The impact of mobile green manures on broccoli (*Brassica oleracea var. italica*) yield and quality

Msc Thesis in Organic Agriculture

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WAGENINGEN UR
For quality of life

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An MSc thesis submitted in partial fulfilment of the requirements for the degree of Master of Science in Organic Agriculture major in Agroecology at Wageningen University, the Netherlands

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EXECUTIVE SUMMARY

This study aims to investigate if the use of mobile green manures can improve nitrogen use efficiency (NUE), yield and quality of broccoli as compared to the use of animal manure under organic farming conditions.

The field experiment was conducted at Droevendaal Farm an organic research facility which is part of WUR in Wageningen, the Netherlands from 1 June 2011- 3 October 2011. The mobile green manures tested were grass clover fresh (GCF), grass clover silage (GCS), and alfalfa pellets (ALP). In terms of manure application, slurry (Slur) and farm yard manure (Man) treatments were included while a non-fertilized control (Con) treatment was added to assess inherent soil fertility served. Furthermore, for grass clover (fresh) and the alfalfa pellets split application treatments (ALP2 and GCS2)were added as well. This to assess if using split applications would enhance the synchronization between soil N supply and crop N demand. A completely randomized block (RCB) design was used with four replicates thus resulting in a total of thirty-two field plots.

Broccoli yield was highest (10.8 ton/ha) with GCF followed by ALP2 (8.4 ton/ha) and ALP (7.7 ton/ha) while Con and Slur had the lowest yield (5.5 and 5.7 ton/ha, respectively). There was no significant difference between single and split application both for silage and alfalfa treatments (at $LSD_{0.05}$= 1.0 ton/ha)

The GCF, ALP2, ALP, and GCS2 treatments had the highest NUE (25.7, 23.2, 17.5, 16.3, 13.5 kg fresh weight/kg applied N) while the slurry has the lowest (2.0 kg/kg) NUE. at $LSD_{0.05}$ = 8.29 kg/kg.

The broccoli head nitrate content was lowest for the Man and Con treatments (101 and 111 mg N/kg, respectively) while the GCS and GCS2 were the highest (339 and 406 mg N/kg, respectively). Other treatments were in between.

1 INTRODUCTION

1.1 Background

The growth of the organic sector during the past decades has been triggered by several factors such as environmental and health concern as related to artificial fertilizer and pesticide usage in conventional farming. On the other hand, there are some issues restricting further organic agriculture growth like product price, availability of organic fertilizer, and organic food safety. Organic animal manure, may not be available locally, its nutrient ratios may not be optimal, while there are also health concerns of potential bacterial contamination particularly when the manure is used in growing vegetable products. As a result, the usage of green manure has recently received increased attention (Kumar and Goh, 2000; Thorup-Kristensen *et al.*, 2003; Scholberg *et al.*, 2010).

Green manure crops are usually grown as part of the crop rotation, either during a short fallow period like in winter or summer green manure or for extended periods for example as two or three year leys. They can also be undersown in a cereal crop (Rayns and Rosenfeld, 2008). However, the need for growing and incorporating green manure prior to the planting of the cash crop at times hampers their effective use in terms of maximum nutrient accumulation. Using mobile green manures system, whereby the green manure may be harvested from another field at a certain time and then either temporarily stored or directly applied to a targeted field enhance flexibility and also use efficiency. As an example, Swedish researchers harvested common reed in a Swedish lake and applied it either as fresh material, compost, or used it first in biogas production and then applied the sludge to the crop field (Hansson and Frederiksson, 2004).

In order to be of practical use, a mobile green manure needs to be rich in nutrients, able to release the nutrient according to the crop demand, and easy to store and handle. Different green manure species and treatment like ensilage or drying may be used that meet these requirements (Sorensen and Thorup-Kristensen, 2011). The matching of nitrogen supply from the green manure and the nitrogen demand of the crop is generally the greatest challenge which involves the matching in time (synchronization) and the matching in place (synlocation) as affected by soil type, precipitation, and root proliferation (Båth, 2000). The possible yield differences between treatments can be explained by the help of NDICEA program (van der Burgt *et al*., 2006), a tool used for enhancing our understanding of in-season nitrogen dynamics in farmers' fields.

While it is generally known that slurry releases nitrogen very fast, it has been found out that farm yard manure can immobilize soil nitrogen during the first three weeks before net mineralization occurs (Mohanty *et al.*, 2011). As for green manures, different plant species and parts have different decomposition/mineralization rate. For example white clover (*Trifolium repens*) leaves decomposes faster than its roots while white clover roots decomposed faster than perennial ryegrass (*Loliumperenne*) shoots during the first 40 days. After this period they decompose very slow (de Neergaard *et al.*, 2002). Thus it is expected that by mixing grass and clover, the overall decomposition rate will be changed.

In terms of broccoli (*Brassica oleracea var. italica*), it has been found that the nutrient demand followed a bell-shaped pattern with the peak in N-uptake occurring at around 30 days after transplanting (Nkoa *et al.*, 2003). It is thus makes sense to apply the green manure before transplanting and as top dressing in the middle of growing period, this to enhance N-synchronization.

An example of synlocation is the incorporation of lucerne at a depth of 30-40 cm. With an Australian clay Sodosol soil this was shown to increase wheat yield while subsoil manuring also improved soil physical properties and increased root growth (Gill *et al.*, 2009).As for broccoli, rooting depth increased with subsoil mineral nitrogen reserves (Thorup-Kristensen, 1993). However, as the recent trend is to apply reduced tillage to the field, subsoil application of the mobile green manure is not really desirable. Furthermore, for sandy soil, the nitrogen is easily leached to the subsoil, thus synlocation may be automatically achieved.

The broccoli growth stages consist of transplanting, vegetative growth, harvesting, flowering, and seed production stage. During the harvesting stage, the inflorescences grow until they have reached their maximum size while still being marketable (Theunissen and Sins, 1984). Thus, the yield of the broccoli is generally related to the head weight while N-supply is the dominant factor determining the yield of broccoli and N-uptake ranges between 150-280 kg N per ha (Thompson *et al.*, 2002). Besides available nitrogen, broccoli yield is also affected by other soil quality parameters. Different manures can have different impact on the soil quality. For example, it was found that in the short term, compost application resulted in stabilization of pH and decrease of water infiltration rate in broccoli fields (Stamatiadis *et al.*, 1999). The incorporation of green manures in the soil will also increase soil organic matter (SOM) which in turn will enhance nutrient retention, improve soil structure, aggregate stability, and soil moisture retention. Increasing SOM also tends to increase earthworm activity (Riley *et al.*, 2008).

The quality of broccoli includes its nitrate content. Fertilizers containing N-forms that first needs to be nitrified such as ammonium sulphate and urea, have lower broccoli head nitrate content, whereas the use nitrate-based fertilizer tends to increase these values (Elwan and

Hamed, 2011). Furthermore, higher applied nitrogen resulted in higher head nitrate content (Erdem *et al.*, 2010).

1.2 Research aim

The research aim is to study if the use of mobile green manures can improve nitrogen use efficiency, yield and quality of broccoli as compared to the use of animal manure under organic farming conditions.

1.3 Research question

a. How is the use of mobile green manures affecting nitrogen utilization, yield and quality of broccoli, earth worm activity, weed growth and soil quality as compared to slurry and farm yard manure?
b. Is split application of mobile green manures an effective management strategy to improve nitrogen use efficiency and broccoli yield?

1.4 Hypothesis

a. Use of mobile green manures will result in more efficient nitrogen use compared to the use of animal-based manures
b. Use of mobile green manures will increase yield and reduce nitrate content compared to use of slurry and farm yard manure.
c. Use of slurry will increase weed density
d. Use of composted manure will increase earth worm numbers
e. Split-application of mobile green manures will result in improved synchronization and thus in higher yield compared to application of all the fertilizers prior to planting.

1.5 Thesis structure

Section 2 of this study will describe the experiment set up as well as field and laboratory methodology used. Section 3 will present the result which will be discussed in section 4. Section 5 will synthesize results and includes an overview table to evaluate the initial hypotheses.

2 MATERIALS AND METHODS

2.1 Site conditions

The experimental site was located at the organically managed experimental farm Droevendaal, the Netherlands. The overall soil pH is around 5.1 and the soil is a sandy soil with 1% clay, 2% silt and 97% sand and has an organic matter content of 4.3%. In specific, the particular field used in the experiment (field 8) has an organic matter content of 3.7% while the total N content in 2010 was 0.121%.The monthly weather data for the field experiment duration (1 June 2011- 3 October 2011) was displayed in Table 2.1.

Table 2.1. Weather condition in Wageningen, the Netherlands during field experiment (data from Haarweg weather station)

Month (2011)	**Average temperature (°C)**	**Total precipitation (mm)**
June	16.5	108.8
July	15.9	140.8
August	17.1	131.4
September	15.9	45.4
October (first 3 days only)	16.7	0.0

2.2 Experimental design

Each plot received a targeted value of 150 kg total N/ha from different soil amendments. The treatments are summarized in Table 2.2. The eight treatments were laid out using a randomized complete block design with 4 replicates. The plot dimensions were 6 × 3 m (18 m^2) with four rows per plot with 75 cm distance between the rows and 50 cm distance between the plants. The complete experimental design is described in Appendix I.

Table 2.2. Experimental factor

No	**Treatment**	**Abbreviation (Code)**
1	Control	CON

2	Slurry	SLUR
3	Farm yard manure	MAN
4	Grass clover-fresh	GCF
5	Grass clover-silage	GCS
6	Grass clover -silage-split application	GCS2
7	Alfalfa pellets	ALP
8	Alfalfa pellets-split application	ALP2

All fertilizer material for the standard (full) treatments and half of it for the split application treatments were incorporated in the upper 15 cm of the soil depth prior to planting. The slurry was incorporated on 7 June 2011 while the other treatments were applied on 8 June 2011. The second half of the split application was applied between two rows 5 week after transplanting (WAT), which was on 22 July 2011.The grass clover was obtained by mowing a nearby grass clover field with a composition listed in Table 2.3. The grass clover-fresh was mown from a 7 cm tall sward and the applications were based on pre-determined dry matter content.

Table 2.3. Grass clover composition

Grass percentage (%)	**Species**	**Cultivar**	**Seeding rate (kg/ha)**
29	*Loliumperenne*	Romark	30
6	*Loliumperenne*	Ambrose	30
64	*Loliumperenne*	Asturion	30
-	*Trifoliumrepens*	Presel wig	3
-	*Trifoliumrepens*	Elles	3

The broccoli (*Brassica oleracea var. italica)* cultivar used in the experiment was Ironman Biologisch KWF. The 6-week old broccoli transplants were planted manually in the field on 16 June 2011 and plants were watered with 10 mm of water to reduce transplant shock. Irrigation was also applied to supplement rainfall in order to reduce the risk of potential crop water stress on the 1 and 3 of June 2011 and 25 mm was applied each time.

On 5 August 2011, an organic pesticide was sprayed since there was an indication of aphid invasion using a mixture of 1 litre of spiritus, 1 litre of soap, and 20 litres of water.

The last harvest was completed on 3 October 2011.

2.3 Field and laboratory analyses

2.3.1 Manure

A representative sample of the manure for each different treatment was taken and dried to determine whether the content was the same as predicted. For the green manure samples, the samples were dried first in the oven for at least 24 hours in 70°C and grinded before analysed. For the slurry and farm yard manure, samples from the cooler room were used (not dried). The dry matter content, mineral nitrogen, total nitrogen, P_2O_5 and K_2O content were then analysed.

2.3.2 Soil

<u>Soil sampling</u>

Soil sampling was carried three times during cultivation (-1, 7 and 14 WAT) and the soil was analyzed for pH, moisture content, P-PO_4,NH_4-N and NO_3-N. The soil sampling for soil organic matter analysis was carried out two times (-1 and 14 WAT) by taking samples for the 0–30 cm soil depth. Soil samples collected prior to manure application were taken from the field margin at both ends of the field using four replicates by mixing ten core samples in each field end (thus 20 core samples for each replicate). The samples were put in the oven for 40°C for five days. Around 300 gram subsamples were taken for each replicate and sieved (2 mm grid size). The final samples to be analyzed only consisted of around 50 gram soil. The same procedure was followed for 7 and 14 WAT samples and in this case samples were taken inside the field plots between the four rows within each 3 m inner plot (1.5 m from both plot edge).

<u>Earthworms</u>

Earthworm samples were taken by excavating a soil volume of 20× 20 × 20 cm block between the four rows within each 3 m inner plot. Two samples in each plot were taken at14 WAT. The earthworm numbers and total earthworm fresh weight for each sample were determined. Average value of earthworm numbers and total fresh weight in each replicate was calculated from the average of the two samples in each replicate. To avoid the inclusion of soil in the fresh weight measurement, the earthworms were washed with water and dried with tissue papers. The specific weight of the earthworms in each replicate was calculated by dividing the total fresh weight of the earthworms in each replicate with average earthworm numbers in each replicate.

2.3.3 Plant

A total of 6 out of 12 plants from the 3 m inner rows (row 2 and 3) of each plot were used for the general growth measurements. The following measurements were taken:

Plant height

Plant height was measured from the soil surface to the uppermost growing point. Plant height measurements were collected three times (3 WAT, 7 WAT, and at harvest).

Leaf number

Leaf number was counted twice (3 and 7 WAT). Only green leaves > 1 cm length were included in the leaf counts. The leave was defined as a green leave if >50% of the area was green.

Leaf area

The leaf area values were measured three times (3 WAT, 7 WAT, and at harvest) using a non-destructive method for 3 WAT and 7 WAT (Stoppani*et al.*, 2003). Length and width at the widest point of all leaves for each plant were measured for calculating leaf area. A general equation to estimate individual leaf area of broccoli was:

Leaf area (cm^2) = $L \times W \times k$ (Equation 1)

Where:

L = leaf length (cm)

W = leaf maximum width (cm)

k = a constant

The constant was calibrated by measuring the leaf length, width, and actual leaf area (measured using a leaf area meter). Around 10-20 remaining broccoli transplants was used for this initial calibration.

At harvest, leaf sub samples were taken from 3 leaves from each plant of 4 harvested plants for sub sample. The leaves in each plant were taken using the following criteria: 1 small leaf that was not fully expanded but greater than approximately 5 cm, 1 recent fully expanded leave, 1 biggest leave, and 1 mature leave but less than 10% yellow/purple. The fresh weight of the leaf sub sample was measured. The leaf blade was then separated from the petiole and the vein and measured using a leaf area meter. The dry weight of the leaf subsample (blade, petiole, and the vein) was measured after oven drying of 70° C for around 24-72 hours. The following formulas were then used to estimate the leaf area in each replicate.

Specific leaf area (SLA, cm^2/g) = leaf area sub sample (cm^2) / dry weight leaf sub sample (g)

Average leaf area (cm^2) = average leaf fresh weight (g) × dry weight content of leaf sub sample (%) × SLA (cm^2/g).

The leaf area sub sample was then grinded after drying for chemical analysis.

Leaf area index (LAI) was calculated based on the product of leaf dry weight with the corresponding SLA value for a specific plot.

Chlorophyll content index (CCI)

CCI measurement was taken twice (7 and 9 WAT).The measurements were using a chlorophyll content (SPAD) meter (SPAD 502, Konica Minolta Sensing, Inc, Osaka, Japan) . Measurements were taken using the most recently matured (fully expanded) leaf..

Head initiation date and harvest date

In general, the heads in a plot were considered to have initiated when more than 6 out of 12 of the plants in the observed inner rows of the plot had a main apex larger than 1 mm in diameter. For the slurry plots, smaller net plots were used due to slurry only being uniformly applied in the central part of the plot. Therefore, in this case the criterion was that 4 out of 8 plants had formed a main apex. Observations were made daily starting at 47 day after transplanting (DAT) until all the plots had formed heads. The harvest date of each replicate was calculated as the approximate harvest time when more than 50% of the plants in the replicate had been harvested.

Head diameter and above-ground shoot fresh matter

The head diameter was determined at harvest by taking the average of perpendicular widest length and width of the head. The above-ground shoots were separated into head, leaves, and stems. For each part, the fresh weight was determined. The harvested head criteria was taken according to the local farmer’s practise to be the main head plus a portion of the main stem until slightly below the lowest floret.

Head and above ground- shoot dry matter (DM)

The DM was determined after drying samples at 70°C for 24-72 hours. The .dry matter (DM) percentage of the head and above-ground shoot were determined based on fresh and oven-dried subsamples weights. To take a stem sub-sample, 4 thin sections each from the upper, middle, and lower of the stem were cut using a chopper in the Unifarm. The stem sub sample from 4 plants in each replicate was then combined (about 200 g subsample in total), dried, and grinded. For the head sub sample, a quarter portion of each head from 4 plants in each replicate was combined, dried, and grinded for chemical analysis.

2.3.4 Weed

Weed density

Two areas of 50 × 50 cm in the 3 m inner rows (row 2 and 3) of each plot were selected and used. A 50 × 50 cm frame was put in the middle of the broccoli plant. Weed density measurements were taken twice (3 WAT and 7 WAT) by counting the number of weeds in each of the sampled areas. Mechanical weeding between the rows and hand-weeding between the plants in a row was done at 4 WAT. Additional hand weeding was done at 7 WAT after the weed density measurements.

Weed ground cover

Two areas of 50 × 50 cm in each of the 3 m inner rows (row 2 and 3) of each plot were selected and used. So there was 4 samples/plot. The sampling was done two times (3 WAT and 7 WAT). A 50 × 50 cm frame was put in the middle of the broccoli plant. The weed ground cover was estimated from 0-100% in 10% increments.

2.3.5 Chemical analysis

Total N in plant parts (head, stem, and leaves) were determined after wet decomposition by adding concentrated H_2SO_4 with an mixed catalyst and hearting to 330°C. The nitrate content of the florets, mineral nitrogen content of the soil and manure samples, and the available P content of the soil was measured after sample extraction with 0.01 M $CaCl_2$. The content was then measured spectrophotometrically by segmented-flow analysis. Available K of the manure sample was determined after extraction with 0.01 M $CaCl_2$ and analysed by flame emission spectrophotometer. The pH was also measured after extraction with 0.01 M $CaCl_2$and measured using a pH meter and combined electrode. Organic matter was determined by loss-on ignition.

2.4 Statistical analyses

SPSS version 19 (IBM, USA) was used to test significant difference of the results between the treatments. Post hoc LSD analysis with P = 0.05 was used to compare significant differences between the treatments.

2.5 NDICEA

To explain the effect of underlying N mineralization dynamics on potential yield differences, the soil and weather condition at the field site as well as the irrigation schedule and final yield were inputted into the NDICEA program (version 6.0.17, Louis Bolk Instituut, the Netherlands). The input values used to initialize NDICEA were based on data from previous field studies conducted in 2008. Some of the soil parameter values (pH, organic matter, and mineral nitrogen) were updated based on the measurement results of this experiment as presented in Section 3.2. The initial t mineral nitrogen value measured in 2011 was based on the measurement of the field margin on 7 June 2011 and it was assumed that values were homogenous across the entire field. While for subsequent pH, organic matter, and subsequent mineral nitrogen values were based on plot-specific values for different treatment based on averaged measurements for corresponding treatments. To convert mineral nitrogen in the top 30 cm of the soil from mg/kg to kg/ha a bulk density of 1.47 g/cm^3 was assumed (this was based on averaged bulk density values from soil samples in the top 20 cm with 6 samples for every 5 cm soil layer, collected for field 8 in 2008). As a result, the following formula is used:

Amount of mineral nitrogen in kg/ha = amount of mineral nitrogen in mg/kg × 3 × 1.47.

The irrigation date and rate were similar for all treatments. On the other hand, the end of the vegetative growth stage (assumed to be the same as head initiation date), harvesting date, fresh yield, dry matter content, dry matter distribution, nitrogen content of different plant parts, and manure properties for different treatment were inputted also to the organic broccoli crop parameters and fertilizer parameters in the NDICEA system.

3 RESULTS

3.1 Manure contents

In a number of cases, the actual N and/or dry matter content of applied fertilizer materials differed from the expected values based on previous sampling and as a result the actual N application rate deviated from the targeted one (Table 3.1). The actual total N application rate ranged from 98 kg N/ha to 204 kg N/ha as compared to the target value of 150 kg N/ha. This was related to the dry matter content of the animal manure being much higher (31%) compared to the expected value of 19%, thus resulting in higher total N application rate. The actual mineral nitrogen contents were in general much lower than the predicted values, except for grass clover fresh, where the actual N concentration was 60% higher than expected. In terms of P-P_2O_5 and K-K_2O contents, values generally lower than expected.

Table 3.1. Predicted values of manure contents

No	Treatments	Dry matter (%)		Nmin(%)		Ntot (%)		$P\text{-}P_2O_5$ (%)		$K\text{-}K_2O$ (%)		Kg N/ha/ application		Organic matter (%)	pH
		Target	Actual	Target	Actual	Target	Actual	Target	Actual	Target	Actual	Target	Actual	Actual	Actual
1	Con	0.0	0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0	0	0.0	-
2	Slur	9.0	8.5	2.50	0.54	5.00	3.71	1.50	0.79	5.40	4.00	150	105	76.3	7.9
3	Man	18.9	30.9	0.43	0.24	2.75	2.17	1.17	0.59	3.56	2.57	150	193	55.8	7.2
4	GCF	23.2	19.0	0.05	0.15	2.50	4.14	1.40	0.41	3.79	1.82	150	204	89.6	5.8
5	GCS	71.5	76.2	0.25	0.01	1.86	1.14	2.93	0.26	2.93	1.92	150	98	93.2	5.3
6	GCS2	71.5	76.2	0.25	0.01	1.86	1.14	2.93	0.26	2.93	1.92	75	49	93.2	5.3
7	ALP	89.5	90.6	0.40	0.04	3.17	2.59	0.89	0.26	3.23	2.17	150	124	86.2	5.8
8	ALP2	89.5	90.6	0.40	0.04	3.17	2.59	0.89	0.26	3.23	2.17	75	62	86.2	5.8

3.2 Soil quality

3.2.1 Nitrogen, soil organic matter, pH, and moisture content

There were no significant difference in terms of soil mineral nitrogen and total nitrogen for the different treatments, except for split application at 14 WAT, which resulted in slightly higher N-NO_3 content (Table 3.2). This made sense since some part of the manures in the split application were added to the soil during the second application (5 WAT), which is closer to the last soil sampling (14 WAT) compared to other treatments where full materials were applied prior to planting. The mineral nitrogen available in the soil in general increased over time as the manure started to decompose in the soil. For the two different measurement dates (7 and 14 WAT), the increasing trends were significant for both N-NO_3 ($F(1,45)= 102.795$, $P < 0.001$; data is not shown) and N-NH_4 ($F(1,45)= 7.647$, $P = 0.008$; data is not shown) as well as for Nmin ($F(1,45)= 19.907$, $P < 0.001$; data is not shown). The total soil N content was 0.10% prior to planting and around 0.11% at harvesting across all treatments (data is not shown).

Table 3.2. Effect of manure treatment on the soil nitrogen contents for different measurement dates.

	-1 WAT			7 WAT			14 WAT		
Treatment	N-NO_3 (kg/ha)	N-NH_4 (kg/ha)	Nmin (kg/ha)	N-NO_3 (kg/ha)	N-NH_4 (kg/ha)	Nmin (kg/ha)	N-NO_3 (kg/ha)	N-NH_4 (kg/ha)	Nmin (kg/ha)
Border	5.95	25.16	31.11						
Con				3.1 a	20.0	23.2	6.9 a	28.4	35.3
Slur				3.5 ab	23.7	27.2	7.4 a	30.2	37.6
Man				3.3 ab	19.9	23.2	8.7 ab	26.2	34.9
GCF				3.6 ab	19.8	23.3	8.0 ab	24.0	32
GCS				5.0 ab	20.2	25.2	8.3 ab	27.1	35.4
GCS2				6.2 b	19.7	25.9	12.5 b	34.8	47.3
ALP				4.8 ab	19.5	24.3	9.2 ab	19.8	28.9
ALP2				5.8 b	24.7	30.4	10.4 b	33.6	44
P-value	-	-	-	0.102	0.939	0.878	0.009	0.765	0.545
$LSD_{0.05}$	-	-	-	ns[1]	ns	ns	2.73	ns	ns

[1] ns = not significant

[2] Different letters within each parameter indicate that means differed significantly ($P < 5\%$) as established by the LSD test.

[3] Each value shown here is an average of four replicates.

There were no significant differences for any of the other soil characteristics among different treatments (Table 3.3). The moisture content in the soil decreased in the second sampling time ($F(1,45)= 256.005$, $P < 0.001$; data was not shown). This was directly related to the rainfall. The pH was more or less constant over time while available soil P values also did not change significantly over time (data was not shown).

Table 3.3. Effect of manure treatment on soil pH, organic matter, phosphorus, and moisture content for different measurement dates

	-1 WAT			7 WAT			14 WAT			
Treatment	pH	OM (%)	$P\text{-}PO_4$ (mg/kg)	Moisture content (%)	pH	$P\text{-}PO_4$ (mg/kg)	Moisture content (%)	pH	OM (%)	$P\text{-}PO_4$ (mg/kg)
Margin	5.67	3.93	1.23							
Con				16.6	5.32	1.93	11.9	5.30	3.98	1.75
Slur				16.3	5.30	1.85	11.9	5.32	3.95	1.73
Man				16.9	5.31	2.18	12.4	5.32	3.82	2.20
GCF				16.2	5.25	2.03	12.0	5.26	3.95	1.80
GCS				16.5	5.38	2.10	12.0	5.33	3.97	1.93
GCS2				16.9	5.32	1.95	12.9	5.35	4.01	1.68
ALP				16.4	5.30	2.08	12.3	5.31	4.00	2.05
ALP2				16.3	5.30	1.80	12.0	5.29	3.88	1.95
P-value				0.966	0.796	0.689	0.900	0.854	0.987	0.337

[1] Different letters within each parameter indicate that means differed significantly ($P < 5\%$) as established by the LSD test.

[2] Each value shown here is an average of four replicates.

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3.2.2 Earthworm fresh weight and number

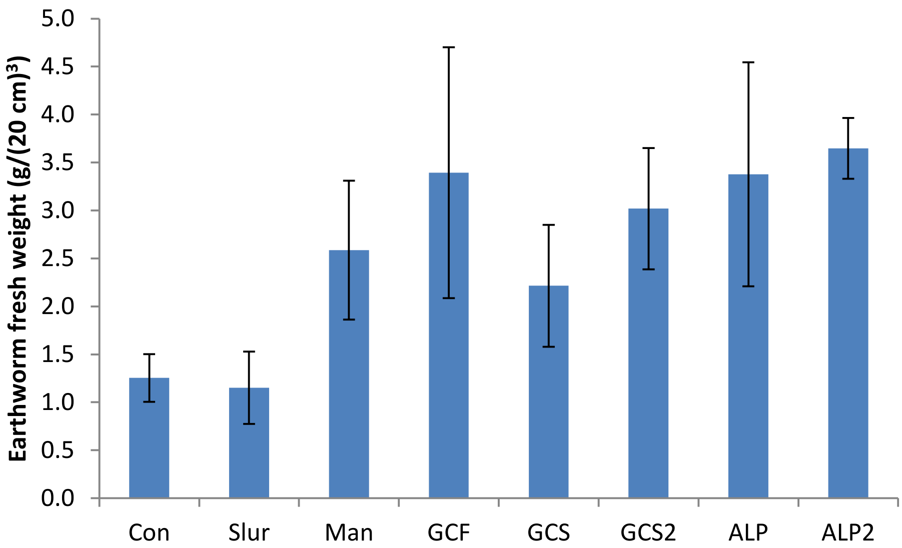
There was no significant difference for earthworm fresh weight , number, and specific weight among different treatments (Table 3.4, Fig. 3.1).

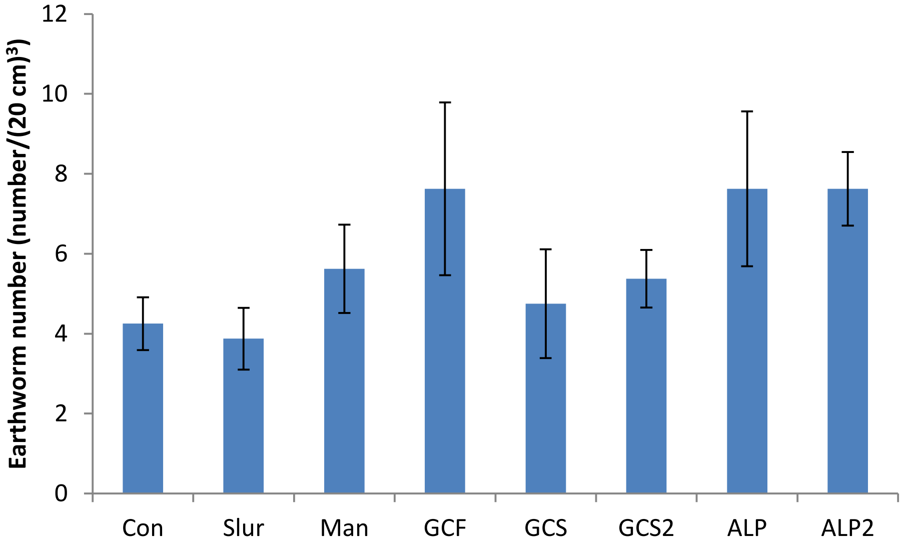
Table 3.4. Earthworm fresh weight, number, and specific weight

Treatment	**Total fresh weight ($g/(20\ cm)^3$)**	**Earthworm number ($number/(20\ cm)^3$)**	**Specific weight (g/earthworm)**
Con	1.25	4.25	0.31
Slur	1.15	3.88	0.29
Man	2.59	5.63	0.45
GCF	3.39	7.63	0.40
GCS	2.22	4.75	0.46
GCS2	3.02	5.38	0.55
ALP	3.38	7.63	0.41
ALP2	3.65	7.63	0.50
P-value	0.232	0.277	0.078

[1] Different letters within each parameter indicate that means differed significantly (P < 5%) as established by the LSD test.

[2] Each value shown here is an average of four replicates.





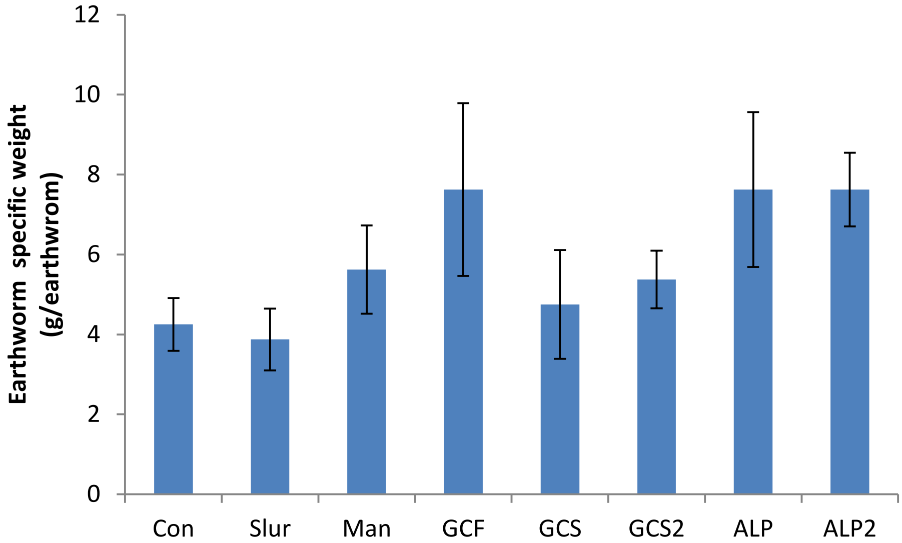


Fig 3.1. Earthworm fresh weight, worm number, and specific weight. Peak to peak error bar shows 1 standard deviation.

The normality of the data was also checked visually by using histogram and P-P plots of the residuals of the statistic model (Figs 3.2 and 3.3). The histogram for the residual of worm number seems to be not so normal, however the P-P plots seems to be more or less normal. To further check the normality more objectively, the Kolmogorov-Smirnov (KS) and Shapiro-Wilk (SW) test were applied to the data (Table 3.5). The results showed the data did not significantly deviate from the normality.

Table 3.5. Normality test for earthworm data

Residual data	**Kolmogorov-Smirnov**(*P*-value)	**Shapiro-Wilk**(*P*-value)
Fresh weight	0.200	0.894

Number	0.200	0.602
Average fresh weight	0.200	0.434

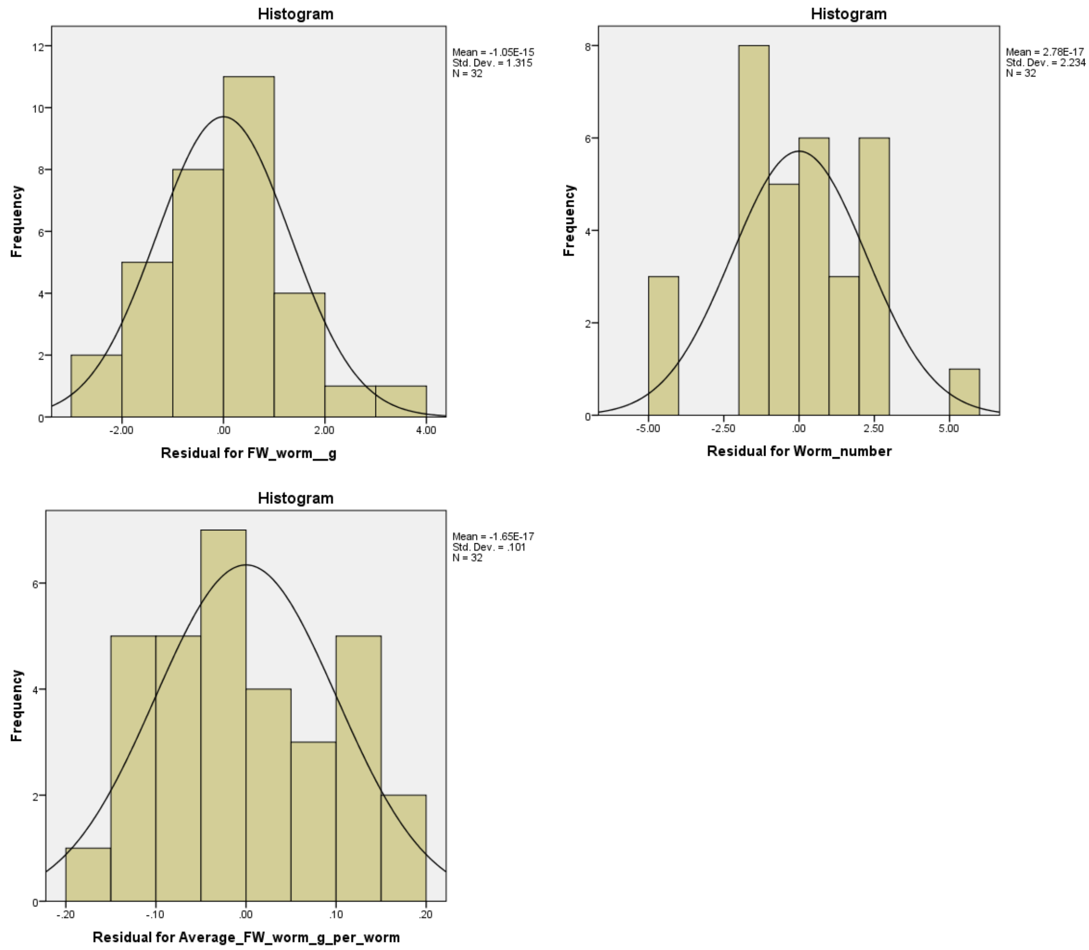


Fig 3.2. Normality histogram for the residual analysis of earthworm fresh weight, worm number, and specific weight (average FW worm (g) per worm).

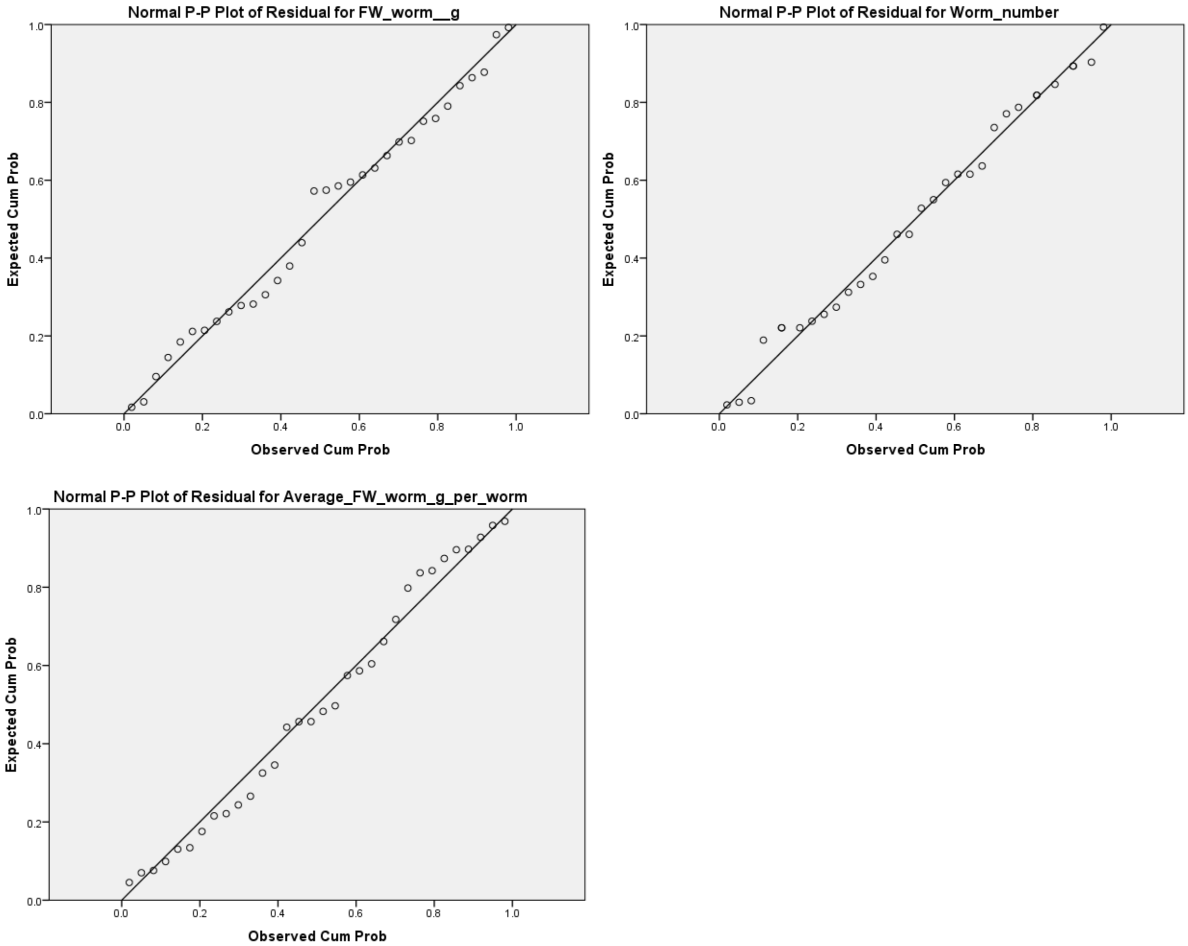


Fig 3.3. P-P plots for the residual analysis of earthworm fresh weight, worm number, and specific weight (average FW worm (g) per worm).

3.3 Crop

3.3.1 Plant height and leaf number

In general, throughout the growing season, the grass clover fresh had the highest plant height followed by the manure and alfalfa treatments (Table 3.6). In contrast to this, both the grass clover silage split and full application plants were shorter. However, at harvesting time, plant heights were similar for al treatments. For leaf number a similar trend was observed (Table 3.6).

Table 3.6. Plant height and leaf number

	Plant height (cm)			Leaf number	
Treatment	3 WAT	7 WAT	Harvest	3 WAT	7 WAT
Con	9.7 bc	18.6 bc	37.0	8.5 b	15.6 b

Slur	9.6 bc	17.7 ab	38.0	8.8 bc	15.1 ab
Man	10.7 c	21.1 cd	36.6	9.6 c	17.6 c
GCF	10.9 c	22.7 d	37.3	9.5 c	20.1 d
GCS	6.5 a	16.6 ab	37.9	6.9 a	14.1 ab
GCS2	7.7 a	16.2 a	37.3	7.4 a	13.8 a
ALP	10.2 bc	20.6 c	38.6	9.0 bc	16.4 bc
ALP2	9.3 b	18.5 b	37.9	8.4 b	16.5 bc
P-value	<0.001	<0.001	0.714	<0.001	<0.001
$LSD_{0.05}$	1.41	2.05	ns[1]	0.82	1.52

[1] ns = not significant

[2] Different letters within each parameter indicate that means differed significantly ($P < 5\%$) as established by the LSD test.

[3] Each value shown here is an average of four replicates, except for plant height at harvest for Con, ALP, ALP2 where it is an average of three replicates

3.3.2 Leaf area and chlorophyll content index

The calibration curve to measure the leaf area non-destructively resulted in a conversion constant of 0.72 for equation 1 (section 2.3.3). This value was then used to convert measured leaf length and width values to estimated leaf area. The predicted leaf area using the conversion constant fitted well with the measured leaf area using leaf area meter (R^2 = 98.5%, Fig. 3.4).

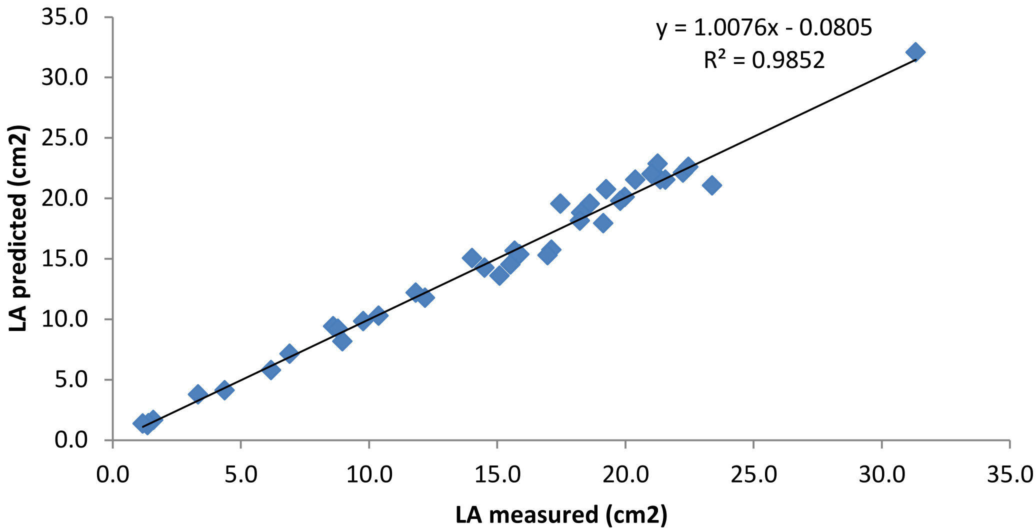


Fig 3.4. Predicted leaf area versus measured leaf area using calibration result k= 0.72 as the conversion constant

The leaf area index in general followed the same trend as the plant height (Table 3.7). The reduction in leaf area at the harvest time was probably because of different method being used to measure the leaf area (indirect method during growing season by measuring the length and width of the lead versus direct method at harvest by taking subsamples of leaf and measure the area using leaf area meter).But it can also be because of the plant progressed towards senescence. The leaf area index (LAI) was calculated based on a plant density of 2.67 plants/m^2. The very small LAI in this experiment was caused by the rather wide spacing being used between plants. This in turn also enhanced weed growth.

Table 3.7. Leaf area and SPAD reading

	Leaf area index (cm^2 / cm^2)			SPAD	
Treatment	3 WAT	7 WAT	Harvest	7 WAT	9 WAT
Con	0.07 b	0.39 ab	0.38 a	63.0 a	69.3 b
Slur	0.07 b	0.44 b	0.43 a	63.1 a	69.3 b
Man	0.12 c	0.65 d	0.59 bc	64.7 ab	65.2 a
GCF	0.13 c	0.91 e	0.83 d	72.6 c	70.1 bc
GCS	0.03 a	0.34 a	0.55 bc	70.5 c	73.4 c
GCS2	0.04 a	0.30 a	0.54 b	63.6 a	72.3 bc
ALP	0.08 b	0.59 cd	0.55 bc	66.8 b	69.8 b
ALP2	0.07 b	0.54 c	0.63 c	68.2 bc	73.3 c
P-value	<0.001	<0.001	<0.001	<0.001	0.001
$LSD_{0.05}$	0.023	0.096	0.093	2.56	3.39

[1] Different letters within each parameter indicate that means differed significantly ($P < 5\%$) as established by the LSD test.
[2] Each value shown here is an average of four replicates.

At 7 WAT the SPAD reading for grass clover fresh and silage were highest while the control, slurry, and GCS split application had relatively low SPAD value. However, at 14 WAT, both GCS full and split application, alfalfa split and full application, and grass clover fresh showed the highest SPAD value while the manure had the lowest SPAD value. Though SPAD readings tended to increase over time, for grass clover fresh treatment, values decreased over time.

3.3.3 Head initiation, head diameter and crop yield

Both GCF and manure treatments initiated heads earlier compared to other treatments and this also translated into earlier harvesting dates (Table 3.8). Use of grass clover silage (both full and split application treatments) resulted in a delay in head initiated and thus also harvesting date compared to all other treatments (about 3 weeks after GCF in average). In terms of head diameter, the GCF, GCS, and alfalfa split treatments had larger head diameters while the size for control and slurry treatments was smallest. The fresh weight of broccoli heads for GCF was greatest followed by both alfalfa pellet treatments while the control and slurry had the lowest head yields. There were no significant differences between full and split application both for silage and alfalfa treatments In general, for other plant parts (stem and leaves) and overall fresh weight, the GCF also had the highest stem fresh while the control and slurry had the lowest weight. Other treatments were somewhere in between.

Table 3.8. Broccoli head initiation day & harvest day, fresh weight of head, stem, leaves, and shoot

Treatment	Initiation day (DAT)	Harvest day (DAT)	Fresh weight (kg/ha)			
			Head	Stem	Leaves	Shoot
Con	58.5 b	77.5 b	5515 a	3568 a	3807 a	12891 a
Slur	58.8 b	75.5 b	5729 ab	4078 ab	4362 ab	14169 ab
Man	50.0 a	69.5 a	7118 b	4635 bc	5997 c	17751 bc
GCF	48.5 a	67.0 a	10756 d	6176 d	9099 d	26031 d
GCS	65.3 c	85.0 c	7114 b	4033 ab	4552 ab	15699 b
GCS2	63.5 c	83.5 c	6839 b	4267 b	5005 b	16110 bc
ALP	55.3 b	75.0 b	7683 bc	4891 bc	5694 bc	18268 c
ALP2	56.3 b	75.5 b	8394 c	5090 c	6279 c	19763 c
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
$LSD_{0.05}$	3.58	4.60	1228.8	623.9	874.9	2315.2

[1] Different letters within each parameter indicate that means differed significantly ($P < 5\%$) as established by the LSD test.

[2] Each value shown here is an average of four replicates

3.3.4 Crop dry weight (DW) accumulation

The head dry weight showed similar patterns as those reported for head fresh weight (Table 3.9). In terms of stem, leaves, and total dry weight, the GCS and GCS2 also showed lowest dry weight similar with the control and slurry treatments while the GCF still showed the highest value.

Table 3.9. Broccoli dry weight of heads, stems, leaves and shoot

Treatment	Dry weight (kg/ha)			
	Head	Stem	Leaves	Shoot
Con	622 a	587 a	628 a	1837 a
Slur	638 a	659 ab	661 a	1958 a
Man	811 b	763 b	935 b	2509 b
GCF	1207 c	992 c	1335 c	3533 c
GCS	740 ab	566 a	676 a	1982 a
GCS2	737 ab	600 a	663 a	2000 a
ALP	839 b	811 b	894 b	2544 b
ALP2	887 b	813 b	989 b	2689 b
P-value	<0.001	<0.001	<0.001	<0.001
$LSD_{0.05}$	131.3	132.7	142.9	359.6

[1] Different letters within each parameter indicate that means differed significantly ($P < 5\%$) as established by the LSD test.

[2] Each value shown here is an average of four replicates.

3.3.5 Broccoli quality and nitrate content

The head nitrate content of the control and manure treatment were the lowest while the GCS and GCS2 were the highest (Table 3.11). Other treatments were in between.

Table 3.10. Broccoli dry weight of heads, stems, leaves and total

Treatment	Broccoli head quality parameters (average value per head)				
	Head diameter (cm)	Fresh weight (g)	Dry weight (g)	Dry matter content (%)	N-NO3 (mg/kg)
Con	11.3 a	207 a	23.3 a	11.3	111 a
Slur	12.0 ab	215 ab	23.9 a	11.2	215 ab
Man	12.7 b	267 b	30.4 b	11.4	101 a
GCF	14.7 c	403 d	45.3 c	11.2	179 ab
GCS	13.9 c	267 b	27.8 ab	10.4	338 b
GCS2	13.0 bc	256 b	27.6 ab	10.8	406 b
ALP	12.9 bc	288 bc	31.5 b	11	245 ab
ALP2	13.3 bc	315 c	33.3 b	10.6	242 ab
P-value	<0.001	<0.001	<0.001	0.216	0.046
$LSD_{0.05}$	1.00	46.1	4.92	ns[1]	192.6

[1] ns = not significant

[2] Different letters within each parameter indicate that means differed significantly (P < 5%) as established by the LSD test.

[3] Each value shown here is an average of four replicates unless for the one missing dry matter content sample each for the ALP and ALP2 where the missing value was calculated from the average value of the other three replicates (this average dry matter content value was then used also for estimating dry weight for the respective sample).

3.3.6 Crop nitrogen content, crop N accumulation, and NUE

In terms of head total nitrogen content, values for manure, GCS, and GCS2 were lowest while the slurry and the control had the highest N content. For stem total nitrogen content, there was no significant difference between treatments. As for the leaves total nitrogen content, the manure and GCF contained less nitrogen while GCS2 had the highest total nitrogen content. This may very well be related with the low dry weight of GCS2 and high dry weight of GCF.

Since the amount of total N applied differed among treatments, standardization was required to warrant a fair comparison among treatments using the following approaches:

NUE = agronomic nitrogen use efficiency = (yield – yield of control treatment)/applied total N

ANR = apparent nitrogen recovery = (total shoot N content – total shoot N content of control treatment)/applied total N * 100%.

The GCF, ALP2, ALP, and GCS treatments had the highest NUE while the slurry had the lowest NUE value. The GCF, ALP2, and ALP treatments also had the highest ANR while the slurry, manure, and GCS had the lowest ANR. Provide that the total N applied was similar (Table 3.1), the GCF clearly resulted in higher yield (higher NUE) and shoot N content (higher ANR) compared to manure treatment (Table 3.8, Table 3.11). Similarly, for similar amount of total N applied, the GCS and GCS2 resulted in higher yield (higher NUE) compared to slurry. However, while GCS2 had higher shoot N content (higher ANR) compared to slurry, there was no significant difference between GCS and slurry treatment (Table 3.8, Table 3.11).

Table 3.11. Broccoli total nitrogen content (TN) head (h), stem (st), leaves (lv), efficiency, and recovery

Treatment	Total nitrogen content				NUE (kg fresh weight/kg N)	ANR (%)
	Head (%)	Stem (%)	Leaves (%)	Shoot (kg/ha)		
Con	3.71 b	1.47	2.14 b	45.0 a	-	-
Slur	3.54 b	1.36	2.08 ab	45.2 a	2 a	0.1 a
Man	3.26 a	1.59	1.84 a	53.4 ab	8.3 ab	4.3 ab
GCF	3.30 ab	1.62	1.83 a	80.0 d	25.7 c	17.2 b
GCS	3.46 ab	1.43	2.44 c	50.1 ab	16.3 bc	5.2 ab
GCS2	3.66 b	1.57	2.66 c	54.2 b	13.5 b	9.4 b
ALP	3.50 b	1.59	2.35 bc	63.6 c	17.5 bc	15 b
ALP2	3.50 ab	1.63	2.18 b	65.7 c	23.2 bc	16.7 b
P-value	0.009	0.501	<0.001	<0.001	0.001	0.001
$LSD_{0.05}$	0.239	ns[2]	0.282: Man[1] 0.261: others	9.69: Man[1] 8.97: others	10.04	8.53: Man[1] 7.90: others

[1]There is one missing leaf sample for the manure treatment, so the LSD differed.

[2] ns = not significant

[3] Different letters within each parameter indicate that means differed significantly (P < 5%) as established by the LSD test.

[4] Each value shown here is an average of four replicates unless for the one missing leaf sample for the manure treatment (average of three replicates)

3.4 Weed

3.4.1 Weed density and ground cover

The weed density before manual and machine weeding (3 WAT) did not show any significant difference (Table 3.12). However, the weed ground coverage for the slurry, manure, grass clover fresh, and alfalfa pellets treatments were the highest while GCS full and split application weed ground coverage was the lowest at 3 WAT. After hand and mechanical weeding at 4 WAT, the grass clover silage and alfalfa split application at 7 WAT showed the lowest weed density and ground coverage while the manure, slurry, GCF, and the other alfalfa treatment had the highest weed ground coverage. In general, the weeding process effectively reduced the weed density and weed ground coverage over time. Moreover, since incorporation of the second application of fertilizer (5 WAT) in the split application to the soil involves very shallow soil tilling which will kill some weed, the split application has lesser weed at the second weed sampling (7 WAT)

Table 3.12. Weed density and ground cover

	Weed density (weed number/m^2)		Weed ground cover (%)	
Treatment	3 WAT	7 WAT	3 WAT	7 WAT
Con	353	157 b	45.0 bc	25.0 b
Slur	310	156 b	56.3 c	31.9 b
Man	316	159 b	62.5 c	35.6 b
GCF	264	161 b	58.8 c	31.9 b
GCS	263	150 b	16.3 a	18.1 ab
GCS2	325	66 a	28.1 ab	8.8 a
ALP	256	158 b	46.9 bc	33.1 b
ALP2	309	92 a	37.5 b	15.0 ab
P-value	0.260	0.001	<0.001	<0.001
$LSD_{0.05}$	ns[1]	45.6	16.35	10.70

[1] ns = not significant

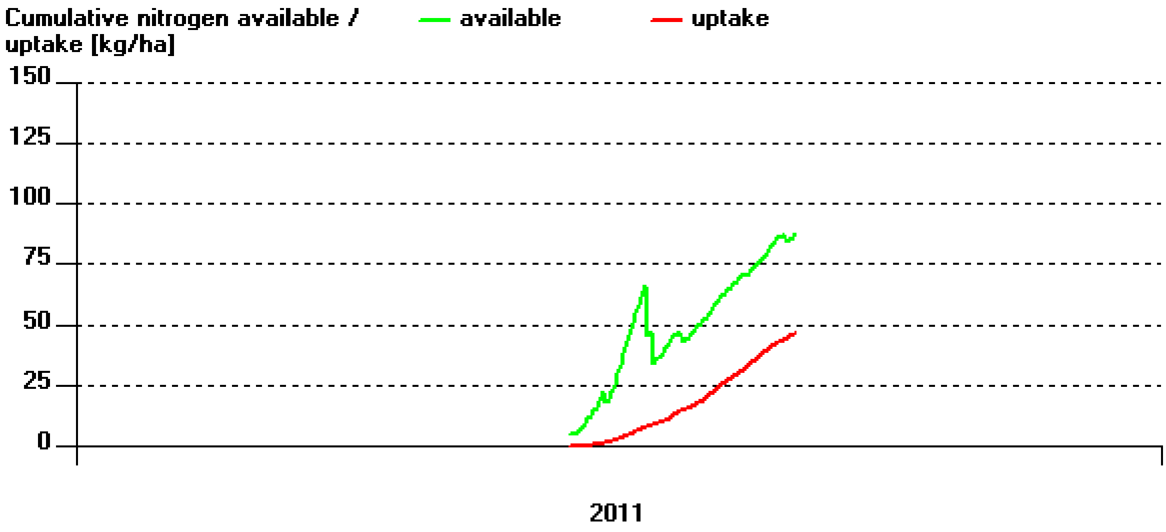
[2] Different letters within each parameter indicate that means differed significantly (P < 5%) as established by the LSD test.

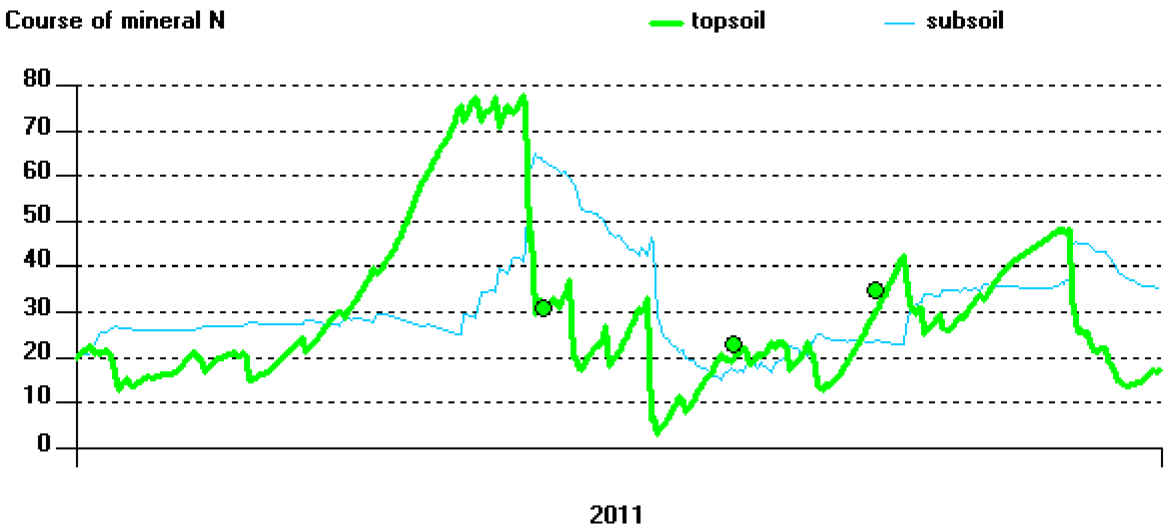
[3] Each value shown here is an average of four replicates.

3.5 NDICEA

3.5.1 Crop N accumulation

The NDICEA results for the control treatment showed that available soil nitrogen exceeded actual plant accumulation. However, actual growth and thus crop N accumulation but also crop N demand may have been greater provided that soil N supply levels would have been higher as is the case in some of the subsequent graphs. This is related to the graph being based on actual yield rather than on expected (targeted) yield for well-fertilized conditions. There was a sharp decrease of available nitrogen near the middle growing period which coincided with high precipitation (Fig 3.5).





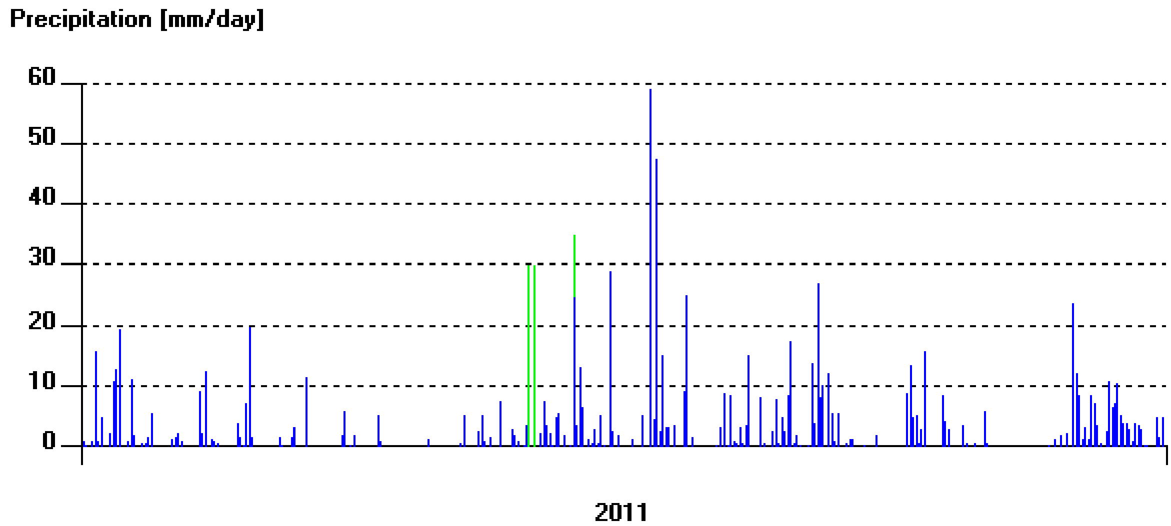
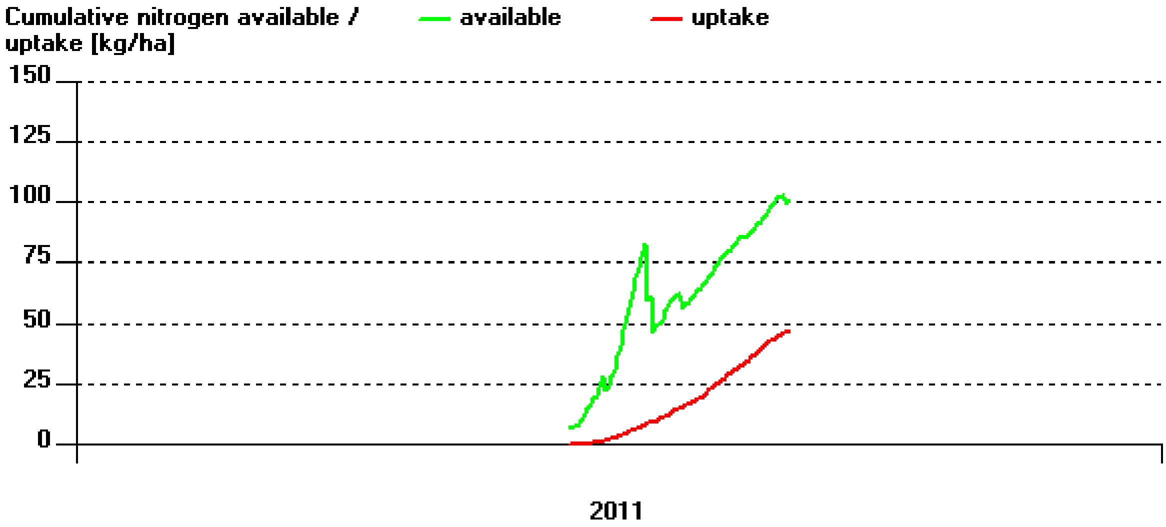


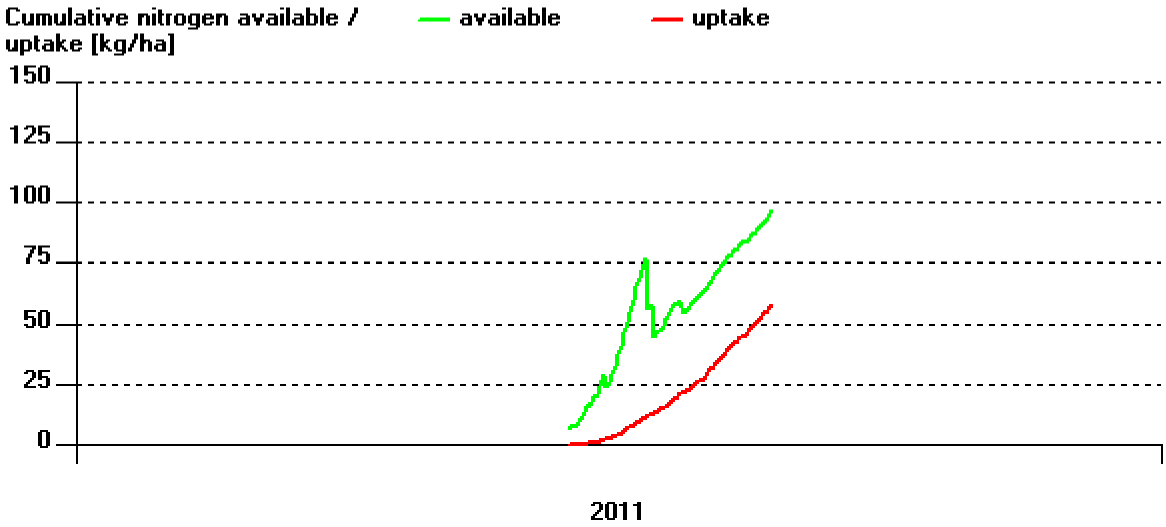
Fig 3.5. Control treatment NDICEA results. Small circles in course of mineral N (kg/ha) graph showed average measured values from 4 replicates (Table 3.2).

Note that average measured values versus predicted values in course of mineral N graphs (Figs 3.5) are not exactly overlapping. This can happen because the program is calibrated against only three different mineral N measurements in time during the growing period. The calibration procedure requires at least five mineral N measurements on different time on the same field. So the accuracy of the result is a bit limited. Nevertheless, the trend of the measured values is similar with the predicted values.

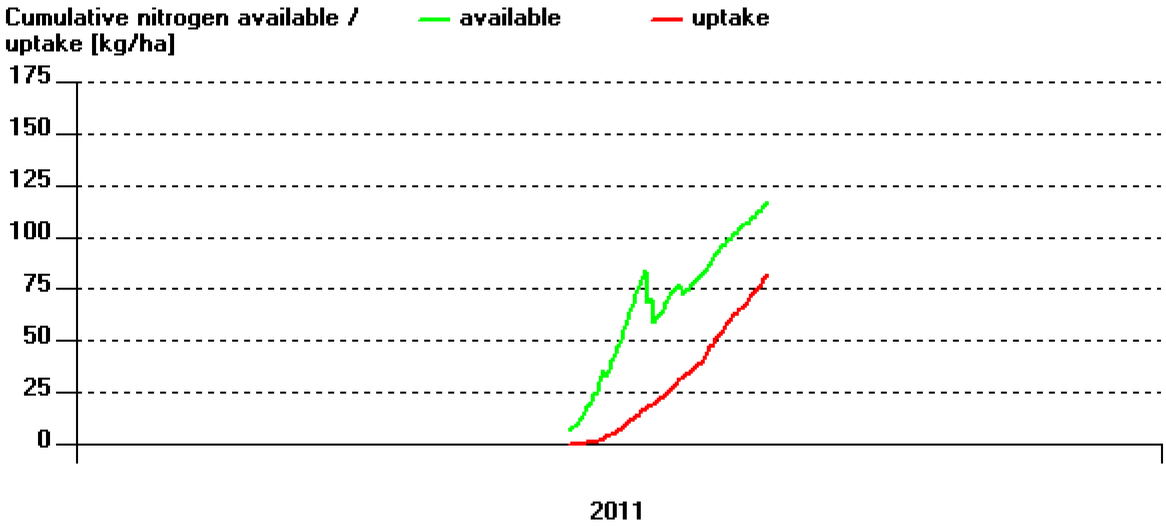
For the other treatments, the shape of nitrogen available versus nitrogen uptake curves were similar in terms of the overall shape although the final available nitrogen cumulative values at harvest ranged between 95 and 125 kg N/ha (approximate value from Fig. 3.6, exact number is not available in the NDICEA version 6.0.17 used in this report), in which ALP2 followed by GCF treatment had the largest amount of soil available nitrogen (Fig. 3.6). The nitrogen available for the plants were above the actual uptake for all treatments and there was a sharp decline in the middle of the growing period which coincided with the period of heavy rainfall.



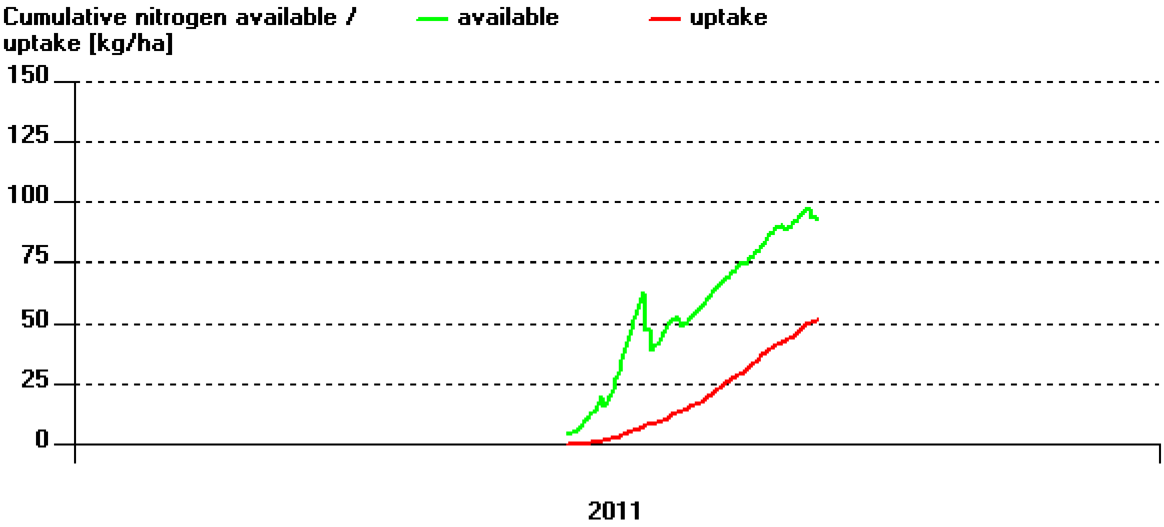
Slurry



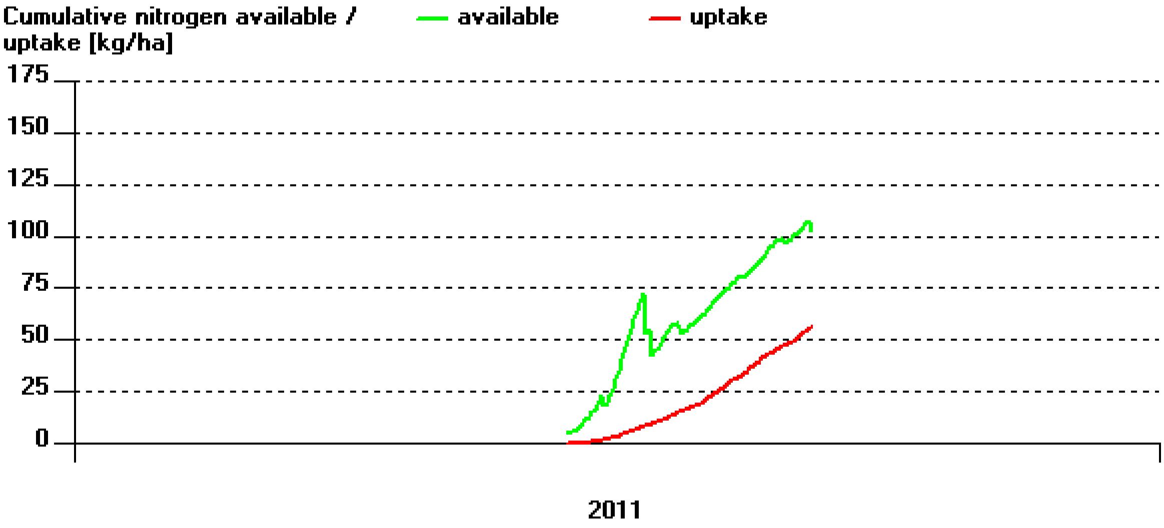
Farm yard manure



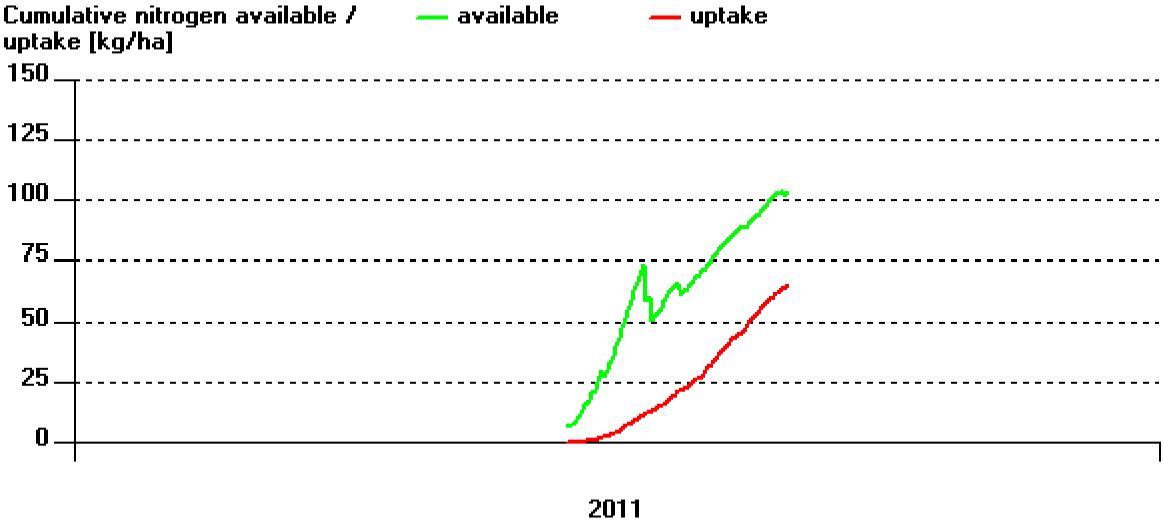
Grass clover fresh



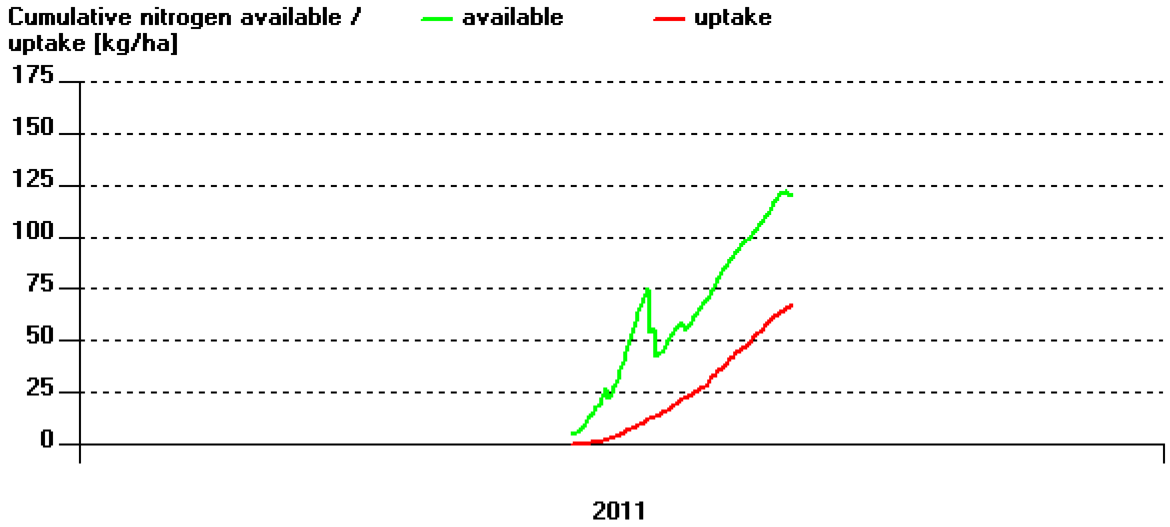
Grass clover silage



Grass clover silage split application



Alfalfa pellet



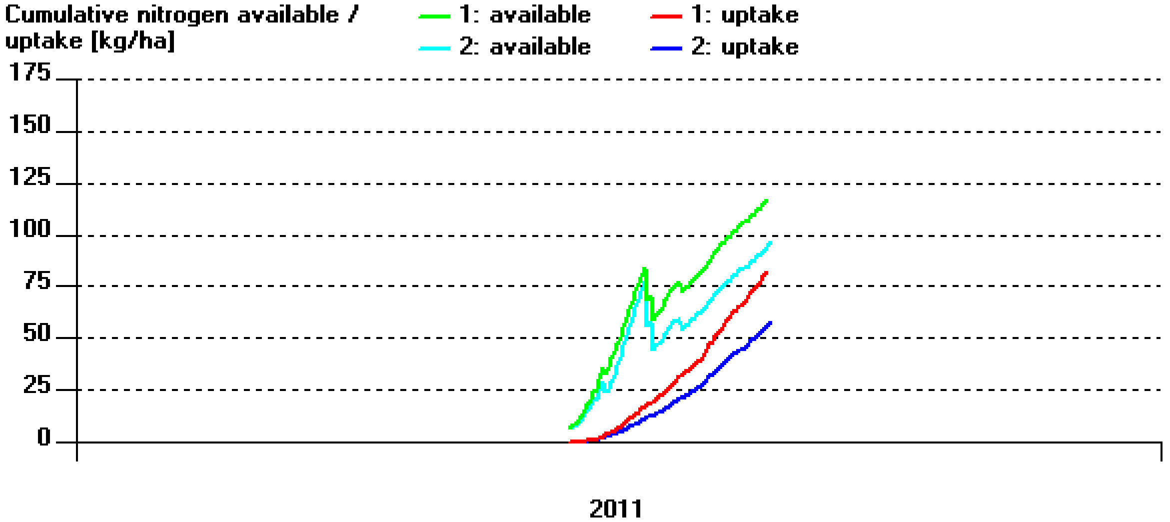
Alfalfa pellet split application

Fig. 3.6. NDICEA results for other treatments

3.5. 2 Soil mineral N content in relation to crop N demand

As the total nitrogen content of the applied organic amendments differed substantially from the expected values (Table 3.1), one meaningful comparison among treatments that can be implemented via NDICEA is to assess temporal mineral soil N dynamics and compare predicted daily values to those measured at specific sampling dates. In this case, the farm yard manure is being compared with the application of fresh grass clover while N release patterns for slurry with those for grass clover silage and alfalfa full or split application.

The first comparison was between farm yard manure and grass clover fresh (Fig. 3.7). It can be seen that at the very initial stage when the manure was applied (almost coincide with the first green dot), the soil mineral nitrogen in the topsoil of the farm yard manure treatment increased sharply while the increase was much slower for the grass clover fresh treatment. This may represent some loss since the plant did not need much nitrogen at the very initial stage of growth. Furthermore, after the sudden decrease of available nitrogen due to heavy rainfall, the grass clover fresh treatment rebounded higher. At the harvest period, it can be clearly seen that the available nitrogen in the farm yard manure treatment cannot support the same yield as the grass clover fresh treatment. Similar trends occurred with the slurry versus alfalfa treatment (Fig 3.8). The slurry treatment lost some nitrogen at the very initial stage while the alfalfa treatment can produce available nitrogen and thus sustain the crop growth at the later stageThe N soil field data itself does not show significant difference (Table 3.2), however from this NDICEA result based on the final yield and N content of the plants, the process of nitrogen availability and uptake by the plant during the growing season can be estimated.



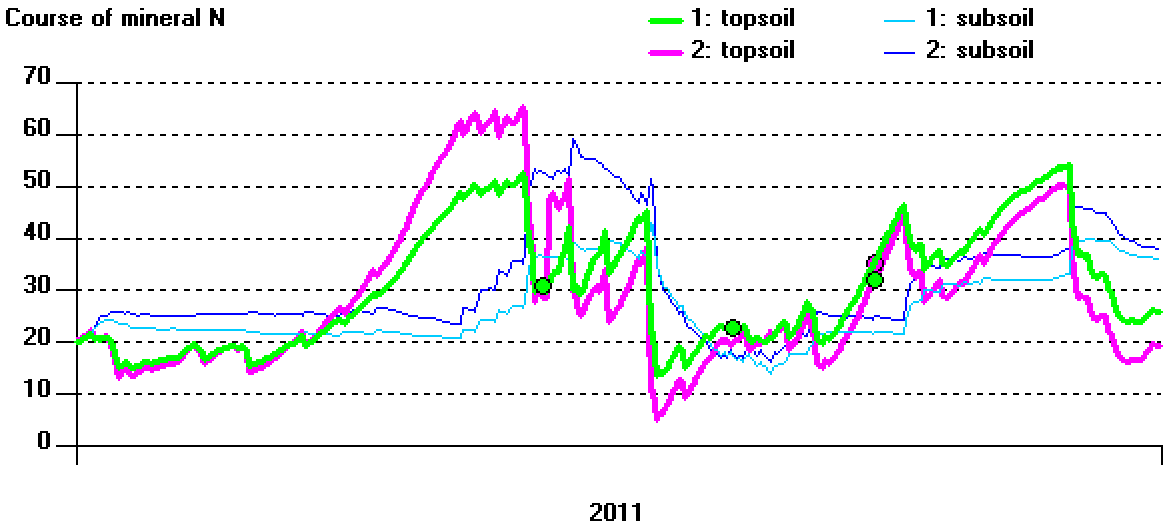


Fig. 3.7. NDICEA results: grass clover fresh treatment (scenario 1) versus farm yard manure (scenario 2). Small circles in course of mineral N (kg/ha) graph showed average measured values from 4 replicates (Table 3.2).

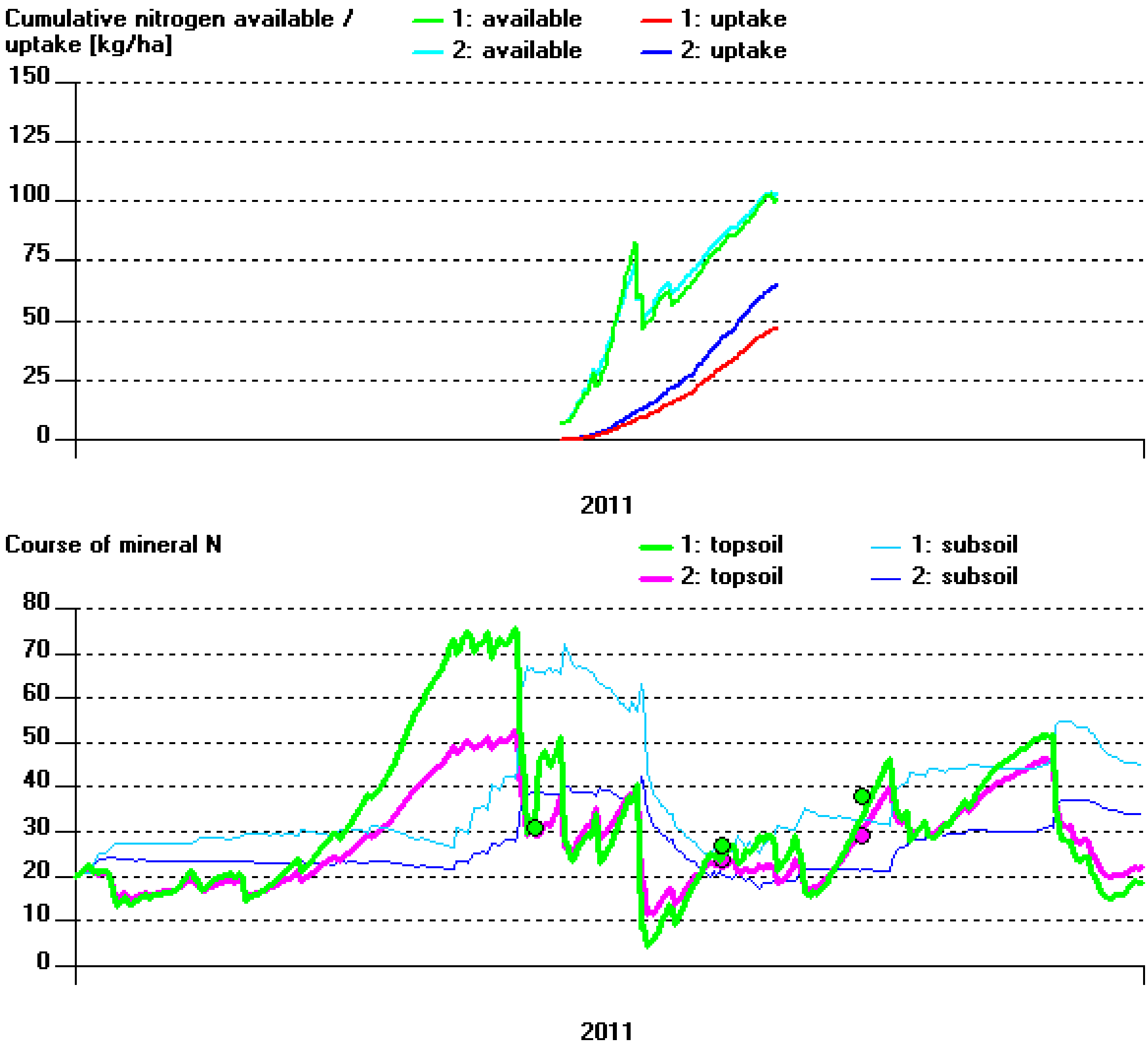
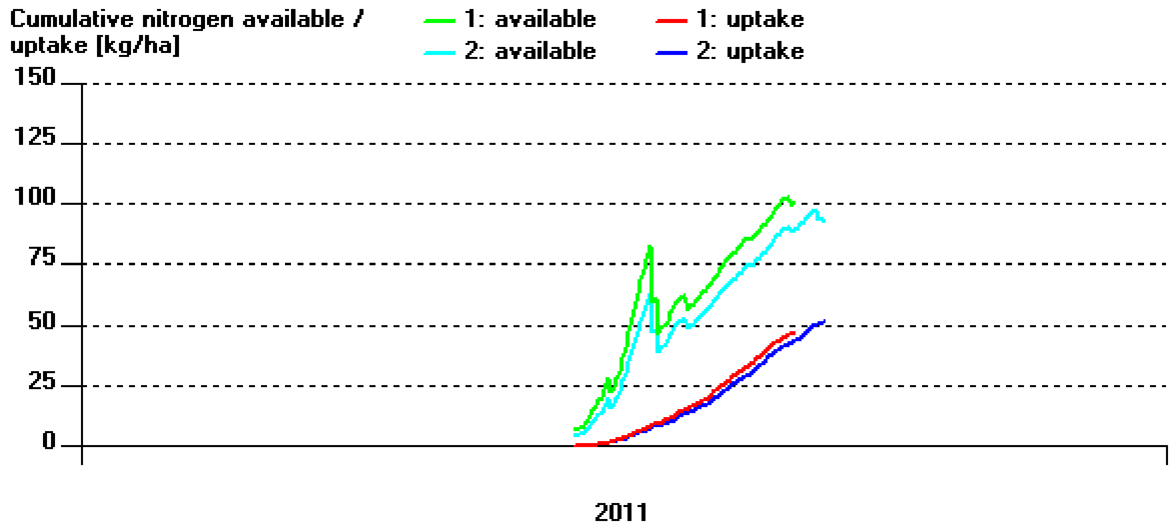


Fig. 3.8. NDICEA results: slurry treatment (scenario 1) versus alfalfa treatment (scenario 2). Small circles in course of mineral N (kg/ha) graph showed average measured values from 4 replicates (Table 3.2).

Another interesting thing to see was the comparison between the slurry treatment versus grass clover silage treatment (Fig. 3.9). The slurry treatment had higher available nitrogen compared to the GCS treatment during initial growth. However, the GCS treatment had longer duration of growth until harvest time which resulted in higher yield in GCS treatment as compared to the slurry treatment (Table 3.7).



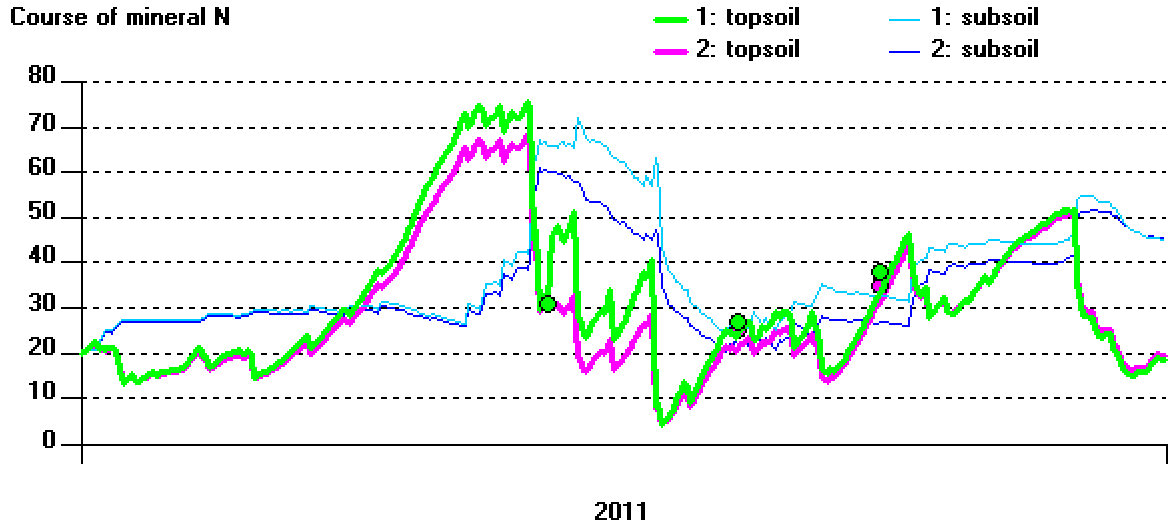


Fig. 3.9. NDICEA results: slurry treatment (scenario 1) versus grass clover silage treatment (scenario 2). Small circles in course of mineral N (kg/ha) graph showed average measured values from 4 replicates (Table 3.2).

The split application did not give a significant higher yield as expected. In fact for the grass clover silage, the yield for split application was slightly lower although not significant as compared to the full application (Table 3.8). The nitrogen dynamics also did not differ much (Fig 3.10) which indicate that decomposition process to produce available nitrogen for the plant may have went very slow.

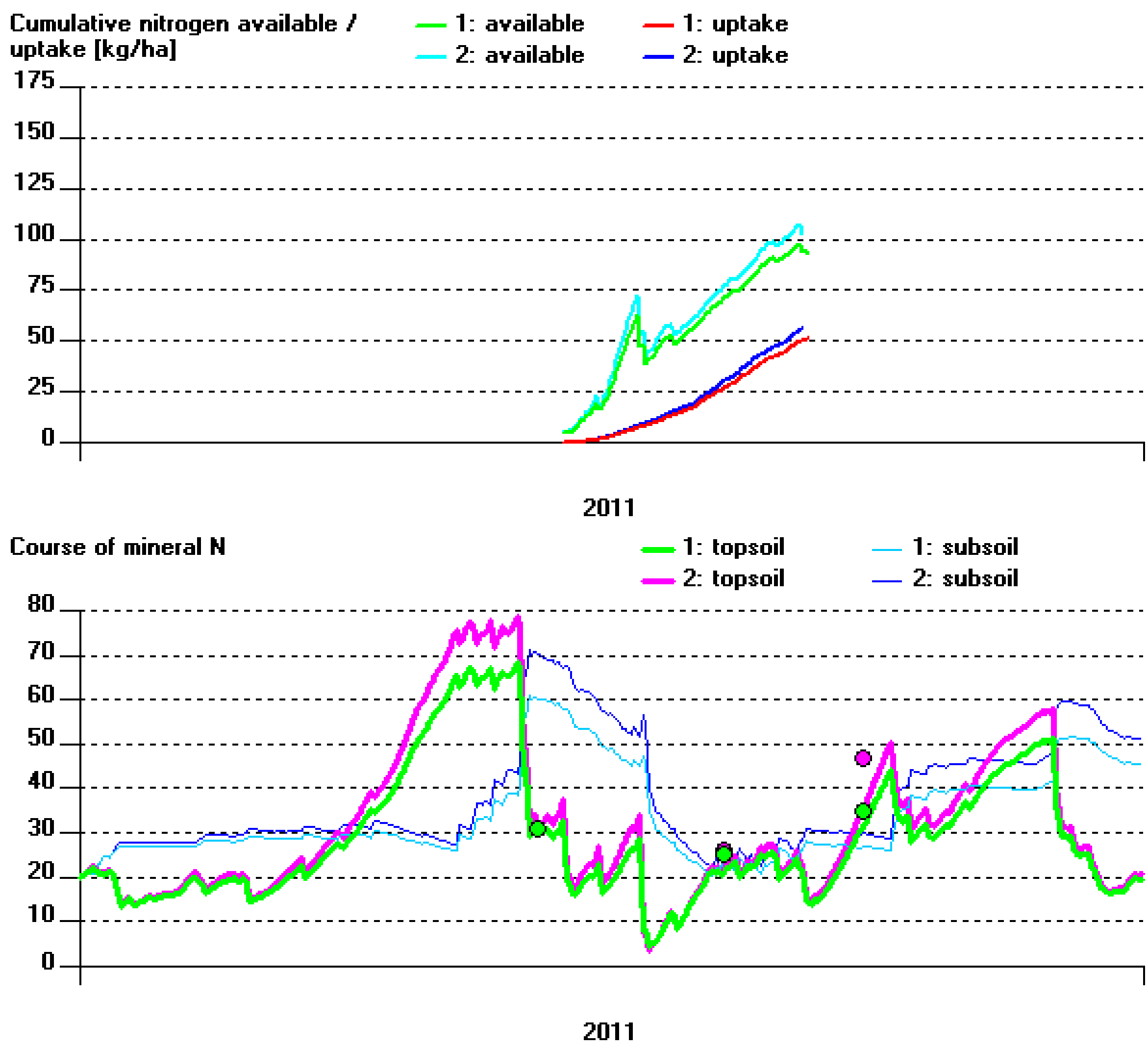


Fig. 3.10. NDICEA results: grass clover silage full application treatment (scenario 1) versus grass clover silage split application treatment (scenario 2). Small circles in course of mineral N (kg/ha) graph showed average measured values from 4 replicates (Table 3.2).

The yield for alfalfa split treatment was higher although not significant as compared to the full application (Table 3.8). From the nitrogen dynamics in the NDICEA, we can see that towards the harvest time, the available nitrogen of the split application was higher than the full application (Fig. 3.11). The leaching was more for the split application.

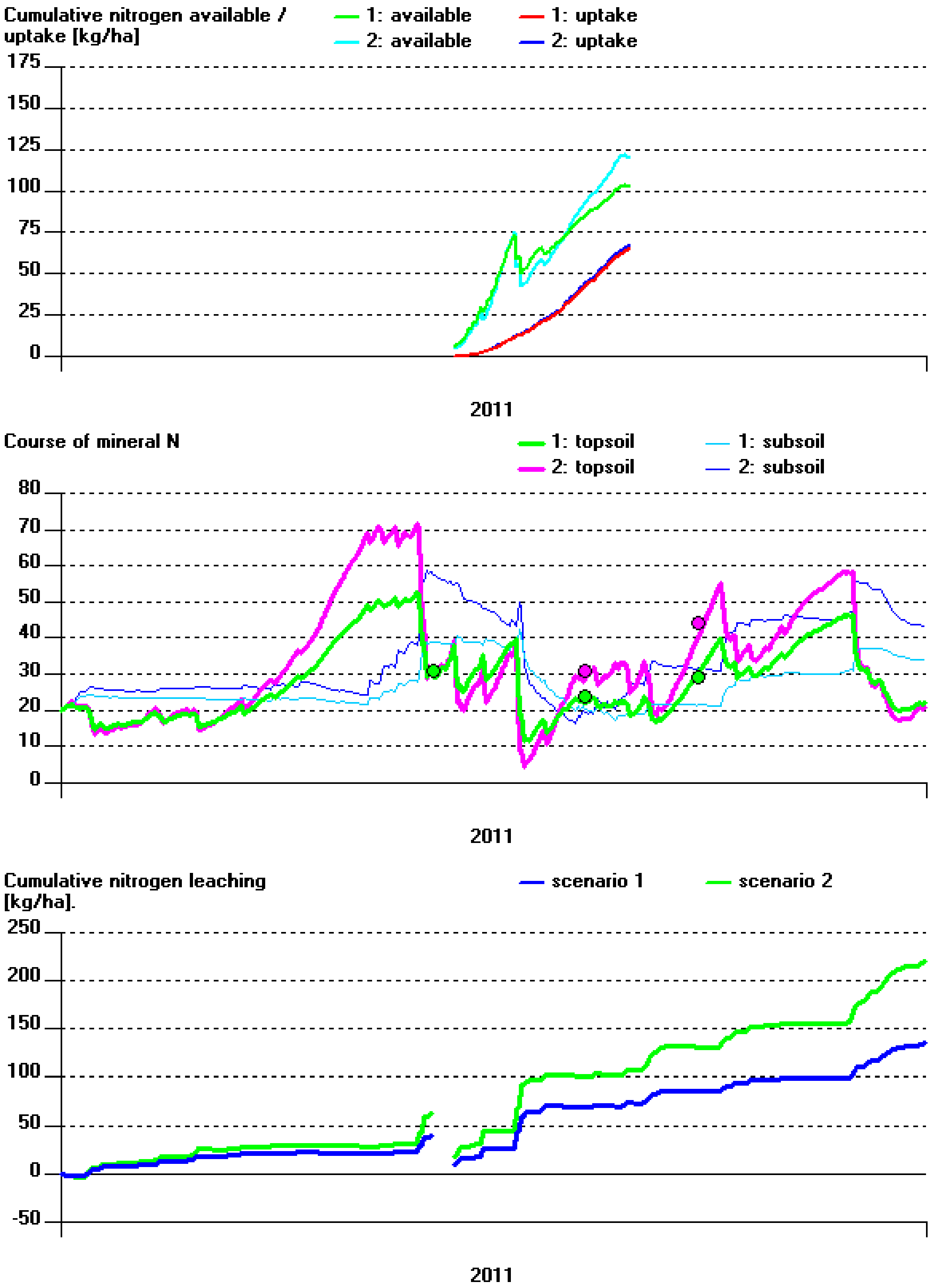
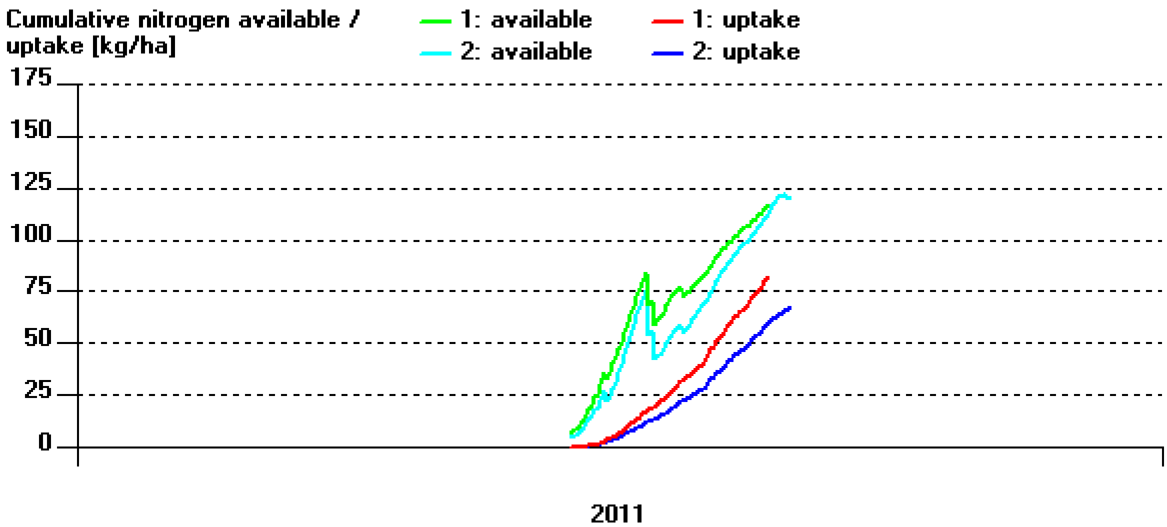


Fig. 3.11. NDICEA results: alfalfa full application treatment (scenario 1) versus alfalfa split application treatment (scenario 2). Small circles in course of mineral N (kg/ha) graph showed average measured values from 4 replicates (Table 3.2).

The available nitrogen at the harvest time for ALP2 was slightly higher than GCF (Fig. 3.12) because the growing duration until harvest is longer for ALP2 since GCF matures faster. The longer growing duration of ALP2 resulted in more time for decomposition and thus the available mineral N will be more for ALP2 than GCF at harvest time. The yield for GCF, however, is higher than ALP2 since in the middle of growing season just after the heavy rainfall the GCF has higher mineral N available (Fig. 3.12).



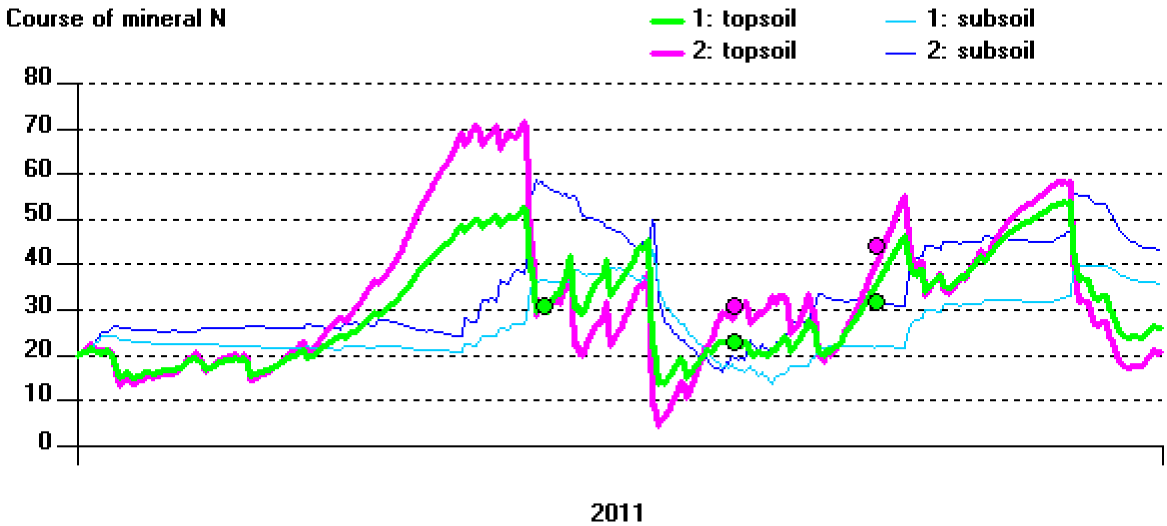


Fig. 3.12. NDICEA results: grass clover fresh (scenario 1) versus alfalfa split application treatment (scenario 2). Small circles in course of mineral N (kg/ha) graph showed average measured values from 4 replicates (Table 3.2).

4 SYNTHESIS OF RESULTS

There is no relationship between yield and head $N\text{-}NO_3$ (Fig. 4.1, $F(1,30) = 0.058$, *P*-value = 0.811).

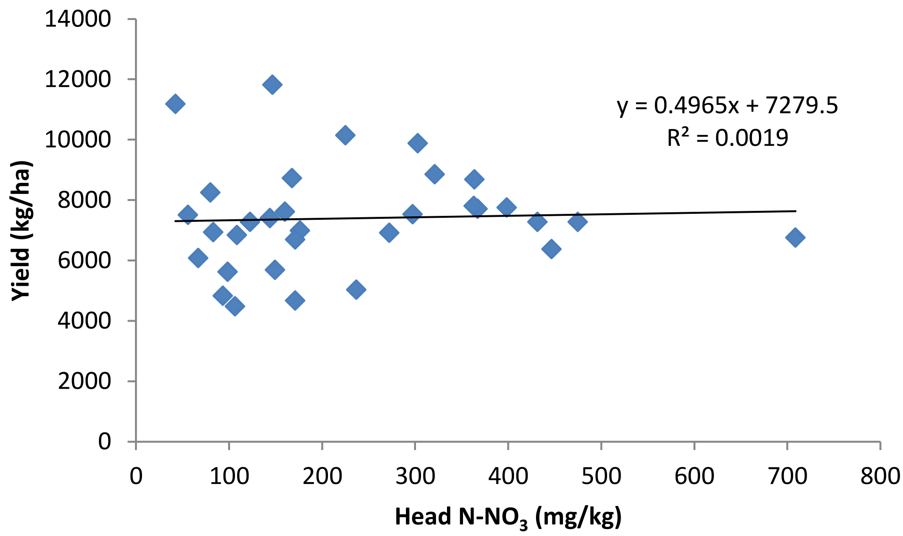


Fig. 4.1. Yield versus Head $N\text{-}NO_3$

There is a clear relationship between yield and total shoot N (Fig. 4.2, $F(1,29) = 200.955$, *P*-value < 0.001, $R^2 = 87\%$). As the total shoot N increases (which means increase of crop N uptake), the yield will also increase.

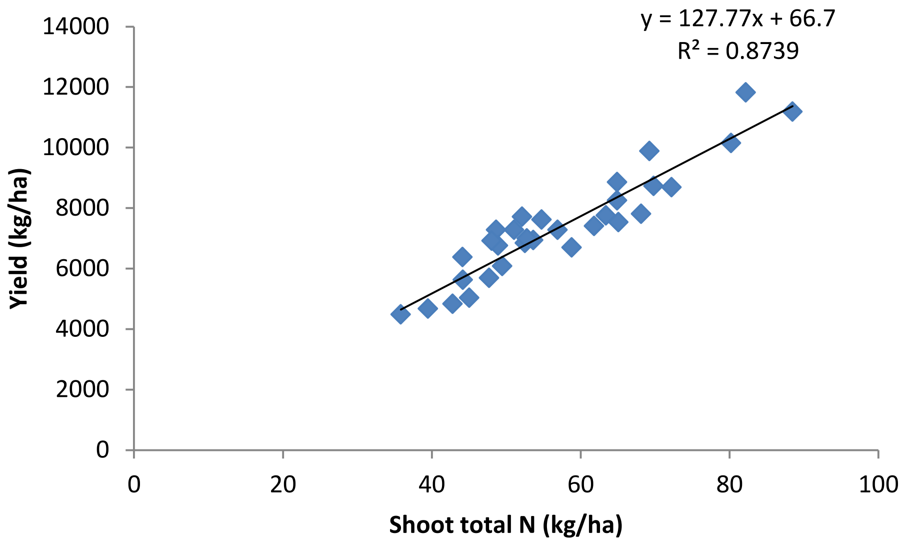


Fig. 4.2. Yield versus shoot total N

There is a relationship between plant height at 3 WAT and weed ground cover at 3 WAT (Fig. 4.3, F(1,30) = 22.866, *P*-value < 0.001, R^2 = 43%). As the weight ground cover increases, the plant height will also increase.

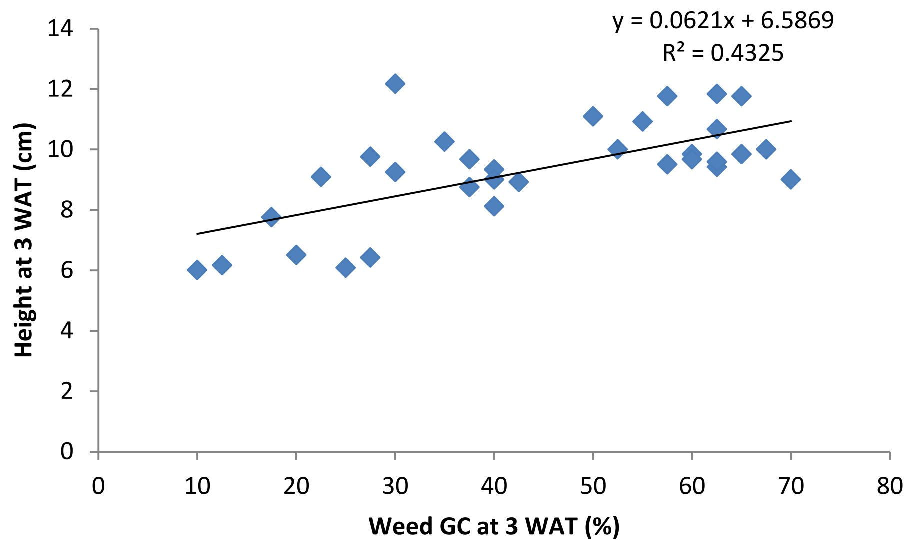


Fig. 4.3. Plant height at 3 WAT versus weed ground cover at 3 WAT

There is no relationship between yield and weed ground cover at 3 WAT (Fig. 4.4, F(1,30) = 0.667, *P*-value = 0.42).

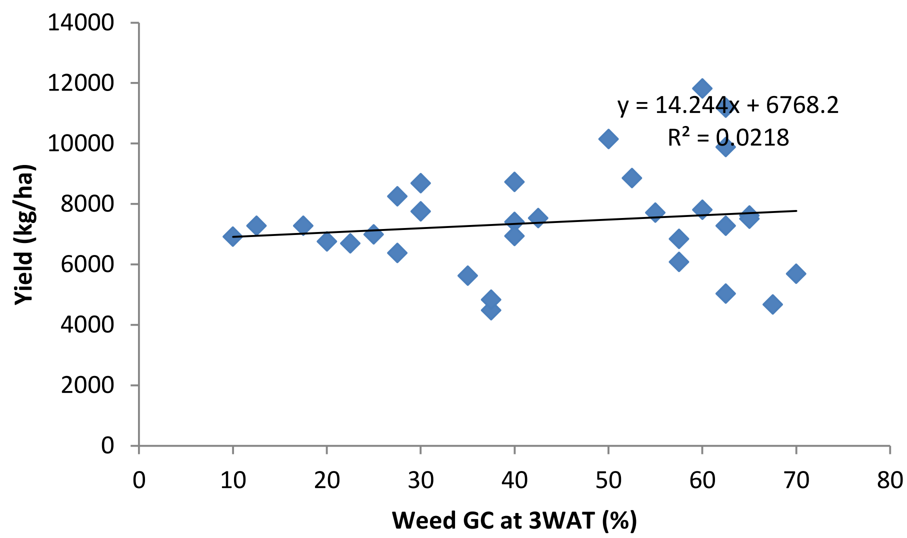


Fig. 4.4. Yield versus weed ground cover at 3 WAT

There is no relationship between yield and plant height at 3 WAT (Fig. 4.5, $F(1,30) = 2.508$, P-value = 0.124).

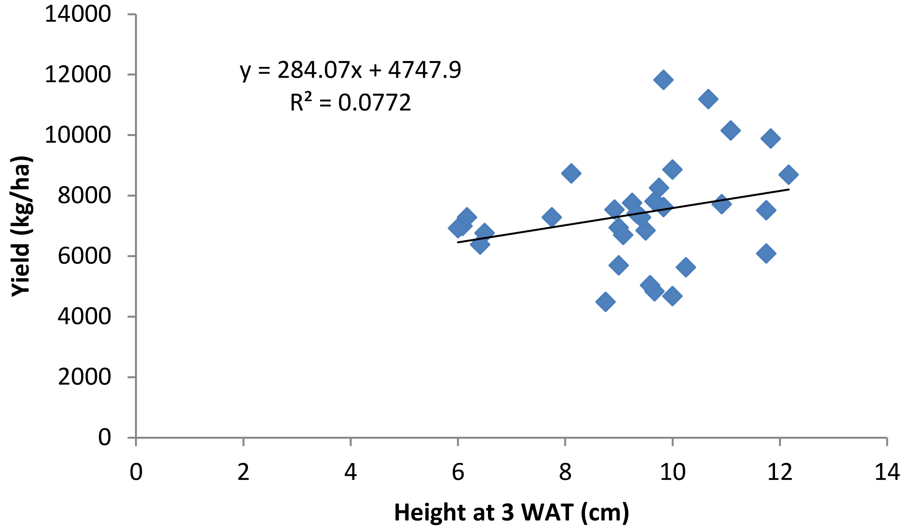


Fig. 4.5. Yield versus plant height at 3 WAT

5 DISCUSSION

The use of mobile green manures in general resulted in significantly more efficient nitrogen use and yield as compared to the slurry (Table 3.8 and 3.11). This is expected to be true since the slurry mineral nitrogen is easier to leach, especially when there is heavy rainfall as experienced during the experiment growing period. Although in average, the NUE is also better for the green manures as compared to the farm yard manure, only the grass clover fresh and alfalfa split application treatments have significantly larger NUE and also yield as compared to the farm yard manure treatment. This is also according to the general knowledge that farm yard manure has more organic matter content as compared to slurry (Table 3.1) which can hold the nitrate better so that it is not so easy to leach and thus can be more available to the plant when needed , which helps especially for autumn growing season (Schröder, 2005). In our case, although the crop is grown at summer period, the weather during the year is cool and wet Fig. 3.5) which is similar to the autumn condition and thus justifies the result that farm yard manure is significantly better than slurry and in our case, has comparable yield with the fresh grass clover and alfalfa which also has high organic matter content

The split application did not really resulted in significantly higher yield and nitrogen use efficiency as expected as compared to the full application. Although the alfalfa split application is on average better than the full application, the grass clover split application in average has lower NUE and yield compared to the full application although not significant. The reason of this is maybe due to the C/N ratio of the green manure. Although we did not analyze the organic carbon of the manure, we analyzed the organic matter of the manure (Table 3.1). Since organic carbon is the main component of the organic matter, it is expected that when the organic matter is high, the organic carbon is also high. If we take simple division of organic matter content and the total nitrogen content, we can see that the slurry, farm yard manure, and grass clover fresh have OM/N ratio between 20-25. While on the other hand, the alfalfa has OM/N ratio of around 30 and GCS of around 80. As has been common knowledge (Kumar and Goh, 2000), high C/N ratio means in general that it takes longer for the material to decompose. In fact, there might even be immobilization of nitrogen at very high C/N ratio which we expect happen in the case of grass clover silage as observed from small growth at the beginning of the growing period. Thus in the future, this C/N ratio needs to be taken into account as well when applying green manure.

The weed ground cover for the slurry and manure are indeed among the highest at 3 WAT (Table 3.11). However, the same can be said for the grass clover fresh treatment followed by the alfalfa and control treatment. This trend is similar as the plant height of those treatments at 3 WAT (Table 3.6). It seems that the weed can utilize the nutrient together with the crop at the early growing period. The inverse case is also true when grass clover silage is used because this resulted in reduced crop growth at the early growing period where as weed growth was also hampered as well. To sum up, at the early growing period the weed growth and the crop growth is more or less proportional (Fig. 4.3). In the later growing period though, since there has already been some weeding done, the result is more mixed. Moreover, since the broccoli is harvested when they reach certain criteria, which means slow growth of the crop at the beginning is compensated with longer developmental time before harvest, the early crop growth and the early weed ground cover cannot be used to predict the yield (Figs. 4.4 and 4.5).

In contrast with the expected outcome, this study did not show that the broccoli head nitrate content of the mobile green manures treatment is less than the slurry and the farm yard manure (Table 3.10). In comparison, Elwan and Hamed (2011) found out that fertilizers containing N forms that are not readily available for crops decreased nitrate content with respect to fast N release fertilizer. So it is expected that the slurry should have the highead hest nitrate content. However, in this study in general, there are no significant differences for the broccoli head nitrate content, except for the grass clover silage which had significantly higher nitrate content compared to the farm yard manure treatment. This may be due to the fact that the grass clover silage treatment did not grow well at the beginning of the growing season. The plant tended to be smaller and thus the nitrate may have been more concentrated in the plant tissue towards the end when availability was high compared to crop demand. On the other hand, for the grass clover fresh, in terms of a relatively large amount of total nitrogen being applied (as compared to other treatment), this did not result in excessively high nitrate content in the heads as compared to other treatments such as manure and slurry which received lower overall N application rates, and this thus seem like positive outcome. In other words, higher yield does not correspond to higher head nitrate content (Fig. 4.1).

The non-significant difference found in the earthworm fresh weight and numbers can be due to the fact that the plots were very small, adjacent to one another, and the experiment time was not long enough. These factors may have resulted in increased variability and although numeric differences were substantial, inherent variability was such that differences were not yet significant. If the plots were larger and more samples were taken, it is expected that some significant difference will show up.

The NDICEA result somehow showed that the actual yield for all the treatments are lower than the potential yield (nitrogen uptake < available nitrogen). This is to be expected since no crop is perfectly efficient in terms of making complete use of available soil N. This may be also be caused by other limiting factors including weeds competing for N uptake . Moreover, it was observed that few plants had some purple leaves which may indicate lack of phosphorus for the expected potential yield. Furthermore, due to the wet weather, the weeding could not be done at the scheduled time, thus the weeds were allowed to grow inside the plots for some time before the weeding could be completed. Weeds of course were taking up some nutrients and also competed for light thereby potentially lowering crop growth, N accumulation and yield. Glass clover fresh treatment had the largest amount of soil available nitrogen at the final harvest time from the NDICEA result (Fig. 3.6). This will translate to the highest N uptake and thus the highest yield among the treatments since yield is proportional to N uptake (Fig. 4.2; Kumar and Goh (2000)).

There was a substantial difference between actual and targeted amount of applied total nitrogen of manure this since initial calculations were based on the previous project (Table 3.1). This shows one disadvantage of use of organic amendments since both the dry matter and nutrient content may be highly variable and values may differ from batch to batch. For experimental purpose, if time permits, it is better to measure the nutrient content of the manure a few days before applying it and have the samples analyzed right away. However, as most organic crop can handle large variation in the nutrient input, the use of mobile green manures in practise is still possible as long as a caution of the C/N ratio of the manure is taken into account. The high C/N ratio of the silage for example has been shown in this study to hamper the yield and delay the harvest by several weeks. Furthermore, the excessive N availability at the beginning of the growth period like in case of slurry is also undesirable, as it causes the weed to grow faster while the crop is still young and thus not in the optimal stage of effectively taking up the nitrogen. Yet, when all these precautions are taken into consideration, it is very promising for example, when farmer can just transfer the grass that he mowed to be used for green manure to another location. It is better though, if there is some cheap and fast ways to analyze the nutrient content before application, especially in developing countries where there is not enough manure since there are many farmers. When there is some reliable measurement and guidelines to follow in applying just enough manure, it is expected that the overall result for the farmers will be optimum. On the other hand, the use of free and available green manures in the nature, for example invasive aquatic plants can be further researched. Furthermore, to prevent the mobile green manure to become weed at its new place, it is necessary that the green manure is harvested before it enters reproductive stage.

6 CONCLUSIONS

The experiment managed to show that some green manures like grass clover fresh and alfalfa are quite promising to replace animal manures, especially slurry which is very commonly use by the farmers in the Netherlands. The yield and nutrient use efficiency are quite high for the grass clover fresh and alfalfa as compared to the slurry. However, not all green manures are suitable for a particular crop. In our case, grass clover silage treatment did not really perform as expected. Further research still needs to be done to determine which green manure is suitable for which crop.

Although the quality part of the crop, in this case the nitrate content did not improved by the use of the green manure, we can also say that the quality of the product can still be maintained even though the nitrogen content of the green manure varies quite a lot. This is especially true for the grass clover fresh, which has the highest actual applied total nitrogen than targeted.

The fact that split application did not perform well as expected must be further researched, especially in terms of decomposition rate and C/N ratio as discussed in the Discussion section before.

7 REFERENCES

Båth, B., 2000. Matching the availability of N mineralised from green manure crops with the N-demand of field vegetables. Doctoral thesis. Agraria 222. Swedish University of Agricultural Sciences, Uppsala, Sweden, 29 pp.

de Neergaard, A., H. Hauggaard-Nielsen, L. Stoumann Jensen, and J. Magid. 2002. Decomposition of white clover (Trifoliumrepens) and ryegrass (Loliumperenne) components: C and N dynamics simulated with the DAISY soil organic matter submodel. *European Journal of Agronomy* 16, (1): 43-55.

Elwan, M. W. M., and K. E. Abd El-Hamed. 2011. Influence of nitrogen form, growing season and sulfur fertilization on yield and the content of nitrate and vitamin C of broccoli. *ScientiaHorticulturae* 127, (3): 181-187.

Erdem, T., L. Arin, Y. Erdem, S. Polat, M. Deveci, H. Okursoy, and H. T. Gültaş. 2010. Yield and quality response of drip irrigated broccoli (*Brassica oleracea* L. var. *italica*) under different irrigation regimes, nitrogen applications and cultivation periods. *Agricultural Water Management* 97, (5): 681-688.

Francescangeli, N., M. A. Sangiacomo, and H. R. Martí. 2007. Vegetative and reproductive plasticity of broccoli at three levels of incident photosynthetically active radiation. *Spanish Journal of Agricultural Research* 5, (3): 389-401.

Gill, J. S., P. W. G. Sale, R. R. Peries, and C. Tang. 2009. Changes in soil physical properties and crop root growth in dense sodic subsoil following incorporation of organic amendments. *Field Crops Research* 114, (1): 137-146.

Hansson, P. -A, and H. Fredriksson. 2004. Use of summer harvested common reed (*Phragmitesaustralis*) as nutrient source for organic crop production in Sweden. *Agriculture, Ecosystems and Environment* 102, (3): 365-375.

Kumar, K., and K. M. Goh. 2000. Crop residues and management practices: Effects on soil quality, soil nitrogen dynamics, crop yield, and nitrogen recovery. *Advances in Agronomy* 68:197-319.

Mohanty, M., K. S. Reddy, M. E. Probert, R. C. Dalal, A. S. Rao, and N. W. Menzies. 2011. Modelling N mineralization from green manure and farmyard manure from a laboratory incubation study. *Ecological Modelling* 222, (3): 719-726.

Nkoa, R., Y. Desjardins, N. Tremblay, L. Querrec, M. Baana, and B. Nkoa. 2003. A mathematical model for nitrogen demand quantification and a link to broccoli (*Brassica oleracea* var. *italica*) glutamine synthetase activity. *Plant Science* 165, (3): 483-496.

Rayns, F., and A. Rosenfeld, 2008. Green manures: an investigation into the adoption of green manures in both organic and conventional rotations to aid nitrogen management and maintain soil structure. A review conducted by HDRA as part of HDC Project FV 299.

Riley, H., R. Pommeresche, R. Eltun, S. Hansen, and A. Korsaeth. 2008. Soil structure, organic matter and earthworm activity in a comparison of cropping systems with contrasting tillage, rotations, fertilizer levels and manure use. *Agriculture, Ecosystems and Environment* 124, (3-4): 275-284.

Scholberg, J.M.S., C. Ter Berg, J. Staps, en J. van Strien. 2010. Minder en anders bemesten: Voordelen van maaimeststoffen voor de teelt van najaarsspinazie: Resultaten veldproef Joost van Strien 2009. Louis Bolk Instituut, Driebergen Zeist, 44 pp.

Schröder, J. 2005. Revisiting the agronomic benefits of manure: A correct assessment and exploitation of its fertilizer value spares the environment. *Bioresource Technolog*y 96, (2), 253-261.

Sorensen, J. N., and K. Thorup-Kristensen. 2011. Plant-based fertilizers for organic vegetable production. *Journal of Plant Nutrition and Soil Science* 174, (2): 321-332.

Stamatiadis, S., M. Werner, and M. Buchanan. 1999. Field assessment of soil quality as affected by compost and fertilizer application in a broccoli field (San Benito County, California). *Applied Soil Ecology* 12, (3): 217-225.

Stoppani, M. I., R. Wolf, N. Francescangeli, and H. R. Martí. 2003. A nondestructive and rapid method for estimating leaf area of broccoli. *Advances in Horticultural Science* 17, (3): 173-175.

Theunissen, J., and A. Sins. 1984. Growth stages of Brassica crops for crop protection purposes. *ScientiaHorticulturae* 24, (1): 1-11.

Thompson, T. L., T. A. Doerge, and R. E. Godin. 2002. Subsurface drip irrigation and fertigation of broccoli: I. yield, quality, and nitrogen uptake. *Soil Science Society of America Journal* 66, no. 1: 186-192,

Thorup-Kristensen, K. 1993. Root development of nitrogen catch crops and of a succeeding crop of broccoli. *ActaAgriculturaeScandinavica Section B, Soil and Plant Science*43: 58–64.

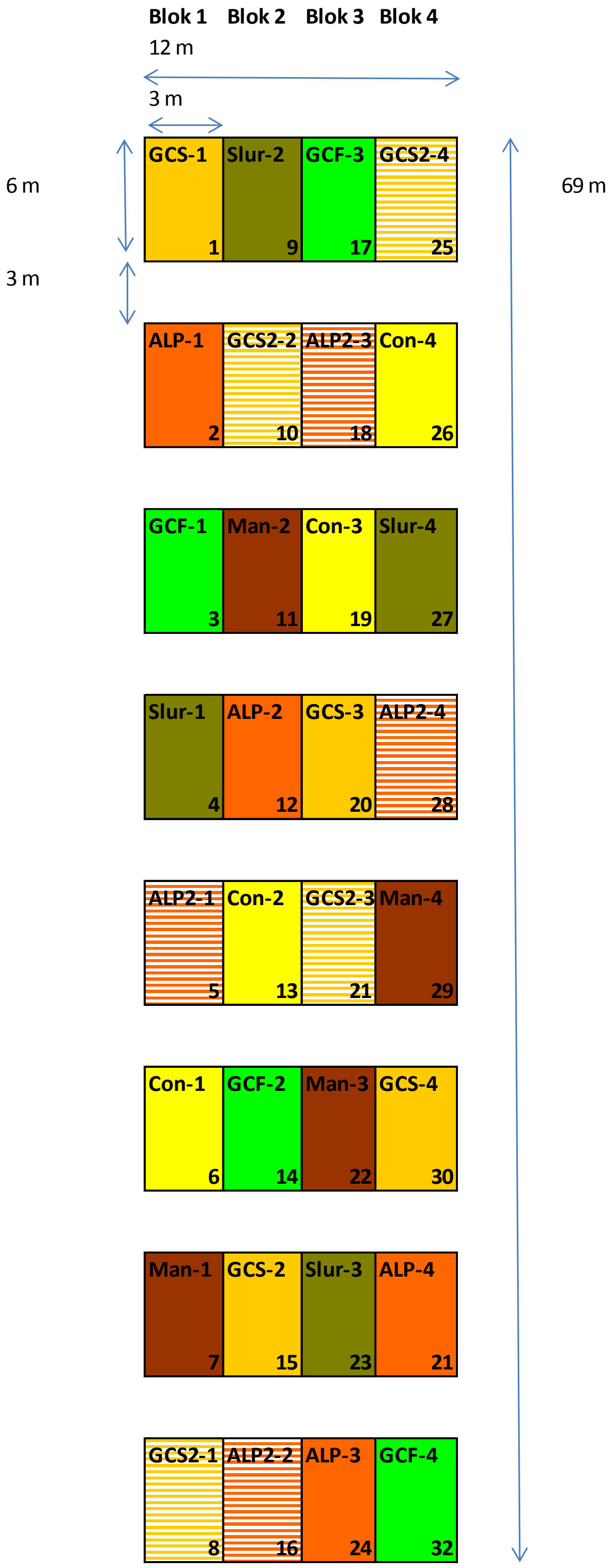
Thorup-Kristensen, K., J. Magid, and L. S. Jensen. 2001. Catch crops and green manures as biological tools in nitrogen management in temperate zones. *Advances in Agronomy* 79:227-302.

van der Burgt, G. J. H. M. , G. J. M. Oomen, A. S. J. Habets, and W. A. H. Rossing. 2006. The NDICEA model, a tool to improve nitrogen use efficiency in cropping systems. *Nutrient Cycling in Agroecosystems* 74: 275-294.

APPENDICES

I Experimental plan

Broccoli (Brassica oleracea var. italica)		
Item	Value	Unit
Cultivar	Ironman Biologisch KWF	
Planting date	16-6-2011	
Row space	0.75	m
# rows	4	
Plant spacing	0.5	
Row length	6	m
Plot width	3	m
Plot area	18	m^2
# replicates	4	
Total area/treatment	72	m^2
# treatments	8	
#plants/plot	48	
#plants/trial	1536	
Total area/trial	576	m^2
N-target	150	kg/ha


Blok 1
Blok 2
Blok 3
Blok 4
12 m
3 m
6 m
3 m
69 m
GCS-1 1
Slur-2 9
GCF-3 17
GCS2-4 25
ALP-1 2
GCS2-2 10
ALP2-3 18
Con-4 26
GCF-1 3
Man-2 11
Con-3 19
Slur-4 27
Slur-1 4
ALP-2 12
GCS-3 20
ALP2-4 28
ALP2-1 5
Con-2 13
GCS2-3 21
Man-4 29
Con-1 6
GCF-2 14
Man-3 22
GCS-4 30
Man-1 7
GCS-2 15
Slur-3 23
ALP-4 21
GCS2-1 8
ALP2-2 16
ALP-3 24
GCF-4 32