20 b	3.12 b	2.73 b	4.09	3.71 b	2.67 b
Alice	5.01 b	3.53 b	3.78 c	3.84	4.14 b	4.09 c
P value						
Clover cultivar	< 0.001	< 0.001	< 0.001	0.124	< 0.001	< 0.001
Clover * manure	0.529	0.487	0.301	0.831	0.740	0.626
LSD (5%)						
Clover cultivar	0.50	0.61	0.74	0.45	0.53	0.83

Within a column, data followed by the same letter are not significantly different at P < 0.05.

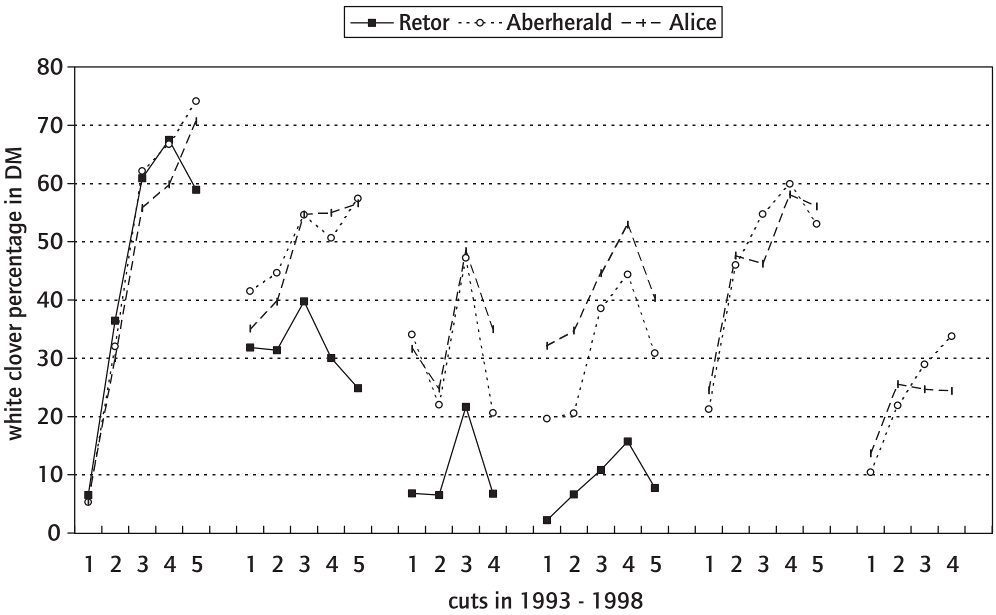

Figure 3.1. White clover content in DM herbage per cut (1993-1998)

Table 3.3. Nitrogen yields(kg N ha^{-1} $year^{-1}$): effect of white clover cultivars averaged over three manure types of the trials 'cutting' (left) and 'grazing' (right) (1994-1996).

Year	**1994**	**1995**	**1996**	**1994**	**1995**	**1996**
Trial	------ **'cutting'** -------			------ **'grazing'** ------		
Mean	365.9	269.4	-	370.8	295.8	-
Clover cultivar						
Retor	343.2 a	195.8 a	-	371.4	271.3 a	-
Aberherald	380.1 b	302.3 b	-	368.4	305.1 b	-
Alice	374.5 b	310.0 b	-	372.6	311.1 b	-
P value						
Clover cultivar	< 0.001	< 0.001	-	0.921	0.001	-
Clover * manure	0.267	0.385	-	0.981	0.585	-
LSD (5%)						
Clover cultivar	16.4	20.2	-	22.2	19.5	-

Within a column, data followed by the same letter are not significantly different at P < 0.05.

with manure type. Under 'grazing' cv. Retor persisted much better than under 'cutting'. In 1995 and 1996 mixtures with cv. Retor had a lower clover yield than mixtures with cvs. Alice and Aberherald ($P < 0.001$). White clover of cv. Alice out-yielded cv. Aberherald in both trials in 1996. From the last cut in 1993 onwards, the percentage of cv. Retor declined and by the end of 1995 it had virtually disappeared (Figure 3.1). In all years mixtures with cvs. Alice and Aberherald contained the highest amount of white clover in DM ($P < 0.001$).
N yields in the harvested herbage were reduced for the mixture with cv. Retor in 1995 for both trials ($P < 0.001$ and 0.001), (Table 3.3) and under 'cutting' also in 1994 ($P < 0.001$).

3.3. Slug damage

The clover leaf area removed by slugs was twice as high for mixtures with cv. Retor compared to the other two cultivars ($P < 0.001$), (Table 3.4). There was no interaction with manure type ($P = 0.530$).

Table 3.4. Percentage of leaf area removed by slugs as an average of three measurements (3rd and 4th cut 1994 and 3rd cut 1995), trial 'cutting'.

	Percentage leaf area removed by slugs (%)
Mean	18.2
Clover cultivar	
Retor	26.2 b
Aberherald	14.9 a
Alice	13.6 a
P value	
Clover cultivar	< 0.001
LSD (5%)	
Clover cultivar	2.8

Within a column, data followed by the same letter are not significantly different at P < 0.05.

3.4. Stolons

Effects of manure types and white clover cultivars on stolon length and weight in 1993-1996 are shown in Table 3.5 for the trial 'cutting' and in Table 3.6 for the trial 'grazing'.

Cv. Aberherald had the highest stolon length and weight in comparison with the other cultivars, except in June 1996, when the results were the same for cvs. Alice and Aberherald. From June 1994 on, cv. Alice outyielded cv. Retor as well.

In the trial ‘grazing’ cv. Aberherald had the highest stolon length and weight in comparison with the other cultivars, except in June 1996, when the results were the same for cvs. Alice and Aberherald. From November 1994 on, cv. Alice outyielded cv. Retor as well. In the trial ‘grazing’ the decline of cv. Retor was somewhat delayed in comparison to ‘cutting’.

Table 3.5. Stolon weight (g m^{-2}) and length (m m^{-2}): effect of white clover cultivar on values in spring (06.1994, 05.1995 and 06.1996) and autumn (11.1993 and 11.1994) in the trial ‘cutting’

Date as month.year	1193	0694	1194	0595	0696	1193	0694	1194	0595	0696
	----- weight in g m^{-2} -----					----- length in m m^{-2} -----				
Mean	224.0	81.1	130.8	80.2	35.0	176.1	111.2	136.6	122.7	54.1
Clover cultivar										
Retor	183.1 a	62.5 a	65.5 a	30.1 a	9.5 a	166.8 a	106.0 b	86.1 a	58.6 a	20.2 a
Aberherald	311.8 b	112.1 b	192.4 c	126.2 c	45.5 b	218.2 b	131.5 c	182.3 c	177.0 c	67.7 b
Alice	176.9 a	68.8 a	134.5 b	84.2 b	50.0 b	143.3 a	96.1 a	141.4 b	132.5 b	74.4 b
P value										
Cultivar	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.003	< 0.001	< 0.001	< 0.001
M x C	0.734	0.064	0.535	0.335	0.254	0.833	0.536	0.508	0.895	0.280
LSD (5%)										
Cultivar	33.7	12.8	20.7	18.0	18.3	24.1	18.9	16.2	31.0	26.1

Within a column, data followed by the same letter are not significantly different at P < 0.05.

Table 3.6. Stolon weight (g m^{-2}) and length (m m^{-2}): effect of white clover cultivar on values in spring (06.1994, 05.1995 and 06.1996) and autumn (11.1993 and 11.1994) in the trial ‘grazing’

Date as month.year	1193	0694	1194	0595	0696	1193	0694	1194	0595	0696
	----- weight in g m^{-2} -----					----- length in m m^{-2} -----				
Mean	208.3	82.0	99.2	53.9	39.2	148.9	113.6	97.5	81.2	61.7
Clover cultivar										
Retor	171.2 a	78.4 a	61.2 a	30.9 a	15.6 a	142.9 a	100.1 a	68.0 a	51.7 a	30.5 a
Aberherald	279.9 b	117.8 b	147.0 c	79.3 c	49.5 b	180.3 b	148.9 b	135.4 b	112.4 c	72.4 b
Alice	173.9 a	80.4 a	89.4 b	51.5 b	52.4 b	123.4 a	91.7 a	89.1 a	79.5 b	82.2 b
P value										
Cultivar	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
M x C	0.902	0.770	0.770	0.044	0.950	0.922	0.917	0.920	0.274	0.979
LSD (5%)										
Cultivar	32.8	23.5	23.5	12.3	16.4	22.4	25.3	22.8	18.5	24.1

Within a column, data followed by the same letter are not significantly different at P < 0.05.

The interaction between manure type and clover cultivar in June 1995 (P = 0.044) for stolon weight was caused by different effects of manure in cv. Retor compared to cvs. Aberherald and Alice. Cv. Retor was lowest in PK and did not show differences between FYM and SLU, whereas the other cultivars were highest in FYM.

Specific stolon weight

Stolons of cv. Aberherald had the highest specific stolon weight (g/m), whereas this was lowest for cv. Retor. Specific stolon weight increased from June till November in all cultivars.
The correlation between stolon length and weight was stronger in May than in November (Table 3.7).

Stolon losses during winter

Differences between May or June and the previous November measurements were regarded as winter losses. The best calculated average regression line of two succeeding winters was curvilinear (Table 3.8). Losses were higher if stolon weight in November was higher. Losses were lowest for cv. Aberherald. Cv. Retor had the highest losses.
At a level in November of about 150 g m^{-2} stolons, cv. Aberherald only lost 37% of weight, whereas cvs. Alice and Retor lost 50% and 60%, respectively.

Table 3.7. Specific stolon weight (g per meter stolon) based om stolon weight (Y in g m^{-2}) and stolon length (X in m m^{-2})

Clover cultivar	May	November
Retor	Y = 0.59 X (R^2 = 0.95)	Y = 1.07 X (R^2 = 0.89)
Aberherald	Y = 0.75 X (R^2 = 0.91)	Y = 1.32 X (R^2 = 0.70)
Alice	Y = 0.67 X (R^2 = 0.92)	Y = 1.16 X (R^2 = 0.71)

Table 3.8. Correlation between stolon weight in November (X in g m^{-2}) and losses during winter as the difference between May/June and November counts.

Retor:	stolon weight losses from Nov. till spring = 0.3587 X + 0.0016 X^2 (R^2 = 0.93)
Aberherald:	stolon weight losses from Nov. till spring = 0.1595 X + 0.0014 X^2 (R^2 = 0.88)
Alice:	stolon weight losses from Nov. till spring = 0.0908 X + 0.0027 X^2 (R^2 = 0.83)

3.5. Discussion

3.5.1. White clover persistency

The main effect of the clover cultivar choice was the reduction followed by a complete disappearance of cv. Retor. The loss of white clover affected average total yield, white clover yield and percentage, N yield and N concentration in the trial 'cutting'. Under 'grazing' the effects on the different yield parameters were delayed for one year and effects were smaller under 'grazing' than under 'cutting'.

In comparison with 'cutting', the average white clover yields and percentage of cv. Retor were higher under 'grazing' for Retor (24% to 16%). Differences between the trials 'grazing' and 'cutting' could be explained by the differences in internal recycling of nutrients. All plots were grazed at the same date and N in urine and dung from grazed animals was spread over all grazed treatments, PK-plots included.

In other comparisons of white clover cultivars, positive outcomes of winter survival and the higher spring yield of cv Aberherald were found by Collins *et al.* (1996). Cv. Huia was used as a control cultivar and differences between cultivars were more pronounced in the colder areas of Europe. In the same period Elgersma and Schlepers (1997) also found a lower yield in mixtures with cv. Retor in comparison to cv. Alice in a three year trial. In the third year the clover percentage in the mixture with cv. Retor still made up 40% of the total DM production, whereas in our trials this was reduced to 8% ('cutting') and 13% ('grazing'). In the subsequent years of production Elgersma *et al.* (1998) again found significant effects from white clover cultivars. Under cutting mixtures with the large-leafed cultivar Alice generally produced the highest total yield, the highest white clover yield and contained the highest N concentration, whereas mixtures with cv. Retor generally showed the lowest figures. In a comparison of leaf damage by invertebrates cv. Retor had the most severe leaf damage compared to cvs. Alice and Gwenda (Li Fengrui, 1999). Schils *et al.* (2000) compared cvs. Alice and Retor under farm conditions. Fields resown with cv. Retor all showed a significant reduction of clover abundance after 2 or 3 years, whereas fields containing cv. Alice all showed a constant level of white clover (40-60%) at the end of each growing season. Therefore, the comparison of clover cultivars under Dutch conditions all with the local standard cultivar Retor showed the same effect of white clover cultivars.

Damage to clover foliage by weevils and slugs was negatively related to the cyanogenic potential of clover (Mowat and Shakeel, 1989). Cv. Retor is described as a low cyanogenic clover, whereas new cultivars such as Alice and Aberherald show very high levels of HCN. Crush and Caradus (1995) investigated the cyanide concentrations of 51 white clover cultivars. The HCN concentrations in cv. Alice (942 $\mu g\ g^{-1}$ DM) were 7.8 times higher than concentrations in cv. Retor. Cv. Alice was one of the eight cultivars where the concentration exceeded 800 $\mu g\ g^{-1}$ DM.

The authors concluded, that cultivars that were agronomically successful in New Zealand were mainly highly cyanogenic.
Because of animal health risks, clover cultivars with medium and high levels of cyanide are not allowed in Switzerland. Thyroid levels in cattle were unaffected by the intake of high amount of cyanide rich clover, but the blood selenium levels (as GSH-pX) were decreased (Lehmann *et al.*, 1990).
In a large field trial, Clements and Murray (1993) calculated an average loss of leaf area in white clover eaten by pest organisms of 12% per year. The main organisms reducing leaf area were Sitona weevils (8%). Slug damage was only responsible for 2% of loss. However, in our trial 14-26% of the leaf area was removed. In our study only the 3rd and 4th cut were examined, whereas the maximum leaf area removed by pest organisms according to Clements and Murray, was found in early spring (18%).

Statistically significant differences between mixtures with cvs. Aberherald and Alice were found in 1996, both for ‘cutting’ and ‘grazing’ and in 1997 and 1998 for ‘grazing’ (data not presented here). White clover yields in spring were higher for cv. Alice. Differences between the two cultivars might be affected by the genetic difference in leaf size. Under a cutting regime a positive correlation was found between clover leaf size on the one hand and white clover yield and total herbage yield on the other (Evans and Williams, 1987; Frame and Boyd, 1987; Elgersma and Schlepers, 1997). However, our trials showed opposite results: under a management of alternate grazing and cutting (‘grazing’) clover of the cultivar with the largest leaf size cv. Alice out-yielded the medium-leafed cultivar Aberherald (Table 3.2). Although cv. Aberherald was selected for a better winter survival, a better performance of cv. Alice after 1996 was found due to severe winter cold in 1995/96 and 1996/97. Cv. Aberherald did not show the expected improved spring yield. In two relatively mild winters, no differences were found in total herbage yield and early spring yield in mixtures with cvs. Aberherald, Abercrest and Menna (Sheldrick *et al.*, 1993). Cv. Menna even showed a tendency to a higher white clover yield, like cv. Alice did in our trial. There was also a better live weight gain by lambs in spring on mixtures with cv. Menna. (Sheldrick *et al.*, 1996).

3.5.2. White clover growth in spring in relation to stolon growth

This study showed that cv. Aberherald had a larger stolon length and weight than cv. Retor, and a greater white clover and N yield. Besides it had a higher N concentration in the first cut (data not shown). However, the total herbage DM yields of the first cut were not affected by clover cultivar. A reduced white clover growth in spring was compensated by increased grass growth. The percentage of stolons lost during winter was lower for cv. Aberherald than for cv. Retor.

Compared with cv. Alice, cv. Aberherald had the highest stolon weight and length in May 1994 and 1995. In May 1996, no differences were found. The comparison of herbage spring yield over the period 1994-1998 did not show any increased herbage spring yield, white clover yield and percentage N or N yield for cv. Aberherald. Mixtures with cv. Alice produced the highest spring yield (1997) and white clover yield in the 1^{st} cut (1996 – 1998), (data not shown). Even after the most severe winters (1995/96 and 1996/97), there were no signs of a better white clover production in mixtures with cv. Aberherald. Sheldrick *et al.* (1993) also did not find any increased yields of mixtures with cv. Aberherald, compared to other clover cultivars (Menna, Abercrest). Stolon length and weight in spring, therefore, were poor indicators for herbage growth in spring. Instead of stolon weight in spring, high levels of total non-structural carbohydrates (TNC) in the stolons in winter were the most important factor affecting early spring yield. Other morphological characteristics were of no importance (Frankow-Lindberg *et al.*, 1996). For Nordic areas it was concluded that TNC levels in autumn were associated with low losses of stolons during winter. Swedish cultivars had a much better ability to accumulate TNC than cv. Aberherald. Collins and Rhodes (1995) used artificial freezing to investigate winter survival of white clover. Results of their trials also suggest that stolon carbohydrate content is an important factor in the overwintering of white clover. Further glasshouse experiments of Turner and Pollock (1998), showed an accumulation of starch in young stolon tissue. The synthesis and maintenance of starch reserves during cold periods was an important factor to explain differences in winter hardiness. Spring growth of white clover was found to be correlated with high levels of stolon starch (Frankow-Lindberg and Frame, 1997).

4. Effects of manure types on herbage yields, mineral yields and botanical composition of grass-clover mixtures with cv. Alice

4.1. Additional methodology

In this chapter the effects of manure type are analysed. Only data from mixtures with cv. Alice will be presented here from all three trials 'cutting', 'grazing' and 'levels'. The effects of manure type in the trial 'levels' will be analysed as an average of the three levels. Except for K yields, effects of manure levels will not be discussed, because as a result of the plot history. Effects of manure levels were confounded with the previous experimental design of different oversowing dates of white clover in 1994.

In the trials 'cutting' and 'grazing', manure and fertiliser were generally applied in March. In accordance with the plan, FYM plots received the manure earlier (14 March) than the SLU-plots (29 March) and PK-plots (26 March). These timings were strategically chosen on the basis of the supposed optimum timing for each manure type. The objective was to apply FYM and SLU at a rate which supplied approximately 100 kg total N ha^{-1} (1993-1995) or 150 kg total N ha^{-1} (1996-1998) (Table 4.1).

In the trial 'levels', on average, the application of FYM (4 March) was about three weeks earlier than the 1st application of PK and SLU. All FYM was applied before the 1st cut, whereas SLU and PK applications were split over the 1st three cuts.
In all trials, the PK plots received the lowest amount of K and an intermediate level of P. The ratio of K to P was highest in SLU and lowest for PK. The SLU treatment received the highest amount of directly available, inorganic N. For FYM the total N amount was equal to SLU.

The botanical composition was assessed once only, in August 1998. Data were used from trials 'cutting', 'grazing' and 'levels'. All measurements were based on visual estimation by an experienced person. All species were listed and the abundance per species was estimated. The sum of all abundance values was 100%.
From an agronomic point of view grass species were divided into three groups: good, moderate and poor. This judgement was based on a mixture of plant characteristics: growth, digestibility and negative elements like being poisonous and stoloniferous (Kruyne *et al.*, 1967 and De Boer and De Gooyer, 1979).

Table 4.1. Overall input of nutrients (kg ha^{1}), trials 'cutting', 'grazing' and 'levels'. For the trial 'levels' values are presented as an average of three application levels.

	--- trials 'cutting' and 'grazing' --- (1993-1998)				--- trial 'levels' --- (1996-1998)			
	Total N	**Inorga-nic N**	**P**	**K**	**Total N**	**Inorga-nic N**	**P**	**K**
Manure type								
PK	0	0	23	146	0	0	35	195
SLU	126	62	16	188	196	98	26	267
FYM	140	22	33	218	203	41	47	323

* after the 2nd cut in 1995 all plots received an extra potassium dressing of 133 kg K ha^{-1}. This amount is included in the average figures

All Dock plants were removed every winter between 1994-1996. The number of removed plants per plot were counted.

4.2. Total herbage dry matter yields

In 1994 and 1995 in the trial 'cutting', no differences were found between the manure treatments (Table 4.2). From 1996 on, when the amount of applied manure was increased by 50%, differences in yield were significant. Plots fertilised with PK always had the lowest yield. Only in 1997 plots fertilised with FYM out-yielded SLU ($P < 0.001$). The overall DM yield for 'cutting' was lowest for PK in comparison to the plots who received FYM or SLU (on average: - 1.21 t DM ha^{-1}; $P = 0.003$).

In the trial 'grazing' plots fertilised with FYM and SLU out-yielded PK in 1995-1998. Differences between FYM and SLU showed a larger yield fluctuation between years than in the trial 'cutting'. The overall DM yields were the same in FYM and SLU. The application of animal manure (FYM or SLU) increased the overall DM yield by 1.27 t DM ha^{-1} ($P = 0.004$) compared with PK fertilised plots.

In the trial 'levels' the yields were highest for FYM and SLU in all years. Only in 1996 plots fertilised with SLU had the same yield as PK fertilised plots. The overall DM yields were not significantly different for SLU and FYM, which both out-yielded PK (on average: + 0.97 t DM ha^{-1}; $P < 0.001$).

Table 4.2. Total yield (t DM ha^{-1} $year^{-1}$) for the trials 'cutting' and 'grazing' (1994-1998) and the trial 'levels' (1996-1998): effects of manure type.

Year	1994	1995	1996	1997	1998	Mean
			------ 'cutting' -------			
Mean	10.60	10.40	9.44	10.61	9.37	10.09
Manure type						
PK	10.11	9.95	8.21 a	9.31 a	8.83 a	9.28 a
Slurry	10.80	10.75	9.82 b	11.05 b	8.92 a	10.27 b
FYM	10.90	10.50	10.30 b	11.46 c	10.37 ab	10.71 b
P value	0.214	0.259	0.008	< 0.001	0.001	0.003
LSD (5%)	1.05	1.08	1.09	0.40	0.60	0.60
			----- 'grazing' -----			
Mean	10.01	10.00	9.66	-	11.07	10.18
Manure type						
PK	9.41	9.42 a	8.50 a	-	9.87 a	9.34 a
Slurry	10.44	10.11 ab	10.68 b	-	11.21 b	10.61 b
FYM	10.18	10.47 b	9.79 b	-	12.00 b	10.61 b
P value	0.252	0.030	0.010	-	0.039	0.004
LSD (5%)	1.40	0.71	1.16	-	1.46	0.64
			--- 'levels' ---			
Mean			10.40	11.64	9.78	10.61
Manure type						
PK			9.94 a	10.76 a	9.17 a	9.96 a
Slurry			10.38 ab	11.98 b	10.01 b	10.79 b
FYM			10.89 b	12.17 b	10.16 b	11.07 b
P value			0.031	< 0.001	0.002	< 0.001
LSD (5%)			0.46	0.32	0.41	0.29

Within a column, data followed by the same letter are not significantly different at $P < 0.05$.

4.3. White clover yields

In the trial 'cutting' there was only a significant effect of manure type in 1994, when the clover yield in FYM out-yielded white clover in PK. In the other years no significant effects of manure type on white clover yields were found (Table 4.3). In 1998 the white clover yield was much lower than in previous years for all manure treatments. Effects in the trial 'grazing' were similar to those in the trial 'cutting'. Overall effects were not found.

In the trial 'levels', the white clover yield was negatively affected by the application of SLU in

1997 and 1998 and FYM in 1998 compared with PK. In 1998 the white clover yield was more than halved, compared to 1996 and 1997.
The overall DM yield was reduced after the use of both types of animal manure (on average: - 1.18 t DM ha^{-1}; P = 0.024).

Table 4.3. White clover yields (t DM ha^{-1} $year^{-1}$) for the trials 'cutting' and 'grazing' (1994-1998) and the trial 'levels' (1996-1998): effects of manure type.

Year	1994	1995	1996	1997	1998	Mean
			------ 'cutting' -------			
Mean	5.01	3.53	3.78	4.52	1.84	3.74
Manure type						
PK	4.46 a	2.98	2.50	3.56	1.88	3.08
Slurry	4.74 ab	3.72	4.52	4.51	1.57	3.81
FYM	5.84 b	3.90	4.31	5.48	2.06	4.32
P value	0.042	0.417	0.259	0.221	0.469	0.203
LSD (5%)	1.06	1.67	2.94	2.38	0.92	1.49
			---- 'grazing' ----			
Mean	3.84	4.21	4.09	-	1.71	3.49
Manure type						
PK	3.27 a	3.66	3.87	-	2.27	3.32
Slurry	3.70 a	3.96	4.47	-	1.40	3.56
FYM	4.54 b	5.01	3.94	-	1.55	3.61
P value	0.021	0.098	0.769		0.350	0.739
LSD (5%)	0.80	1.33	2.27		0.85	0.96
			---- 'levels' ----			
Mean			5.25	5.63	2.16	4.38
Manure type						
PK			5.61	6.34 b	3.26 b	5.17 b
Slurry			4.64	4.97 a	1.73 a	3.78 a
FYM			5.51	5.59 ab	1.50 a	4.20 a
P value			0.223	0.019	0.005	0.024
LSD (5%)			1.31	0.83	0.87	0.91

Within a column, data followed by the same letter are not significantly different at P < 0.05.

4.4. Nitrogen, phosphorus and potassium yields

4.4.1. N yields

In 1994 and 1995 no differences were found between the manure treatments in the trial 'cutting' (Table 4.4). From 1996 on, higher amounts of manure (Table 2.1) were applied and differences in N yield were observed, except in 1998. Plots fertilised with PK always had the lowest yield. In 1997 plots fertilised with FYM out-yielded SLU (+ 33.7 kg ha^{-1}; P = 0.001). The overall N yield was highest for both FYM and SLU treatments (+ 51.6 kg N ha^{-1}; P = 0.025) compared to PK fertilised plots.

Table 4.4. Total nitrogen yields (kg N ha^{-1} year^{-1}) for the trials 'cutting', 'grazing' (1994-1998) and trial 'levels' (1996-1998): effects of manure type.

Year	1994	1995	1996	1997	1998	Mean
			------ 'cutting' -------			
Mean	374.6	310.0	296.3	359.0	244.7	316.9
Manure type						
PK	353.8	291.0	242.0 a	308.8 a	217.0	282.5 a
Slurry	375.5	323.5	324.6 b	367.3 b	255.8	329.3 b
FYM	394.5	315.5	322.2 b	401.0 c	261.3	338.9 b
P value	0.114	0.367	0.031	0.001	0.164	0.025
LSD (5%)	39.5	53.7	63.6	33.4	52.5	38.6
			--------- 'grazing' ----------			
Mean	373.2	313.7	297.9	-	282.7	317.0
Manure type						
PK	345.6	296.6	271.5	-	273.6	296.8 a
Slurry	387.3	316.1	329.5	-	295.7	329.4 b
FYM	386.7	328.2	292.8	-	278.6	324.8 b
P value	0.091	0.126	0.150	-	0.510	0.040
LSD (5%)	43.5	31.9	65.2	-	46.2	25.4
			--------- 'levels' --------			
Mean			337.2	409.5	255.7	330.0
Manure type						
PK			324.5	388.9 a	258.3 ab	321.5
Slurry			340.5	433.8 b	271.5 b	338.8
FYM			346.7	405.8 a	237.5 a	329.8
P value			0.365	0.003	0.038	0.293
LSD (5%)			36.2	18.4	24.3	24.3

Within a column, data followed by the same letter are not significantly different at P < 0.05.

In the trial 'grazing' no effects from the manure treatment were found. However, the overall N yield was increased after application of SLU and FYM (on average: + 30.3 kg N ha^{-1}; P = 0.040) compared to PK fertilised plots.

In the trial 'levels', the use of SLU positively affected the N yield in 1997 compared to PK and FYM and to FYM only in 1998. The N yield was much lower in 1998 than in earlier years. There were no overall effects of manure type on N yield.

Table 4.5. Total phosphorus yields (kg P ha^{-1} $year^{-1}$) for the trials 'cutting' and 'grazing' (1994, 1996-1998) and the trial 'levels' (1996-1998): effects of manure type.

Year	1994	1995	1996	1997	1998	Mean
			------ 'cutting' -------			
Mean	46.3	-	32.3	39.8	36.7	38.7
Manure type						
PK	46.7	-	29.5	37.3	36.3	37.4
Slurry	46.2	-	33.8	40.3	34.0	38.6
FYM	46.0	-	33.5	41.8	39.8	40.2
P value	0.988		0.101	0.154	0.130	0.378
LSD (5%)	8.5		4.4	4.7	6.0	4.7
			---------- 'grazing' ----------			
Mean	45.3	-	32.4	-	45.0	41.6
Manure type						
PK	42.9	-	29.3	-	43.0	38.4 a
Slurry	46.3	-	35.6	-	44.8	43.0 b
FYM	46.8	-	32.3	-	47.3	43.5 b
P value	0.172		0.093		0.411	0.024
LSD (5%)	4.8		5.9		7.3	3.6
			----------- 'levels' ---------			
Mean			37.9	44.1	38.9	39.7
Manure type						
PK			36.7	41.2 a	38.7	38.4 a
Slurry			38.1	45.2 b	38.6	39.5 ab
FYM			38.9	45.9 b	39.3	41.2 b
P value			0.122	0.002	0.767	0.022
LSD (5%)			2.2	1.9	3.3	1.8

Within a column, data followed by the same letter are not significantly different at P < 0.05.

4.4.2. P yields

In the trials 'cutting' and 'grazing' there were no significant differences among manure treatments in any year (Table 4.5). In the trial 'grazing', however, the overall P yields were higher in both FYM and SLU than in the PK treatment (on average: + 4.9 kg P ha^{-1}; P = 0.024).

In the trial 'levels', the P yield was increased in 1997 after the use of both SLU and FYM compared to PK. The overall P yields were lowest for PK and highest for FYM (+ 2.8 kg P ha^{-1}; P = 0.022).

4.4.3. K yields

In the trial 'cutting' there were significant differences among manure types on K yields in 1994 (Table 4.6). Generally, in all years except in 1998, K yields in FYM and SLU were similar. In 1998 FYM out-yielded both SLU and PK. The overall K yields were highest in FYM in comparison with SLU (+ 29.1 kg K ha^{-1}; P < 0.001). Plots fertilised with SLU also out-yielded PK (+ 40.0 kg P ha^{-1}; P < 0.001).

In the trial 'grazing' no effects of manure type on K yields in 1994 and 1995 were found. In 1998 the K yield was highest in plots fertilised with FYM (P = 0.004). In 1995 K yields in SLU fertilised plots were higher than in FYM (P = 0.021). There were no overall differences between manure types. The average K yield in the trial 'grazing' was higher than in the trial 'cutting' (+ 56.6 kg K ha^{-1}), which was due to the recycling of urine and faeces in the trial 'grazing'.

In the trial 'levels', the K yield in 1996 was higher for FYM than for SLU fertilised plots. In 1997 all manure types had a different K yield, FYM was highest, SLU moderate and PK had the lowest K yield. In 1998 FYM out-yielded both SLU and PK. The overall K yields were highest for FYM (+ 63.4 kg K ha^{-1}; P < 0.001), whereas SLU did not affect the K yield compared to PK.

In the trial 'grazing', there was a recycling of N, P and K via animal dung and urine, but this did not affect total N and P yields or N and P concentrations in the herbage (Figure 4.1 and 4.2). The concentration of K in herbage was higher for 'grazing' than under 'cutting' (Figure 4.3). Under 'cutting', the K concentration decreased during the growing season from 27 till 17 g K kg^{-1} DM.

In the trial 'levels', in 1997 and 1998 the K yields were highest after application of the high level of manure and lowest at the low application level (data not shown). The overall increase of K was + 50.5 kg K ha^{-1} from the low to the medium level, and + 36.5 kg K ha^{-1} from the medium to the

Table 4.6. Total potassium yields (in kg K ha^{-1} $year^{-1}$) for the trials 'cutting' and 'grazing' (1994-1998) and the trial 'levels' (1996-1998).

Year	1994	1995	1996	1997	1998	Mean
			------ 'cutting' -------			
Mean	258.4	228.1	216.6	235.3	230.3	233.7
Manure type						
PK	224.5	201.3 a	186.8 a	192.8 a	181.5 a	197.4 a
Slurry	277.6	242.0 b	226.3 b	249.5 b	191.5 a	237.4 b
FYM	273.0	241.0 b	236.8 b	263.8 b	318.0 b	266.5 c
P value	0.205	0.006	0.004	< 0.001	< 0.001	< 0.001
LSD (5%)	70.0	21.6	22.8	22.9	26.1	17.6
			------- 'grazing' -------			
Mean	301.2	270.7	270.9	-	311.4	290.3
Manure type						
PK	274.9	252.9	249.4 a	-	242.0 a	260.5
Slurry	322.1	276.7	321.9 b	-	311.2 b	304.0
FYM	306.5	282.4	241.6 a	-	381.0 c	306.4
P value	0.361	0.163	0.021		0.004	0.081
LSD (5%)	75.4	34.4	50.4		56.1	45.1
			------ 'levels' --------			
Mean			287.4	312.6	274.8	287.9
Manure type						
PK			285.3 ab	283.3 a	230.9 a	263.2 a
Slurry			273.8 a	309.2 b	251.9 a	270.4 a
FYM			303.1 b	345.4 c	341.7 b	330.2 b
P value			0.032	0.001	< 0.001	< 0.001
LSD (5%)			20.2	22.3	21.2	18.6

Within a column, data followed by the same letter are not significantly different at P < 0.05.

high level of application ($P < 0.001$). There was an interaction between manure type and manure level for K yield in 1998 ($P = 0.035$). In FYM, K yields were not affected by the level of application, whereas both other manure types showed significant effects of increased levels of application. Compared to all other yield measurements, only the K yields were affected by an increased level of manure application.

4.5. 1st cut yields

On average, plots fertilised with PK had a lower overall DM yield than SLU-plots (+ 0.71 t ha^{-1}; $P < 0.001$) or FYM-plots (+ 0.50 t ha^{-1}). In 1998 herbage yield was very high. There was an

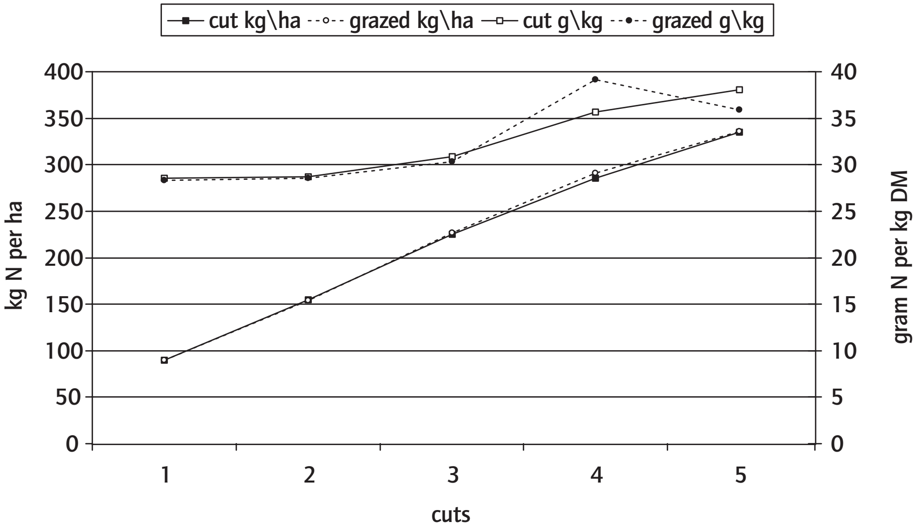

Figure 4.1. Accumulated N yield [1] and N concentration in DM of each cut in the trials 'cutting' and 'grazing' as an average of three manure treatments (1994, 1995, 1996, 1998)

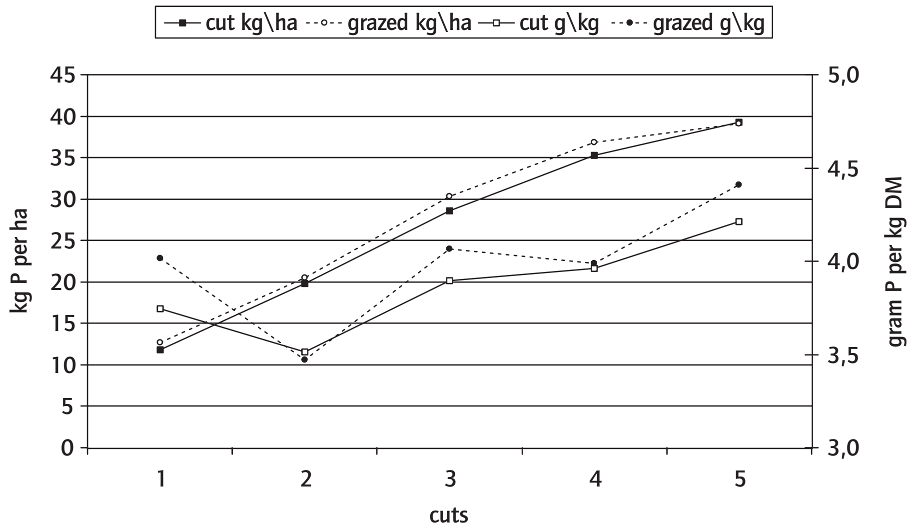

Figure 4.2. Accumulated P yield [1] and P concentration in DM of each cut in the trials 'cutting' and 'grazing' as an average of three manure treatments (1994, 1995, 1996, 1998)

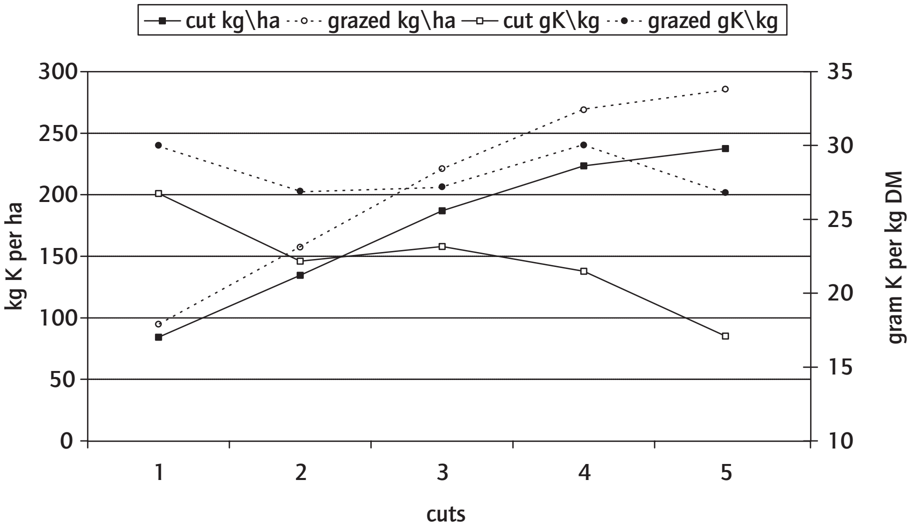

Figure 4.3. Accumulated K yield [1] and K concentration in DM of each cut in the trials 'cutting' and 'grazing' as an average of three manure treatments (1994, 1995, 1996, 1998)

interaction with year (P = 0.003). White clover yields and white clover contents in DM herbage were unaffected, see Table 4.7.

The interaction between manure type and year for DM yield (Table 4.8) depended on the time of manure application and weather conditions in winter and spring (Chapter 5.5). In 1994 and 1996 no effects of manure application were found. The lack of effects in 1994 may have been due to late application in spring and in 1996 because of a severe winter followed by a delayed timing of application. Significant effects of manure application on early spring growth were found in years with a mild winter and an early application of manure (1995, 1997 and 1998). In 1995 and 1998 there were no differences between SLU and FYM, whereas in 1997 SLU outyielded FYM (+ 0.86 t ha^{-1}; P = 0.003).

White clover yields and percentages in DM were on average not affected by manure (Table 4.7). Only in 1995 clover yields were lower for SLU (- 0.37 t ha^{-1}; P = 0.002) than PK and FYM. After 1995 white clover yields were reduced and in 1998 clover yields were very low, mainly because of the high herbage yield (4.42 t ha^{-1}). Especially the high total herbage yield for FYM in 1998 negatively affected the white clover percentage in DM (P = 0.001). Clover percentage in herbage DM had the same result in 1995 (- 17.1%; P = 0.002).

Table 4.7. Yields of the first cut: effect of manure type on the total DM yield, white clover yield and clover percentage in DM, N, P and K yield; trial 'cutting' for mixtures with cv. Alice (1994-1998)Table 4.6. Total potassium yields (in kg K ha^{-1} year^{-1}) for the trials 'cutting' and 'grazing' (1994-1998) and the trial 'levels' (1996-1998).

	Total	---- White clover ----		N	P	K
	---- t DM ha-1 ---		%	--- kg ha-1 ---		
Mean	3.05	0.79	22.3	88.7	11.5	83.7
Manure type						
PK	2.65 a	0.77	29.1	75.2 a	10.3	66.0 a
SLU	3.36 b	0.75	22.8	100.7 b	12.7	95.4 b
FYM	3.15 b	0.85	27.0	90.1 b	11.4	89.8 b
Year						
1994	2.52 a	0.89 bc	35.7 b	93.6 c	12.1 c	79.2 b
1995	3.32 b	1.05 c	32.1 b	97.6 c	N.A.	62.7 a
1996	2.35 a	0.75 ab	32.3 b	70.8 a	7.8 a	66.1 a
1997	2.67 a	0.65 ab	25.9 b	84.2 b	10.4 b	81.8 b
1998	4.42 c	0.61 a	14.2 a	97.3 c	15.5 d	129.0 c
P value						
Manure	0.003	0.800	0.527	0.003	0.083	0.001
Year	< 0.001	0.007	0.001	< 0.001	< 0.001	< 0.001
M x Y	0.003	0.149	0.227	0.251	0.199	< 0.001
LSD (5%)						
Manure	0.35	0.36	13.0	10.7	2.2	10.6
Year	0.24	0.25	10.3	8.4	1.4	8.8

N.A. = not available
Within a column, data followed by the same letter are not significantly different at P < 0.05.

Table 4.8. Annual herbage DM yields of the first cut (t DM ha^{-1}): effect of manure type in the trial 'cutting' for mixtures with cv. Alice (1994-1998)

	1994	1995	1996	1997	1998
Mean	2.52	3.32	2.35	2.67	4.42
Manure type					
PK	2.31	2.86 a	2.16	2.00 a	3.94 a
SLU	2.63	3.65 b	2.60	3.44 c	4.51 b
FYM	2.62	3.44 b	2.29	2.58 b	4.80 b
P value	0.113	0.027	0.231	0.003	0.018
LSD (5%)	0.36	0.54	0.57	0.59	0.52

Within a column, data followed by the same letter are not significantly different at P < 0.05.

On average, N yields were highest for SLU and FYM in comparison to PK (P = 0.003), (Table 4.7). However, after 1995 SLU always out-yielded PK for N yield (Table 4.9). In 1995 and 1998 SLU also out-yielded FYM.
The lowest N yield was found in 1996. Manure application was delayed in that spring.
On average, P yields were not affected by manure type (Table 4.7). P yields were correlated with herbage DM yield. The lowest P yield was reached in 1996. Only in 1997 SLU out-yielded both FYM and PK (+ 4.9 kg P ha^{-1}; P = 0.020).

Table 4.9. Yields of the first cut: effect of manure type per year on N yield in the trial 'cutting' for mixtures with cv. Alice (1994-1998)

	1994	1995	1996	1997	1998
Mean	93.6	97.6	70.8	84.2	97.3
Manure type					
PK	85.3	88.1	58.2 a	64.4 a	95.4 b
SLU	98.5	105.2	85.6 b	98.0 b	116.3 c
FYM	97.1	99.5	68.6 a	90.1 b	80.0 a
P value	0.096	0.414	0.016	0.010	< 0.001
LSD (5%)	13.3	29.7	16.1	18.4	9.8

Within a column, data followed by the same letter are not significantly different at P < 0.05.

K yields were highest for SLU and FYM (P = 0.001). K yields were also correlated to the herbage DM yield, but K yields had an interaction with year (P < 0.001), see Table 4.10.
In all years, except 1996, K yields in PK-plots were the lowest. In 1996, however, K yields were lowest for FYM as well. K yields were higher for SLU than for PK. In 1997 SLU also outyielded FYM, which was correlated to the DM yield (Table 4.8). In 1998 FYM out-yielded SLU, which again was correlated to herbage DM yield.
Effects of manure on N concentrations were not significantly different, but PK tended to have the lowest N concentrations (not shown). N concentrations were highest in 1994 and lowest in 1998 (P < 0.001). The low level in 1998 was correlated to the high herbage DM yield. Also P concentrations were not different among manure types; P concentrations were highest in 1994 (not shown). K concentrations were correlated to K yields. The use of SLU or FYM increased K concentrations in herbage (P < 0.001). There was an interaction with year (P < 0.001) (not shown).
K concentrations were lowest in spring 1995 (1.87 g K kg^{-1} DM). An extra fertiliser dressing of K was applied after the 2nd cut for all treatments, to maintain the clover in the sward. In most

years K concentrations were lowest for PK (1994, 1995, 1997 and 1998), although not always significantly so. In 1996, however, K concentrations were lowest for FYM (P = 0.001).

4.6. Botanical composition

After 6 years of treatment 25 species were found in the three trials together. The number of grass species was 13, legumes 1 and herbs, 11. Most species were perennial plants, *Polygonum aviculare* and *Poa annua* were annual weed plants (see Appendix 1).
In the comparison of plots with cv. Alice the number of species was the same for all manure treatments in the trials 'cutting', 'grazing' and 'low level' (Table 4.11). The abundance of the 'good' grass species was highest for PK and SLU (P = 0.002). The abundance of 'good' grass species was negatively correlated to the white clover abundance, which were highest for FYM (P = 0.004). In the trial 'grazing' the amount of white clover was lowest (P = 0.048). The amount of 'poor' grass species was highest for SLU (P < 0.001) and for the Trial 'grazing' (P < 0.001). The higher value was mainly because of the amount of Poa annua, which was highest for SLU (P < 0.001) and trial 'grazing' (P < 0.001). In all treatments only Docks were found in FYM (P < 0.001). Dandelion was also highest in FYM plots (P = 0.002).

In the trial 'cutting' the number of Dock plants removed from the plots tended to be higher for SLU than for PK and FYM (Table 4.12).

4.7. Yield residues after grazing

In the trial 'grazing', herbage residues after each grazed cut are shown. Manure was only applied before the 1st cut in spring (Table 4.13).

In neither of the cuts results were affected by the white clover cultivar (P = 0.323 for all data; data not shown). The overall residues after grazing were negatively affected by FYM (P = 0.026), (Table 4.13). The interaction with cut number was highly significant (P < 0.001) and effects of manure applied before the 1st cut in spring were only found for the 1st and the 2nd cut. Effects on the 1st cut residues were higher (P = 0.002) than on the 2nd cut (P = 0.042). The herbage residues after the application of FYM were + 149% and + 69%, respectively.

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[2] This classification is related to characteristics of production capacity, digestibility and negative elements like poisonous and stoloniforous of each grass species Kruyne *et al.* (1967), (see Appendix 1).

Table 4.11. Botanical composition of trials 'cutting', 'grazing' and 'low levels' as an average of manure type: number of species per plot, total abundance and abundance of white clover, addition of abundance of 'good', 'moderate' and 'poor' species [2] and Herbs, abundance of Poa annua (Pa), Taraxacum spec. (Dandelion) and Rumex obtusifolius (Docks).

	No species	Total	White clover	Good	Mode-rate	Poor	Herbs	Pa	Dandelion	Docks
	Plot^{-1}	---------- abundance as percentage ----------								
Mean	9.3	88.3	29.8	43.6	15.7	4.6	0.3	4.4	5.3	0.5
Manure										
PK	9.6	88.8	27.4 a	51.7 b	13.0	3.5 a	0.5	3.4 a	3.0 a	0.0 a
SLU	8.9	89.6	22.8 a	46.6 b	19.0	6.6 b	0.3	6.3 b	5.0 a	0.0 a
FYM	9.5	86.7	39.2 b	32.6 a	15.0	3.8 a	0.2	3.6 a	7.9 b	1.5 b
Trial										
Cutting	9.6	88.3	33.4 b	42.7	14.1	4.3 b	0.5	4.0 b	5.0	0.4
Grazing	9.0	88.8	22.8 a	47.0	14.8	7.0 c	0.4	6.8 c	6.5	0.8
low level	9.4	87.9	33.2 b	41.2	18.1	2.6 a	0.1	2.6 a	4.4	0.4
P value										
Manure	0.361	0.312	0.004	0.002	0.112	<0.001	0.330	<0.001	0.002	<0.001
Trial	0.494	0.911	0.048	0.481	0.328	<0.001	0.209	<0.001	0.235	0.262
M x T	0.548	0.347	0.331	0.682	0.826	0.649	0.641	0.551	0.847	0.258
LSD (5%)										
M and T	1.0	4.0	9.5	10.1	5.8	1.5	0.5	1.3	2.5	0.6

Within a column, data followed by the same letter are not significantly different at P < 0.05.

4.8. Discussion

4.8.1. Effects of manure on yield

The comparison of yields between the trials was hampered by the absence of measurements in 1997 for trial 'grazing' compared to 'cutting' and a shorter range of measurements for the trial 'levels'. Nevertheless, the overall total herbage yield was highest in the trial 'levels'. In all trials, the use of FYM and SLU increased total herbage yields compared to PK (on average + 1.26 t DM ha^{-1}). Application of FYM in 1997 showed that FYM could even out-yield SLU, but positive

Table 4.12. Total number of removed Dock plants between 1994-1996 in the trials 'cutting' and 'grazing', averages of three white clover cultivars (Retor, Aberherald and Alice)

	'cutting'	'grazing'
	No of plants plot^{-1}	
Mean	1.2	1.3
Manure		
PK	0.5	1.1
SLU	2.3	0.7
FYM	0.9	2.0
P value	0.073	0.164
LSD (5%)	1.6	1.4

Table 4.13. Effects of herbage residue after grazing; manure type as an average of three white clover cultivars Retor, Alice and Aberherald in trial 'grazing' 1994-1996. Yields in t DM ha^{-1}

Cut number	1st	2nd	3rd	4th	5th	All
Mean	273	286	418	278	241	287
Manure type						
PK	163 a	213 a	456	246	201	235 a
SLU	251 a	286 ab	397	301	254	285 ab
FYM	407 b	360 b	400	287	269	343 b
P value						
Manure (M)	0.002	0.042	0.581	0.138	0.234	0.026
Cut number						< 0.001
M x Cut number						< 0.001
LSD (5%)						
Manure	91	107	149	59	91	70

Within a column, data followed by the same letter are not significantly different at P < 0.05.

results were affected by favourable weather conditions and a high FYM quality.
Effects of applied manure on the first cut yield (Tables 4.7 and 4.8), however, showed that in all trials the herbage and N yields of the 1st cut were highest for SLU and lowest for PK (on average + 0.76 t DM ha^{-1} and + 22.0 kg N ha^{-1}). Although differences in herbage yield between SLU and FYM were significant, on average the extra herbage and N yields after SLU were small (+ 0.25 t DM ha^{-1} and + 16.5 kg N ha^{-1}).
The average total white clover yields were higher in the trial 'levels' than in 'cutting' and 'grazing'. This difference was also found for the average white clover percentage (42% to 37% and 34% in DM herbage respectively). White clover yields were reduced (- 1.08 t DM ha^{-1}) by the use of animal manure in the trial 'levels' and white clover was replaced by a larger amount of grass (+ 2.02 t DM ha^{-1}; P = 0.001) (data not shown). In the other trials, no significant effects were found, although in the trial 'cutting' the average white clover yield was increased by the use of animal manure (+ 0.99 t DM ha^{-1}). Differences, however, were only significant in 1994. In the PK plots, the white clover content was 52% in the trial 'levels' and only 33% and 36% in the trial 'cutting' and 'grazing', respectively (data not shown). The lower levels of applied fertiliser P and K in these trials (Table 2.2) might have mainly reduced white clover growth, because soil pH was similar for all trials.
The expected negative effect of N on white clover growth, irrespective of its organic or inorganic origin, was also found in several other trials (Buchgraber, 1983 and 1984; Van der Meer and Baan Hofman, 1999). In contrast with our trials Van der Meer and Baan Hofman (1999) could

distinguish between separate effects of either N, P and K. Schils and Sikkema (1992) distinguished between the N-effect of slurry application and the fragmentation of the stolons by the slurry injector on the white clover growth. He concluded that negative effects of slurry were explained as an effect of the amount of inorganic N. On a sandy soil, Van der Meer and Baan Hofman (1999) only found a negative effect of shallow injection on white clover in the 1st time of applied spring slurry in a grass-clover sward sown in May the year before. After recovery of the clover, effects of shallow injection could be explained from the level of inorganic N. This is confirmed by our findings. It was only in 1993, in the year of establishment, that SLU negatively affected white clover growth, although slurry was applied after the 1st cut. The more negative results in the 1st year after sowing seems to be more correlated to young clover plants with only a primary root instead of stoloniferous plants.
Another negative effect of the slurry application technique might be caused by wheel damage if soil conditions in spring are wet. Slipping and soil compaction from slurry application negatively affected total yields and yields in spring under wet conditions in a young sward on a clay soil (Humphreys *et al.*, 1997). In our trial on a sandy soil we could not detect any visual damage to the sward or the soil. On the other hand, under practical circumstances the application of FYM in early spring might cause wheel damage, when it is done by heavy machinery. Within an organic farming system it was found that increased tractor traffic significantly reduced the yield, the number or earthworms and N efficiency of the system (Hansen and Engelstad, 1999).
In our trials negative effects of applied manure on white clover yields were only found in the trial 'levels'. The low level of applied P and (mainly) K in the trial 'cutting', compared to the trial 'levels', led to a positive effect of FYM and SLU, because of the higher amount of K. The negative effect within the trial 'levels' was related to the higher and probably sufficient level of K in this trial (see Chapter 5.3).

The average N yields were only slightly higher under 'levels' (+ 13 kg N ha^{-1}) than in the other trials. They were similar for 'cutting' and 'grazing'. N yields were increased in the trials 'cutting' (+ 51.6 kg N ha^{-1}) and 'grazing' (+ 32.1 kg N ha^{-1}) by the use of both types of animal manure, but were unaffected in the trial 'levels'. N concentrations in herbage DM were very similar (31.2-31.4 g N kg^{-1} DM) in all trials (data not shown). The overall N content tended to be lowest in FYM in the trial 'levels' (- 2.6 g N kg^{-1} DM; $P = 0.058$) compared to the PK plots. The same tendency was found in the other trials, although differences were small (data not shown). The lower concentration of N in FYM might be caused by the lower level of inorganic N in FYM, compared to SLU. Another possibility is that N from the soil pool was used by microbes for the destruction of carbon in FYM.
Within each trial the N yields were determined by a combination of the grass and the white

clover yield and their respective N concentrations. Grass yield and grass N concentration are affected by the amount of applied N. Irrespective of the differences in average yield of white clover, there were hardly any differences in N yield, which showed a replacement of clover N by grass N. Grass N yields were increased by application of manure N. A comparison of clover DM yield and the average N yields within the PK-plots showed an increased N yield (283, 297 and 330 kg N ha^{-1}), if white clover yields increased (3.08, 3.32 and 5.17 t DM ha^{-1} respectively).

The P-yields were lowest for PK in all trials compared to FYM plots (+ 3.1 kg P ha^{-1}) and only in the trial 'grazing' did FYM out-yield SLU in P yield. The P yields were correlated with the total herbage yield. The P concentration in DM herbage was negatively affected by the herbage DM yield and positively by the recycling of urine and dung. The PK plots had the highest P concentrations, which was combined with the lowest DM yield. The P concentrations in the PK plots were lowest in the trial 'levels', intermediate in the trial 'cutting' and highest in the trial 'grazing' (respectively, 3.7, 3.9 and 4.1 g P kg^{-1} DM). All average P levels were sufficient for an optimal herbage growth. The levels were far above the levels of about 2 g P mentioned by several authors (Andrew, 1960; Rangeley and Newbould, 1985).

The K yields showed a different pattern in all three trials. The average K yield under FYM was always highest. Under 'cutting' FYM out-yielded SLU (+ 29.1 kg), whereas SLU out-yielded PK (+40 kg); under 'grazing' effects were not significant, because of the high variation in this trial caused by the deposition of urine and dung by the grazing animals. However, on average both FYM and SLU were very similar and out-yielded PK (+ 44.7 kg). In the trial 'levels' FYM out-yielded both PK and SLU (+ 63.4 kg). K yields were mainly affected by the level of applied K. In the trial 'levels' it was only for K that increasing levels of application showed an increased K yield and K concentration in DM, irrespective of the type of manure.
The K concentrations in herbage DM were lowest in the trial 'cutting' and highest in the trials 'grazing' and 'levels' (23.1, 28.5 and 27.1 g K kg^{-1} DM respectively), (data not shown). In the trial 'cutting' and 'levels' the K concentrations were lowest for PK ($P = 0.002$ and $P < 0.001$) (data not shown). Therefore it was assumed that a low average herbage K concentration and especially the reduction of herbage K during the season, reduced the growth of white clover.

4.8.2. Interaction of manure type and level of K application

It was expected (Evans *et al.*, 1987) that a large-leafed cultivar like Alice would have performed better under the cutting regime (trial 'cutting') than a regime of cutting plus grazing (trial 'grazing'). The return of N in excreta and the behaviour of grazing animals will reduce the amount of clover in grazed swards (Wilkins, 1985). Nevertheless in the trial 'grazing', white clover yields and clover percentages in DM were higher than in the trial 'cutting'. Additionally the low productivity of white clover in the PK plots of the trials 'cutting' and 'grazing' was in conflict with expectations. Normally N in animal manure should have reduced white clover growth. Negative effects of manure were only found in the trial 'levels', where a higher amount of PK, FYM and slurry was applied. In the trial 'cutting', however, clover yields were low in plots that did not receive any N. The lowest level of applied K in the PK plots reduced white clover more than the extra N in the SLU and FYM plots. Shortages of K suppressed the white clover development for PK in trial 'cutting'. The low herbage mineral concentration and the visual plant symptoms showed that the level of applied K restricted the growth of white clover. K shortages were higher in trial 'cutting' than in trial 'grazing'. The repetitive application of P and K in the trial 'levels' improved white clover growth by 100% compared to the trial 'cutting' (Table 4.3: 1996-1998). Chapman and Heath (1988) investigated the effect of cattle slurry on a mixture of grass-white clover. At inadequate levels of soil K, slurry was beneficial to the clover content of the herbage, although the overall slurry N negatively affected clover content and yield. This was also shown by González-Rodríguez (1993) in experiments on a sandy-loam soil. Higher levels of applied K (0, 83, 167 kg K ha^{-1}) led to a doubling of the white clover yield. Effects were highest at a low or zero level of N application. In a comparison of equal levels of diluted urine and dry NPK fertiliser, Castle and Drysdale (1963) found positive effects of urine, in comparison to NPK fertiliser, on herbage yield and clover content of a newly established mixture of meadow fescue and white clover. In these trials, the soil K level declined during the three experimental years and K concentrations in herbage were different between the types of manure. However, this could be explained by the different levels of applied K, which were about 52% in the fertiliser treatment compared to the diluted urine. From the data presented by Castle and Drysdale (1963), a positive correlation between the K concentration in DM herbage (X in g kg^{-1} DM) and the white clover percentage in DM (Y as %) at the end of the trial was calculated: $Y = 1.99 + 10.71\ X$ ($R^2 = 0.70$). Therefore at the same level of applied N, the amount of applied K also affected the growth of white clover. At low levels of K clover development was reduced.

In a grass-clover mixture with cv. Retor on a sandy soil (1993-1995), Van der Meer and Baan Hofman (1999) could distinguish between effects of P and K on total, white clover and N, P and K yields. In the 3rd year, plots not receiving any K had a clover content of only 1.5%, whereas the content of plots not receiving any P was 5.5%. The control plots had clover levels of 18.9% (moderate PK level) or 25.1% (high PK level). P concentrations in herbage were 4.1, 3.3, 4.2 and 4.2 g P kg^{-1} DM, respectively of the treatments no K, no P, moderate PK and high PK. P concentrations in white clover herbage were even lower at a level of respectively 3.1, 2.6, 3.0 and 3.1. K concentrations in herbage DM were 16.7, 30.8, 28.5 and 31.8 g K kg^{-1} DM and in the white clover only 7.7, 32.0, 31.5 and 35.2. Effects on white clover yield were therefore strongly

correlated with both the amount of K and P application. The reaction on K shortages, however, was even stronger than on P shortages. Evans *et al.* (1986) showed the strong relationship between white clover growth and K shortages. K was the primary limiting factor in the soil. In treatments without any applied K, white clover yield was negligible after 2 years, whereas the absence of P fertilisation showed a much later and less vigorous reaction on clover growth. Mean concentrations for P and K in white clover were 17 and 3.8 g kg^{-1}, respectively, as critical values for maximum plant growth. Baily and Laidlaw (1998) showed in a pot experiment that in the phase of establishment, the shoot and leaf development of white clover were more adversely affected by P than by K deficiency. The persistence of white clover in established swards, however, was reduced more by K deficiency than by low or inadequate P supplies. There was a considerable reduction in leaf size, number of growing points, stolons and root masses at deficient K levels. In a 65 year experiment in permanent pastures on a dry sandy soil, De la Lande Cremer (1976) found herbage yields in between 6.7 and 10.1 t DM ha^{-1}. K yields, however, were very constant at 152-160 kg K ha^{-1} and P yields fluctuated with the total herbage yield. The level of P in herbage was sufficient (0.40-0.41 g kg^{-1}) and the K levels were low (16-17 g kg^{-1}) when N was supplied and moderate (24 g kg^{-1}) when no N fertiliser was used. The level of K restricted the herbage production of all treatments. The results of De la Lande Cremer are very similar to our results, although K levels were higher in our trials. In a survey of Swiss organic farms, Mäder *et al.* (2000) showed a negative trend in soil K concentration after conversion towards organic. Other major elements and trace elements were unaffected.
In a small additional experiment in 1998 (trial 'cutting; unpublished results) plots with cv. Aberherald received an extra K dressing at a level of 60 kg ha^{-1} after the 2nd and 3rd cut. Compared with control plots of cv. Alice a significant increase in total herbage yield, grass yield, P and K yield and K concentration in herbage DM was found (data not shown). White clover contents in herbage DM increased as well, but only significantly in the last cut of 1998.

4.9. Effects of manure on herbage residues after grazing

Herbage residues after grazing were highest for FYM applied in spring. However, effects were only found in the 1st and 2nd cut after spring application, which might be because of the smell and structure of FYM in the lower part of the sward. Effects were not found later in the season and visually we could not find any remains of FYM.
In our 'grazing' trial animals could choose between the manure plots. In a normal agricultural practice, however, animals cannot choose and effects might be smaller. There may be no effects on total herbage yield by manure type in practice, because in practice grazing is followed by a herbage cut and higher residues are harvested in the following cut or removed when topping the sward after grazing.

5. Effects of manure types on soil fertility and soil fauna

5.1. Additional methodology

In this chapter the effects of manure type are analysed. Effects of manure type on soil fertility are described for the periods 1994-1999 (trial 'cutting' and 'grazing') and 1997-1999 (trial 'levels').

For the trials 'cutting' and 'levels' nutrient field balances were calculated as input per ha in manure minus output in the herbage. Losses caused by leaching or build up in soil organic matter were ignored. In 1995 P yields were not measured. The positive correlation between herbage yield and P yield was used to estimate the P offtake in 1995.

To evaluate the efficiency and timing of manure application the apparent N efficiency (ANE) was calculated in relation to results of the first cut. Rainfall and temperature data in the 1st two weeks after manure application were used to analyse differences between years. The T-value was related to the date of application.

5.2. Soil fertility parameters

The average results for the trials 'cutting' and 'grazing' in soil pH, soil organic matter and soil P showed a proper development which was related to the manure type (Table 5.1 and Figures 5.1-5.4). Although the data were not statistically analysed, the differences in average mineral input in 1993-1998 were reflected in the average soil fertility levels in 1994-1999. Average soil-P and soil-K levels (Table 5.1) showed the same ranking as the average mineral input (Table 2.1, Chapter 2). In the trial 'cutting', the levels of fertility declined further than in the mixed regime of grazing and cutting (trial 'grazing'), (Table 5.1). Under 'grazing' the output of minerals was reduced, because of internal recycling of dung and urine.
The increase of soil organic matter (OM) and the decline in soil pH were different for all treatments. FYM showed the largest and PK the smallest increase in soil OM. Differences in increase were related to the level of input of organic matter. In FYM the pH level remained at the same level, whereas in PK and SLU soil acidity increased.

Table 5.1. Trials 'cutting' and 'grazing': initial level of soil fertility and average soil fertility (layer: 0-5 cm; 1994-1999) as organic matter (OM), soil acidity (pH), P (P-Al) and K (K-HCl); above average value of the trials 'cutting' and 'grazing'; below average value of the three manure treatments [3].

	OM %	pH-KCl	P-Al mg P_2O_5/100 g	K-HCl mg K_2O/100 g
Initial value in 1993	3.0	5.4	44.0	13.0
Average value 1994-1999				
PK	3.9	4.8	37.2	9.3
SLU	4.2	5.0	33.8	10.3
FYM	4.7	5.4	38.3	12.6
Average of manure types (1994-1999)				
Trial 'cutting'	4.0	5.0	34.1	8.8
Trial 'grazing'	4.5	5.2	38.8	12.7

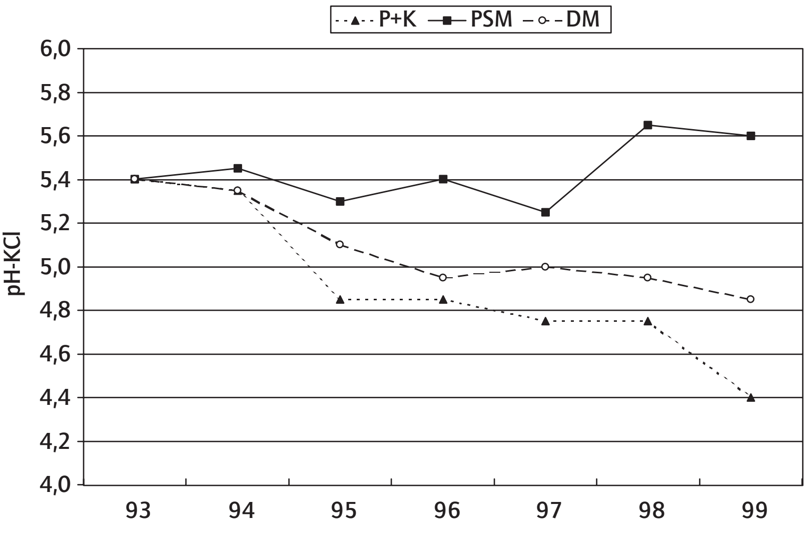

Figure 5.1. Development of soil acidity as pH-KCl as an average of the trials 'cutting' and 'grazing'

[3] Soil fertility parameters were not statistically analysed, because only a single soil sample was taken per manure treatment per year. Results, therefore, should be treated as an illustration of a process.

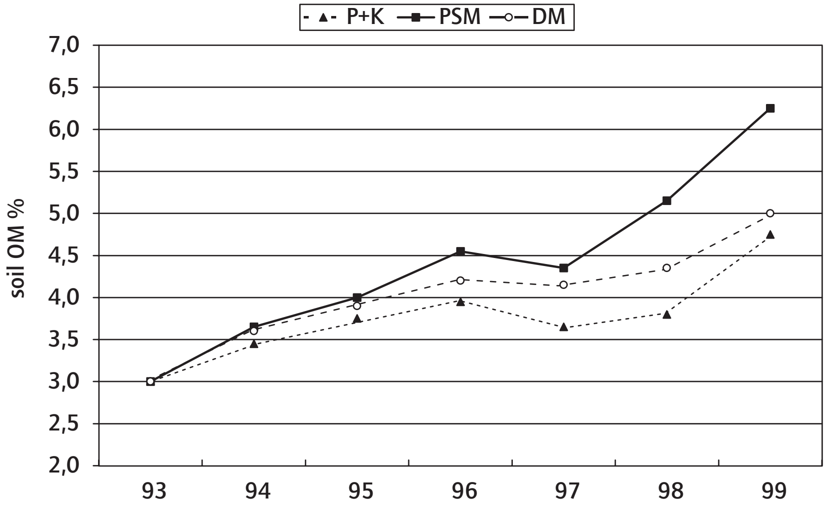

Figure 5.2. Development of soil organic matter as an average of the trials 'cutting' and 'grazing'

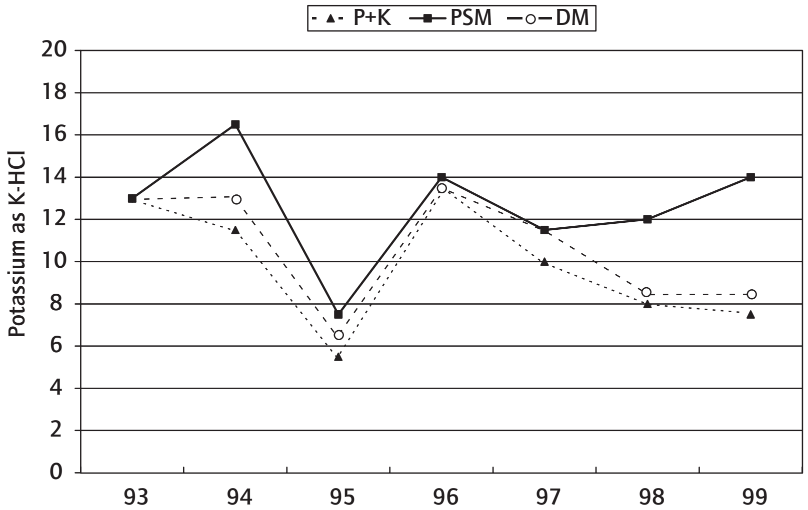

Figure 5.3. Development of soil fertility as K-HCl as an average of the trials 'cutting' and 'grazing'

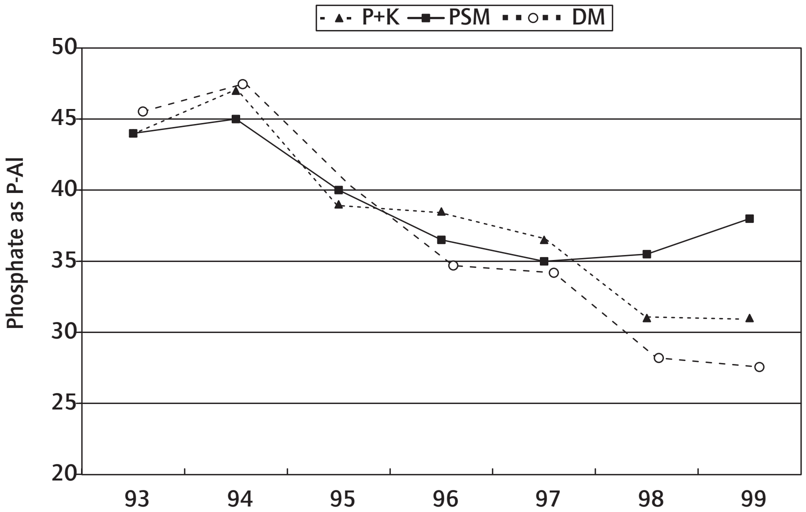

Figure 5.4. Development of soil fertility as P-Al as an average of the trials 'cutting' and 'grazing'

The initial soil fertility levels and the average levels of trial 'levels' in 1997-1999 are presented in Table 5.2.

The pH was highest in the FYM and lowest in PK-plots. The initial pH differences (4.9-5.4) were

Table 5.2. Trial 'levels': initial soil fertility (average of 1995 and 1996) and average soil fertility (1997-1999) for three manure types.

	pH-KCl	OM %	P-Al mg P_2O_5/100 g	K-HCl mg K_2O/100 g
Initial level (1995 – 1996)				
PK	4.9	3.7	39.0	12.5
SLU	5.1	3.9	37.5	10.0
FYM	5.4	4.0	42.5	12.5
Average of three levels (1997 – 1999)				
PK	4.6	4.2	34.9	9.0
SLU	5.0	4.3	27.3	8.7
FYM	5.6	5.3	39.1	13.1

further increased during the experiment (4.6-5.6). PK led to a lower and FYM to a higher pH. These effects on pH were consistent with the development of soil pH in the trials 'cutting' and 'grazing' (Table 5.1).
The amount of soil OM increased for all treatments. The largest increase, however, was found for FYM, which was consistent with the other trials (Table 5.1).

SLU had the lowest average soil-P and soil-K levels (1997-1999). On average the soil-P levels were decreased by10 points in all SLU-levels (data not shown). The medium and high levels of applied PK or FYM, resulted in similar or even higher soil-P levels in the top soil compared to the initial level (data not shown). The decrease of the soil-K level was largest in the PK plots (- 3.5 points). For FYM the soil-K level increased for both the medium and the high level of application (data not shown).

The results on changes in soil fertility were correlated with the different amounts of applied minerals for each treatment (Table 2.2, Chapter 2). A higher level of manure or fertiliser led to an increase of P and K levels in the soil.

5.3. Field balances for P and K

The output of P was equal for all manure treatments. Differences in P input ha^{-1} caused differences in P balances between the three manure treatments. The ranking for K input and K output was the same. FYM had the highest in- and output and PK the lowest (Table 5.3). K outputs reflected the amount of applied K, which was lowest in PK and highest in FYM (Table 2.1, Chapter 2). The field K balance was negative and similar for all manure treatments. The ratio of K and P input in manure was highest for SLU, because of its low level of P. PK and FYM showed on average the same K/P ratio. The K/P ratio in herbage was lowest for PK and highest for FYM.

Only at the highest K input level was the average K application sufficient to compensate for removal by the herbage (Table 5.4) The most negative field balance was found at the low level of PK. The application of P in SLU was lowest and FYM showed on average a surplus of P. At the high manure level, on average the P input showed a surplus as well.

Table 5.3. P and K field balance and the ratio between K and P in manure and herbage of the trial 'cutting' (average of 1994-1998).

	Input/output in kg ha-1					K/P-ratio		
	--------- P --------		-------- K ---------		-------- Manure --------		-------- Herbage --------	
	Input	**Output**	**Balance**	**Input**	**Output**	**Balance**	**Input**	**Output**
Manure type								
PK	24	39	-15	158	198	-40	6.6	5.1
SLU	19	39	-21	200	237	-37	10.5	6.1
FYM	34	40	-6	226	266	-40	6.7	6.7

Table 5.4. P and K input and output ha^{-1} and balances and K/P ratio in manure and in herbage: manure types as averages of three manure levels (average of 1996-1998).

	Input/output in kg ha^{-1}					K/P-ratio		
	-------- P -------		------- K --------		-------- Manure --------		-------- Herbage --------	
	Input	**Output**	**Balance**	**Input**	**Output**	**Balance**	**Input**	**Output**
Manure type								
PK	35	39	-3	195	266	-71	5.6	6.8
SLU	26	40	-14	267	278	-10	10.3	7.0
FYM	47	41	5	323	330	-8	6.9	8.0
Manure level								
Low	26	38	-12	188	247	-59	7.2	6.5
Medium	36	41	-5	262	292	-30	7.3	7.1
High	46	41	5	335	335	0	7.3	8.2

Table 5.5. Relationship between mineral yield or mineral concentration and mineral input in the trial 'levels'; data of three manure types at three increasing manure levels as an average of 1997 and 1998 (for each equation N = 9)

	Yield (Y) as kg ha^{-1}	Concentration (C) as g in DM herbage
Potassium (K):	Y = 152 + 0.49 I (R^2 = 0.88)	C = 17.2 + 0.03 I (R^2 = 0.83)
Phosphorus (P):	Y = 38 + 0.09 I (R^2 = 0.38)	C = 3.8 + 0.00 I (R^2 = 0.02)

The ratio of K to P in herbage was lowest for PK and SLU. Compared to P the provision of K was much higher in FYM. However the ratio of K/P in manure was highest for SLU. Increasing levels of manure application increased the K/P ratio in herbage. The output of K increased relatively more than the output of P.

In the trial 'levels' for all manure treatments three increasing levels of P and K were used. K yields (as Y in kg ha^{-1}) and K concentrations in herbage (C as g kg^{-1} DM) were significantly related to the K input (I as kg ha^{-1}). The effect did not depend on manure type. The relation was poor or even absent for P (Table 5.5).

5.4. N efficiency

To evaluate the efficiency of the manure applied in spring, the apparent N efficiency (ANE) of the 1st cut yield was calculated for SLU and FYM as an average of the trials 'cutting' and 'grazing' (Table 5.6).
On average, the calculated ANE was twice as high for FYM as for SLU. However, differences between years were even larger and showed a range between 1.5 and 31.4 kg DM kg^{-1} inorganic N. For FYM, rainfall in the first two weeks after application positively affected the herbage yield (1995 and 1998). However, effects were poor in 1994 although there was enough rainfall. Similarly lack of rainfall after slurry application negatively affected herbage yield. In the drier years (1994 and 1996) N-effects on yield were lowest. The lowest ANE for SLU was in 1994, when the application of slurry was delayed till the 3rd week of April, because of rainfall in March and April.
In Table 5.6 the T-value has been calculated for the day of manure application. In 1996 the temperature sum at the day of FYM application was below the advised T-value of 200. Spring N yields were poor, but the results were also affected by the low amount of rain.

Table 5.6. Apparent nitrogen efficiency (ANE): extra yield in kg DM of the first cut kg^{-1} inorganic N of SLU and FYM compared to PK (average of mixtures with cv. Alice under 'cutting' and 'grazing'), the T-value at the time of manure application, and the precipitation during the first two weeks after application.

	1994	1995	1996	1997	1998	Mean
Apparent nitrogen efficiency						
SLU	1.5	14.9	8.3	17.7	13.0	11.8
FYM	13.5	31.4	6.5	16.6	29.2	22.1
T-value (°C)						
SLU	553	259	192	326	490	364
FYM	406	210	106	241	345	262
Rainfall (mm)						
SLU	6.9	41.0	8.2	25.1	17.3	19.7
FYM	67.8	52.7	0	8.9	75.4	41.0

5.5. Discussion

5.5.1. Effects of manure on soil fertility

The application of FYM to the sward increased soil organic matter faster than did slurry. A higher level of soil organic matter is expected to be positive for the development of white clover with regard to moisture in the soil, but negative from the point of view of nitrogen release. In the long-term experiments at Park Grass (Rothamsted Experimental Station), Cooke (1976) showed that the maximum variation in soil-N level was caused by manuring (0.21-0.35 %N) and by liming (0.21-0.32 %N).

On the humid sandy soil of our experiments, the application of slurry decreased the pH of the top soil. An increased acidity on sandy soils might be caused by the nitrification of ammonia (Van Faassen and Van Dijk, 1985). FYM maintained or even increased soil pH at the initial level of 5.4, which is an acceptable level for white clover (Frame and Newbould, 1986). In the long term, acidification will negatively affect the clover growth and the uptake of P and K from the soil. Extra liming might be needed to maintain an acceptable level of soil pH.

Low levels of soil pH (pH in water below 5.0) and calcium levels reduced nodulation of white clover (Andrew, 1976). In grass-clover swards Sheldrick *et al.*(1990) found a better P uptake in herbage at higher input levels of lime. In a comparative study with the same amounts of applied N, P and K in diluted urine and Nitro-chalk, superphosphate and muriate of potash, Drysdale (1965) explained different reactions on white clover growth by, among others, the reduced pH, because of the use of a combination of superphosphate and Nitrochalk. Urine with a pH of 8.6, on the other hand, increased the pH and K concentration in the top-soil and a positive effect on the clover development was found. Floate *et al.* (1981) described the interaction between soil pH and availability of K on deep peat soils. A low K supply was found when soil pH (water) was lower than 5.0. K deficiency was less acute when extra lime was applied. In our trials the ranking of the average soil K level after 6 years of manure application (Table 2.1) was related to the level of manure input and herbage output, but also to the ratio of K and P in the herbage (Table 5.3). PK plots always showed the lowest and FYM the highest levels. The supplied K did not depend on the manure type. In comparison to SLU and FYM, the shortage of K was highest in the PK plots. Therefore, the level of K application seemed to be more important than the difference in soil pH for the different manure types.

In our trials the negative effects of a decreased soil pH after the use of PK on white clover growth was primarily explained by the low amount of applied K (trials 'cutting' and 'grazing'). The low soil pH of the PK plots after 6 years of application (pH = 4.4 in spring 1999), also might have reduced the white clover growth. The overall growth of white clover in the trial 'levels', however, was high compared to the other manure treatments, despite the decreasing soil pH.

Changes in soil pH, but also in soil OM might have interacted with the availability of the major elements P and K.

The ranking of manure type for the soil P level in 1999 was the same as the ranking for the P field balance. SLU was lowest, PK was moderate and FYM had the highest figures. The SLU plots had the highest soil P depletion. The P output, however, was not restricted by the level of soil P or the input via manure P, which indicates that P was still not the most restrictive major element for plant growth. Shortages of P and K were also reflected in the botanical composition measured after six years of manure application. The vegetation was changed in the direction of the *Lolio-cynosuretum*, a vegetation type of the less intensive pastures (Westhoff and Den Held, 1969). The presence of non-sown species like *Leontodon autumnalis, Rumex acetosa, Festuca rubra, Anthoxanthum odoratum* and *Holcus lanatus* were signs of a decreased level of soil fertility.

5.5.2. Assessment of nutrient status in soil and herbage

To predict a sufficient dose of mineral supply needed for plant growth, chemical soil nutrient measurements can be used. However, data of soil analyses are not reliable for an indication of nutrient availability. They depend on the method of extraction (Salomons, 1998), the buffering capacity of nutrients in the soil (During and Duganzich, 1979), the depth of sampling in relation to the depth of plant roots and on differences in the absorption rate of nutrients between plant species (Dunlop *et al.*, 1979). To estimate the soil K status in grassland, some form of exchangeable K is used (Spencer and Govaars, 1982). With the sampling method used in this experiment (0-5 cm depth and HCl as extractant), the soil K level of 8 in spring was too low and the white clover showed significant K shortages and even died. An extra K dressing in 1995 led to a higher soil K level in 1996 (Figure 5.3). The higher average input from 1996-1998, still caused a gradual yearly decrease of soil K, because of a negative field balance. The higher K input in the FYM plots was reflected in a higher soil K level at the end of the trial and a higher offtake of K in herbage (trials ‘cutting’ and ‘levels’). Nevertheless the K balance for SLU and FYM was the same, which was caused by a lower K concentration in DM herbage in the SLU plots. However, based on these data it was not possible to define the range of normal and luxurious consumption of K.

A factor affecting the estimation of the mineral supply for grassland is caused by differences in the mineralisation of P and K from the manure applied. The efficiency of K and P in shallow injected slurry on grassland, are the same as for artificial fertiliser K and P, if based on the uptake of P and K (Van Dijk, 1989a and b). They found that the uptake of slurry P was higher if the injection in spring was earlier. Most of the P applied in spring was taken up only from the 2^{nd} cut onwards. However, the mobility of K in the soil was much higher and the uptake of K was already 25% both in the 1st and the 2nd cut. In a 22-years experiment carried out in a typical *Arrhenaetherum*-meadow, comparing solid farmyard manure, slurry and artificial fertilisers, Künzli and Geerling (1973) also concluded that the P and K uptake was similar for all types of fertiliser.

Instead of the estimation of soil minerals, the nutrient status of white clover can be checked by means of the nutrient concentrations in plant tissue. With the critical value method (Jones and Sinclair, 1991) concentrations in plants are indicated as deficient, just adequate or safe excess. In legume based pastures in New Zealand those values have already been estimated for several regions. Problems with the comparison of results are caused by the stage of development in relation to the age of leaves (Wilman *et al.*, 1994), weather conditions and time of measurement. Recently, however, Morton *et al.* (1999) concluded that pasture yield response could be predicted by the level of available soil P, S and K, the amount of soil reserve K, and P, K and S concentration in mixed herbage. A level of 24 g K kg^{-1} DM in mixed herbage was mentioned as a critical level for maximum yield.
In a soil deficient in P, K and Ca and consisting almost entirely of sand (92%), Andrew (1960) could find clear responses as optimum curves for P and K between mineral concentration in white clover and dry matter yield. In pot experiments critical concentrations were defined as the turning point with levels for P, K and Ca of 2.3, 11 and 10 g kg^{-1} DM respectively. In field experiments the same levels were found for P and K (2.3 and 12 g kg^{-1} DM). Higher applications of P and K led to luxury consumption. Visual symptoms of P deficiency were not accurate for the diagnosis of P nutrition, except for acutely deficient plants. Deficiency of K, however, showed a clear relationship with the level of K application. Clover plants grown at levels lower than the optimum all showed visual symptoms of K deficiency. Increased levels of Ca above the critical level showed a small increase in yield. In K deficient pastures Spencer and Govaars (1982) found a response in white clover growth to added K at a critical level of 15 g kg^{-1} DM, which corresponded with a level of 12 g kg^{-1} DM in total herbage. Rangeley and Newbould (1985) also calculated critical levels for P, K and Ca of respectively 2.0, 10 – 15 and 20 g kg^{-1} DM. The results for P and K showed much similarity with other research findings. In this study there were overall differences in K concentration between the three trials (data not shown). Differences in between years were large. The most critical situation was found in 1995, when the K concentration in DM herbage in the first and second cut was only respectively 19 and 15 g K per kg DM (trial 'cutting'). In the second cut the clover died. After a potassium dressing the level increased till 32 g K per kg DM in the third cut and signs of K shortages were fully disappeared.

5.5.3. Field mineral balances

The field balance calculations (Table 5.3) showed a firm relationship between K yield and K application level, irrespective of manure type. A relationship with the P application level was not found. Therefore K uptake was regulated by the level of applied manure-K, whereas the P uptake was more related to the herbage yield.

Based on the experience in these trials, on this soil type an input of about 350 kg K ha^{-1} and 42 kg P ha^{-1} is necessary in a full cutting regime, to reach a zero field mineral balance. On the other hand, on sandy soils the buffer capacity for K was low and risks of leaching for N and K were high. Therefore some extra input is needed for building up soil fertility (organic matter and soil life) and to compensate for leaching during winter. For this type of soil with a small K buffer capacity, an even distribution over the growing season of K in farm waste, manure, effluent or fertiliser is important, otherwise this might lead to luxury consumption and undesired levels of K in the herbage (Younie *et al.*, 1994) or to low K levels at the end of the growing season.

5.5.4. Timing of manure application

The low clover yields in 1998 were due to a combination of an effective and early manure N application (28 Feb), a high FYM quality in terms of inorganic N amount (39 kg N ha^{-1}), the high winter and spring temperatures and the low precipitation during the growing season. Although the growing season in 1998 started very early, the total annual herbage yields were low. The early FYM application and the ideal growing circumstances in spring, however, were not followed by an earlier date of cutting. Therefore the spring yields were too high (4.8-5.0 t DM ha^{-1}), although the date of cutting was normal (19 May). The application of slurry was also very effective and 1st cut herbage yields ranged from 4.5-5.0 t DM ha^{-1}). The extreme high spring yields were associated with a very low white clover yield in the 1st cut. In the 2nd and 3rd cut a higher clover yield in the SLU and PK treatments compensated for the lower clover yields of the 1st cut. Plots fertilised with FYM showed a delayed regrowth. The higher annual DM yields in FYM and SLU were negatively correlated with the white clover yield and the white clover percentage. In 1998 FYM and SLU yielded only half the amount of white clover in comparison with PK. Therefore the reduced white clover yield was mainly caused by the poor cutting management of 1998 and not by changes in, for instance, soil pH or soil nutrient status.

The lower white clover yield in 1998 dramatically decreased the N yield for FYM and SLU. Therefore the N content in herbage of the PK plots was higher. The white clover content is one of the factors affecting the average N content. Additionally a higher level of available N in SLU could significantly increase the N content in comparison to FYM. This effect was significant mainly in the first two cuts.

Frame (1987) and Fisher and Wilman (1995) showed that the combination of applied N in spring and a long interval between two defoliations were harmful for clover persistency. Applied N increased the number of grass tillers, reduced the number of white clover growing points and the amount of leaves in the upper part of mixed canopy (Laidlaw and Withers, 1998). Wilman and Fisher (1996) suggested that the risk of applied fertiliser N in out-competing white clover is higher than the risk of delayed defoliations. In our trial, applied N in spring 1998 in the SLU and FYM plots had the same negative effects on clover, although the date of application was 4 weeks later for SLU. PK plots without N application showed significant higher white clover levels in spring. In a young grass-clover sward on a sandy soil (Van der Meer and Baan Hofman, 1999), deep injection negatively affected white clover yield compared to shallow injection. However, effects were explained by differences in amount of applied inorganic N, which was twice as high for the deep injected plots. The date of cutting was the same for all plots and the double dose of applied N led to a very high grass yield and a reduced white clover growth. These findings are comparable to our results in 1998 and show that the date of manure application, the amount of applied N and the growing circumstances should be further optimised for each manure type separately. The date of the 1st cut herbage also should not be fixed, but should depend on the herbage mass present.

Bussink (1999) analysed the relationship between the T-value and the timing and level of fertiliser N on grass. A total of 5000 records were collected between 1960 and 1983. It was concluded that the optimal timing of fertiliser N application for grazing was at a T-value of 200, but for cutting this was at 300. Heavy rainfall or a cold period in the first two weeks after application reduced the effect of fertiliser N. The soil fertility in terms of P and K, soil type and temperature during fertiliser application, were important factors in explaining differences between years. The level of N was not important for the effect on yield (see also Prins *et al.,* 1988). Wightman *et al.* (1996) showed that timing of slurry application with regard to rainfall is important for the sward responses. Responses were found in above ground application of slurry and simulated rainfall within 3 h after application. Smothering of leaves negatively affected white clover development, but was avoided in our trial because slurry was injected. Another aspect of rainfall, however, is the reduction of ammonia volatilisation under cool and wet aerial conditions. In our trials we could find some evidence that rainfall after spring application affected N efficiency of applied N (Table 5.6).

On a sandy soil Schils (1997) showed that a spring application of 100 kg fertiliser N ha^{-1} increased the first cut yield with 11.1 kg DM per kg applied N, which is comparable to the results obtained with SLU application in this experiment (Apparent Nitrogen Efficiency in Table 5.6). Differences between years were large and levels of ANE could be contradictory to each other, depending on timing of manure application, soil conditions in spring and weather. Although rainfall was high in 1994 the effect of FYM was poor and the ANE was low (13.5 kg DM per kg N applied). Late application in 1994 and also in 1996 reduced the N effect for FYM and also SLU. In the winters with a high average temperature (1994/95 and 1997/98) the ANE of FYM was much higher than in other years. In 1998 FYM was applied about three weeks earlier than in other years.

The higher N response of FYM than of SLU might be affected by several factors. Firstly the timing of application was on average two weeks earlier than for SLU. A second possibility is that the lower level of inorganic N in FYM in comparison to SLU led to higher DM responses. In a range of DM responses per year of 3 to 30 kg DM kg^{-1} N, the highest responses were found at N rates of 30 to 60 kg N ha^{-1} (Frame and Newbould, 1986). The third possibility is that the methodology of determination of easily degradable N in FYM is not adequate and therefore inorganic N might be underestimated.

De la Lande Cremer (1953) also calculated the N-effect of FYM on herbage yield. The most efficient use of FYM was found after spring application. A low level of application led to higher N efficiencies. The highest N efficiencies were found on sandy soils: 26.5% or 23.5 % of total N in relation to ammonium nitrate at a level of 20 and 40 t FYM ha^{-1} , respectively. Combinations of FYM and fertiliser N did not show a yield improvement. Increased levels of either FYM or fertiliser had the same effect on yield.

6. Interrelationship of results: N-fixation per ton white clover

In this chapter additional calculations are made based on the measurements discussed in previous chapters. Two questions were raised. The first question concerns the amount of N fixation in a sward. There are several reasons to assume an optimum curve instead of a linear relationship. If the amount of N that is cycling in the grass-clover system increases, the amount of fixation will decrease. Another mechanism for controlling the amount of clover is the ratio of grass to clover in the sward. At higher amounts of clover, there is less grass available to act as a nitrogen sink.
The results from previous chapters can also be used to discuss the question whether organic grassland production should be based on manure input or on N fixation.

6.1. Additional methodology

To calculate the amount of N fixation per tonne of white clover, data were used from the trial ‘cutting’ of the pure grass (data not shown) and the grass-clover plots (cv. Alice) between 1994-1998 and from trial ‘levels’ of the low level of manure application between 1996-1998 (Chapter 4, Tables 4.3 and 4.4). For each calculation, 52 yield measurements were used. These grass-clover plots showed a range of clover yields (0.5-8 tonne). Relations were calculated separately for the three manure types as polynomial relationships.

To calculate the contribution of manure N versus fixed N, the clover N contribution was calculated as the difference between grass-clover and grass yield or N yield for each of the manure types. The manure N contributions was calculated as the difference in grass-clover herbage or N yield between SLU or FYM results on one hand and PK results on the other hand.

6.2. N yield in relation to white clover yield

The relationship between white clover yield (X as tonnes DM ha^{-1}) and N yield (Figures 6.1-6.3: kg N ha^{-1}) was affected by the amount of directly available manure-N in the different treatments.

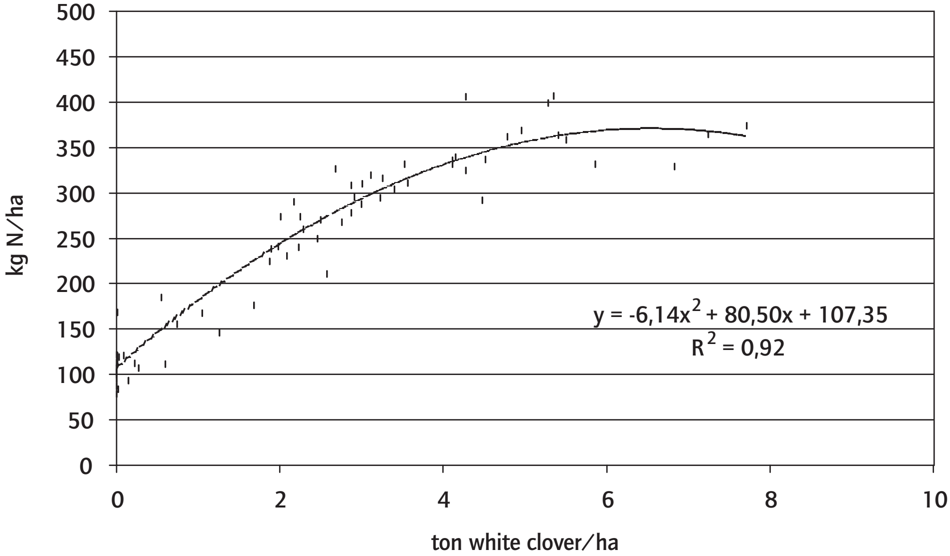

Figure 6.1. Polynomial relationship between white clover yield (t DM ha^{-1}) and total nitrogen yield (kg N ha^{-1}) with PK

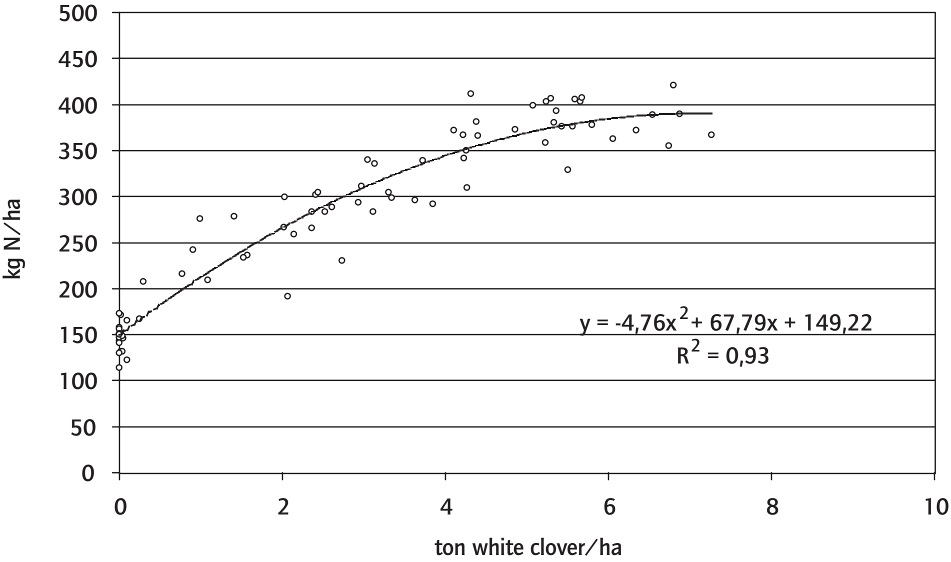

Figure 6.2. Polynomial relation between white clover yield (t DM ha^{-1}) and total nitrogen yield (kg N ha^{-1}) with FYM

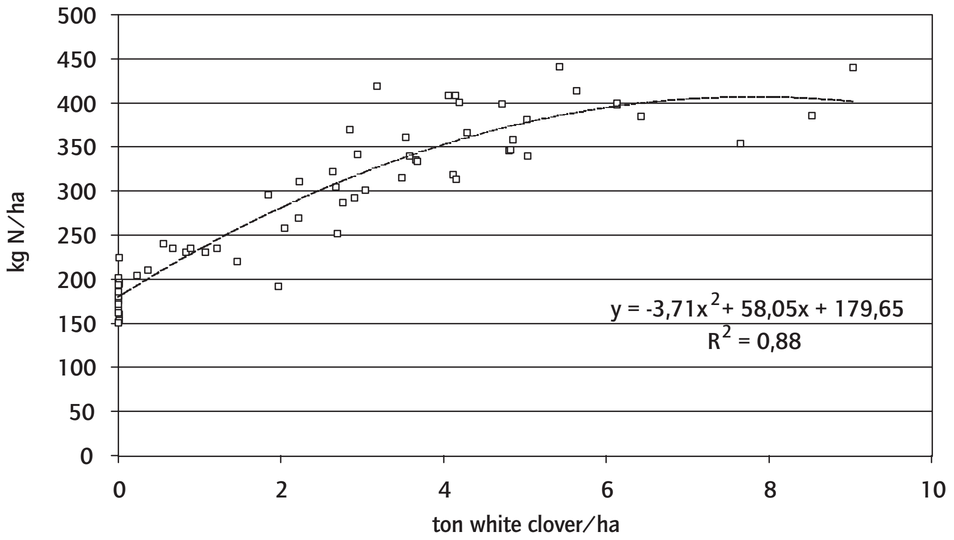

Figure 6.3. Polynomial relation between white clover yield (t DM ha^{-1}) and total nitrogen yield (kg N ha^{-1}) with SLU

In all regression models all values had $P < 0.001$ level of significance.

The polynomial relationship showed decreasing contributions of white clover to N yield if the amount of inorganic N increased. In the PK plots the highest N yield was at a level of 370 kg N ha^{-1}, which was reached at a yield of 6.8 t white clover ha^{-1}. The optimum levels for FYM and SLU were more similar at respectively 8.1 and 8.3 t white clover ha^{-1} and both yielded 400 kg N ha^{-1}. To reach a maximum N yield, white clover contents in herbage yield were much higher than for the maximum DM yield. Clover contents in DM were respectively 68, 85 and 90%.

6.3. Manure N versus fixed N

The presence of white clover as well as the applied animal manure increased herbage yields and N yields (Table 6.1). To evaluate the importance of white clover versus animal manure as N source, N yields of grass-clover and pure grass stands were compared (Table 6.1 top: clover effect) and within the grass-clover plots animal manure (FYM and PK) were compared to PK (Table 6.1. bottom: manure effect).

Clover effect

In the PK-plots without any applied N, herbage yields on average were increased by 4.3 t ha^{-1}, because of the presence of white clover (Table 6.1 top). Where animal manure was used the yield difference between grass-clover and grass was reduced to 2.2 (SLU) and 3.7 t ha^{-1} (FYM). The reduction of DM yield was highest in SLU where the highest amount of inorganic N was applied. Similar effects could be calculated for the N yields ha^{-1}. The extra N yield as a comparison between grass-clover and grass was highest in PK (+ 197 kg N ha^{-1}) and lowest in SLU (+ 142 kg N ha^{-1}), whereas FYM showed an intermediate level (+ 181 kg N ha^{-1}).

Manure effect

In the trial 'cutting' the level of white clover in PK was low and animal manure increased the amount of white clover. Within the grass-clover plots a comparison of manure types with (SLU and FYM) and without nitrogen (PK) showed an increase in yield in SLU of 0.9 t ha^{-1} and in FYM of 1.4 t ha^{-1} (Table 6.1. bottom). Differences in N yields were 47 and 56 kg N ha^{-1} , respectively. In the trial 'levels' the yield of white clover was reduced, when animal manure was applied. The increase in N yield after manure application in grass-white clover, therefore, was very low, because of an interaction between increased N input of animal manure and decreasing levels of white clover. Differences in N yields were only 8 and 2 kg N ha^{-1} for respectively SLU and FYM.

Table 6.1. Yield differences of grass-white clover versus grass and N [1] for each manure treatment (top) and grass-clover yield differences between animal manure (as SLU or FYM) and only PK application as t DM ha^{-1} and kg N ha^{-1} (bottom). Data from trial 'cutting' (1994-1998) and from trial 'levels' at the low manure level (1996-1998)

Effects of clover	**---- yield in tonnes DM ha-1 ----**			**---- yield in kg N ha-1 ----**		
	PK	**SLU**	**FYM**	**PK**	**SLU**	**FYM**
Extra yield						
Trial 'cutting': 94-98	3.84	2.21	3.61	174.1	148.1	192.0
Trial 'levels': 96-98	4.73	2.24	3.81	219.2	135.4	170.2

Effects of manure	**---- yield in tonnes DM ha-1 ----**			**---- yield in kg N ha-1 ----**		
		SLU	**FYM**		**SLU**	**FYM**
Extra yield						
Trial 'cutting': 94-98		0.99	1.43		46.8	56.4
Trial 'levels': 96-98		0.86	1.37		7.7	1.9

6.4. Discussion

6.4.1. N-fixation figures for white clover

The calculation of N fixation can be done by direct methods, using ^{15}N and by indirect methods, where pure grass stands are compared with grass-clover plots. Ennik (1982) summarised several authors who used this comparison technique and concluded that on average the level of apparent fixed nitrogen was 55 kg N t^{-1} white clover (range of 40-65). On a river clay soil Elgersma *et al.* (1998) and on a sandy soil Elgersma *et al.* (2000) calculated about the same range of N fixation, between 52-65 kg N t^{-1} clover, with significant differences between cultivars. On a sandy soil, Van der Meer and Baan Hofman (1999b) investigated several slurry application techniques, timing of application, PK and NPK fertiliser treatments and their effects on N yields of pure grass and grass-clover swards. Differences between N sources and application techniques were negligible and the N fixation was calculated as on average 46 kg t^{-1} clover. In this study, the level of N fixation based on a linear relationship was lower. The calculated N fixation decreased, if applied inorganic N in spring increased. For PK, FYM and SLU, fixation levels were respectively 45, 40 and 34 kg N t^{-1} white clover.

All authors mentioned linear regression relationships between white clover yield and clover fixed N. Such a linear relationship will only be expected at lower levels of clover. However, instead of a linear relationship there are several reasons to look for an optimum relation as a polynomial model. If the clover amount in the sward increases, the N sink as accompanying grass decreases and therefore fixed nitrogen will be recycled by the clover. The optimum relationship between N ha^{-1} and white clover ha^{-1} found for PK, FYM and SLU fits the idea that the behaviour of white clover in a mixed sward is affected by the nitrogen soil pool. Increasing levels of external N sources will not only lead to higher grass yields and lower clover yields, but also to reduced N fixation (Nesheim *et al.*, 1990). In urine spots the amount of fixed N was reduced to half of the amount for spots without urine over a four month period (Vinther, 1998). N fixation by clover is least if the soil N pool is at a sustainable high level of N (Davidson and Robson, 1986). This might be the case if animal manure is used: a constant mineralisation of organic N may lead to reduced N fixation. A greater reduction in N fixation would be expected for FYM than for slurry, because of the higher level of organic N in FYM. Murphy (1985) suggested, that in fertile soils, nitrate levels might limit N fixation by inhibiting nodule formation or by reducing the nodule activities. Therefore the amount of fixed N is reduced at higher levels of soil nitrate.
On the other hand, increased levels of white clover will lead to increased total DM and N yields. However, when the amount of white clover in the sward increases, the N fixation will decrease because of the increased amount of N in the soil pool and the turnover from clover fixed nitrogen

to grass. Fixation of N will decrease if the level of soil mineral N (via manure, mineralisation of soil OM or decomposition of white clover roots and buried stolons) increases. At low levels of white clover the sink for organic N will be the accompanying grass. When the clover yield of a mixed sward increases, the transfer of N to grass will decrease, because of the lower grass tiller density. White clover will take up its own fixed decomposed organic N. The transfer of nitrogen below ground from clover to grass was estimated on average as 70 kg N ha^{-1} $year^{-1}$ (range 3 to 102) (Ledgard and Steele, 1992).
This non-linear relationship between N fixation, decomposition, turnover and uptake of mineral N will lead to a reduction of N fixation if the amount of clover in the sward increases. The 1^{st} tonne of white clover will give the highest amount of fixation and this was calculated for PK, FYM and SLU respectively at levels of 70, 63 and 52 kg N t^{-1} white clover. At the optimum level of white clover, which was derived from the polynomial model, the N fixation level was only 7 kg N t ha^{-1} white clover for all manure applications.
This wastage in N response was confirmed by the work of Riffkin *et al.* (1999). In a survey of 71 sites, growth factors for grass-clover were associated with level of N fixation. On sandy loam soils, soil K, the number of rhizobia, the total soil N and the density of the nematode *Pratylenchus* spec. accounted for 72% of the variation in N fixation. The negative relation to total soil N supports the idea of decreasing N fixation at higher N levels.

6.3.2. Productivity effects of white clover-N versus animal manure-N

On commercial organic farms on sandy soils high levels of SLU were often used to maintain the production level of the grass-clover (Baars and Van Ham, 1996). Poor persistency of the white clover could be caused by inappropriate cultivar choice (Tables 3.1 and 3.2, Chapter 3).
Productivity in terms of dry matter yield and N yield of organic swards relies much more on the yield of white clover than on the input of manure. Clover yields in these trials depended on the level of P and K in each manure treatment in relation to the amount of N in the manure.
The increased yield of grass-clover compared to grass (clover-effect) was much higher than the increase of animal manure in relation to PK (manure-N-effect). Losses of white clover, because of management, therefore, will result in a higher dependency of external manure N. If a grass yield is required at a comparable level to the yield from grass-clover only applied with PK fertiliser, an amount of animal manure of over 50 tonnes or m^3 ha^{-1} is necessary. Effects of the absence of clover on N yield were even worse than on herbage yield. Differences between trial 'cutting' and 'levels' were caused by the larger depletion of soil K in the trial 'cutting'. If the soil P and K levels were increased, like in the trial 'levels', fixed N almost completely replaced manure N.
To reach a maximum N yield, a very high white clover content up to 90% was necessary. From an agronomic point of view, such levels are far from optimum. At these high levels, losses of N by

leaching will increase and forage losses at harvest will increase. The optimum levels found for herbage yield (43-65% in DM) are also higher than mentioned for an optimum grass-clover sward (30-40% and under sheep grazing (15-20%). Under cutting, however, levels up to 50-60% are acceptable. Differences in clover economy of organic swards were also distinguished by Newton (1995) in the UK. He found a significantly lower clover content in permanent pastures in comparison with leys (19.4% v 33.4%).
Dyckmans (1989) compared the differences in herbage yield of pure grass plots and grass-clover mixtures of resown fields at 9 sites in Germany. He used a range of N fertilisers between 0 and 300 kg N ha^{-1}. Effects of increasing N were significant in the grass plots (R^2 = 0.90), but were poor for the grass-clover plots (R^2 = 0.32). The average yield increase per kg N was 26.3 kg DM kg^{-1} N for the grass plots and only 8.5 kg DM kg^{-1} N for the grass-clover mixture. So the dependence on external nitrogen is much higher in the plots without clover and the maintenance of white clover in a mixture is therefore very important.

Calculated N-effects of animal manure in grassland

Based on long-term data of Palace leas meadow plots (UK: Coleman *et al.*, 1987) we have calculated the relationship between average yield and applied level of N. For our calculation only plots that received FYM, NPK or additional P and K were used. The relationship between applied N (X in kg total N ha^{-1}) and yield in the 1^{st} cut (Y in kg DM ha^{-1}) was significant :
Y = 18.9 X + 3198 (R^2 = 0.99).
In a five year trial in Austria, Buchgraber (1983) investigated effects of different types of FYM (fresh, short composting, long composting; 70-120 kg total N ha^{-1}) and slurry (aerated, not aerated, diluted; 100-120 kg total N ha^{-1}) in relation to PK and NPK fertiliser (0-250 kg N ha^{-1}). The cutting regime was only 3 times per year and the trial started when the pasture was 10 years old. Applied N (X) increased total herbage yields (Y) to 9.8 kg DM kg^{-1} N. The increase of total yield and decrease of white clover did not depend on the choice of manure or fertiliser. We have calculated the following relationships based on average yield data:

Y = 6923 + 9.8 X (R^2 = 0.81)

N reduced legumes yield (Z) (mainly white clover) by 4.5 kg DM kg^{-1} N:

Z = 1267 – 4.7 X (R^2 = 0.75)

From average yield data of Van der Meer and Baan Hofman (1999) we could also calculate a significant relationship between inorganic N application applied before the first cut in spring

($X_{inorg-first}$ from 0-120 kg N ha^{-1}) as shallow or deep injected slurry, or fertiliser N before the 1st cut in relation to the white clover yield (X) measured as the average yield of the 2nd and 3rd year at the same level of K:

$$Z = 2712 - 10.7\, X_{inorg-first}\ (N = 6, R^2 = 0.98)$$

They showed, that a higher level of applied P and K increased white clover yields at the same level of N application. Additionally, surface spread diluted or undiluted slurry showed higher clover yields at the same level of N. Losses of ammonia during application were probably higher for surface spread slurry. Diluted slurry yielded as much as deep injected slurry, the clover level however was significantly higher. Grass growth was very profitable because of slurry dilution.
In several plots of Van der Meer and Baan Hofman (1999), extra N was applied after the 2nd cut. With regard to the total inorganic N application per year ($X_{inorg-total}$) the decrease of white clover in relation to the total amount of applied inorganic N was poorer compared to spring application:

$$Z = 2793 - 7.5\, X_{inorg-total}\ (N = 6, R^2 = 0.74)$$

Total DM yields (Y), however, showed a higher correlation with total inorganic N application $year^{-1}$ than with the application level of inorganic N before the 1st cut:

$$Y = 8390 + 21.6\, X_{inorg-first}\ (N = 6, R^2 = 0.75)$$
$$Y = 8497 + 15.5\, X_{inorg-total}\ (N = 6, R^2 = 0.83)$$

Effects of animal manure can be seen as the sum of separate effects of the mineral composition. Effects therefore can be positive or negative. The results show that effects of animal manure in a young grass-clover sward can be understood primarily as an effect of applied N, if P and K levels are sufficient. In our trials, white clover yields were negatively (trial ‘levels’) or positively (trial ‘cutting’) affected by the use of animal manure. At higher levels of P and K in trial ‘levels’, repeated application of slurry reduced the white clover content of the sward. At insufficient levels of P and K, animal manure application might increase white clover growth, because of the positive effects of extra P and K. In that case, the positive effect of higher K application using animal manure is more important than the negative effects of inorganic N.