Plant-Based Biogas Production for Improved Nutrient Management of Beetroot

in Stockless Organic Farming

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Cover: Experimental biogas reactor, biogas car, effluent supply before drilling and in growing beetroots (photo: Kjell Christensson and Anita Gunnarsson)

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Abstract

Transition from a nutrient management system based on green manure (GrM system) to one based on biodigested plant material produced within the crop rotation (BG system) was investigated in crop sequences including clover-grass, beetroot and cereals. The overall hypothesis was that transition would improve nitrogen (N) availability. In field experiments on sandy soil, harvested clover-grass ley had lower N content in clover and biomass produced than GrM-ley. The residual N effect of clover-grass ley harvested twice (2H) or three times (3H) was 42 and 74 kg N ha^{-1} less than that of GrM-ley considering uptake in beetroot and mineral N in soil at harvest. Expressed as inorganic fertiliser equivalents the reduction was 52 and 80 kg N ha^{-1}, respectively. Net inorganic N equivalents (from effluent plus pre-crops) were simulated for three crop sequences: (A) green manure ley, beetroot, winter rye; (B) harvested ley, beetroot, winter rye; and (C) harvested ley, spring barley, beetroot, where B and C represented BG systems and A a GrM system. For three hectares with the entire crop sequence A, B and C, net inorganic N equivalents were 73, 74 and 128 kg N, respectively. Net inorganic N equivalents in BG systems with 2H- and 3H-ley did not differ significantly. When the whole increase in net inorganic N equivalents was used for beetroot following barley, marketable beetroot yield increased by 1.7 Mg ha^{-1} (12%) in the BG system with 2H-ley and by 5.8 Mg ha^{-1} (34%) with 3H-ley compared with beetroot grown without digestate fertilisation following a GrM-ley. Fertilisation with a moderate level of effluent of beetroot directly following harvested ley gave unexpectedly low yield responses. Compositional nutrient diagnosis (CND) using norms derived from aeroponic experiments with 22 treatments with dynamic nutrient supply and partial least squares (PLS) were synonymous in showing K as more growth-limiting than N at early growth stages. Growth limitation was more severe in effluent-fertilised beetroot following harvested ley than following barley. Pot experiments showed an apparent net mineralisation of organically bound N in digestate of 12%. The overall conclusion was that a BG system can greatly improve N efficiency. However, as the nutrient buffering capacity in sandy soil is low, inappropriate use of the effluent, *e.g.* at an unsuitable point in the crop rotation, can negate the N efficiency benefits.

Keywords: Beta vulgaris var. *conditiva* Alef, residual N effect, pre-crop, clover-grass, biogas, anaerobic digestion, effluent, digestate, slurry,

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Dedication

Till Per som höll ut ända till slutet men som aldrig kommer att läsa ens sammanfattningen.

I am not young enough to know everything
James Matthew Barrie

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List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Gunnarsson, A., Bengtsson, F. & Caspersen, S. (2010). Use efficiency of nitrogen from biodigested plant material by ryegrass. *Journal of Plant Nutrition and Soil Science* 173, 113-119.

II Gunnarsson, A., Lindén, B. & Gertsson, U. (2008). Residual nitrogen effects in organically cultivated beetroot following a harvested/green manured grass-clover ley. *Journal of Plant Nutrition* 31, 1355–1381.

III Gunnarsson, A., Lindén, B., and Gertsson U. 2011. Biodigestion of plant material can improve nitrogen use efficiency in a red beet crop sequence. *HortScience* 46(5), 765-775.

IV Gunnarsson, A., Asp, H., and Gertsson U., Growth-limiting nutrients in beetroot fertilized with biodigested plant material. In manuscript.

Papers I-III are reproduced with the permission of the publishers.

The contribution of Anita Gunnarsson to Papers I-IV in this thesis was as follows:

Paper I: Was responsible for the scope, aim and general planning of the study. Co-author Siri Caspersen was responsible for planning the practical procedures for the pot experiment. Co-author Fredrik Bengtsson performed the main practical work, assisted by Gunnarsson and Caspersen. Was responsible for data analysis with the assistance of Bengtsson. Wrote the manuscript assisted by Caspersen.

Paper II: Was responsible for the scope, aim and general planning of the study, assisted by co-authors Ulla Gertsson and Börje Lindén. Was responsible for field experiments with practical help from the staff at the Swedish Rural Economy and Agricultural Society in Halland, Dept. of Field Experiments. Conducted most field sampling and preparation of plant material with the assistance of Elin Carlsson. Was responsible for all data analysis. Wrote the manuscript assisted by co-authors.

Paper III: As Paper II.

Paper IV: Field experiment part: As Paper II. Image analysis of roots was performed by John Löfkvist. Aeroponic part: Was responsible for the scope, aim and general planning of the study, assisted by Olof Hellgren and by co-author Ulla Gertsson. Was responsible for all data analysis. Wrote the manuscript assisted by the co-authors (Ulla Gertsson and Håkan Asp). Technical support with the aeroponic growth units was provided by research engineer Göran Nilsson.

Abbreviations and Synonyms

BG system	Biogas nutrient management system with its N supply originating from biodigested plant material
CH_4	Methane
CND	Compositional nutrient diagnosis (for interpretation of plant nutrient status)
COD	Chemical oxygen demand (parameter used as a rough indicator of organic matter in *e.g.* wastewater)
CSTR	Continuously stirred tank reactor (for biogas production)
CVA	Critical value approach (for interpretation of plant nutrient status)
DAS	Days after sowing
DM	dry matter
DRIS	Diagnosis and recommendation integrated systems (for interpretation of plant nutrient status)
GrM-ley	Non-harvested clover-grass ley used for green manure purposes
GrM system	Green manure nutrient management system with its N supply originating from clover-grass green manure ley
HRT	Hydraulic retention time (for describing biogas technology)
K_{crit}	Critical K concentration in plant
K_{max}	Maximum K concentration in plant
LCA	Life cycle assessment
N, P etc.	Chemical abbreviations for elements
N_{dfa}	The proportion of N in a leguminous crop derived from fixation of atmospheric N_2
N_{crit}	Critical N concentration in the plant
N_{min}	Soil mineral N (ammonium and nitrate N)
N_{org}	Organically bound N
$N_{R\text{-}ley}$	Expression for supply of NH_4-N in digestate needed by beetroot

	with barley pre-crop to reach the same yield level as beetroot following ley
NUE_{min}	N uptake efficiency of mineral N (*i.e.* for digestate, NH_4-N and not the organically bound N was considered. For the inorganic fertiliser used in the pot experiment, all mineral N was in the form of NO_3-N) In Paper I the NUE concept was called *N use efficiency* but now, in secion 2-4, *N uptake efficiency* is used, as according to a suggestion by Weih *et al.* (2011) *N use efficiency* should be used as an overall expression
OLR	Organic loading rate (for describing feeding rate to biogas reactor)
PLS	Partial least squares
STP	Standard temperature and pressure (0 °C, 1 bar) (used *e.g.* as standard when expressing biogas or methane yield)
STR	Solid retention time (for describing biogas technology)
VFA	Volatile fatty acids
VS	Volatile solids (*i.e.* dry matter minus ash; in some studies volatile solids are denominated organic matter)
2H-ley	Clover-grass ley harvested twice
3H-ley	Clover-grass ley harvested three times

Synonyms

Biogas residues = digestate = biogas effluent

In Papers I-IV, biogas residues or biogas effluent is used but in this covering essay digestate is used, as that tends to dominate in the later scientific literature.

Beetroot = red beet (*Beta vulgaris* var. *conditiva* Alef.) or *Beta vulgaris* L. subsp. v*ulgaris* (Garden beet group). 'Red beet' is used in Paper III.

1 Introduction and theoretical framework

1.1 Challenges in N self-sufficient stockless cropping systems

A nitrogen (N) self-sufficient cropping system depends on biological N_2 fixation. Biological N_2 fixation on agricultural land amounts to an estimated 50-70 million tonnes per year (Herridge *et al.*, 2008), compared with 130 million tonnes of industrially fixed fertiliser N in 2007/2008. Globally, supply and demand for N is continuously increasing (FAO, 2008). The use and production of industrially fixed N is continuously increasing in agriculture, but in organic farming biological N_2 fixation is the main N source. Green manure and legumes are recommended in organic crop rotations by the International Federation of Organic Agriculture Movements (IFOAM, 2005). IFOAM also recommends that nutrient resources be used in a sustainable and responsible manner; that nutrient losses from the farm to the environment be minimised; and that nutrients be used in such a way and at such appropriate times and sites that their effect is optimised. Biological N_2 fixation has many potential benefits, such as reduced fossil energy use and less CO_2 and N_2O emissions; improved long-term fertility; positive soil structure effects; *etc.*, although there is a risk of N losses in the post-harvest period (Jensen & Hauggaard-Nielsen, 2003). Based on a literature review, Crews & People (2005) suggest that in rainfed agriculture, crops recover more of the N from synthetic N fertilisers but a higher proportion of legume N is retained in the soil and thus N losses tend not to differ greatly between these sources. Similarly, Torstensson *et al.* (2006) found that a conventional cropping system without leguminous and cover crops grown on a very sandy soil (91% sand and 2% clay in the 30-60 cm soil layer) leached 39 kg ha^{-1} $year^{-1}$, whereas an organic crop rotation with one-third of the acreage in green manure (red clover + grass) leached 34 kg N ha^{-1}.

However, when the leaching was expressed as a percentage of total N removal by the crop, the green manure crop rotation leached 59% more. Choice of cropping measures affects N use efficiency and losses of N in green manure. For example, leaving a red clover green manure over winter instead of incorporating it in the autumn, before sowing of winter wheat, reduced leaching from 102 kg ha^{-1} to 26 kg ha^{-1} (Stopes *et al.*, 1995). However, leaving crop residues on top of soil during late autumn and winter causes NH_3 losses, which may correspond to 5-16% of the N content of the residues, compared with almost insignificant losses when incorporated into the soil (De Ruijter *et al.*, 2010). Total ammonia volatilisation in that case was related to C/N-ratio and N concentration of the plant material and was negligible from plant material with N concentration below 2%, but was 10% of the N content of plant material with 4% N. Altogether, most research indicates that improvements in N efficiency in nutrient management systems based on green manure is an important challenge - both in respect of increased harvests and of reduced leaching.

In order to increase the N use efficiency of green manure, different measures have been tried. Growing the crop in rotovated strips of green manure was tested by Riley & Brandsœter (2001). However, they observed reduced yield in beetroot from 39 to 8 Mg ha^{-1} and in cabbage from 41 to 10 Mg ha^{-1} when these crops were grown in strips between red clover green manure strips, compared with soil ploughed in spring, even though the red clover sward in the strips was mowed frequently. Using fresh red clover as mulch increased the yield of beetroot and white cabbage planted in spring-ploughed soil with barley and undersown clover as pre-crop (Riley *et al.*, 2003; Riley & Dragland, 2002; Riley & Brandsœter, 2001). However, N recovery was only 2-16% in beetroot and 8-27% in white cabbage (supply of mulch from 263 to 587 kg N ha^{-1} to both crops; white clover, red clover, alsike clover or cocksfoot as mulch). The better response for mulch in cabbage was explained by the longer growing season. Båth *et al.* (2006b) found somewhat higher N recovery of red clover mulch (243 kg ha^{-1}) in cabbage: 28% based on total uptake in the above-ground parts of the white cabbage plants. In leek, N recovery was 16% of the 400 kg N supplied in mulch of mixed clover-grass (Ekbladh, 1995).

Fresh biomass is not available in time to be needed for most crops in Sweden. For that reason, studies have been performed with silage from N-rich ley as fertiliser, but N delivery proved to be very low. From applications of silage as mulch to an uncropped arable soil, only 1-6% appeared as an increased amount of mineral N in the topsoil within 110 days after mulching (Larsson, 1997). This can be compared with 9-10% recovery of the amount of N supplied in fresh grass. The grass and the silage had approximately the same N concentration (2.1-2.2%). In another approach, Wivstad (1997) incorporated biomass preserved as hay and silage into soil in a pot experiment with spring wheat. During 126 days, 38% of N in the hay but only 29% of that in the silage was mineralised, *i.e.* recovered in wheat plants including roots and as soil mineral N. The recovery of N from inorganic fertiliser was 70% in the same experiment. In conclusion, none of the measures for improving the N use efficiency of green manure was really successful in giving acceptable short-term effects. Of course there may be other reasons for mulching, such as weed suppression and soil water conservation. However, mulching has not been frequently implemented in organic farming in Sweden, probably due to the benefit being too small and the costs for harvesting, preserving and spreading the biomass too high.

In general, best management practice (BMP), with the focus on N efficiency, needs to be more innovative. There is a need to recognise that in some situations, radical changes in farming systems may be the only solution (Shepherd & Chambers, 2007). The results reviewed above indicate that this may be the case for organic stockless cropping systems. Transition from a 'green manure farm' to a 'biogas farm', or rather from a green manure nutrient management system (GrM system) to a biogas nutrient management system (BG system) is a possible approach investigated in this thesis. To evaluate the impact on nutrient use efficiency when changing from one nutritional management system to another, many aspects need to be taken into account (Neeteson *et al.*, 2003). Transformation from a GrM system to a BG system involves many changes at the field level. The following sections in this chapter provide a theoretical framework for some of the factors that may be important when interpreting the results from the experimental work presented in Papers I-IV.

1.2 Clover-grass ley – factors of importance for expected impacts of cutting versus harvesting

In Sweden, most green manure crops on farms with cereals in the crop rotation consist of clover and grass undersown in cereal in spring in the year before the green manure year. That type of green manure is referred to here as a green manure ley (GrM-ley). As biological N_2 fixation is the major N source in stockless organic farming (and on organic farms with cattle), it is of high concern to keep a high content of leguminous species in the ley. The normal seed rate is 15-20 kg ha^{-1}, of which 30% is clover and the rest is grass. GrM leys are normally unfertilised and therefore the grass growth is N-limited in contrast to the clover, which results in a typical stand with 60-70% of biomass (dry matter) as clover.

This section presents some existing knowledge on topics of importance for predicting the effects of changing ley management from cutting and leaving the biomass to removing it. Gosling & Rayns (2005) list some management factors and their effects on N_2 fixation in green manures (Table 1). (It is unclear whether the list concerns proportion of N in clover derived from fixation of atmospheric N_2 (N_{dfa}) or total amount of fixed N_2 per hectare.) However, as shown below, the reaction of species in a crop mix of clover and grass depends on many factors and is not easy to predict.

Basic morphological and physiological differences between the plants affect the nature of competition between the species in a mixture such as a clover-grass ley. In general, legumes are poor competitors with grasses for light, nutrients and water (review by Haynes, 1980). Legumes with their planophile leaves adsorb a great deal of light with only a few layers of leaves, while with grasses light is distributed more evenly throughout the leaf canopy. Legumes also appear to have a physiological need for higher light intensity than grasses for maximum growth rates. The ranking for some common ley species concerning sensitivity for light reduction is as follows: Italian ryegrass (not sensitive) < red clover < white clover < lucerne. Italian ryegrass (*Lolium multiflorum*) has its maximum growth rate at 71% of daylight, red clover needs 100%, white clover 185% and lucerne 251%. The competitive disadvantage of legumes is increased by the fact that pasture grasses are generally taller and have often faster growth rate and can thus overtop and shade legumes. Cutting frequency and intensity is therefore important in order to maintain the legume component of a pasture.

Grasses generally have longer, thinner, more finely branched roots than clovers and can thus explore a greater volume of soil (Evans, 1977). For example, Evans (1977) showed that perennial ryegrass had 10% longer roots per kg dry weight, roots with 69% more branches and a volume within the root hair cylinder that was 6 times larger than that of white clover. Shoot/root ratio did not differ between the species. These differences could give grasses a competitive advantage over clovers when there is a shortage in their supply of nutrients (particularly P, K and S) and water. The roots of legumes generally have a cation exchange capacity (CEC) twice that of grass roots (review by Haynes, 1980). A plant with high CEC may adsorb relatively more divalent cations (such as Ca) than grasses, with low CEC. This may be a partial explanation for the poor ability of legumes to compete with grass for K.

Table 1. *Management practices and their effect on N_2 fixation by legumes in leys. From Gosling & Rayns (2005)*

Practices which: Increase N_2 fixation	Reduce N_2 fixation
Add P and K	Add manure
Cut and remove material	Cut and mulch
Mix with grass*	Graze
Short-term leys	Monocropping legumes
	Long-term leys

* As the proportion of clover in the sward declines, overall N_2 fixation of the sward declines

Nitrogen fertilisation generally reduces the clover/grass ratio but time of fertilisation and choice of cultivar are important. In a mixture with 25 kg ha^{-1} ryegrass and 4 kg ha^{-1} white clover, fertilisation with 30 kg N ha^{-1} per cut (5 cuts; biomass removed) reduced the clover proportion from 42 to 12% during the first harvest year (Nassiri & Elgersma, 2002). Ryegrass had its highest growth rate in the first part of the growing period, whereas white clover grew faster in the summer. The N fertilisation effect on total DM production was therefore largest in the first cut (Nassiri & Elgersma, 2002). A large-leaved white clover cultivar (Alice) was better able to withstand the negative effect of repeated N application, and even increased its proportion in the second harvest year (Elgersma *et al.*, 2000; same experiments as reported by Nassari & Elgersma, 2002). The difference between the large-leaved cultivar and the small-leaved was due to the fact that the N fertilisation increased the petiole length in the large-leaved variety (Nassiri & Elgersma, 2002). Transfer of clover-derived N to grass was larger in the N fertilised swards, ranging from 50 kg N ha^{-1} transferred from clover to ryegrass in the non-fertilised sward to 114 kg N ha^{-1} with N fertilisation, as a mean for a two-year period (Elgersma *et al.*, 2000).

The impact of supplying nutrients other than N on clover-grass competition may vary. Up to a certain level, grass will benefit more than clover from an additional nutrient supply. Above that level, however, the clover will benefit more than the grass. Thus research has shown that K either increases clover yield and clover/grass ratio (Campillo *et al.*, 2005; soil low in K) or decreases it (Høgh-Jensen *et al.*, 2001). Furthermore, Campillo *et al.* (2005) showed no negative effect on the white clover fraction in a mixed ley when N was supplied, if K supply was sufficient. Baines (1988) showed interaction reactions of a clover-grass crop with N, P and K supply, cutting frequency, grass species and clover types.

The amount of N_2 fixed per ha is normally linearly proportional to total N in legume biomass if the crop is not fertilised (Carlsson, 2005; Carlsson & Huss-Danell, 2003). The proportion of N in clover derived from fixation of atmospheric N_2 (N_{dfa}) in clover-grass mixtures is only reduced by mineral fertiliser N if applied at high rates. Up to a level of 200 kg N ha^{-1} applied to clover-grass leys, the effects on N_{dfa} are small and inconsistent (Carlsson, 2005). The N_{dfa} mainly varies due to climate and species. For white clover a good estimate for N_{dfa} in mixture with ley with N application of less than 200 kg N ha^{-1} is 32 kg N ton^{-1} legume DM. The corresponding figures for red clover and lucerne are 26 and 21 kg N ton^{-1} legume DM, respectively (Carlsson, 2005). Fertilisation with a complete fertiliser containing P, K, Mg, S and micronutrients, but not N, has no impact on N_{dfa} in the clover in a mixed sward (Campillo *et al.*, 2005).

Nitrogen fixation in a mixed clover-grass ley is often higher if the ley is harvested compared with cut for self-mulching. N_2-fixation by leguminous plants has been shown to increase by 83% in harvested ley compared with green manure ley with the same seed mixture of grass and legumes (Loges *et al.*, 2000a; soil with 14% clay; mean for mixtures with white clover-grass, red clover-grass and lucerne-grass). The advantage for harvested ley was only 19% for a white clover-grass mixture (from 209 to 248 kg fixed N ha^{-1}), compared with a +128% increase of fixed N as a mean for red clover-grass and lucerne-grass leys (from 145 to 331 kg fixed N ha^{-1}) (Loges *et al.*, 2000b). In another study, the estimated N_2 fixation increased by 35% (from 370 to 498 kg N ha^{-1}) and total above-ground N uptake by 8% (from 369 to 397 kg ha^{-1}) in harvested clover/lucerne-grass ley compared with GrM-ley (Stinner *et al.*, 2008). Legume proportion increased from 88 to 94%. The study was performed on a silty loam with 25-30% clay.

Knowledge of factors affecting competition between grass and legumes in a mixed ley is important in order to understand what happens with a change from green manure leys to harvested leys. Due to differences in physiology between grasses and clover, but also between different types of *e.g.* white clover, it is probable that the balance between grass and clover after cutting or harvesting a ley may differ depending on K supply and type and cultivar of clover. The reaction of N_2 fixation to harvesting or cutting a ley differs between species: red clover and lucerne-grass leys increase their N_2 fixation after harvesting much more than white clover-grass leys.

1.2.1 Nitrogen mineralization in general

Nitrogen mineralisation from plant residues is less in cropped soil than in soil without a crop, as microorganisms seem to prefer C from root exudates rather than from crop residues (Nicolardot *et al.*, 1995). This is one of the reasons why results from incubation experiments cannot be quantitatively transferred to the situation in soil with a growing crop. In the following section both results from incubation experiments with and without a crop are presented.

Mineralisation rate for plant material added to soil is mainly correlated with percentage of N in residues, although other quality parameters are also involved (Thorup-Kristensen *et al.*, 2003; Yadvinder-Singh & Khind, 1992). The impact of N concentration can be illustrated by data from Marstorp & Kirchmann (1991) concerning net mineralisation from six green manure legumes in incubation and pot experiments. Mineralisation within 16 weeks was, as expected, correlated both to percentage of N and C/N ratio, with the highest percentage of supplied N mineralised from white clover or black medick and the lowest from Persian clover or Egyptian clover. Mineralisation of N from red clover, Egyptian clover and subterranean clover was somewhat lower than expected from the fitted regression line, whereas mineralisation from black medick was somewhat higher (Figure 1). In treatments with red clover, Persian clover and Egyptian clover, N was immobilised during the first day and the level of inorganic N did not reach the level of the unfertilised control until approximately two weeks. The initial decrease correlated well with the C/N ratio in the legumes. Wivstad (1997) found similar relationships for red clover, white clover, yellow sweet clover and perennial ryegrass with different percentages of N in dry matter, but mineralisation was less when incorporated biomass was in the form of silage rather than hay (Figure 2a and b).

Inspired by Kolenbrander (1974), Granstedt (1995) used a simple model for explaining apparent N mineralisation from green manure within one year of ploughing. The model assumed that 35% of C in the residues was humified (*i.e.* a humification coefficient of 0.35) and that the humus had a C/N ratio of 10. The same model was found to be useful for estimating the N mineralisation in the first two years after ploughing under harvested leys (Granstedt & L-Baeckström, 2000). For more sophisticated N mineralisation models, explaining N dynamics from day to day, other quality parameters in the plant material need to be taken into account. Regression models using data from stepwise chemical digestion (SCD) were found to give a better empirical prediction than those using only C/N ratio for detailed mineralisation pattern (Bruun *et al.*, 2005). Using the same data set as Bruun *et al.* (2005), Jensen *et al.* (2005) found water-soluble N content to be important for initial net N mineralisation, and from day 22 total plant N and neutral detergent-soluble N were most correlated with N mineralisation.

Again using the same data set, Henriksen *et al.* (2007) concluded that both near-infrared (NIR) spectroscopy and measurement of total N concentration offer good, cost-effective alternatives for prediction of N mineralisation if they are calibrated with SCD data.

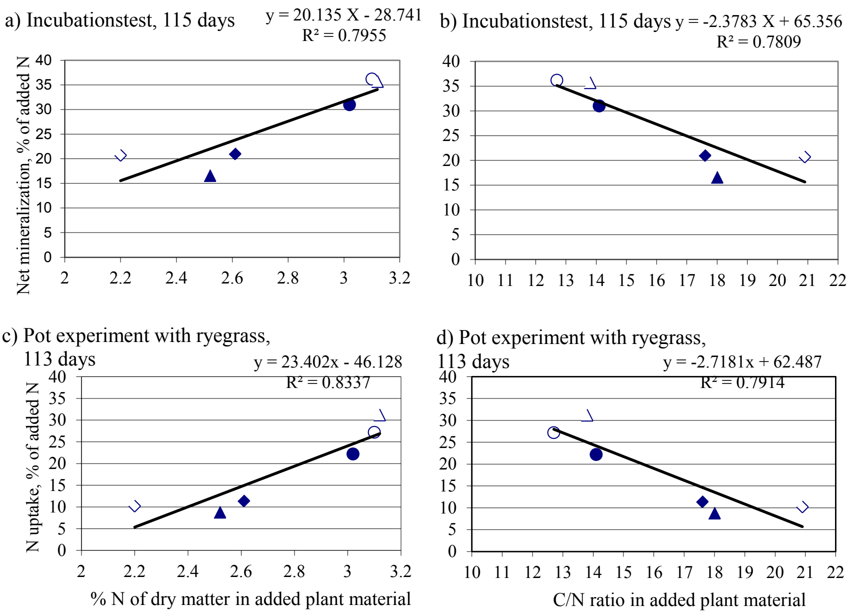


Figure 1. Net mineralisation after 115 days in an incubation study, with 25 °C, on soil without plants (a and b) and N uptake in ryegrass on day 113 in pot experiments (c and d) with six green manure legumes used as fertilisers. Filled circles = subterranean clover, empty circles = white clover, empty triangles = black medick, filled triangles = Egyptian clover, empty squares = Persian clover, filled squares = red clover. Modified from Marstorp & Kirchmann (1991).

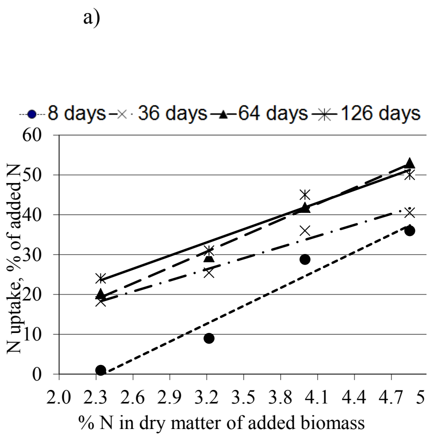


Figure 2a. Relationship between N mineralisation measured as soil N_{min} plus N uptake in wheat (incl. roots) and N% in dry matter of hay from different species with different N concentration at 8, 36, 64 and 126 days from incorporation. Temp: 17/10 °C 16/8 hours day/night, Light: 330 µmol m2 s-1. Linear equations: 8 days: 14.97X-35.2 (R2=0.96); 36 days 9.29X-3.4 (R2=0.98); 64 days: 13.3X-11.9 (R^2=0.99); 126 days: 11.0X-2.2 (R^2=0.96). Modified from Wivstad (1997).

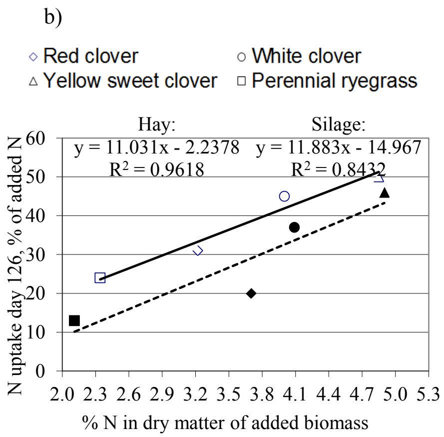


Figure 2b. Relationship between N mineralisation after 126 days measured as soil N_{min} plus N uptake in wheat (incl. roots) and N% in dry matter of four different species conserved as hay and silage. Filled symbols = silage; open symbols = hay. Temperature and light as described in fig 2a. Modified from Wivstad (1997).

For modelling N behaviour from residual N effects of plant material, many models are available. A recently revised model is EU-Rotate_N, in which temperature, soil water dynamics and root development of the crop are taken into account (Rahn *et al.*, 2010b; Rahn, 2007). Root depth differs between crops, which causes different residual N effects depending on the following crop. Beetroot, sweetcorn and celeriac roots reached 1.55-1.8, 0.6-0.9 and 0.4-0.6 m, respectively, (the range refers to two different years) (Christiansen *et al.*, 2006). Roots of beetroot extended 0.5 m deeper when a green manure crop was incorporated in autumn than in spring, as roots can grow deeper if N is available further down in the soil profile. Beetroot with 1.5 m deep roots was listed as a crop with an intermediate root system in comparison with leek (0.5 m deep roots), ryegrass and barley (0.75 m), fodder radish and white cabbage (2.25 m) and a chicory catch crop (>2.5 m) (Thorup-Kristensen, 2006). Both the studies reported by Christiansen *et al.* (2006) and Thorup-Kristensen (2006) were performed on a soil with 70% sand and 12-19% clay in the soil layers from 0-2.5 m, thus representing a soil with good potential for a deep root system. Other studies indicate that root development is ensured in sandy soils only if they contain a minimum of 2% organic matter or 6% clay (review by Heinonen, 1985, p 51). Thus the differences in root depth between crops may be less on other soil types than those used by Christiansen *et al.* (2006) and Thorup-Kristensen (2006).

Mineralisation of organic matter is affected by inorganic salts. In an incubation study, Campino (1982) showed that NO_3-N increased by up to 65% with K application to the soil compared with a control without added K. The K fertiliser effect on fixed or exchangeable ammonium was not the main explanation. Omar & Ismail (1999) also found that the overall effect of the addition of inorganic salts on mineralised N was promotive. However, there may be interactions on soil organic matter mineralisation with different combinations of added salts. Such interactions were shown for NaCl and Na_2SO_4, possibly through salt-induced changes in the microbial community (Li *et al.*, 2006).

The data provided in this section can help interpret results presented in Paper II about residual N effects and are thereby also of importance for interpreting results presented in Papers III and IV regarding:

- N mineralisation rate after incorporation into soil of different clover species with different N concentrations, *e.g.* N from white clover mineralises rather rapidly, mainly due to the high N concentration.
- Models for estimating residual N effect, *e.g.* that used by Granstedt (and in Paper II) for predicting first-year mineralisation of green manure and harvested ley.
- Importance of root depth of the following crop for its ability to use mineralised N from the pre-crop. Of interest for the root studies on beetroot in Paper IV.
- Methods for improving use of N in green manure ley, *e.g.* ensiling or haymaking to allow biomass to be used as fertiliser in the following year.
- Importance of K for N mineralisation (Paper II).

1.2.2 Residual effects of harvested ley and green manure ley - field experiments

In a review, Lindén (2008) estimated the first-year residual N effect (defined as N usable for a following cereal crop) from harvested clover-grass ley to be 35-40 kg N ha^{-1} and from green manure with red clover-grass mixture and white clover grass mixture to be 50-60 and 60-80 kg N ha^{-1}. Torstensson

(1998) studied residual effects in rye and barley of eight different ley types and a ryegrass cover crop. The topsoil was sandy, containing 8-9% clay and 5% organic matter, and the subsoil (30-90 cm) contained <1% clay and organic matter. Three of the leys were not harvested but used as directly incorporated green manures. For all pre-crop types the highest N uptake in the following crop was achieved following late autumn incorporation. Compared with a barley pre-crop, it was (kg N ha^{-1}): +40 for harvested red clover-grass, +69 for red clover-grass as green manure, +36 for harvested pure red clover, +88 for white clover grass green manure and +101 for pure white clover as green manure. Thus, white clover had a higher N residual effect than red clover, which is in accordance with the higher immobilisation and lower net mineralisation of N from red clover than from white clover already mentioned (Wivstad, 1997; Marstorp & Kirchmann, 1991). The advantage in N uptake for late autumn compared with spring incorporation was (kg N ha^{-1}) +3 for barley pre-crop, +8 for white clover-grass green manure, +19 for harvested red clover-grass, +24 for pure white clover green manure and +27 for red clover-grass green manure (Torstensson, 1998). The only possible comparison between first-year harvested ley and green manure of the same species was for red clover-grass. The residual N effect of harvested red clover-ley, measured as total N uptake in cereals, was 7, 40 and 7 kg N ha^{-1} with incorporation in early autumn (3 Sept), late autumn (13 Nov) and spring (31 March), respectively. (Here winter rye was sown following early incorporation and barley following incorporation in late autumn and spring.) Leaving the red clover ley biomass for green manure purpose instead of harvesting it increased N uptake by 32, 29 and 21 kg ha^{-1} for early and late autumn and spring, respectively. SMN analysis in 0-90 cm soil layer in November year 1 (ley year) indicated lower leaching risk for harvested red clover-grass than when used as green manure, for all three incorporation times. Differences in SMN were small between red clover and white clover green manures at that time.

In a field experiment with organically cultivated potatoes, a full-season green manure pre-crop increased yield compared with barley + undersown clover-grass. In contrast to results for cereals (Torstensson, 1998), potato yields were not affected by whether the biomass was cut and left on the soil surface for its green manure effect or removed (Båth *et al.*, 2006a). However, in 2 of the 3 years the green manure crop was poorly established. In the only year when it was well established, according to cultivation practices used by most Swedish organic farmers, the potato yield was 7 Mg ha^{-1} (-16%) lower and the N yield in potato tubers was 26 kg ha^{-1} (-20%) lower where ley biomass was harvested than where it was left in the field.

Hansen *et al.* (2005) reported that the residual effect of a harvested clover-grass ley was larger following first-year ley than following second-year ley, but the opposite was found by Granstedt & L-Baeckström (2000). Hansen *et al.* (2005) attributed the reduced effect to organic N being easier to mineralise if formed more recently. This was indeed also observed by Granstedt & L-Baeckström (2000), as the third-year ley had a substantially lower first-year residual N effect than the second-year ley although a larger amount of N was incorporated with the third-year ley. The explanation was not only to be found in a lower N concentration due to reduced clover content, but also to the fact that the third-year ley had a humification coefficient of 0.4, in comparison with 0.35 for harvested first- and second-year leys. Similarly, Granstedt (1995) was able to predict the first-year apparent N mineralisation from different one-year green manure crops by using a humification coefficient of 0.35.

In conclusion, factors of importance for residual N effects include:

- species
- N-concentration
- time of incorporation
- harvested ley or green manure ley
- age of ley

1.2.3 Calculation of N use efficiency, residual effect and fertiliser value

Nutrient efficiency can be expressed in several ways depending on the main focus of the study (Weih *et al.*, 2011). For practical farming or advisory service organisations, mineralisation of crop residues needs to be translated into some expression of fertiliser value. The literature contains a mix of different ways to evaluate fertiliser value. For example, Schröder *et al.* (1997) evaluated the first-year fertiliser value of cover crops by their effect on: (1) economic optimum N rates; (2) marketable yield; and (3) N yield when no mineral fertiliser N was supplied. In a split-plot experiment with cover crops and a control without cover crop as main plots, five N fertiliser levels (including a 0-level) were applied to the following crop in sub-plots. This is an unusually ambitious way of studying residual N effect, but it showed that the N fertiliser value was lower when evaluated in terms of economic optimum N rates and highest in terms of effect on N yield when no mineral fertiliser was supplied.

A more common way of studying residual N effects in field experiments is to compare yield level and/or N uptake between the pre-crop or cover crop in question and a control, which is often a cereal without a cover crop (e.g. Wallgren & Linden, 1994). The comparison is typically made without

N fertilisation and/or with a low N supply. To translate this to fertiliser value with reference to inorganic fertiliser N, a correction must be made for apparent N recovery of the inorganic fertiliser. This is different for different crops. Schröder *et al.* (1997) found *e.g.* 40% N recovery of inorganic fertiliser N in potatoes and 70% in sugar beet. For sugar beet, the tops were included but for potatoes only the N in all tubers was included. Lindén (2008) used a factor of 0.75 for apparent N recovery when transforming increased N uptake to fertiliser value of the studied pre-crop or cover crop compared with a cereal pre-crop. This factor 0.75 referred to total apparent N recovery in cereal grain, straw and roots.

Another 'comparative' method to describe the fertiliser value of a pre-crop or crop residues is to supply increasing levels of inorganic N to the 'control' (often cereals). By measuring the marketable yield following the tested pre-crop, cover-crop or green manure (A) and using the N response curve with the control pre-crop (B), it is possible to calculate how much inorganic N is needed to reach the same yield with pre-crop B as in the non N-fertilised crop with pre-crop A. This is a less common, but sometimes used method according to a review by Lindén (2008; p 9). It assumes that the only effect of A is on N supply, and thus that the following crop is only 'moved to the right' on a yield versus N response curve and that the top yield is the same after pre-crop A as pre-crop B. However, this is not always the case in practice, as illustrated by Engström & Lindén (2009) in comparisons of N response curves for winter wheat with winter oilseed rape, peas and oats. The optimal N rate for winter wheat following oilseed rape was 25 kg ha^{-1} less than following oats, but yield at optimal N supply was 7% higher following oilseed rape. Thus, parameters other than N supply were also of importance for yield.

The different ways of establishing and expressing residual N effects described above can be used in interpreting the results from the different methods used in Papers II and III.

1.3 Introduction to biogas production

The application of anaerobic biotechnology dates back to at least the 10^{th} Century BC, when the Assyrians used it for heating bath water. In 1776 Volta recognised that the anaerobic process results in conversion of organic matter to methane gas. A further historical review is given by Khanal (2008; chapter 1). The description of the steps in the biogas process below originates mainly from Gujer & Zehnder (1983), updated by material from Khanal (2008).

The transformation of organic solids or complex organic macromolecules, *e.g.* proteins, carbohydrates (polysaccharides) and lipids, into end-products such as methane (CH_4) and carbon dioxide (CO_2) is accomplished through a number of metabolic stages mediated by several groups of microorganisms (Khanal, 2008; chapter 2; Gujer & Zehnder, 1983). A schematic diagram of the various steps and the bacterial groups involved in anaerobic digestion of complex waste is given in Figure 3. The fermentative bacteria (group 1 in Figure 3) are involved in the hydrolysis, fermentation and anaerobic oxidation steps. The hydrolysis step can be rate-limiting for wastes containing lipids or significant amounts of particulate matter (*e.g.* sewage sludge, animal manure and food waste). The hydrogen acetogenic bacteria group (group 2) metabolises C in the intermediary products into acetate, hydrogen (H_2) and CO_2. The acetotrophic or acetoclastic methanogens (group 3) are involved in the generation of methane from acetate. This catabolic process (the acetotrophic pathway) contributes up to 72% of the total methane generation (Gujer & Zehnder, 1983 *cit.* Khanal, 2008, chapter 2). Since methane is largely generated from acetate, acetotrophic methanogenesis is the rate-limiting step in anaerobic wastewater treatments. The remaining methane is generated by the hydrogenotrophic methanogens (group 4). The synthesis of acetate from H_2 and CO_2 by homoacetogens (group 5) has not been widely studied, but seems to be of minor quantitative importance as an acetate synthesiser.

As shown in Figure 3, biogas production is a multistep process in which a diverse group of microorganisms is involved. In terms of pH optima there are two groups of bacteria: the acid producers (acidogens) with a pH optimum between 5.5-6.5, and the methane-producers (methanogens) with their pH optimum between 6.8-7.4. Since methanogenesis is considered the rate-limiting step where both groups of bacteria are present, it is necessary to maintain the reactor pH close to neutral. If an anaerobic treatment fails, *e.g.* due to unsuitable environmental conditions or biomass washout from the reactor, it may take several months for the system to return to normal operating conditions because of the extremely low growth rate of methanogens (Khanal, 2008, chapter 1).

Biogas processes are normally run under mesophilic conditions (optimum 35-40 $^{\circ}$C) or thermophilic conditions at about 55 $^{\circ}$C. However, the process can still operate at 10 $^{\circ}$C (Khanal, 2008, Chapter 2).

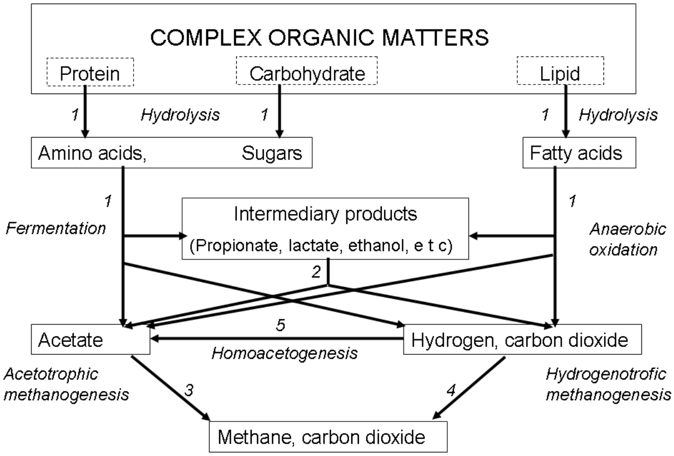


Figure 3. Conversion steps in anaeriobic digestion of complex organic matters. The number indicates the group of bacterias involved in the process. 1 = Fermentative bacteria group, 2 = Hydrogen acetogenic bacteria group, 3 = Acetotrophic methanogens, 4 = Hydrogenotrophic methanogens, 5 = Homoacetogens (Modified from Khanal, 2008 and Gujer and Zehnder, 1983)

Many types and concepts for agricultural biogas plants have been applied. A sub-division can be made into: (i) one-stage or two-stage process; (ii) dry or wet digester; (iii) batch or continuous digester; (iv) attached or non-attached digester; (v) high or low rate digester; and (vi) digesters with a combination of different approaches (Nizami & Murphy, 2010). In Germany, which is the largest biogas producing country in the world, nearly 90% of modern biogas reactors use wet fermentation (below 10% dry matter) with vertical continuously stirred tank reactors (CSTR), most operating at mesophilic temperatures (Weiland, 2010). In a single-stage anaerobic system the acidification and methanogenesis stages take place in the same reactor. In a two-stage anaerobic system, originally proposed to treat high solid organic waste, the acidification stage and the methanogenesis stage are separated. The two-stage system was originally developed by Travis in 1904 (Khanal, 2008; chapter 1). An evaluation of 61 farm biogas plants has shown that two-stage digestion results in higher gas yields and a reduced residual methane potential of the digestate (Gemmeke *et al.*, 2009, cited by Weiland, 2010). However, this technology is mainly applied for municipal and industrial waste and solid manure, and only a few results are available for energy crop digestion.

In a review, Nizami & Murphy (2010) explained and evaluated various anaerobic digesters, with the focus on their application to grass silage. They concluded that:

- Much work needs to be undertaken to ascertain optimal digester configurations from production of grass biomethane.
- The CSTR system appears to be safe technology if the mixing system is adapted to deal with the tendency for grass silage to float. Loading rates of approximately 1.4 kg VS m^{-3} day^{-1} are mentioned.
- There may be significant benefits from using leach beds followed by a high-rate digester (*i.e.* two-stage sequential batch digester connected to a high-rate bioreactor). High methane yield (0.39 m^3 methane $(kg\ VS)^{-1}$ added as ryegrass + white clover silage (50/50)) has been reported with this system (Lehtomäki & Björnsson, 2006).

According to Buswell & Mueller (1952) and modified by Richards *et al.* (1991), the biogas process can be expressed as:

$$C_nH_aO_bN_c + \left(\frac{4n-a-2b+7c}{4}\right) H_2O \longrightarrow$$

$$\left(\frac{4n-a-2b+3c}{8}\right)CH_4 + \left(\frac{4n-a-2b+5c}{8}\right)CO_2 + cNH_4^+ + cHCO_3^-$$

where the subscript letters refer to the number of elements in the molecule.

From this equation, the theoretical methane yield can be accurately predicted if the chemical composition is known. For example, it can be useful for many laboratory studies with simple substrates. The expression shows that the more biomass degraded to methane, the higher the ratio of NH_4-N to total N. For more complex substrates, maximum theoretical methane production is estimated based on chemical oxygen demand (COD) of the substrate, which is a rough indicator of the total organic matter: 1 g COD produces 0.35 L CH_4 at standard temperature and pressure (STP). STP is a temperature of 273.15 K (0 °C, 32 °F) and an absolute pressure of 100 kPa (14.504 psi, 0.986 atm, 1 bar). At STP, one mol of a gas occupies 22.4 L (Khanal, 2008, chapter 2). The theoretical methane yield is far above that obtained in practice. For example, based on chemical composition the theoretical methane production from primary solids and secondary sludge should be 0.7 and 0.5 m^3 methane $(kg\ VS)^{-1}$, respectively (Khanal, 2008, chapter 2) but 0.26 m^3 CH_4 $(kg VS)^{-1}$ is considered a practically feasible yield in full-scale biogas production from sewage sludge (i.e. ~0.20 m^3 kg DM) (Linné *et al.*, 2008).

In order to maximise the potential benefits of a biogas reactor and prevent process failure, monitoring of key parameters is needed. The level of VFA in the reactor has been shown to be the single variable that can predict gas yield, methane yield, cellulose conversion efficiency and hemicellulose efficiency when digesting lucerne silage (Nordberg *et al.*, 2007). Thus, VFA alone as explanatory parameter was able to predict those factors almost as efficiently as multivariate regression models with 23 or 7 measured input variables (Nordberg *et al.*, 2007). A combination of acetate, propionate and biogas measurements was suggested by Boe *et al.* (2010) as an indicator of disturbances in the anaerobic digestion process. These measurements can be made on-line. In a study on 18 full-scale centralised biogas plants, most were found to be operating in a stable way, with VFA concentration below 1.5 g L^{-1} (Angelidaki *et al.*, 2005). Reasons for increased VFA level were found to be high ammonium + ammonia level (>4 g L^{-1}), high loading rate, temperature instability, substrate mixture variation, insufficient mixing, co-substrate which affects pH balance and substrates with too high N or S content. Those authors also recorded a residual methane potential of between 6 and 33% of the methane produced in the biogas plant. If the after-storage was performed at low temperatures (below 15-20 °C) the activity nearly ceased. The VFA turnover was more temperature-sensitive than the hydrolysis activity, which led to increased VFA level in downstream low temperature post-digestion systems despite some biogas recovery (Angelidaki *et al.*, 2005).

The above description of the biogas process in principle, different types of reactors and elementary concepts and entities in various technologies used for biogas and digestate production is important for understanding and analysing the biogas technology used in Papers I-IV.

Box 1

Methane yield from different organic materials

The literature contains a range of information on the biogas yield from different materials. However, it is important to note that data on biogas yield from individual materials almost always come from experiments at laboratory or pilot scale. Experiments at laboratory scale are often performed under optimal conditions and when turning to a larger scale biogas yield per amount of fed organic material declines. Different experiments can also be performed with different reactor configurations and varying process parameters, such as temperature, stirring, organic loading rate and retention time.

Large-scale production of biogas is often based on a mixture of materials which makes it impossible to obtain data on biogas yield for each individual material. Co-digested materials create a more optimal environment for the microorganisms, which means that the biogas yield increases significantly compared with when the different materials are digested individually.

When the potential for Swedish biogas production was investigated, Linné *et al.* (2008) tried to get as realistic values as possible for methane yield, taking the explained complications above into account. Expressed as m^3 CH_4(ton DM)$^{-1}$ at standard temperature and pressure (STP), Linné *et al.* (2008) assumed the following yield as practically realistic: Slurry from cattle: 150; Solid manure from cattle: 150; Deep litter from cattle: 135; Slurry from pigs: 200; Slurry from sows: 200; Solid manure from pigs: 150; Deep litter from pigs: 135; Manure from poultry: 150; Sheep and horse manure: 120; Foliage from sugar beet and potatoes: 280; Discarded potatoes: 330; Clover-grass: 330; Pea (straw and pods): 190; Straw: 160. To convert this to m^3 CH_4 per ton VS the factor 70-80% VS in DM is typically valid for manure and 85-95% for plants.

One m^3 methane at STP has an energy content of ~ 10 000 kWh and is equivalent to ~1.1 L petrol (c.f. Clementson, 2007).

Box 2

Concepts and abbreviations normally used in biogas literature

Organic loading rate (OLR), solid retention time (SRT) and hydraulic retention time (HRT) are important expressions in the biogas context. OLR describes the feeding rate to the digester and is normally expressed as kg of volatile solids (VS) per m^3 active reactor volume and day. Volatile solids corresponds to ash free dry matter; concentration of VS is obtained by determining the amount of ash in the substrate normally by heating the substrate in 550 ℃ for 5 hours. In wastewater treatment the expression volumetric organic loading rate (VOLR) is frequently used instead of OLR and is often expressed as chemical oxygen demand (COD) m^{-3}. SRT is the time that the organic substrate stays in the reactor and it must not be too short as the metanogens have a relatively low biosynthesis rate. If the SRT is too short the methanogens will be washed out. Therefore technology has been developed where the hydraulic phase in the reactor has a shorter retention time than the solid phase, *i.e.* the SRT/HRT ratio is high. For economic reasons it would be advantageous as high OLR and short SRT as possible, provided that the methane production is not reduced. However, a well functioning biogas process puts limitations on OLR and SRT which have to be taken into account when constructing a biogas reactor.

1.4 Fertiliser effect of organic wastes, particularly biogas residues

Much research has been done on the fertiliser effect of biogas residues (also denominated digestate or biogas effluent in scientific articles) based on farm manure, but less as regards plant-based digestate. In order to provide a general understanding of this area, the following sections review studies on biodigested and non-digested animal manure, biodigested household waste, and plant-based digestates.

1.4.1 Waste treatment and storage of manure – impact on some key characteristics

Different deliberate or non-deliberate treatments of waste may affect the short-term or long-term N fertiliser value. Digestate has both similarities and differences with other organic fertilisers. In studies on the fertiliser value of digestate, the comparisons deal typically with: (i) mineral fertiliser with or without nutrients other than N; (ii) non-biodigested but at least partly anaerobically stored manure; or (iii) composted municipal waste.

Kirchmann & Witter (1992) quantified the differences between fresh, anaerobically and aerobically stored pig and cattle faeces (Table 2) and showed that pH increases during both anaerobic and aerobic storage, probably in connection with ammonification during decomposition. The main form of N in fresh and aerobically stored faeces from pigs and cattle is organically bound N, whereas a high ammonium N concentration is significant after anaerobic storage. As a result of the mineralisation of organically bound N to ammonium N, the C/N_{org} ratio in anaerobically stored material is higher than that in the fresh material. In contrast, in aerobically stored faeces the C/N_{org} ratio is reduced due to C losses exceeding N losses.

Table 2. *Characteristics of fresh and anaerobically or aerobically stored animal manure. Means for cattle and swine faeces. Modified from Kirchmann & Witter (1992)*

	pH	C/N_{org}	NH_4-N/N_t	NO_3-N/N_t	Organic matter
Type of manure		ratio	ratio	ratio	losses, %
Fresh	7.0	17.2	0.05	0.0000	0
Anaerobic	7.9	20.6	0.51	0.0000	23
Aerobic	7.7	11.6	0.02	0.0006	40

The anaerobic storage in the study by Kirchmann & Witter (1992) was not in a biogas digester and therefore the relationships between different bacteria groups involved in anaerobic digestion were not balanced for optimising the methane production. Thus steps 3 and 4 (Figure 3) were probably only partially completed.

From other works it is possible to compare biogas-digested manure with fresh (raw) manure or anaerobically stored but undigested manure. The differences between biogas-digested manure and fresh manure and between biogas-digested manure and anaerobically digested manure normally go in the same direction: digestion increases NH_4-N/N_{total} ratio and pH and decreases the percentage of DM (Tables 3 and 4) (Möller *et al.*, 2008a; Loria *et al.*, 2007; Schröder *et al.*, 2007; Kirchmann & Lundvall, 1993; Dahlberg *et al.*, 1988). However, some exceptions occur. [For instance DM concentration increased in one retention time of two in a study presented by San *et al.* (2003) and in one experiment presented by Loria & Sawyer (2005). Moreover, pH did not change in one experiment with cattle slurry mixed with starch at a retention time of 29 days, but changed as expected with 56 days of retention (Clemens *et al.*, 2006) (Table 3)]. In general, the difference in NH_4/N_{total} ratio is larger between biodigested and fresh manure than between biodigested and anaerobically stored manure (Kirchmann & Lundvall, 1993; Dahlberg *et al.*, 1988). However, changes in NH_4/N_{total} ratio were small between fresh and biodigested manure in the studies by Loria & Sawyer (2005) and Loria *et al.* (2007).

Volatile fatty acids (VFA) are known to increase N immobilisation (Kirchmann & Lundvall, 1993) or to increase denitrification (Paul & Beauchamp, 1989) when mixed into soil. As the acids are degraded to methane in the biogas process (Figure 3), VFA should be lower in digestate compared with anaerobically stored but undigested manure. This was shown by Kirchmann & Lundvall (1993) but the VFA level in their digested manure was 22 g L^{-1} which is 15 times higher than the level in a healthy biodigester (<1.5 g VFA L^{-1} according to Angelidaki *et al.*, 2005). San *et al.* (2003) also reported higher VFA levels in digestate than would be acceptable in an active biodigester (Table 3), but N immobilisation and fertiliser effects were not studied in detail in that work. Chantigny *et al.* (2004) compared digested and undigested pig slurry with 0.7 and 9 g VFA L^{-1} in field experiments. However, in contrast to Kirchmann & Lundvall (1993) and Paul & Beauchamp (1989), the observations did not indicate larger N immobilisation or denitrification in the undigested slurry, although

VFA level was more than 10 times larger than in the digestate. In the other studies cited in Tables 3 and 4, the VFA level was not presented.

Storage of digestate normally takes place at lower temperatures than used in biodigesters (normally 35 °C for manure). As already mentioned, turnover of VFA is more temperature-sensitive than hydrolysis activity (Angelidaki *et al.*, 2005), which explains the accumulation of VFA in stored digestate.

In summary, comparison of digestate from biogas digestion of animal manure with fresh animal manure or manure stored anaerobically or aerobically showed that the pH in digestate is higher, especially compared with anaerobically stored manure. In digestate and anaerobically stored manure the NH_4-N/N_{total} ratio is higher than in fresh manure. Biogas digestion normally decreases the percentage of DM and the C/N_{org} compared with anaerobically stored manure. The increase of pH in digestate is probably attributable to increased ammonia and organic acid production in the first steps in the biogas process (Figure 3) being degraded to methane and CO_2 in the methanogenesis step which only occurs to a small extent during unstirred anaerobic storage.

The characteristics of digestate from biogas digestion of animal manure described can be partly applied to digestate from plant-based biogas production, which is less studied.

Table 3. *Characteristics of digestate from biogas digestion with swine manure-based feedstocks and of the corresponding raw manure used as feedstock or (figures in italics) swine manure stored more or less anaerobically but without controlled biogas production (N_t= total nitrogen, DM = dry matter, N_{org} = organically bound N, VFA = volatile fatty acids)*

Type	NH_4-N/N_t Ratio	pH	DM %	OM. % of DM	C/N_{org}	VFA g L^{-1}	Reference
Digested	0.76	7.60	7.14	-	21.1	22.1	Kirchmann & Lundvall, 1993
Fresh (= input in digester)	0.58	7.40	10.1	-	18.9	24.0	Kirchmann & Lundvall, 1993
Anaerobically fermented	*0.73*	*7.00*	*9.80*	-	*21.4*	*37.3*	*Kirchmann & Lundvall, 1993*
Digested, 10 days retention, the same daily input of biomass	0.40	6.80	1.60	66.20	-	18.3	San *et al.*, 2003, exp 1
Undigested fresh manure mixed with water	0.04-0.08	6.60	1.86	88.17	-	-	San *et al.*, 2003, exp 1
Digested, 20 days retention, the same daily input of biomass	0.45	6.90	3.10	70.90	-	20.3	San *et al.*, 2003, exp 1
Undigested fresh manure mixed with water	0.04-0.08	6.60	3.58	87.15	-	-	San *et al.*, 2003, exp 1
Digested, 30 days retention, the same daily input of biomass	0.53	7.10	4.60	75.20	-	20.9	San *et al.*, 2003, exp 1
Undigested fresh manure mixed with water	0.04-0.08	6.70	7.28	90.38	-	-	San *et al.*, 2003, exp 1
Digested, 10 days retention, same DM concentration in input	0.50	6.90	1.95	74.00	-	14.0	San *et al.*, 2003, exp 2
Digested, 20 days retention, same DM concentration in input	0.53	7.08	2.90	73.00	-	12.3	San *et al.*, 2003, exp 2
Digested, 30 days retention, same DM concentration in input	0.60	7.05	2.20	72.00	-	11.0	San *et al.*, 2003, exp 2
Undigested fresh manure mixed with water	0.20	6.80	2.80	81.10	-	4.2	San *et al.*, 2003, exp 2
Digested	0.69	8.1	3.2		8.4	0.7	Chantigny *et al.*, 2004
Anaerobically stored	*0.69*	*7.7*	*5-9*		*13.1*	*9.0*	*Chantigny et al., 2004*
Digested	0.56	8.10	12.0	-	-	-	Loria & Sawyer, 2005
Undigested raw manure⋆	0.50	7.80	11.0	-	-	-	Loria & Sawyer, 2005
Digested	0.76	8.17	14.4	-	-	-	Loria *et al.*, 2007
Undigested raw manure⋆	0.69	7.67	18.9	-	-	-	Loria *et al.*, 2007

⋆ from a reception-transfer pit before entering the digester

Table 4. *Characteristics of digestate from biogas digestion with cattle manure-based feedstocks and of the corresponding raw manure used as feedstock or (figures in italics) cattle manure stored more or less anaerobically but without controlled biogas production (N_t= total nitrogen, DM = dry matter, N_{org} = organic N) (data on VFA not presented in these studies)*

	NH_4-N/N_t	pH	DM	OM	C/N_{org}	Reference
	Ratio		%	% of DM		
Digested	0.59	-	-	-	14.8	Schröder *et al*., 2007
Undigested stored slurry	*0.52*	-	-	-	*15.8*	*Schröder et al., 2007*
Digested	0.63	-	5.4	-	-	Dahlberg *et al*., 1988
Fresh manure taken from the alley scrapers*	0.41	-	14.3	-	-	Dahlberg *et al*., 1988
Diary manure stored (earthen storage basin	*0.48*	-	*12.5*	-	-	*Dahlberg et al., 1988*
Digested, HRT 29 days	0.68	7.6	2.3	63	-	Clements *et al*., 2006
Digested, HRT 56 days	0.66	7.8	2.3	62	-	Clements *et al*., 2006
Undigested slurry (probably fresh**)	0.55	7.4	3.3	71	-	Clements *et al*., 2006
Digested with starch added, HRT 29 days	0.65	7.6	2.8	63	-	Clements *et al*., 2006
Digested with starch added, HRT 56 days	0.67	7.8	2.3	63	-	Clements *et al*., 2006
Undigested slurry + starch	0.53	7.6	4.4	79	-	Clements *et al*., 2006
Digested	0.53	7.8	9.2	64	16.0	Möller *et al*., 2008
Undigested slurry stored in closed boxes	*0.43*	*7.0*	*11.3*	*70*	*21.4*	*Möller et al., 2008*

*) Material containing large amounts of bedding avoided

**) At the quality for feeding the digester

1.4.2 Impact of waste treatment and storage of animal manure on N fertiliser value

The first-year fertiliser effect of digested liquid manure is often higher than that of undigested but anaerobically stored manure if NH_3-N losses are avoided (Schröder *et al.*, 2007; Kirchmann & Lundvall, 1993; Dahlberg *et al.*, 1988). Schröder *et al.* (2007) reported a fertiliser replacement value of 58% of total N for digested and 52% for undigested cattle manure after one growing season following injection of manure into permanent grassland. The comparison was with surface-applied calcium ammonium nitrate (CAN). The first-year fertiliser replacement value corresponded well to the NH_4-N/N_{total} ratio (Table 4). Within four years of yearly application, however, the corresponding figures were 69 and 66% and the difference between digested and undigested manure was no longer statistically significant. This can be interpreted as larger absolute mineralisation of organically bound N from the undigested than from the digested manure and can be explained by the supply of organic N being larger for the undigested than the digested manure as NH_4-N/N_{total} ratio was larger in the digestate. An additional explanation is that C in the digested manure was more stabilised than that in the undigested manure.

In an incubation study (10 g soil + 40 g sand) Kirchmann & Lundvall (1993) recovered all supplied NH_4-N plus net mineralisation corresponding to 37% of organic N within 70 days from supply of biodigested pig manure. The recovery of NH_4-N in undigested slurry, within the same time span, corresponded to the amount of NH_4-N added at the start of the experiment, thus no net mineralisation at all of organic N. One explanation for the lower N recovery from the undigested manure was the higher VFA content (Table 3), causing higher N immobilisation within the first 1-3 days of the experiment. Dahlberg *et al.* (1988) measured yield of grain and total biomass of wheat in a pot experiment (1.5 kg soil pot^{-1}) and found no significant yield increase from fertilisation with biodigested cattle slurry compared with stored undigested cattle slurry. Both fertilisers were supplied at the same level of 'available N' estimated as NH_4-N + 0.3 × organic N. Consequently, as NH_4-N/N_{total} ratio was higher in the digestate (Table 4), the yield response per kg added total N from the digestate was larger than from the undigested slurry.

In contrast to Schröder *et al.* (2007) and Dahlberg *et al.* (1988), Loria & Sawyer (2005) and Loria *et al.* (2007) could not show any higher N fertiliser value of digested than of undigested manure. In both studies the comparison was between digestate and raw manure, collected in the reception pit before entering the digester, thus not anaerobically stored, which gives a more correct system comparison. Loria & Sawyer (2005) compared the same application level of total N in digested or raw swine manure with inorganic fertiliser N in an incubation experiment using 1 kg soil. The recovery of N as NO_3 within 112 days was almost 92% for the mineral fertiliser at the highest fertiliser level and about 83% of total N for the manure, with no difference between digested and raw manure. Knowing that the NH_4-N/N_{total} ratio was 0.56 and 0.50 in the manures (Table 3), the net mineralisation of organic N can be computed to 61% in the digestate and somewhat higher (66%) in the raw manure. In a field experiment Loria *et al.* (2007) compared the same application level in fresh weight of digested and raw swine manure as fertiliser to maize and observed no significant differences in fertiliser value between digested and raw manure. However, with the same application level of fresh weight both the amount of NH_4-N supplied and total N differed between the treatments. This makes it difficult to compare the N fertiliser value. Furthermore, the design was split-split-plot, with raw and digested manure in main plots. With this statistical design it is very difficult to find statistically significant differences between the parameters in the main plots, in this case between the manure types.

In experiments where ammonia losses are not avoided, the fertiliser value is frequently not better in digested than anaerobically stored manure. On average, in a system study comparing biodigested and undigested liquid manure, used on non-leguminous crops in a crop rotation, the yield increase for non-leguminous crops was only 2% (non-significant) (Möller *et al.*, 2008a). The result is based on a mean for 5 crop positions in the rotation, where 3 of the 5 crops were winter cereals. The manure was surface-applied in spring in growing stands of autumn-sown winter wheat, rye and spelt. For spring wheat and maize, part of the supply was also surface-applied after crop emergence. Only in spring wheat, where 80% of the manure was supplied before ploughing, was a significantly higher yield (+11%) observed for digested than for anaerobically stored manure. The lack of differences in yield of the winter cereals was explained by larger NH_3-losses in treatments fertilised with digestate, where pH was 7.8 compared with undigested manure with pH 7.0 (Table 4). The differences in ammonia losses from supply of digested and undigested manure are illustrated for winter wheat in another paper on the same experiment (Möller & Stinner, 2009). In another study, supply of biodigested swine manure to bare soil resulted in higher NH_3 volatilisation during the first 6 hours than supply of undigested stored swine manure (Chantigny *et al.*, 2004). However, from day 1 volatilisation was higher from undigested manure. The suggested explanation was a slower infiltration rate of the undigested slurry, as shown by *e.g.* Sommer *et al.* (2006).

Most programmes used as tools for determining the fertiliser value of manure consider the two factors ammonia losses of manure ammonium N, and mineralisation of manure organic N in the year of application (Thompson *et al.*, 1997). When NH_3 losses are taken into account, the remaining NH_4-N is typically assumed to be equivalent to commercial N fertiliser. In a recently developed model for ammonia losses, temperature and slurry pH values were the main drivers of ammonia volatilisation, although many other parameters such as DM concentration and infiltration rate of NH_4-N were also considered in the model (Gericke *et al.*, 2012).

The parameters NH_4-N, NH_4-N/N_{total} ratio, % DM, pH and VFA are all important for N fertiliser value. From Tables 3 and 4 it is obvious that there are large differences in characteristics of the digestates used in different studies. In digested swine manure the ranges for NH_4/N_{total} ratio, pH and % DM in the digestate were 0.40-0.76, 6.8-8.2 and 1.2-14.4, respectively. In digested cattle manure the corresponding ranges were 0.53-0.68, 7.6-7.8 and 2.3-9.2, respectively. San *et al.* (2003) showed that NH_4-N/N_{total} ratio, pH, % DM, % of organic matter in DM and VFA changed with retention time in the digester (Table 3). Biogas production also changed with retention time, with biogas yield being higher at 20 and 30 days retention time than at 10. The large variations in the characteristics of different digestates show that statements about N fertiliser value must not be too general, but must be accompanied by information about the quality of digestate. Other factors that affect fertiliser value include whether: (i) the fertiliser is surface spread or tilled into the soil; (ii) the fertiliser value is expressed as first-year effect or long-term effects with repeated application; and (iii) the fertiliser value is expressed in terms of NH_4-N or total N or a mixture of both, assuming *e.g.* 30% of N_{org} to be available in the first year (Dahlberg *et al.,* 1988).

The data presented above refer to animal manure. However, the general conclusions that can be drawn about any residues from properly managed biogas processes are that high NH_4-N/total-N ratio and low amount of water-soluble C and VFA will give a high N fertilisation effect, but the risk of NH_3 losses must be taken seriously as pH is normally high.

1.4.3 N fertiliser value of household waste

In many studies referred to when discussing the fertiliser value of biogas residues, one or several non-biogas digested organic materials are compared with biodigested materials of another origin and/or with mineral fertilisers.

Household waste digestate (Table 5) is often compared with composted household waste to which other organic materials are added in order to reach a proper recipe for the compost process: *e.g.* Båth & Rämert (2000) compared biodigested household waste with compost of the same material, but with addition of autumn leaves to reach a C/N-ratio of 30. Larsen *et al.* (2007) compared digestate of digested bark chips mixed with mesophilic decomposed sewage sludge and kitchen organic waste (1/1/2) with composted municipal solid waste and garden waste (3/2) in a pot experiment with a barley crop; and Luxhøi *et al.* (2007) used similar products in an incubation experiment studying C and N mineralisation.

Likewise, Svensson *et al.* (2004) compared source-separated household waste from Stockholm co-digested with food residues from *e.g.* restaurants with composted household waste from Västerås, mixed with chopped park and garden waste before composting. A feature in common for most of these studies is that the fertiliser value or short-term N mineralisation is higher from biogas effluent than from the composted organic materials supplied with the same total N rate. Svensson *et al.* (2005) showed that the yield response (cereal grain) and N yield were correlated with mineral N, and not with total N, supply. Båth & Rämert (2000) obtained 37% higher yield of leek and 20% higher N uptake when fertilising with biodigested household waste (Table 5) than with compost, although total N supply was 3.5 times larger with the compost. Those authors reported an initial immobilisation of N from the digestate (incubation study) during the first week and no net N mineralisation from the organically bound N during an entire 24-week incubation period. The immobilisation corresponded to 25-60% of added mineral N, with the lower immobilisation obtained in sandy soil (50% sand, 11% clay) and the higher in clay soil (5% sand, 47% clay). Luxhøi *et al.* (2007) reported N immobilisation in soil amended with digestate (which they denominated anaerobically digested municipal solid waste; Table 5), corresponding to approximately 85% of added mineral N within 3 weeks. However they obtained net N mineralisation corresponding to approximately 2% of added organic N at 16 weeks from the start (incubation study with a sandy loam soil, 50 g soil per vessel). Larsen *et al.* (2007) reported no biomass increase in barley supplied with biogas residues compared with compost (the same or similar substrate as Luxhøi, 2007). They provide no information about biogas production but as the residues had 62% dry matter and the NH_4/total-N ratio was only 0.18 there may have been problems in the digestion process and the immobilisation reported by Luxhøi (2007) may be explained by a high level of VFA and

Table 5. *Characteristics of digestate originating from digesters fed with substrates from plants, or mixes of different origin. (N_t= total nitrogen, DM = dry matter, N_{org} = organic N) (data on VFA not presented in the studies cited)*

Origin	NH_4-N_t ratio	pH	DM %	C, % of DM	C/ N_{org}	Total N, % of DM	Reference
Water hyacinth†, high N in plant material	- φ	-	-	43 φ	12 φ	3.9 φ	Moorhead *et al.*, 1987
Water hyacinth†, low N in plant material	- φ	-	-	42 φ	16 φ	2.7 φ	Moorhead *et al.*, 1987
Plant biomass (maize, oats and kale)	-	8.0	2.5	42	-	2.8	Ross *et al.*, 1989
Clover-grass silage	0.57	-	5.0	37	7.0	5.3	Elfstrand *et al.*, 2007
Clover-grass silage	0.51	8.3	12.5	40	14.7	5.8	Fauda, 2011
Maize and wheat silage and other substrate	0.59	8.2	6.8	43	14.5	4.2	Fauda, 2011
Maize and wheat silage and other substrate	0.56	8.2	5.2	40	9.9	9.2	Fauda, 2011
Cattle slurry + maize	0.61	8.2	7.4	34	15.8	4.9	Fauda, 2011
Cattle and swine slurry + maize + wheat silage + other substrate	0.60	8.1	5.7	38	12.6	7.4	Fauda, 2011
Cattle slurry + maize + other substrate	0.70	8.3	6.1	41	11.4	11.5	Fauda, 2011
Slurry not characterised + poultry manure + maize	0.68	8.4	6.3	36	9.5	11.9	Fauda, 2011
'Dedicated crops'. Liquid phase from mechanically separated digestate	0.63	8.3	4.5	16	4.3	10.1	Grigatti *et al.*, 2011
-"- -"- . Solid -"- -"- -"- -"- -"-	0.04	8.2	81	49	35	1.4	Grigatti *et al.*, 2011
Liquid¥: Clover/grass, oilradish/vetch, pea-& cereal straw	*0.71*	7.7	2.5	36	12.1	10.2	Stinner *et al.*, 2008
Solid¥ : -"- -"- -"- -"- -"- -"-	0.15	-	18.3	43	25.6	2.2	Stinner *et al.*, 2008
Kitchen waste (65% potato, 15% carrot, 13% meat meal, 7% bone meal)	0.69	8.7	3.0	30	8.8	11.0	Båth & Rämert, 2000
-"- -"- -"- -"- -"- -"- -"- -"- -"- -"- -"- -"-	0.63	9.4	5.9	25	7.6	9.0	Båth & Rämert, 2000
Woodbark chips, sewage sludge, kitchen waste (1:1:2 w:w)	0.18	-	63	27	13	1.5	Luxhøi *et al.*, 2007
Source-separated household+restaurant food waste	0.61	-	-	36	5	1.7	Svensson *et al.*, 2004

†) Not total effluent but screened (1 mm) sludge ***φ)*** Results presented as % of freeze-dried screened sludge. NH_4-N not analysed. Presumably most NH_4-N lost during freeze drying, but to be exact the C/N ratio is for C/N_{total} and not for C/N_{org}. ¥) Liquid and Solid phases from a two-stage digester. ~59% of total N in the liquid phase. Values for solid phase are mean for digestate from clover/grass, oilradish/vetch, pea and cereal straw

other short-chain fatty acids.

The above examples from digestates based on different kinds of household wastes show that the fertiliser value or short-term N mineralisation is higher from biogas effluent than from composted organic materials supplying the same total N rate. Although a system comparison of N efficiency between composted and biodigested matter is not available, the results indicate that at least the short-term N efficiency is better from digested than from composted household wastes.

1.4.4 N fertiliser value of plant-based biogas residues

Very few studies have examined the fertiliser effect of biogas residues of digested pure plant material. Three studies focus on N mineralisation (Moorhead, 1988; Moorhead *et al.*, 1987) or fertiliser effect (Fauda (2011) or both (Grigatti *et al.*, 2011), while four other studies focus more on the system level, but with experiments performed as small-plot field trials: a 6-year project in New Zealand (Ross *et al.*, 1989), a 2-year project in Sweden (Båth & Elfstrand, 2008; Elfstrand *et al.*, 2007), a 5-year project in Sweden (Gissén & Svensson, 2008) and a 4-year project in Germany (Michel *et al.*, 2010; Möller, 2009; Möller & Stinner, 2009; Möller *et al.*, 2008a; Möller *et al.*, 2008b; Stinner *et al.*, 2008).

Moorhead *et al.* (1987) compared mineralisation of organically bound N in fresh and biodigested water hyacinth with either low or high N concentration (1.0 and 3.4% N of DM, respectively) (Table 5). After incubation (50 g soil per sample) for 90 days, mineralisation of organic N in fresh water hyacinth accounted for 3 and 33% of applied N in biomass from plants with low and high N concentration, respectively, and 8% of applied organic N in sludge from biodigested plants. Thus, those authors found no difference in mineralisation of organically bound N in digestate depending on whether the digestion was made with substrate from plants with high or low N concentration. No information was presented on NH_4-N/N_{total} ratio in the digestate.

Fauda (2011) studied the fertiliser value of three pure plant-based digestates, digestates based on a mix of manure and plants, and undigested cattle slurry (Table 5) in comparison with an inorganic N fertiliser treatment (NH_4NO_3). Both short- and long-term N availability was studied in a pot experiment with perennial ryegrass lasting for 309 days. Nitrogen supply was equal in terms of mineral N. This means that the total N supply in treatments with digestate and undigested manure was larger than in the mineral fertiliser treatment. Nine weeks after first fertilisation, N offtake by ryegrass shoots with digestate as fertiliser was equal to or greater than that with mineral fertiliser. The mineralisation of organic N during this period was correlated with C/N_{org} in the organic fertiliser (Figure 4). After five successive fertiliser applications, the N use of supplied NH_4-N was higher from most of the organic fertilisers than from the NH_4NO_3-N in the mineral fertiliser. This clearly indicates N release from the organic N in digestate and cattle slurry.

Grigatti *et al.* (2011) studied N mineralisation of slurry derived from plant-based digestate mechanically separated into a liquid and a solid phase. The liquid phase had 4.5% DM concentration with 16% C of DM and a C/N_{org} ratio of 4, and the solid phase had 81% DM with 49% C of DM and a C/N_{org} ratio of 35 (Table 5). The two phases of digestate and a treatment with urea were supplied with the same total N dose in a pot experiment with ryegrass, together with an unfertilised control. Within 112 days the apparent recovery, in shoots and roots, of NH_4-N was 80% from the liquid phase and 91% from urea, thus with a value for the liquid slurry corresponding to 88% of that of the urea fertiliser. As the digestate was incorporated into the soil, NH_3 losses are not plausible. Obviously net immobilisation of N in the liquid phase occurred during the experimental period. In the solid phase of the digestate, N recovery in the ryegrass corresponded to all NH_4-N plus about 3% of organic N. Thus the liquid phase immobilised N, although the C/N_{org} ratio was 4, and the solid phase mineralised N despite a C/N_{org} ratio of 35.

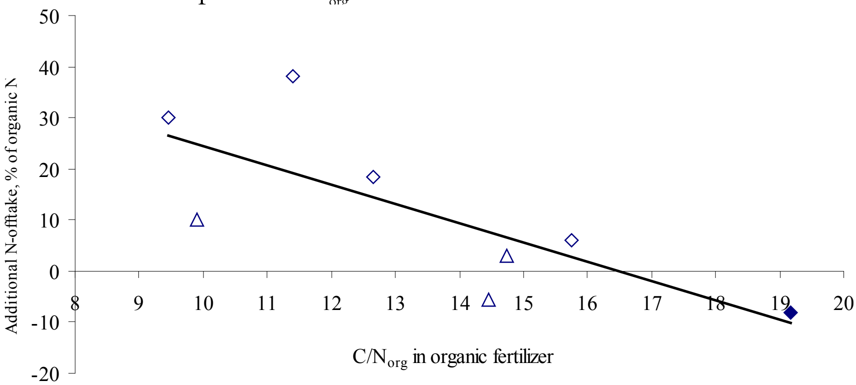


Figure 4. *Results 9 weeks after supply of organic N: Relationship between N mineralisation of organic N in 6 organic fertilisers and C/N_{org} ratio in the fertilisers. N mineralisation was measured as N offtake by ryegrass leaves. Equation was $y = -3.7887x + 62.424$ (R^2 0.57). Results for C_{org}/N_{org} were similar ($y = -3.6739x + 56.046$; R^2 0.61). Filled squares = undigested cattle manure, unfilled squares = digested manure and codigested plant biomass. Triangles = digestate based on pure plant biomass. Modified from Fauda (2011).*

As the fertiliser dose was based on total N, the C supply became very low with the liquid slurry. The immobilisation of N indicates that most of the C in this phase must have been easily available for the microorganisms. Very rapid microbial use of C was confirmed in an incubation study (20 g soil; 70 days) with the same slurry, in which very intense CO_2 emission was recorded within 24 hours of supply. To fit CO_2 emission from the liquid phase to a mathematical model, a two-component model was needed assuming one labile and one stable C pool, respectively representing 33.0 and 30.6% of added C. The model showed extremely different C mineralisation rate constants, corresponding to a half-life of 0.37 days for the labile and 16.9 days for the stable C pool. The immobilisation of N, although the C/N_{org} in total DM was 4, can be explained by the C in the labile pool having a very high C/N_{org} ratio, which is the case if C in this pool is mainly present as VFA, ethanol or sugar (*cf.* Figure 3).

Another interesting result reported by Grigatti *et al.* (2011) was that roots as a percentage of total plant biomass amounted to 38% in the treatment fertilised with the solid phase of slurry, which was higher than for both the unfertilised control (30%) and the urea or liquid slurry treatments (25 and 26%, respectively).

The studies cited above by Moorhead *et al.* (1987) and Fauda (2011) show the same range for mineralisation of organically bound N (0-10% of that supplied to soil). The results from Fauda (2011) indicate that about 10% of organic N in plant-based digestate mineralises if the C/N_{org} ratio is 10, but that mineralisation is small or negative (immobilisation) at C/N_{org} of 14-15. Grigatti *et al.* (2011) showed that the relationship between C/N_{org} and N mineralisation/immobilisation is not applicable for each phase of mechanically separated slurry.

1.4.5 System-orientated studies of fertilisation regimes using plant-based biogas residues

In a pioneer project in New Zealand, fertilisation with digestate from biodigested crops was compared with inorganic fertiliser with N, P, K, S and Ca in ratios similar to those in the effluent and with an unfertilised control (Ross *et al.*, 1989). Application rate of digestate was based on the measured total N content. The total application of N, P, K and S during the growing period of the crops (maize, oats and kale) was intended to return the amounts of these elements removed by the previous crop. The inorganic fertiliser was dissolved in water and the same volume of water and digestate was used for each treatment, including a treatment with water only. The fertilisers were spread every 3-4 weeks. Dry matter yield ranged between 10 and 15 Mg ha^{-1}. No differences in crop yields in any of the treatments were observed over the 6-year period of the trial. The reason suggested for lack of significant yield differences was high nutrient reserves in the soil. (The history of the site included 7 years of grazed pasture of ryegrass and clovers.)For the last three harvests of the crops in the experiment, however, the N concentration was higher in the plots fertilised with digestate than in those with inorganic fertiliser. In soil studies in the last experimental year, available N and potentially available organic N were significantly higher in the digestate treatment than in the treatments with water only or inorganic fertiliser. This indicates long-term effects of organic N in the digestate, confirming the results presented by Schröder *et al.* (2007).

Elfstrand *et al.* (2007) and Båth & Elfstrand (2008) (the same experiment) used effluent from biodigested red clover ley as fertiliser in a field experiment with leek. They found a total N recovery in plants (excl. roots) and soil (N_{min}; 0-60 cm soil layer) of 40% of supplied NH_4-N in the best effluent treatments, whereas N recovery of inorganic N fertiliser was about 95% of the supply. The effluent was supplied after planting and was only incorporated superficially with a hand rake (S. Elfstrand, pers. comm. 2008) and thus NH_3 may have been lost from the surface.

The experiment by Elfstrand *et al.* (2007) was part of a field experiment where different alternatives for use of red clover green manure were studied in leek. The focus was on yield and nutrient uptake, land use efficiency and microbiology. The treatments were: (1) digestate from digested red clover; (2) direct incorporation of red clover as green manure; (3) mulch consisting of fresh red clover biomass; (4) compost based on red clover biomass + straw biomass (85/15 on dry weight basis); (5) mineral fertiliser; and (6) an unfertilised control. In all treatments except direct incorporation of red clover, barley was the pre-crop.

In addition to the direct incorporation treatment, three levels of each organic fertiliser were included: (i) the same amount of total N; (ii) the same amount of C; and (iii) the same amount of available N as with directly incorporated red clover. Levels of nutrients in the treatment with inorganic fertiliser were (in kg ha^{-1}) 190 N, 40 P, 142 K and 21 S, where the N dose approximately corresponded to total N in directly incorporated clover (incl. roots).

Final yield of leek (in October) was not significantly different in the treatments with equal total N supply (i and iii) or equal C supply (ii), but N, P and S concentration increased in response to higher amounts of slurry and compost amendments (Elfstrand *et al.*, 2007). The results strongly indicate that N was not the most growth-limiting parameter. However, with supply of 2-4 times as much total N or C with mulch or with supply of compost corresponding to >4 times as much N or 2 times as much C as in directly incorporated red clover, leek yield increased from 44-47 Mg fresh leek ha^{-1} to almost 60 Mg ha^{-1} (Båth & Elfstrand, 2008). This again confirms that growth was less limited by available N than by lack of organic matter, probably due to unfavourable soil physics.

From a system point of view, the conclusion of the work was that the most area-efficient use of clover biomass in terms of producing leek was a moderate supply of digestate (corresponding to 68 kg NH_4-N ha^{-1}) (Båth & Elfstrand, 2008). This was more area-efficient than direct incorporation, mulching or composting of the clover. Elfstrand *et al.* (2007) showed that there were differences in abundance of bacteria and fungi and of enzyme activities and concluded that direct incorporation of a red clover crop was best for enhancing and sustaining a high microbial biomass and high rates of enzyme activities in the soil. However, a correct system level comparison between a GrM fertilisation management system and a BG fertilisation management system was not possible, as a treatment with digestate supply to a crop following after harvested red clover was lacking (all treatments except direct incorporation had barley as pre-crop).

Gissén & Svensson (2008) compared a GrM system and a BG system in a 5-year organic crop rotation with clover-grass, sugar beet, spring wheat, pea and winter wheat. The clover-grass was used as green manure in the GrM system. In the BG system clover-grass was harvested and digested together with beet foliage and the digestate was returned as fertiliser to the non-leguminous crops in the crop rotation. Preliminary results show that yields were 12, 22 and 18% higher for sugar beet (sugar yield), spring wheat and winter wheat, respectively, in the BG system than in the GrM system. Protein concentration in spring wheat was improved from 12.7 to 14.4% and in winter wheat from 9.9 to 11.0%.

In Germany, three nutrient management systems for stockless agriculture were compared: (1) common green manure practice, *i.e.* biomass from clover-grass ley, cover crops and crop residues were left on the ground (further denominated GrM system); (2) biogas digestion of biomass from clover-grass ley, cover crop and straw from cereals and peas (further denominated BG system); and (3) as (2), but with external substrate (clover and maize) added to the digester supply of plant-based digestate (further denominated BG-ES system). External substrates corresponded to 40 kg total N ha^{-1}. A two-step percolation reactor was used for biogas digestion, whereas Ross *et al.* (1989) and Elfstrand *et al.* (2007) used one-step digesters. The crop rotation was clover-grass, potatoes, winter wheat, spring peas, winter wheat, spring wheat. In both the BG and BG-ES systems, the solid effluent was applied in the winter before potatoes and spring wheat. In BG the liquid biogas effluent was supplied to both winter wheat crops and to spring wheat, whereas in BG-ES some liquid effluent was also applied to potatoes. The results from these German experiments are published in seven articles (Michel *et al.*, 2010; Möller & Stinner, 2010; Möller, 2009; Möller & Stinner, 2009; Möller *et al.*, 2008a; Möller *et al.*, 2008b; Stinner *et al.*, 2008), and those concerning BG compared with GrM are summarised in Table 6.

The mean yield of marketable products from non-leguminous crops was 10% higher in the BG system than in the GrM system (Stinner *et al.*, 2008). Yield effect in the different crops was: +1% potatoes, +9% winter wheat 1, -7% peas, +17% winter wheat 2 and +25% in spring wheat, with statistically significant differences between the BG and GrM systems for spring wheat and winter wheat 2. The yield increase was explained by better and more even allocation of N in the BG system and higher input via N_2 fixation, lower N losses as N_2O and probably higher N availability of digested manure in comparison with the same amount of N in undigested biomass. In the BG-ES system (i.e. with added external substrates), yield of non-leguminous crops was not significantly different from yield in the GrM system. Lodging was observed in one of the winter wheat crops and potato yield was 9% lower than in the GrM system (although the difference was statistically non-significant). Total N uptake in ley was 8% higher in the BG system and the legume percentage was 93% compared with 83% in the GrM system (Stinner *et al.*, 2008). Biological N_2 fixation in the ley crop was computed from a model considering DM yield, legume content and above-ground biomass management (harvested or mulched). For clover-grass, including the year of under-sowing, N_2 fixation was 428 kg ha^{-1} in the GrM nutrient management system and 551 kg N ha^{-1} in the BG system – thus an increase of 135 kg ha^{-1} (32%) compared with the GrM system.

As mentioned, cover crops were included in both crop rotations. The total N_2 fixation (model computed) for the three positions of cover crops (oil radish + common vetch) in the six-year crop rotation was 20% larger in the GrM system (214 kg fixer N_2 per 3 ha)than in the BG system (178 kg fixer N_2 per 3 ha) (Möller *et al.*, 2008b). The difference was explained by differences in straw handling: the straw was left in the GrM system and removed in the BG system. The result was a combination of increased competitiveness of the vetch versus oil radish and (according to the algorithms used) increased N_{dfa} (proportion of legume N derived from the atmosphere). In the cover crop following peas (also oil radish + common vetch), N_2 fixation was 5 kg N ha^{-1} greater when crop residues were harvested than when left in the field, *i.e.* N_2 fixation was higher in the BG system.

Table 6. *Summary of important key factors in a German field experiment comparing system effects of a stockless organic crop rotation with (BG system) and without (GrM system) biodigestion of biomass from clover-grass ley, cover crops and straw. 'Change' refers to difference in the BG system compared with the GrM system. (Michel et al., 2010; Möller, 2009; Möller & Stinner, 2009; Möller et al., 2008a; Möller et al., 2008b; Stinner et al., 2008)*

Parameter	Change
Measured in the experiment	
Marketable yield of non-leguminous crops	+10%
Total N uptake in clover-grass ley	+8%
Soil mineral N at end of season (indicator of N leaching risk)	-17%
Surplus in field N balance (NH_3 losses excluded due to lack of data)	-12%
N_2O emission	-38%
Computed using models or reference data as complement to measured data	
Biological N_2 fixation, total in crop rotation	+14%
Biological N_2 fixation in clover-grass ley	+32%
Biological N_2 fixation in cover crops	+20%
Surplus in C balance	-31%
Fossil energy balance	+12.3 GJ ha^{-1}⋆
Greenhouse gases, total	-65%
CO_2	-54%
N_2O	-21%
CH_4	+10%
NH_3	4%
Acidification	+377%
Terrestrial eutrophication	+300%
Potential nitrate leaching	-8%

⋆ +6.3 GJ in BG system and -6.0 GJ in GrM system. Only electrical energy produced in a combined heat and power (CHP) plant was considered. The heat used in the biogas plants was assumed to be produced from biogas in the CHP unit.

The N_2 fixation for the whole crop rotation, including one year of clover-grass ley (undersown in cereal), three years with a cover crop (oil radish + vetch) and one year of peas, was computed to be 120 and 137 kg

N ha^{-1} in GrM and BG systems, respectively, as a yearly mean for the six-year crop rotation, *i.e.* an increase of 14% without using more area for legumes (Möller, 2009).

Mineral N in the 0-90 cm soil layer at the end of the growing season was used as an indicator of the leaching risk. It was 9 kg ha^{-1} less (-17%) in the BG system than in the GrM system as a mean for all positions in the crop rotation (43 and 52 kg N ha^{-1}) (Möller & Stinner, 2009). Nitrogen balance, before taking into account losses by NH_3 volatilisation, leaching and denitrification, showed a surplus of 53 kg N ha^{-1} in the GrM system and 46 kg ha^{-1} in the BG system, *i.e.* a reduction of 13% (Möller, 2009). The leaching risk after autumn incorporation of cover crops was larger in the GrM system, in which biomass was left, than in BG system, in which biomass was removed for biodigestion (Möller *et al.*, 2008b). With winter incorporation, the leaching risk was estimated to be equal (*i.e.* no differences in N_{min} irrespective of whether cover crops were removed or not), but the amount of mineral N at the beginning of the following growing season was larger in the GrM system, where cover crop biomass was left.

The NH_3 losses from the liquid digestate within four days after spreading were about 8% of applied total N (Möller & Stinner, 2009). No data were presented for losses from solid digestate in that study, but Möller (2009) presented total NH_3 losses after digestate spreading to be 87 kg N for all six years of the crop rotation. The NH_3 losses from the clover-grass plant material after in-season cutting were not measured or estimated.

Fertilisation with liquid effluent in the BG system resulted in a strong increase in N_2O emissions (Möller & Stinner, 2009). Although incorporation of GrM clover-grass ley and cover crops with narrow C/N ratios caused N_2O emissions, the N_2O emissions were 38% lower in the BG system than in the GrM system.

Carbon supply to soil was 60% less in the BG system than in the GrM system but C balance in both systems was highly positive: 433 kg humus C ha^{-1} in GrM and 300 kg C in BG (Möller, 2009). The balances showed that the supply was 2.6 and 2.1 times larger than the soil humus degradation by fertility demanding crops. The authors commented that: "growth of crops in organic farming systems is very often N limited, and not limited by the soil C inputs".

A life cycle assessment (LCA) was carried out using data from the field experiments with the organic GrM and BG cropping systems supplemented with some literature data when needed (Michel *et al.*, 2010). (The work also included five cropping systems for animal farms, which are not further mentioned in this review.) The categories considered were energy use, climate change, acidification, eutrophication and groundwater pollution and the unit used was 1 hectare, with one year as the time frame.

The energy balance for fossil energy from the BG system ended on a surplus of 6.3 GJ ha^{-1} (Michel *et al.*, 2010). In the GrM system, where no energy was produced, the balance ended on -6 GJ ha^{-1}, which represented the consumption of fossil energy for the resources needed for plant production.

Greenhouse gases (GHG) were reduced from 1181 to 448 CO_2-equvalents ha^{-1} (-65%) with transition from the GrM system to the BG system (Michel *et al.*, 2010). Of the four emission types in the GHG concept, CO_2 and N_2O contributed to a reduction in total CO_2 equivalents compared with the GrM system (-54% and -21%, respectively), whereas CH_4 and NH_3 contributed to an increase (+10% and +4%, respectively).

Due to changes in NH_3, NO_x and SO_2 emissions, acidification was almost 4 times higher in the BG system than in the GrM system (27.9 and 7.4 SO_2 equivalents ha^{-1} × $year^{-1}$, respectively (Michel *et al.*, 2010). Terrestrial eutrophication potential increased by a factor of 4 (from 1.4 to 5.6 PO_4-equivalents).

Potential nitrate leaching, according to the LCA, was 8% less in the BG system (49 compared with 53 kg N ha^{-1}) (Michel *et al.*, 2010). This was less than the 17% difference between soil mineral N in autumn mentioned earlier (Möller & Stinner, 2009). In the LCA, the potential leaching was assessed by the farm gate balance based on a method developed by the German Soil Science Association. The components in the farm gate balance were N input by biological fixation, seeds and biogas substrates and output by sold plant products and gaseous losses. For the BG system with purchased substrates for the digester, the LCA method indicated a very large increase (56%) in potential leaching compared with the GrM system, whereas the soil mineral N in autumn was 8% less than in the GrM system. Michel *et al.* (2010) expressed criticism of the farm gate method, which is commonly used for LCA studies, and noted that cover cropping only slightly influences the farm gate balance although soil mineral N content is strongly influenced. They concluded that the farm gate balance is a weak indicator for assessing groundwater pollution.

As mentioned above, NH_3 losses from mulched clover-grass leys or cover crops were not measured in the experiments. Due to lack of reliable data (published data range 2.7 to 45% of biomass N according to the authors) Michel *et al.* (2010) chose not to include those losses in the LCA. However, their inclusion would boost values for the GrM system in the inventory categories GHG emissions, acidification and eutrophication. The LCA also included other uncertainties and for a full understanding the full article must be read.

The LCA concluded that digestion of green manure ley, cover crops and crop residues in organic stockless cropping systems has the potential to reduce mainly net emissions of GHG, whereas the effects per unit area on impact categories such as eutrophication and acidification potential are rather small.

The work reviewed above showed advantages for crop production and N efficiency in BG systems compared with GrM systems. The yield increase was somewhat larger in the five-year crop rotation in the Swedish experiments than in the six-year crop rotation in the German experiments (+17% and +10%, respectively, as a mean for non-leguminous crops) (Gissén & Svensson, 2008; Stinner *et al.*, 2008). The studies by Ross *et al.* (1989) and Elfstrand & Båth (2007) are examples of growing sites where N is not the most growth-limiting factor and therefore yield increases are not statistically significant. The conclusion must be that BG systems increase N efficiency and productivity in the crop rotation when N is the most limiting growth factor, but that is not always the case. The German experiments have been analysed from many points of views (Table 6). Before generalising the results, one must consider that the German experiments were based on digestate from two-stage digestion with a solid and a liquid phase, whereas the digestates used by Gissén & Svensson (2008), Ross *et al.* (1989) and Elfstrand & Båth (2007) were from wet one-stage processes. Furthermore, cover crops were harvested and digested in the German experiments but not in the other works.

1.4.6 Final reflections about studies concerning fertiliser effects

Studies of biogas digestate from plant material often lack the information needed for judging the possibilities of generalising the results and transferring them to other situations. For example, one or more of the parameters VFA, pH, NH_4-N/total-N ratio, C/N_{org} are often missing (Tables 3-5). Often there is too little information about the biogas process. If gas production is sub-optimal, the NH_4-N/total-N ratio will be reduced. For example, the loading rate may affect gas production per kg VS and thereby also NH_4-N/total-N ratio. Thus Zauner & Kuntzel (1986) reported 0.32 m^3 methane kg^{-1} VS from lucerne and residues with NH_4-N/total-N ratio 0.82 and with one loading rate and retention time, but 0.28 m^3 methane kg^{-1} VS from lucerne and residues with NH_4-N/total-N ratio 0.69 when the loading rate was increased and retention time reduced. San *et al.*

(2003) showed increasing NH_4-N/total-N ratio with increasing retention times (Table 3).

Information about the scale of the digester may be of interest. When producing digestate in a small-scale reactor, for example, it may be easier to keep the biogas process optimal *e.g.* by using very small pieces of plant material and having very efficient stirring, thereby achieving very high gas production and NH_4-N/total-N ratio. This may be the explanation for the higher NH_4-N/total-N ratio in digestate from plant material with clover or lucerne as presented by Zauner & Kuntzel (1986) using a biogas reactor with 4-16 L volume (NH_4-N/total-N ratio 0.69-0.82) than reported by Båth & Elfstrand (2008) and Elfstrand *et al.* (2007; the same experiment). The latter used a biogas reactor with 30 m^3 volume (~11 m^3 active volume), giving a NH_4-N/total-N ratio of 0.57. In this thesis, a reactor with 80 m^3 volume giving a NH_4-N/total-N ratio of 0.38-0.46 was used (Papers I-IV). In a commercial full-scale biogas reactor the process may be better optimised than in 'half-scale' research reactors, but less optimised than in a small-scale laboratory reactor.

1.5 Beetroot

1.5.1 Classification and botany

Within the taxonomic system of binomial nomenclature established by Carl Linnaeus, all cultivated beets are currently considered to be within the subspecies *Beta vulgaris* subsp. *vulgaris*. In terms of cultivar groups, beet crops are divided into Garden beet, Fodder beet, Sugar beet and Leaf beet. Within the horticultural scheme of classification, the concept of cultivar is often used. A certain cultivar is an assemblage of cultivated plants that is clearly distinguished by any characteristics (*e.g.* morphological or chemical) which, when reproduced by sexual or asexual means, retains these distinguishing characteristics. Variety is often used synonymously with cultivar, but the terms have different meanings. In binomial nomenclature, botanic variety refers to a fixed rank below the subspecies. Cultivar is a category without rank as long as it comes below the taxonomic rank to which it is assigned (Nottingham, 2004; chapter 1 & 3).

In Papers II-IV in this thesis the nomenclature *Beta vulgaris* var. *conditiva* Alef. is used. *Beta* is the genus, *vulgaris* the species epithet and *conditiva* the variety epithet according to Friedrich Georg Christoph Alefeld (1820–1872; Wikipedia). It would be more correct to write *Beta vulgaris* L. var. *conditiva* Alef. where the L indicates that *Beta vulgaris* is the Linnaean binomial nomenclature. However, nowadays *Beta vulgaris* L. subsp. v*ulgaris* (Garden beet group) is the preferred form.

Beetroot varieties are divided into four groups depending on root shape: circular, conical, cylindrical and flat (Balvoll, 1999; p 113).

1.5.2 History, use and health aspects

Beta vulgaris was initially valued for its leaves and for the fleshy elongated leaf midribs that characterise chard. Leaf beets have been popular food plants in Europe, North Africa and the Middle East since the start of recorded history. *Beta vulgaris* is described in Greek texts from 400-300 BC. The Greeks ate the leaves but the roots were used medically. The Romans were the first people to become interested in the root of *Beta vulgaris,* both as a medicine and as a food, and were the first to cultivate beetroot. By 300 AD the first recipes for preparing the root had appeared. At that time, the roots of beet were black or white, long, thin and roughly turnip-shaped. By the end of the 15th Century cultivated forms of *Beta vulgaris* could be found throughout Europe. In contrast to the Romans, who also primarily took the root medically, from the 16th Century onwards people consumed beetroot mainly as a vegetable. In the 16th Century a beet type with red roots began to be described. For example, a shorter thicker form of beetroot described by an Italian source in 1586 is generally recognised as the forerunner of the modern beetroot. When this beet type spread to France, Germany and England it was called Roman beet. During the 16th and 17th Century Roman beet and possibly other early long-root types became variable in leaf and root morphology due to hybridisation with leaf beets, chards and other beetroot cultivars. By the 19th Century, a wide range of cylindrical, flat and globular varieties had been introduced to growers, particularly in Northern Europe (Nottingham, 2004; chapter 2 & 6).

As mentioned above, beetroot has long been considered a medicinal plant. In the Roman era it was used to treat fever, constipation and other ailments. More recently, it has been considered interesting because of its high content of the antioxidant betalain, which is the characteristic pigment in beetroot. Betalain has been identified as being a dietary antioxidant. Many other health aspects of beetroot are described by Nottingham (2004; chapter 6).

Beetroot has one of the highest nitrate concentrations recorded in vegetables. Contents above 2500 mg NO_3^- kg^{-1} are common and maximum levels in commercial beetroot range from 3000 to 4500 mg NO_3 kg^{-1} in countries with limits on nitrate level in beetroot (Santamaria, 2006). Although recent reports show that the blood pressure lowering effect of beetroot juice can be explained by a high NO_3^- content (Webb *et al.*, 2008), experts generally agree that high levels of nitrate in vegetables should be avoided (Santamaria, 2006).

1.5.3 Physiology and fertilisation

Beetroot is classified as a long-day crop concerning flowering but is day-neutral concerning underground storage organs (Swiader & Ware, 2002; p 52). Flowering takes place in the second year. During the first year, beetroot can accumulate very large amounts of dry matter, as leaf blade development and storage root growth can both continue almost indefinitely, providing continuously available sinks (Tei *et al.*, 1996). The minimum temperature for seed germination is 2.1 °C, with the applicable temperature range for germination from 3-17 °C, and a heat sum of 119 day-degrees is needed for emergence (Bierhuizen and Wagenvort, 1974, cited by Taylor, 1997; Bierhuizen & Wagenfoort, 1974). The base temperature for growth is 5.6 °C (Brewster & Sutherland, 1993) and Tei *et al.* (1996) stated that DD (temperature sum above 5.6°C) and DD combined with light interception (named efficient day-degrees, EDD) could well explain the total yield of a beetroot crop, but not the partitioning.

Smit & Groenwold (2005) found beetroot to be medium fast in terms of root growth, with the first roots to reach 30 cm and 60 cm depth occurring 36 days and 53 after sowing (DAS), respectively. Maximum root density was reached at those depths at 59 and 66 DAS, at a thermal time of 990 and 1140 day-degrees. This was similar to potatoes.

Beetroot has a pH optimum of 6-7.5 and shows a high response to the micronutrients Fe, Mn, Cu, B and Mo and a medium response to Zn (Swiader & Ware, 2002). The P recommendation by Balvoll (1999) is 30-40 kg ha^{-1}, which is 10 kg P ha^{-1} lower than recommended for carrots. Swiader & Ware (2002) recommend the same level of P application to beetroot and carrots (25-75 kg P ha^{-1} depending on plant-available P in soil). The K recommendation is 120-210 kg ha^{-1} according to Balvoll (1999) and 45-185 kg ha^{-1} according to Swiader & Ware (2002), with both sources giving equal K recommendations for beetroot and carrots.

The N recommendations are at the level 80-140 kg N ha^{-1} (Balvoll, 1999), or 85-110 kg N ha^{-1} (Swiader & Ware, 2002), for which Swiader & Ware consider a yield level of 25 Mg ha^{-1}. Takácsné Hájos *et al.* (1997) reported that optimum N fertilisation to irrigated beetroot in Hungary was as low as 70 kg N ha^{-1} with a yield of 27 Mg ha^{-1}. Greenwood *et al.* (1980b) in the UK reported a higher response for N fertilisation, with an optimum of 245 kg N ha^{-1} for a yield of 62 Mg ha^{-1}. Ugrinović (1999) found an increase in marketable beetroot yield from 0 to 225 kg N ha^{-1} with increasing 75-kg N fertilisation steps. At high N supply, root quality was reduced because of higher nitrate content and lower contents of minerals, ascorbic acid and red betanine pigments (Ugrinovi, 1999). Nitrate concentration at the 225 kg N level was 1310 mg kg^{-1} fresh weight, which is still far below the 3000 mg kg^{-1} maximum level applied for commercial beetroot in Germany (Santamaria, 2006). Nitrate content increases following late sowing (Feller & Fink, 2004). With an early sowing date (April), nitrate N content was <563 mg kg^{-1} fresh weight even with a supply of 250 kg N ha^{-1} and yield level at 250 kg N ha^{-1} was 90-100 Mg ha^{-1}. With a late sowing date (July), the nitrate level was 3027 mg kg^{-1} fresh weight and yield was ~40 Mg ha^{-1} at an N supply of 250 kg ha^{-1}. A medium sowing date (June) was intermediate as regards yield and nitrate level.

Feller & Fink (2002) present calculated N_{min} target values for about 30 field vegetable crops. Beetroot had a calculated N_{min} target value of 227 kg N ha^{-1} (soil sample depth 60 cm) based on the total N uptake in the crop of 268 kg N ha^{-1}, apparent N mineralisation from sowing to harvest (140 days) of 61 kg and an estimated amount of residual N in soil of at least 20 kg ha^{-1}. Yield data are not presented in that study, but Feller & Fink (2004) reported beetroot yield levels of 79-85 Mg ha^{-1}, for which the optimal N target value was estimated to be 175-250 kg N ha^{-1}.

The critical N concentration (N_{crit}) in beetroot and in other crops declines with increasing biomass (Zhang *et al.*, 2009; Greenwood *et al.*, 1990). The critical N level in beetroot is reported to $1.53(1+3e^{-0.26W})$, where W is the dry weight of the entire plant excluding fibrous roots and N_{crit} is critical % N in crop dry weight. This equation is used in the EU-Rotate_N model for simulation of N in soil and crop, including a total of 60 cash crops (Rahn *et al.*, 2010a; Rahn *et al.*, 2010b). [In EU-Rotate_N the equation for critical N level in beetroot is a slightly modified version of that published by Greenwood *et al.* (1998) of $N_{crit} = 1.35(1+2.53e^{-0.26W})$].

Optimal NPK concentrations at final harvest in beetroot are reported to be 2.40% N, 0.45% P and 2.46% K in total plant excluding fibrous roots for a yield level between 12.3 and 13.5 Mg ha^{-1} (Greenwood *et al.*, 1980a; Greenwood *et al.*, 1980b). These results were obtained in two field experiments, in 1970 and 1972.

Similarly to N_{crit}, both P_{crit} (Ziadi *et al.*, 2007; maize; Greenwood *et al.*, 1980a; several crops) and K_{crit} (Greenwood & Stone, 1998; Greenwood *et al.*, 1980a; several crops) decline with increasing biomass. The critical P concentration is less affected by increasing biomass than N_{crit}. This can be due to the fact that plants consist of growth-related tissue and storage-related tissue, with the latter increasing relative to the former during growth, but with the P/N ratio being lower in the former. As a mean for several crops tested in 38 field experiments, the ratio between P and N concentration was 0.085 in growth-related tissue and 0.17 in storage-related tissue, while within the tissue type the P/N ratio remained approximately constant throughout growth (Greenwood *et al.*, 2008). The ratio reported for growth-related tissue was similar to that found in leaves of many wild plant species, and even micro-organisms and terrestrial and freshwater autotrophs.

Both critical and maximum K concentration were shown to be proportional to critical N concentration throughout the growth of 16 test vegetable crops (Greenwood & Stone, 1998). For beetroot the relationship obtained between maximum K concentration expressed in milliequivalents (100 g)$^{-1}$ dry matter ($K_{maxmeq,}$) was:

$$K_{maxmeq} = 96.4 \text{ x } 0.619\ (1+2.53\ e^{-0.26W})$$

where 0.619 and 2.53 are crop-specific constants experimentally determined for beetroot (0.619 is the ratio K_{maxmeq}/N_{crit} for K_{maxmeq} determined from field experiments with different K supply and N_{crit} according to previously established relationships between biomass and critical N. Furthermore, critical K ($K_{critmeq,}$) was established to be 59.52% of $K_{maxmeq,}$ which gives the equation:

$$K_{critmeq,} = 0.5952(96.4 \text{ x } 0.619\ (1+2.53\ e^{-0.26W}))$$

Using the molar weight of K (39.1 g mol^{-1}), the relationship can be presented as critical percentage of K in dry matter instead of as meq. (100 g)$^{-1}$ dry matter:

$$K_{crit\%}=0.5952(96.4\times(39.1/1000)\times0.619\,(1+2.53e^{-0.26W}))$$

The ratio between $K_{crit\%}$ and $N_{crit\%}$ and $K_{max\%}$ and $N_{crit\%}$ for beetroot amounts to 1.02 and 1.78, respectively, according to the models for N and K presented by Greenwood & Stone (1998). These models are illustrated in Figure 5.

Greenwood & Stone (1998) also showed that the total cation concentration expressed as meq. (100 g)$^{-1}$ dry matter was linearly related to critical N concentration.

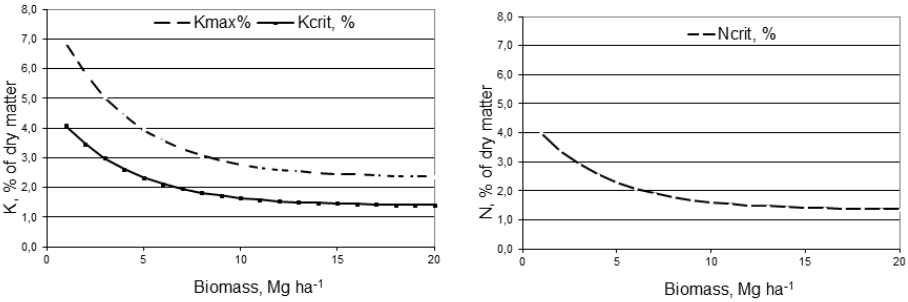


Figure 5. Illustration of the equations for maximum and critical K concentration in % of dry matter ($K_{max\%}$ and $K_{crit\%}$) (to the left) and for $N_{crit\%}$ (to the right) according to the equations for beetroot presented in Greenwood & Stone, 1998. (As the equation for $N_{crit\%}$ is slightly modified in Zhang *et al.* (2009) compared with Greenwood & Stone (1998), the illustration for $N_{crit\%}$ in the diagram is slightly different from Figure 1 in Paper IV.)

1.6 Tools for interpreting multinutritional status of crops

Nutrient analysis can be a tool for interpreting the nutritional status of crops. One method is to compare the levels of individual nutrients with a reference value (CVA = critical value approach). This method has several limitations. In order to consider the interaction between the various nutrients a method called DRIS (Diagnosis and Recommendation Integrated Systems) has been used, but in recent years another technique called CND (Compositional Nutrient Diagnosis) has been developed (Lucena, 1997). In many studies, the CND approach has been shown to be better than the CVA and/or DRIS methods in providing a proper convergence of nutrient imbalance and harvest (Kumar *et al.*, 2004; rhizome of tumeric; Kumar *et al.*, 2003; leaves of tumeric; Khiari *et al.*, 2001a; potato; Raghupathi & Bhargava, 1998; pomegranate; Parent *et al.*, 1994a; potato). CND is also considered easier to interpret than DRIS on the basis of plant physiological knowledge and is easier to calculate when many nutrients are to be taken into account (Parent *et al.*, 1994b; carrots).

The CND method takes into account the concentration of all the nutrients analysed including a filling value (R), so that the sum of all nutrient concentrations + R = 1 (or 100 if concentrations are expressed as a percentage). For each substance, the ratio to the geometric mean concentration of all substances (including R) is calculated and this ratio is logarithmised. The value obtained is called the row-centred log-ratio. The row-centred log ratios for all nutrients in each sample of a population have been shown to be particularly suitable for statistical analysis with multivariate methods. A detailed theoretical background on the reasons for using row-centred log-ratios and filling value R is given by Parent & Dafir (1992), based on statistical theories for compositional data analysis (Aitchison, 2003).

Norm values for CND are calculated from a reference population originating from nutritional experiments or from samples from a number of fields where the harvest is known. Based on the CND norm, a 'nutrient imbalance index' can be calculated for each nutrient in the studied sample: the higher the value (positive or negative), the larger the imbalance of the element (Khiari *et al.*, 2001b). If the CND imbalance index has a high positive value for a certain nutrient, that nutrient may have been present in excess during crop growth, while if the imbalance index has a high negative value the nutrient may have been sub-optimally supplied. Note that in Paper IV also a multivariate statistic method called Partial Least Squares is used for calculating equations that give the best fit for nutrient concentrations (expressed as row-centred log ratios) as explanatory variables and yield or growth rate as dependent variables. In such PLS equations a high positive PLS coefficient means that the higher the concentration of a certain nutrient, the higher the yield. Thus, the nutrient was deficient in many of the samples in the population used for the PLS analyses. Consequently, a PLS coefficient with a high negative value indicates that the nutrient has been present in excess in many of the samples in the population used for the PLS analyses, creating confusion with the CND results.

The statistical methods described are for handling data from plant nutrient experiments where more than one element has to be taken into account. This is necessary in system research about nutrient management systems, especially in organic farming where there is a limited possibility of adding easily available nutrients that are not the main focus (see Paper IV).

2 Objectives and hypotheses

The overall aim of this work was to generate information that could inspire agricultural enterprises to improvement N use efficiency in organic stockless farming systems.

Specific objectives were to investigate and quantify the impact on crop production and N use efficiency of converting from a green manure nutrient management system (GrM system) to a biogas nutrient management system (BG system).

When ley biomass is fed into a biogas digester, N mineralisation takes place. The starting hypotheses in Paper I were:

- I-1 The fertiliser effect of mineral N from plant-based digestate is equal to that of mineral fertiliser if N-losses are avoided.
- I-2 The fertiliser effect of organically bound N (*i.e.* total N minus NH_4-N) in plant-based digestate is negligible.

On converting from a GrM system to a BG system, the ley biomass is harvested instead of being left for its green manure effect. The starting hypotheses in Paper II were:

- II-1 The total N amount in clover biomass increases if the biomass is harvested compared with being left in the field as green manure.
- II-2 A clover-grass ley has a smaller residual N effect on a following beetroot crop if the biomass is harvested three times compared with all biomass being left in the field as green manure.
- II-3 A clover-grass ley harvested twice has a better residual effect than one harvested three times.
- II-4 If the third and final harvest of the ley is omitted, the residual N effect on the following crop is as large as if all cuts of green manure were left in the field.

The total effect of a transition from a GrM system to a BG system includes many changes and choices. With results from these field experiments together with other research we modelled a system where the acreage of ley in the crop rotation was unchanged (one year of three) and where the amount of biogas effluent produced in the crop rotation was returned. Thus the starting hypotheses in Paper III were that harvesting the ley and beet foliage for biodigestion and returning the digestate as fertiliser will:

- III-1 Improve the N supply to beetroot and cereals in the crop sequence and
- III-2 increase the marketable beetroot yield,
- III-3 without increasing the content of unused plant-available N in soil and
- III-4 without jeopardising product quality in terms of excessive nitrate (NO_3-) concentration.

On converting from a GrM system to a BG system optimisations are needed to reap the maximum benefits in terms of crop production and N use efficiency. The starting hypotheses in Paper IV were thus that:

- IV-1 Using correct N targets increases the benefits of a biogas nutrient management system
- IV-2 In transition to a biogas management system, nutrients other than N may become growth-limiting.

3 Methodological aspects

An overview of the methods used is provided in this section (for further details see Papers I-IV). The section adds some details that were not mentioned in the papers and explains and discusses some of the choices made.

One pot experiment (Paper I), a two-year field trial repeated twice (Papers II, III and IV) and a series of aeroponic experiments (Paper IV) were carried out. The pot experiment was used for determining short-term (40 days) and long-term (6 months) fertiliser values of effluent N in terms of inorganic N equivalents. The results from the two-year field experiments were used for modelling three-year crop sequences with and without BG systems. The results of the aeroponic experiments were used for establishing the optimal nutrient proportions for beetroot and as the norm in compositional nutrient diagnosis (CND) when analysing field experiment data for identification of the most limiting nutrients for growth. PLS analysis was also used for this purpose.

3.1 Biogas effluent production

The biogas effluent used in the field and pot experiments was produced in an 80 m^3 experimental biogas reactor managed for research purposes by the Department of Biotechnology at the Lund University research station in Billeberga, Sweden. The biodigester was fed with 1/3 beet leaves (DM) and 2/3 clover-grass ley biomass. Most of the plant material was ensiled, but a minor proportion of the beet leaves were fresh. A small amount of cereal straw was mixed with the beet leaves in order to avoid losses of plant sap during the ensiling process. The silage was mixed with water in a feed mixer wagon (SEKO, Italy) before being pumped into the reactor. The length of the plant pieces in the silage was approximately 4-5 cm for the ley when pumped into the reactor and smaller for the beet leaves.

In autumn 2002, when the biodigester was started, it was inoculated with sludge from another anaerobic biodigester using beet foliage. Volatile fatty acids increase the risk of denitrification and N immobilisation in the effluent when added to soil (Kirchmann & Lundvall, 1993; Paul & Beauchamp, 1989). In order to avoid disturbance in the methanogenesis process and therefore accumulation of VFA, the organic loading rate, as a mean for the feeding period, was only around 0.5 kg VS day^{-1} m^{-3} active biodigester. This is very low compared with loading rates suggested as optimal in many other studies (Bohn *et al.*, 2007; Nordberg *et al.*, 2007; Zauner & Küntzel, 1986). For example, 0.9-1.8 kg VS day^{-1} m^{-3} is typically used at sewage treatment plants or 2.7-3.6 kg VS day^{-1} m^{-3} in co-digestion plants where manure and food waste are treated (Christensson *et al.*, 2009). However, a feeding rate of 0.5 kg VS day^{-1} m^{-3} with pure ley biomass was also used as a mean for a two-month period when evaluating a full-scale reactor in Sweden (Edström *et al.*, 2005).

Feeding was performed once or twice a week and tap water was added gradually for dilution. In total, the biodigester received approximately 70 m^3 of water and biomass in each year. Feeding always ceased for a minimum of four weeks before use of the effluent as fertiliser in the experiments. The pH and concentrations of NH_4-N, VFA and lactate were measured weekly during the feeding period (October to March) in 2002/2003. The pH averaged 7.4, with no trend over time (Figure 6). The reduction in pH on 18 February coincided with reactor feeding on 17 February after a period of two months without feeding. Levels of VFA, mainly acetate and propionate, increased from 18 February (Table 7). Acetate both increased and decreased faster than propionate, as is usual after an overload (Boe *et al.* 2010). However, biogas production increased, which showed that the process was not disturbed although VFA levels were somewhat higher than the 1.5 g L^{-1} found to be a safe level by Angelidaki *et al.* (2005). In the final effluent used as fertiliser, the levels were below the detection limit (0.01 g L^{-1}) after the feeding period in both years (L. Björnsson, pers. comm. 2008). Biogas production was stable with an estimated methane yield, measured at atmospheric pressure, of 0.21 m^3 and 0.25 m^3 kg^{-1} VS in 2003 and 2004, respectively. Gas production was measured with an on-line gas volume meter. The methane content of the biogas produced was analysed off-line with a gas chromatograph.

The stirring equipment was not efficient enough, especially in the first year, for the plant material used, and a crust of organic material was formed and floated on top of the reactor content. This probably reduced both the biogas production and the mineralisation of organic N in the plant material used for feeding. The stirring was improved in the second year, but was still not as efficient as one could wish. In order to get a representative digestate as fertiliser, the top of the digester was lifted and the digester contents were stirred with equipment normally used for stirring slurry before spreading it on fields. This was done immediately before each of the three spreading times per year.

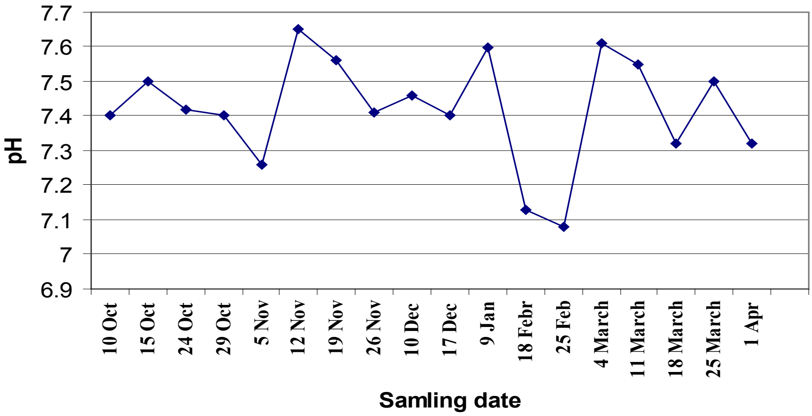


Figure 6. pH during biodigestion in the experimental biogas reactor during the first winter (2003/2004) (Magnusson, 2003).

The difficulty we experienced with insufficient mixing of the long fibrous ley biomass is a recognised problem when ley is used as a biogas crop in a one-step digester (Edström *et al.*, 2005; Nordberg & Edström, 1997; Nordberg *et al.*, 1997). Edström *et al.* (2005) reported that mixing the ensiled forage crop in a mixer wagon seemed to reduce the density of the silage and made it more inclined to float on the surface than when fed directly from a tractor loader into the mixture reservoir.

3.2 Pot experiment (Paper I)

Italian ryegrass (Lolium multiflorum Lam. cv. Fredrik) was grown in 11 dm^3 large pots (surface area 3.57 dm^2, depth 31 cm, soil layer depth 28 cm) for 172 days (almost 6 months). Treatments were fertilised with two levels of biogas effluent (digestate), BE_{75N} and BE_{150N}; the indices 75N and

Table 7. *Volatile fatty acids (VFA) and lactic acid measured during the feeding period in the experimental biogas digester during the first winter (2002/2003), mg L^{-1}. ud = undetectable = <10 mg L^{-1}). Published with the permission of Lovisa Björnsson, Lund University*

Date	Lac-tic	Ace-tic	Pro-pio-nic	Iso-buta-nic	N-buta-nic	Iso-vale-ric	N-vale-ric	Sum
10-Oct	706	423	360	15	25	39	18	1586
15-Oct	*ud*	61	350	*ud*	84	7	*ud*	502
29-Oct	*ud*	52	11	*ud*	*ud*	8	*ud*	71
05-Nov	229	149	32	*ud*	*ud*	*Ud*	128	538
11-Nov	*ud*	234	216	*ud*	*ud*	10	57	517
19-Nov	*ud*	23	*Ud*	*ud*	*ud*	*Ud*	*ud*	23
26-Nov	*ud*	109	*Ud*	*ud*	*ud*	13	*ud*	122
06-Dec	*ud*	278	120	8	*ud*	16	83	505
12-Dec	*ud*	*Ud*	*Ud*	*ud*	*ud*	*Ud*	*ud*	*ud* 0
17-Dec	1068	376	58	*ud*	18	*Ud*	342	1862
09-Jan	*ud*	*Ud*	*Ud*	*ud*	*ud*	*Ud*	*ud*	0
17-Feb	226	186	52	*ud*	36	*Ud*	*ud*	500
18-Feb	*ud*	882	439	53	90	38	*ud*	1502
25-Feb	18	2528	1449	58	265	121	249	4670
04-Mar	*ud*	1030	2572	67	*ud*	141	*ud*	3810
11-Mar	*ud*	727	2268	137	*ud*	16	*ud*	3148
19-Mar	*ud*	23	*Ud*	*ud*	*ud*	*Ud*	*ud*	23
25-Mar	*ud*	2315	637	*ud*	223	79	1714	4968
01-Apr	*ud*	1480	1965	*ud*	*ud*	70	*ud*	3515
In May before use as fertiliser								*Ud*
Mean from October to 1 April								1466
SD from October to 1 April								1699
CV from October to 1 April								116%

150N reflecting the supply of NH_4-N as mg dm^{-2} pot surface area (or to kg ha^{-1}). The digestate came from the same digester as the digestate used in the field trials. For comparison, an inorganic, nitrate-based fertiliser was applied in other treatments (IF_{75N} and IF_{150N}). An unfertilised control was also included. The inorganic fertiliser was mixed from pure mineral salts to get a fertiliser with the same nutrient proportions to N as in the digestate when only considering the NH_4-N in the digestate and not the organic N. Excess Ca was chosen in IF to balance ion composition. The salts used were K_2SO_4, KH_2PO_4, NaCl, $NaNO_3$, $Ca(NO_3)_2$, KNO_3, $Mg(NO_3)_2$, $Fe(NO_3)_3$, $Mn(NO_3)_2$, $Zn(NO_3)_2$, $CuCl_2$, Na_2MoO_4, H_3BO_3 and acetic acid (HNO_3).

The reason for mixing our own inorganic fertiliser was to exclude undesirable salt effects on N mineralisation (cf. Omar & Ismail, 1999; Campino, 1985; Campino, 1982) as the digestate was rich in salts. Another reason was to avoid interaction between N use and the availability of other nutrients.

It would have been interesting to have added a treatment with a pure NH_4-N based fertiliser although the mixture of other nutrients would then have differed as regards elements other than Ca from the content of the digestate (*e.g.* higher S or Cl). Another possibility could have been to use urea together with a commercial PK-fertiliser. It would of course also have been interesting to have treatments with *e.g.* pig slurry and cow slurry in the experiment. However, time and money were limited.

Soil solution samplers were used to measure N content in the soil solution in the experiment (Figures 7 and 8). The technique is interesting but we had to be careful when drawing conclusions as we were unsure whether the soil moisture content was exactly equal in all treatments. The technique would have been better combined with monitoring the soil moisture during the sampling period, *e.g.* using a calibrated TDR (Time Domain Reflectometry) meter.

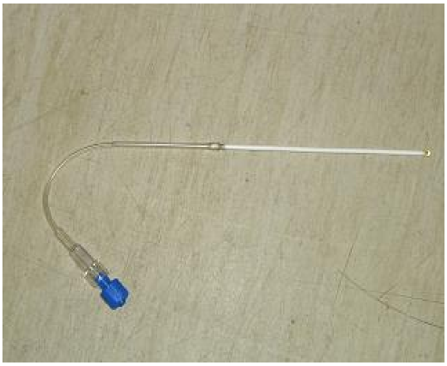


Figure 7. Soil solution sampler (Rhizon SMS). Size: 10 cm. (Photo: Fredrik Bengtsson)

Figure 8. Pot experiment in climate chamber. Two soil solution samplers put into each pot. (Photo: Fredrik Bengtsson)

On the day the experiment was started, laboratory testing revealed that the soil pH(H_2O) was as high as 8.1. As the high pH meant a large risk for NH_3 losses, especially from the digestate-fertilised pots, we placed a thin layer of peat on top of the pots with the aim of preventing these NH_3 losses.

For further details about sampling, analyses *etc.* see Paper I.

3.3 Field experiment (Papers II-IV)

The priority when searching for an experimental field was to find an organic crop rotation with an existing first-year clover-rich clover-grass ley, where irrigation was possible and where machinery for beetroot growing and skilled field experimental staff were available. The soil at the experimental site found was very sandy, which was not a priority but certainly affected the results.

The design included 11 treatments placed in totally randomised plots within the blocks. Handling two-year field experiments with totally randomised plots and with different crops in the first year demands experienced field personnel, which were luckily on hand. The field experiment was situated about 150 km from the biogas research station. The digestate was transported from the digester by a truck and loaded into a band spreader and applied immediately (see picture on the front cover). The digestate was incorporated into the soil within one hour of spreading by harrowing or interrow cultivation.

The treatments applied to beetroot were (Table 8):

- Five treatments with unfertilised barley as pre-crop
    - with four fertiliser regimes with increasing amounts of digestate (1 N-t to 4 N-t) and with one control treatment without digestate but with Kali vinasse and Besal for supplying the plants with K and Na (for further details see Tables 1-3 in Paper IV). As the fields were fertilised with surplus P with farm manure during the years before the experiment, no other nutrients than those in the Kali vinasse (mainly K and S) and Besal (NaCl) were supplied in the control.
- Two treatments each comprising one green manure ley (GrM ley), ley harvested twice (2H-ley) and ley harvested three times (3H-ley) as pre-crop
    - with one fertiliser regime (Low N-t) with digestate and one as control in which the digestate was replaced only by Kali vinasse and Besal

The ley species were white clover (of the broad-leaved cultivar Alice), red clover cv Sara and perennial ryegrass cv Tove (10/20/70% based on seed weight).

In both beetroot cropping years, the treatments with ley pre-crops were fertilised with a level of Low N-t corresponding to the same N target value as one of the beetroot treatments with barley pre-crop. In the first beetroot year (2003), the lowest N target value (1 N-t) was used as the mutual N target value for all pre-crops and in the second beetroot year (2004) the second lowest N target value was chosen as the mutual N target value. The reason for this change was that the yield response for the low N target was unexpectedly low in 2003 and we suspected that there were suboptimal levels of nutrients other than N, especially during the first growth period before digestate was supplied for the second time. We considered three alternatives when planning for the next beetroot year: (i) changing nothing; (ii) keeping the low N target level as the mutual level but adding some Kali Vinasse and Besal before sowing; and (iii) using the second lowest N target level as the mutual level instead of the lowest and thereby getting an increased K and Na supply. None of the alternatives was perfect, but we chose (iii).

In Paper III the concept 'N supply including residual N of ley' is used and in Paper IV 'plant available N' (PAN) is introduced. These two concepts are somewhat different (Table 9) and are explained in the respective papers. For beetroot with barley pre-crop, 'N supply including residual N of ley' is exactly the same as the amount of NH_4-N supply with digestate, but for the other pre-crops the higher residual N from ley than from barley is taken into account and therefore the N amount is higher. For PAN, total N uptake and soil mineral N (N_{min}; sum of NO_3-N and NH_4-N) in the 0-60 cm soil layer at harvest in the unfertilised treatments are taken into account.

A fair judgment of system effect needs to take at least 3 years into account. In Paper III, despite the 2-year field experiments, we therefore simulated the following 3-year crop sequences for consideration of residual effects, with the crops included in the field trials being marked in bold type:

- GrM system, Sequence A: Year 1) **GrM–ley**; 2) **beetroot**; 3) winter rye.
- BG system, Sequence B: Year 1) **harvested ley**; 2) **beetroot**; 3) winter rye.
- BG system, Sequence C: Year 1) harvested ley; 2) **spring barley**; 3) **beetroot**.

The aim was to simulate how both beet and cereals were supplied with N produced in the crop sequences as a result of pre-crop effects.

3.4 Aeroponic experiments

In order to obtain CND norms (*cf.* section 1.6) for beetroot plants, treatments with different nutrient supply were established in systems with culture solutions continuously sprayed on the roots (aeroponics; Ingestad & Lund, 1986) (Figure 9). The resulting CND norm was used for calculating nutrient imbalances in the field experiments in order to investigate the nutrient status of nutrients other than N with regard to pre-crops and fertilisers.

The aeroponics system used was constructed on the theoretical basis that the rate of nutrient supply rather than the concentration is a driving force for growth and that conclusions about nutrient response should be based on studies with plants in stable physiological condition (Ingestad & Ågren, 1995; Ingestad, 1982).

In the vast majority of plant studies where nutrient solution techniques are used, the nutrient levels are allowed to swing wildly from feast to famine (Epstein & Bloom, 2004) and the evaluation of relations between nutrition and growth becomes erroneous (Ingestad, 1982). This was the reason for our choice of the technique suggested by Ingestad & Lund (1986). The method is further described in Paper IV, where the expression 'nutrient solution' is used. However, Ingestad & Lund (1986) preferred to use 'culture solution' to refer to the experimental technique with continuous supply of nutrients from stock solutions into the growth unit, in order to distinguish the method from the more common way of working with nutrient solutions with wildly varying nutrient levels.

The different treatments from which the CND norms were established were designed to investigate different research questions, all with the purpose of establishing optimal conditions for growth of beetroot plants (for a fuller description, see Table 4 in Paper IV). In total, 22 treatments were used for establishing the norms. A few more treatments were tested, but in the procedure of producing CND norms (*cf.* section 1.6) we only used those with a stable relative growth rate (RGR; the concept is explained in Paper IV). This was in order to avoid drawing conclusions from plants that were not in stable physiological conditions. Ingestad & Ågren (1995) used the following definition for stable physiological conditions: $r^2 > 0.99$ for the linear regression of logarithmised biomass from the studied samples on the Y-axis and time (days) on the X-axis; standard error of the mean <10% for the internal concentrations at the different harvests. We used $r^2 < 0.99$ as our limit but still kept treatments 21 and 22 (Table 4 in Paper IV) with $r^2 =$ 0.986 and 0.981, as they represented plants with suboptimal N supply but with other nutrients in relevant proportions to each other. Nutrient internal concentration, expressed as ratio to N concentration, in some cases followed a clear sigmoid curve ending at a stable level corresponding to the nutrient ratio to N in the stock solution (*e.g.* as for K in treatment 12 illustrated in Figure 10). In other cases the stability was less obvious (examples are shown in Figure 10). However, we used a mean for the nutrient concentration in the last two harvests in the aeroponic experiments

Table 8. Treatments in the field experiments. Effluent = digestate from plant-based biogas production

Treat-ment	Pre-crop (during the first experimental year)	Fertilisation regime during beetroot year	Abbreviation: Pre-crop /fertili-sation regime
I	Ley, used as green manure	Kali vinasse and Besal†	GrM-ley/0 EF†
II	Ley, harvested 2 times	–“– –“– –“– –“–	2H-ley/0 EF†
III	Ley, harvested 3 times	–“– –“– –“– –“–	3H-ley/0 EF†
IV	Spring barley with undersown ryegrass	–“– –“– –“– –“–	Barley/0 EF†
V	Ley, used as green manure	Effluent to low N-target††	GrM-ley/Low N-t††
VI	Ley, harvested 2 times	–“– –“– –“– –“–	2H-ley/Low N-t ††
VII	Ley, harvested 3 times	–“– –“– –“– –“–	3H-ley/Low N-t ††
VIII	Spring barley with undersown ryegrass	Effluent to 1 N-target†††	Barley/1 N-t †††
IX	–“– –“– –“– –“– –“–	Effluent to 2 N-target†††	Barley/2 N-t †††
X	–“– –“– –“– –“– –“–	Effluent to 3 N-target†††	Barley/3 N-t †††
XI	–“– –“– –“– –“– –“–	Effluent to 4 N-target†††	Barley/4 N-t †††

† 0 EF = without addition of effluent, Na supply with Besal: 40 kg ha^{-1}. K supply with Kali vinasse: 100, 150 and 200 kg K ha^{-1} to beetroot following GrM-ley, barley and harvested ley, respectively.

†† The N-target used for beets following clover-grass leys (Low N-t) were the same N-target as in treatment VIII in 2003 and as in treatment IX in 2004.

†††1, 2, 3 and 4 N-t = Fertilisation to beetroot determined according to increasing N targets for soil mineral N (N_{min}) plus effluent NH_4-N. 1 N-t was the treatment with the lowest N target value and 4 N-t the highest. For detailed information about intended N target levels and actual N target values, see Paper IV.

Table 9. Supply of NH_4-N with effluent (kg ha^{-1}), N supply including residual N of ley (kg ha^{-1}) (as in Paper III) and plant-available N at final harvest (kg ha^{-1}) (PAN; as in Paper IV) for the total growing period of beetroot. Effluent = digestate from plant-based biogas production

Treat-ment	Pre-crop[†]	Fertili-Sation regime[††]	2003 NH_4-N supply with effluent	2003 N supply incl.res. N of ley	2003 PAN	2004 NH_4-N supply w. effluent	2004 N supply incl.res. N of ley	2004 PAN	Mean for 2003 and 2004 NH_4-N supply w. effluent	Mean N supply incl.res. N of ley	Mean PAN
I	GrM-ley	0 EF	0	78	159	0	22	90	0	50	125
II	2H-ley	0 EF	0	31	113	0	4	72	0	18	93
III	3H-ley	0 EF	0	30	111	0	13	81	0	22	96
IV	Barley	0 EF	0	0	82	0	0	68	0	0	75
V	GrM-ley	Low N-t	18	129	177	35	69	124	27	99	151
VI	2H-ley	Low N-t	27	98	140	80	79	152	54	89	146
VII	3H-ley	Low N-t	37	28	148	92	77	173	65	53	160
VIII	Barley	1 N-t	37	37	119	49	49	117	43	43	118
IX	Barley	2 N-t	104	104	186	106	106	174	105	105	180
X	Barley	3 N-t	130	130	208[†††]	181	181	249	156	156	229[†††]
XI	Barley	4 N-t	192	192	270[†††]	211	211	278[†††]	202	202	274[†††]

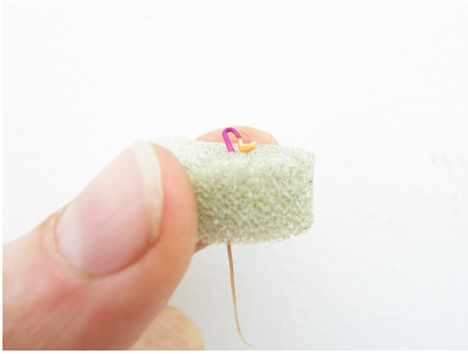
[†] GrM-ley = green manure ley; 2H-ley and 3H-ley = ley (for biogas production) harvested two or three times.

[††] 1, 2, 3 and 4 N-t = Fertilisation to beetroot determined according to increasing N targets for soil mineral N (N_{min}) plus effluent NH4-N where 1 N-t is the treatment with the lowest N target and 4 N-t that with the highest. Low N-t was used for clover-grass leys where the N target was the same as in treatment VIII in 2003 and treatment IX in 2004. For detailed information about intended N target levels and actual N target values see Paper IV. 0 EF = no effluent supply.

[†††] PAN for treatment VIII to XI should be the same as NH_4-N supply with effluent + PAN for treatment IV. Deviations are caused by rounding off and by the fact that we wanted to keep the values for N supply and PAN equal in this table and in Table 3 in Paper III and Tables 7 and 8 in Paper IV.

when running the PLS analyses on aeroponic data and also when producing the CND norm.

In order to establish stable physiological conditions according to the definition by Ingestad & Ågren (1995), it is more or less necessary to work with plants that are in exponential growth. Therefore the experiments were ended at about the 6-7 true leaf stage of the beetroot plants and, as the CND norm is derived on analyses of the last two sampling occasions, the plants had between 4 and 7 true leaves. Therefore the norms were basically expected to be useful for interpreting data from early growth of beetroot in the field experiments.



↑ Beetroot plant ready to be put in the growth unit for aeroponic experiments

↑ Four growth units, 8 days after transplanting

↑ Plants grown with free access to nutrients 24 days after transplanting. (Treatment 19 in Paper IV)

↑ Beetroot with fibrous roots, lifted out from the growth unit.

← Computerised monitoring of pH and electrical conductivity (EC) every 10 minutes and addition of stock nutrient solutions for either pH upregulation or EC regulation into the culture solution according to a specified setpoint value – or, when relative addition rate (RAR) was used: stock nutrient solution added according to specified RAR.

Figure 9. Photos from the aeroponic experiments. (Photo: Anita Gunnarsson).

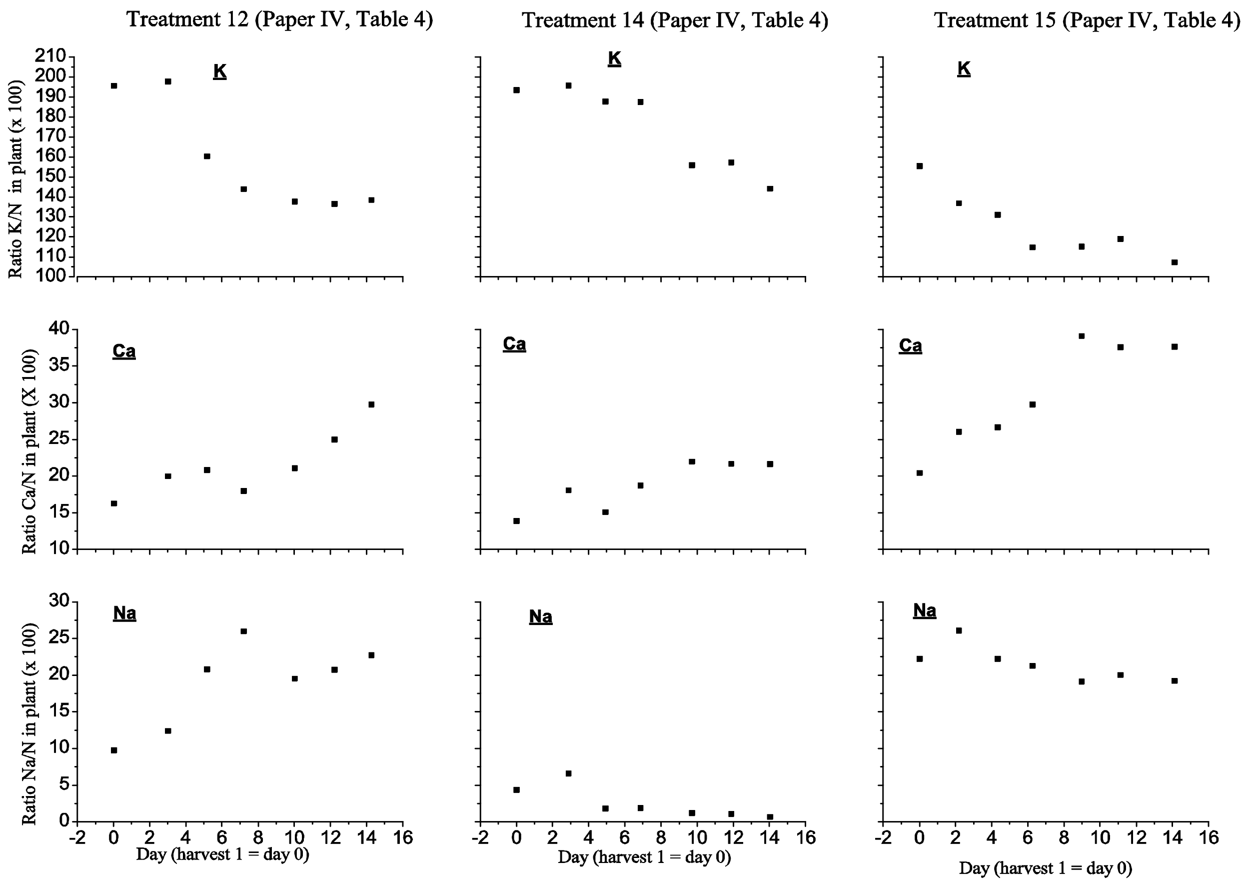


Figure 10. Illustration of different dynamics for ratio of K, Ca and Na concentrations in relation to N concentration (×100) in beetroot plants in three treatments of aeroponic experiments (Paper IV).

4 Results and discussion

The system effect of a transition from a green manure nutrient management system (GrM system) to a biogas nutrient management system (BG system) could be assumed to be equal to the sum of the fertiliser effects of: (i) digestate based on on-farm produced plant material from different crops in the crop rotation; and (ii) changes in residual effect when harvesting the ley and other crop materials instead of using it for green manure purposes. The structure of this chapter is based on that assumption, with sections dealing in turn with N fertilisation effect of digestate; residual effects of ley; possible impacts of nutrients other than N; and system effects on N in the crop rotation.

4.1 Growth promotion and N use efficiency of plant-based digestate

4.1.1 N uptake in the pot experiment

In the pot experiment (Paper I), dry matter production and N uptake in ryegrass foliage in treatments fertilised with biogas digestate (BE) were lower than in those with inorganic fertiliser (IF) at the first cut, *i.e.* 40 DAS. The percentage difference was greater at the 75 N fertilisation level than at the 150 N level (-11% and -4%, respectively). In the following cuts (81, 136 and 172 DAS), the biomass and N uptake were greater in the treatments fertilised with digestate. Therefore the accumulated biomass and N uptake (Figure 11) and N uptake efficiency (Figure 1 in Paper I) did not differ at the end of the experiment (day 172).

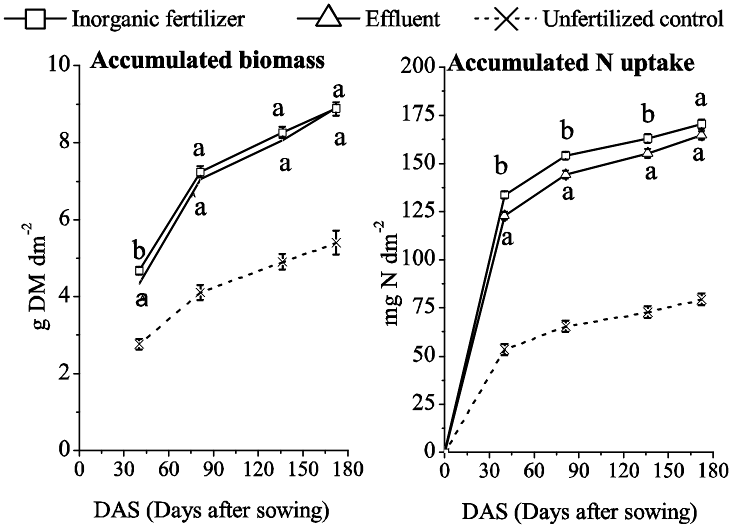


Figure 11. Accumulated biomass (left) and N-uptake (right) in ryegrass foliage. Means for 75 and 150 N for effluent (= digestate) and inorganic fertiliser treatments. Error bars show standard error of the mean. Different letters refer to significant differences between effluent and inorganic fertiliser at 5% level, analysed with two-way Anova (Paper I).

The rather large differences in yield and N uptake at 40 DAS were surprising, as the nitrification rate could have been expected to be fast enough to prevent major differences between BE and IF due to N source (NO_3-N in IF and NH_4-N in BE). Nitrification rate differs greatly depending on *e.g.* soil texture and climate (Lindén *et al.*, 2003; Honeycutt *et al.*, 1991; Lindén, 1982; Malhi & McGill, 1982), soil C concentration (Berg & Rosswall, 1985) and other not always predictable factors (Maag & Vinther, 1996) and can be stimulated by roots (Klemedtsson *et al.*, 1987). Under field conditions when soil moisture is not optimal and ammonium N is supplied to the surface, soil N can be unused for a long period due to low nitrification rate and the lack of root growth in the dry surface soil (Lindén, 1982). However in our case, the water content was kept optimal and the digestate was thoroughly mixed into the soil before filling it into the pots. Under those conditions, according to data presented in Honeycutt *et al.* (1991), 50% of added NH_4-N could be expected to be nitrified within 15-25 days.

Possible reasons for the unexpectedly lower yield in the BE treatments at the first cut were (Paper I): (i) growth rate with BE being lower due to NH_4 as the initial N source, compared with NO_3 as the N source in IF; (ii) the root fraction (biomass and N) being greater in BE than in IF already at the first cut; (iii) immobilisation in the BE treatment being higher or remineralisation lower or slower than expected; (iv) NH_3 losses or denitrification losses being greater with BE than with IF; and (v) nutrients other than N limiting growth more in BE than in IF. Although the clay content was only 11%, some NH_4 may also have been fixed in the clay colloids, giving reduced N availability in BE both for the plants and for the nitrifying microorganisms. The first two reasons can be considered the most probable, but the suspicion remains that VFA in the digestate may have been involved (reason iii), causing high N immobilisation. When the digestate was collected, the digester had been unstirred, unfed and unheated for a long period. According to observations by Angelidaki *et al.* (2005), VFA accumulate at low temperatures as their turnover is more sensitive than the hydrolytic activity. Table 3 (section 1.5.3) shows that high levels of VFA (>1.5 g L^{-1}) are more common in digestate used in experiments than low levels (<1.5 g L^{-1}). If the VFA level in our digestate was 10-20 g L^{-1} the immobilisation would have been about 190-360 mg N pot^{-1} in the BE_{150N} treatment, according to interpolation using a linear relationship presented in Kirchmann & Lundvall (1993). With 3.57 dm^2 pots, that corresponds to an N immobilisation of 53-106 kg N ha^{-1}. Although remineralisation occurred rather rapidly, the N immobilisation may have been more important than differences caused by NH_4-N in BE and NO_3-N in IF. [The mean cumulative net N mineralisation was found to be zero after 8 days at 25 ℃ in the experiment by Kirchmann & Lundvall (1993.) Adapted to the temperature in the pot experiments (16 °C), remineralisation would have reached the zero level on day 18 (calculated using data for temperature impact from Heumann & Böttcher (2004)].

The root fraction was larger in BE than in IF treatments at the end of the experiments. Grigatti *et al.* (2011) presented higher root proportion at 112 DAS in Italian ryegrass, fertilised with the solid phase of digestate, than in the unfertilised control. However, that study and Paper I overlooked the importance of analysing VFA and therefore it is impossible to determine whether the high root proportion was a consequence of N shortage due to initial N immobilisation caused by high level of VFA, or whether some factor in the digestate triggered increased root proportion. For example, when present in low concentrations, root initiation and elongation might be enhanced by the high-molecular-weight fraction of organic matter, especially fulvic acids, and also by some phenols in the low-molecular-weight fraction (Marschner, 1995; review; chapter 14, p 522-523). At higher concentrations, however, a low-molecular-weight fraction instead inhibits root growth. This is particularly true for phenolic and short fatty acids.

4.1.2 N uptake efficiency in the pot experiment (ryegrass) and field experiments (beetroot)

The N uptake efficiency of digestate NH_4-N (NUE_{min}) in ryegrass foliage (excl. stubble) was 0.76 in the pot experiment (Figure 1 in Paper I). In the field experiment, NUE_{min} ranged from 0.36 to 0.70 in beetroot (foliage and storage roots) following barley, as a mean for the two experimental years (Figure 12). This was calculated on original data for each plot and not from treatment means from Table 3 in Paper III. The pot experiment was run for 172 days, whereas the field experiment was harvested at 110 DAS (mean for the two years). From Figure 1 in Paper I, it can be interpolated that NUE_{min} in ryegrass at 110 DAS was slightly above 0.7, *i.e.* it was at approximately the same level as the NUE_{min} in the 'best' treatment of the field experiment. This is rather surprising as: (i) ryegrass in the pot experiment had a more even plant distribution and consequently a more even root distribution than the row-drilled beetroot and therefore should have found it easier to deplete the soil of digestate N; (ii) more NH_3 losses may have occurred in the field although soil tillage was performed within one hour from digestate supply; (iii) some N leaching is highly probable in the field experiment in 2004; and (iv) N uptake in the fibrous roots was not included for beetroot in the field experiment but was for ryegrass in the pot experiment.

In the field experiment in 2003, NUE_{min} for digestate was significantly lower in treatments with 1 N-t fertilisation than in the 2 to 4 N-t regimes with barley as pre-crop (Figure 12d). Normally a lower N supply would give higher NUE, which was the case in 2004 (Figure 12e). The differences in NUE_{min} are also illustrated in Figures 13 and 14 by the biomass yield at 32 DAS and marketable root yield at final harvest in the different treatments. The low NUE_{min} in the 1 N-t treatment indicates that K (or S) supply may have been too low in 1 N-t in 2003 compared with in 0 EF, where K and S were supplied with Kali vinasse. At the higher rates of digestate (2 to 4 N-t), the supply of K and S (and not only N) increased to an acceptable level. As already mentioned in section 3.3, those observations caused us to change the target value for 'low N-t' in beetroot following ley from the 1 N-t level to the 2 N-t level in 2004. Despite that change, in 2004 the NUE_{min} in beetroot following 3H-ley was still very low, (Figure 12a and b). This was despite the fact that digestate NH_4-N supply was more than double that in 2003 and thus the K supply was also much larger.

In contrast to the low NUE_{min} in 3H-ley, the small amount of fertiliser with digestate N supplied to beetroot following GrM and 2H-ley gave an extremely good response in growth in 2003, resulting in NUE_{min} >1 (Figure 12a). The differences in NUE_{min} between pre-crops were confusing. They may have been caused by direct impacts of different plant nutrient balances as influenced by the different pre-crops, but also by beneficial effects such as releasing chelating agents due to the addition of plant material in GrM ley and 2H-ley (Yadvinder-Singh & Khind, 1992).

4.1.3 N utilisation efficiency in the field experiments (beetroot)

The overall N use efficiency is both a question of how efficiently the crop can use N in soil and fertiliser (N uptake efficiency) and how efficiently the plant can produce desirable plant products from the N taken up (N utilisation efficiency) (Weih *et al.*, 2011). Beetroot with ley pre-crops had lower N utilisation efficiency than beetroot following barley (Table 10). Beetroot following barley tended to be more efficient the higher the N target value. Some of the mineralisation from ley residues obviously came too late to increase the root yield. Changing beetroot position to follow barley instead of ley therefore improved N utilisation efficiency.

The possibility to change an organic crop rotation from growing beetroot directly after ley to growing it after cereal is dependent on the availability of an efficient N fertiliser approved for organic farming. This is offered by the use of digestate. Thus, the positive N effect of transition from a GrM system to a BG system is not only due to the direct effect of better synchronisation of N supply from clover-grass, but also to the larger flexibility in choice of position for N-demanding crops in the crop rotation.

4.2 Residual N effects of harvested ley and green manure ley

The residual N effect of clover-grass was 32-52 kg N ha^{-1} less when the ley was harvested twice (2H-ley) instead of being used as a green manure ley (Table 11). When it was harvested three times (3H-ley), the residual effect was 50-80 kg N ha^{-1} less than that of a GrM-ley. The range depends on how residual effect is expressed, as discussed further below.

Different approaches for studying residual N effect are used in the literature (see section 1.2.3). In Paper II, the residual N effect of ley is expressed as: (i) increase in N uptake in the entire beetroot crop; or (ii) increase in N uptake in the entire beetroot crop plus soil mineral N. With

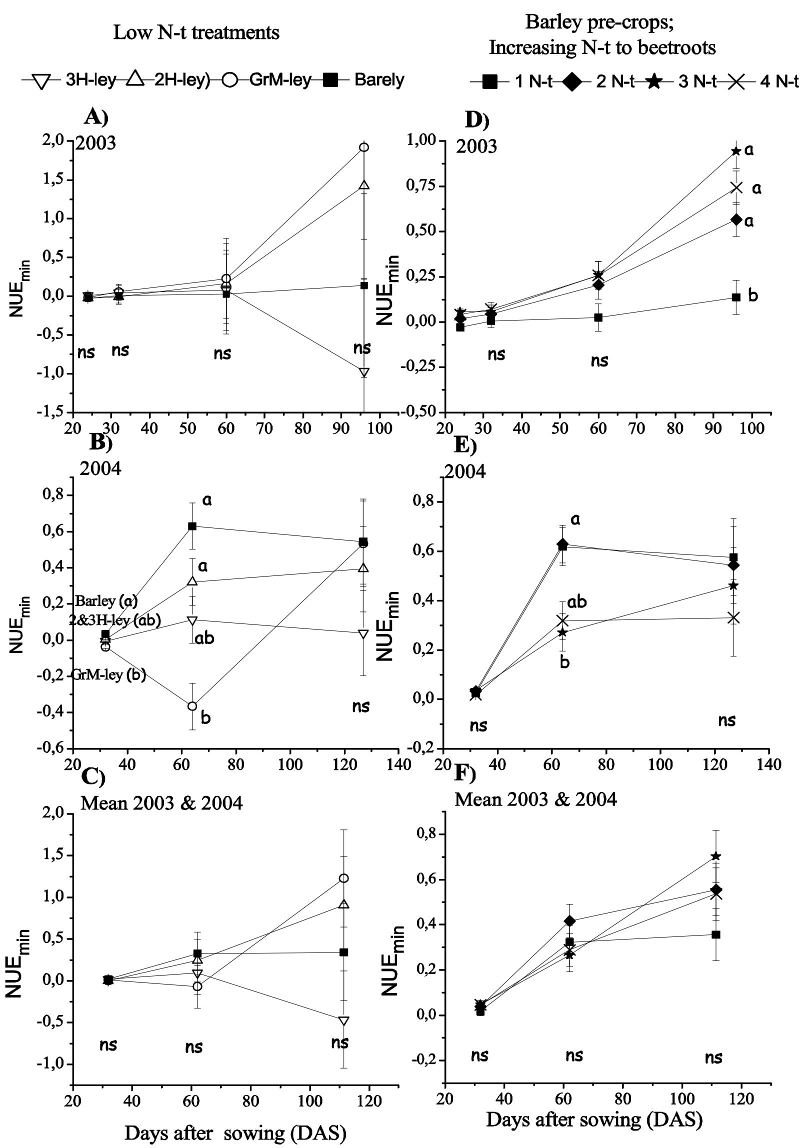
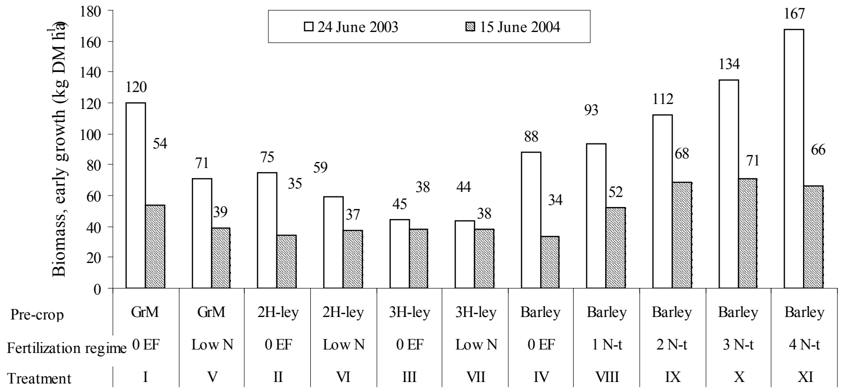


Figure 12. Nitrogen uptake efficiency (NUE_{min}) in beetroot in field experiments [(N-uptake in foliage and storage roots of beetroot fertilised with digestate (=effluent) minus N-uptake in unfertilised beetroot with the same pre-crop)/NH_4-N supply with effluent]. A, B and C = low N-target fertilisation regime following all four pre-crops; D, E and F = all four N-target regimes following barley pre-crop. Error bars show standard errors of means. Different lower case letters show statistically differences at the 5% level according to Tukey's test.



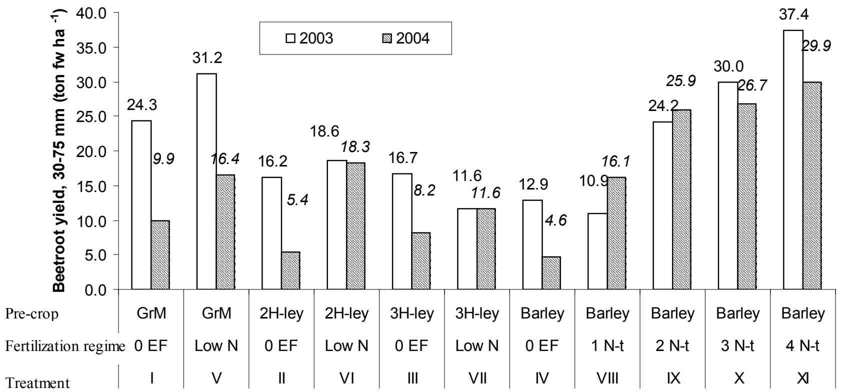


Figure 14. Marketable root yield, fresh weight at final harvest in 2003 and 2004.

both approaches, 'increase' refers to the difference between beetroot grown after ley compared with after barley (Table 11; Figure 2 in Paper II). This is a common way of expressing residual N effect. Furthermore, in Paper II the residual effect is based on a mean for unfertilised beetroot and beetroot fertilised with digestate to a low N target value.

Table 10. Efficiency of beetroot in allocating the N in the plant to the food product (N utilisation efficiency). Mean for 2003 and 2004 in field experiments

Treat-ment	Pre-crop	Fertilisation regime	N supply incl. res. N of ley kg ha^{-1}	N utilisation efficiency N in root/ N in total plant Ratio	N utilisation efficiency Change towards unfertilised
IV	Barley	No N-t	0	0.48	
II	2H-ley	No N-t	18	0.45	
III	3H-ley	No N-t	22	0.45	
VIII	Barley	1 N-t	43	0.52	+
I (control)	GM-ley	No N-t	50	0.44	
VII	3H-ley	Low N-t	53	0.49	+
VI	2H-ley	Low N-t	88	0.53	+
V	GM-ley	Low N-t	99	0.48	+
IX	Barley	2 N-t	105	0.52	+
X	Barley	3 N-t	156	0.53	+
XI	Barley	4 N-t	202	0.55	+
	p-value			*0.40*	
	SEM			*0.04*	

In Paper III a different approach is used. In that study the residual N effect was computed in order to get inorganic N equivalents to describe how much mineral fertiliser is needed by beetroot with a barley pre-crop to reach the same yield as beetroot following ley (further denominated $N_{R\text{-ley}}$) (Table 11). For this reason, the response function for digestate NH_4-N supply to beetroot following barley was used (Figure 1 in Paper III). The results obtained by these different ways of expressing residual effects are both correct, but it is important to understand the differences. The reason for using the second method for modelling system effects (Paper III) was to obtain a mutual unit in inorganic N equivalents. We could then summarise the positive fertiliser effect of digestate and the reduction in the residual effect of ley when harvesting the biomass and compare it with the residual effect of GrM ley.

The results from the two different methods of computing the residual effect are presented in Table 11. The content is partly from Figure 2 in Paper II and Table 5 in Paper III, but some information has been added.

The negative $N_{R\text{-ley}}$ values in Table 11 may be difficult to understand, but are caused by the low yield increase of digestate to beetroot following harvested ley, as mentioned in section 4.1.2. The results indicate that fertilising a beetroot crop with biogas effluent up to a certain (low) N target value, with harvested ley as a pre-crop, is less efficient than using the effluent on beetroot after barley. (For more in-depth information see Figure 15 and the last 20 rows in column 3, p. 771 of Paper III.)

The results on residual N effect, expressed as A-Soil-N (Apparent soil N; eq. 2 in Paper II), including N uptake plus N_{min} in the 0-90 cm soil layer, should be the most useful information when generalising to other soil types, especially for crops with deep root systems. However, $N_{R\text{-}ley}$ should be most useful when communicating with beetroot growers with sandy soils.

The differences in residual effect between GrM-ley, 2H-ley and 3H-ley were larger in treatments V, VI and VII, with digestate-fertilised beetroot, than in treatments I, II and III. The results were unexpected and possible reasons are discussed in Papers II, III and IV and in sections 4.1.1 and 4.1.2.

The differences in residual N effects from harvested ley compared with ley cut during the season depend mainly on the amount of biomass left in the field and in the soil and the C/N in the biomass. A model test indicated that the residual N effect from the summer cutting in GrM ley (*i.e.* cuts in June and July) corresponded to 40-70% of the potential effect (Table 7 in Paper II). This was more than expected.

The clover ratio is of importance, as a high clover ratio increases N in ley biomass and a high N concentration increases N mineralisation rate. We had expected an increased proportion of clover in harvested ley according to observations by Loges *et al.* (2000) and Stinner *et al.* (2008), as explained by the fact that GrM cuttings left on the soil serve as N fertiliser, which normally decreases the clover proportion in mixed leys. The lack of differences in clover proportion in Paper II is explained by the circumstance that K may have limited clover growth in harvested but not in GrM leys (Campillo *et al.*, 2005). The fact that we used a broad-leaved white clover cultivar (Alice) may also be of importance, as large-leaved white clover cultivars are better able to withstand the competitive effect of N application (*cf.* Nassiri & Elgersma, 2002; Elgersma *et al.*, 2000).

Table 11. Overview of residual N effect of ley (kg ha^{-1}) depending on the calculation model used in Papers II and III Barley is used as the zero level. Mean for 2003 and 2004

Treat-ment	Pre-crop	Fertilisation regime	As in Paper II ⋆ N in entire crop ⋆⋆⋆⋆	A-soil-N⋆⋆⋆ N uptake + N_{min} 0-60 cm	N uptake + N_{min} 0-90 cm	As in Paper III⋆⋆ Inorganic N equivalents (N_{R-ley} in Paper III)
I	GrM-ley	No N-t	36	50	69	64
II	2H-ley	No N-t	10	18	38	27
III	3H-ley	No N-t	16	22	25	22
V	GrM-ley	Low N-t	70	73	96	65
VI	2H-ley	Low N-t	32	35	43	-2
VII	3H-ley	Low N-t	-11	-11	-7	-52
I & V	GrM-ley	Mean	53	61	83	65
II & VI	2H-ley	Mean	21	27	40	13
III & VII	3H-ley	Mean	3	5	9	-15

⋆) In Paper II (Figure 2) only a mean for No N-t and Low N-t is presented.

⋆⋆) In Paper III (Table 5), treatments II and III were used for crop sequence C and treatment VI and VII for crop sequence B when calculating N_{R-ley}. N_{R-ley} for GrM-ley in the Low N-t regime was not presented in Paper III.

⋆⋆⋆) A-soil N as defined in Paper II, *i.e.* N-uptake plus N_{min} at harvest. Deduction made for 50% of digestate supplied in low N-t fertilisation regimes when 0-60 cm soil layer was included and 60% when 0-90 cm soil layer was included

⋆⋆⋆⋆) Deduction made for 60% of digestate supplied in low N-t fertilisation regimes.

4.3 Optimum nutrient supply to beetroot

The PLS and CND analyses presented in Paper IV showed that K was more growth-limiting than N at early growth stages, with more severe limitation in digestate-fertilised beetroot following ley than following barley (Table 10 in Paper IV). In addition, B, Cu, Fe and possibly Mn and P may have been involved as growth-limiting elements. Removal of P with the 3H-ley pre-crop was double the removal with barley including straw. For the other nutrients the removal was 3 (Cu), 3 (Zn), 3 (S), 5 (Mg), 6 (Na), 8 (K), 10 (Mn), 12 (Fe), 16 Ca), and 17 (B) times larger with ley than with barley. (Removals are presented in Paper II, except for Fe.)

The results presented in Paper IV also indicated 70 N was an optimal N target before sowing and that N_{min} in the 0-30 cm soil layer measured before sowing was not fully available for the plants until the first sampling date (at 32 DAS). This was probably a greater disadvantage in digestate-fertilised beetroot following ley than following barley.

In Paper IV, the results from the aeroponic experiments were used for producing CND norms for explaining yield variation at 32 DAS and at harvest in the beet crop in the field experiments. For the field experiments the summarised squared nutrient imbalance indices (CND R^2) with aeroponics as norm, but with Mn excluded, explained 26-29% of the plant biomass 32 DAS (Figure 3 in Paper IV). It was very surprising that this index, derived in such an artificial environment, was better at explaining biomass production than the CND R^2 based on the field experiment in the other year (Figure 3 in Paper IV). Of course the CND norm based on 22 nutrient treatments needs to be improved. However, as it was produced under controlled environment conditions concerning light and temperature, it would be possible to add more experiments to the series, preferably starting with more tests for establishing optimum P and Mn proportions.

Degree of explanation (R^2) for correlations between the CND index and final harvest was low (2003; R^2=10%; P=0.04) or non-existent (2004; P=0.9). The lower R^2 for final harvest was expected, as the aeroponics experiments were intentionally finished at the 6-7 true leaves stage, in order to keep the plants in the exponential growth period. To produce CND norms useful for identifying nutrient imbalances at late growth stages, the aeroponics experiments would have to be extended to include a full first-year growth period for the beetroot.

4.4 System N effects

Transition from a green manure nutrient management system to a biogas nutrient management system increased the inorganic N equivalents from 73 to 128 kg N for a crop sequence with 1 ha of ley and 1 ha of beetroot, *i.e.* an increase of 56 kg inorganic N equivalents (Table 5 in Paper III; mean for 2H- and 3H-ley). This is within the range found for other crop rotations by Stinner *et al.* (2008) and Gissén & Svensson (2008), but more than that found by Elfström & Båth (2007) and by Ross *et al.* (1989).

Using the net increase in inorganic N equivalents in 2H-ley (+26 kg) and 3H-ley (+ 85 kg) according to Paper III (Table 5; crop sequence C), the yield increase compared with beetroot following GrM-ley would be +12% and +34% (using the equations for yield response of inorganic N in beetroot following barley, see Figure 1 in Paper III). In our field experiments (Paper II-IV) we only harvested or sampled plants that were not affected by wheels. However, when applying digestate to growing beet, there will be a yield reduction due to the damage by wheels of the tractor and the spreading equipment. If the digestate is spread with a 12 m wide spreader and 2 rows per 12 m are not sown but only used as tramlines for the spreader and the row spacing is 50 cm, the yield reduction would theoretically be 8% (not taking into account compensatory growth by plant rows close to the tramlines, but that could also be a disadvantage if the roots become oversized). The calculation indicates that all digestate from the 2H-ley biogas system (*i.e.* 80 kg NH_4-N) would be better used before sowing than with a split application. In the 3H-ley system the amount of NH_4-N in digestate was 116 kg from 1 ha of ley. That exceeds the amount that can be spread at one time if the NH_4-N content in the digestate is as low as in our case (1.3 kg NH_3-N ha^{-1}). Therefore the potential yield increase must be reduced for the tramline effect when presenting the figures to producers or communicating the results to growers in one way or another.

In Paper III, the increases in beet yields of 33 and 53% for 2H and 3H-ley, respectively, refer to effects if all digestate is used for beetroot following barley and no consideration is taken of the reduced residual effect of harvested ley. Therefore the yield increases of 12 and 34% mentioned above should be used in communications about system effects.

The increase mentioned above of 56 kg inorganic N equivalents was valid for the biogas system with the simulated crop sequence harvested ley, barley, beetroot (denominated C in Paper III), but not for the crop sequence harvested ley, beetroot, barley (denominated B). In crop sequence B there was no positive N effect of the transition. Deeper analysis of the nutrient situation (Paper IV) indicated that the negated benefit in crop sequence B could have been caused by the circumstances that: (i) K limited growth more than N; and (ii) the benefit on early growth of high soil mineral N level in spring after ley crops was overestimated, as N may have leached down too deep for the small beetroot plants. However, if all available digestate N had been used by the beetroot crop in crop sequence B, it is possible that the benefit from the transition would have been equally good as in crop sequence C.

The fertilisation effects observed in Papers II-IV could have been further improved if imported organic waste had been fed into the digester. The reason for not working with added organic waste was that we wanted to refine the study. In practice, the best way for a farmer with stockless organic production to improve both the economic and N nutrient system benefits by a transition to a biogas farm system is to co-digest clover-grass ley with *e.g.* kitchen waste or waste from the food industry. The biogas process is often referred to as being stabilised by co-digestion. By co-digestion of off-farm products the input and output to the farm of nutrients other than N can be kept in balance without draining limited natural resources.

A change to ley with more red clover and lucerne may increase both biomass and N_2 fixation, as N_2 fixation has been shown to be larger in harvested red clover or lucerne-grass mixtures than in harvested white clover-grass. However, N_2 fixation in green manure leys is larger with white clover-grass than red clover-grass and lucerne-grass leys (Loges *et al.*, 2000b).

More efficient biogas digestion, *i.e.* by better stirring equipment and finer chopping of ley material, would have increased the NH_4-N/total-N ratio in the digestate and also the inorganic N fertiliser equivalents. In our experiments (Paper I.IV), we used a wet one-step batch-fed experimental reactor. For commercial use, biogas production with wet continuous digestion technology is today considered a mature technology. However, this technology is probably not optimal for clover-grass. Some recent studies have concentrated on the development and optimisation of better technology for biogas production from grass (Nizami *et al.*, 2012; Nizami & Murphy, 2011; Singh *et al.*, 2011; Nizami & Murphy, 2010; Nizami *et al.*, 2010; Lehtomäki & Björnsson, 2006). Biogas production varies greatly between chosen technologies for biogas production, but it is not only reactor technology that needs optimisation. Time of harvesting, ley species and method of ensiling may also be of importance (Nizami *et al.*, 2009). There is good reason to believe that system effects on N use efficiency in the cropping system vary with technology for biogas production and with the cultivation technique for clover-grass ley production for biogas purposes compared with green manure purposes.

The improved N effect shown in this thesis is more of a case study rather than a final answer to the system effects. Differences in soil, land use and biogas technology will hopefully show that the positive system effect obtained in the simulated crop sequence with harvested ley, barley and beetroot was at the low end of what is practically achievable for a skilled organic farmer.

Measures that could have changed the system N effect in Papers I-IV are:

- More efficient wet biogas process (better stirring and cutting equipment) => increased degradation of C => increased NH_4-N/total N ratio => increased system N effect
- Two-stage process: if the process is more effective in degrading C the system N effect should be increased, provided that N losses from the solid phase of digestate during storage are prevented.
- K fertilisation to the ley in the BG system (especially on sandy soils) => increased growth of legumes (and N_2 fixation) of the ley to be harvested => more biomass to digest => more digestate => increased system N effect
- 2-year ley instead of 1-year ley => larger residual effect from harvested 2-year ley than from 1-year ley (Granstedt & L-Baeckström, 2000) => increased system N effect
- Co-digestion of imported organic waste => possibility to prevent reduced yield in beetroot following harvested ley if the digestate is partly moved to other crops in the crop rotation => better system N effect also in the BG crop sequence C (harvested ley, beetroot, barley)
- Imported K on sandy soils if needed to get full improved utilisation of the increase in available N obtained by transition to a BG system from a GrM system
- Optimised cultivation technology of ley for biogas purposes (species, time of harvesting, *etc.*). The residual N effect of ley may then change, as well as the amount of NH_4-N available with the digestate.

4.5 Conclusions and answers to the hypotheses

The following conclusions and answers to the starting hypotheses for Papers I-IV formulated in chapter 2 were possible:

I-1 The fertiliser effect of ammonium N from plant-based digestate was, for a 6-month period, equal to the fertiliser effect of added nitrate-based inorganic fertiliser if N losses are avoided. The short-term effect (6 weeks) was less than that of a NO_3-N fertiliser.

I-2 The fertiliser effect of organically bound N was 12% of supplied organic N within the 6-month period. This was not statistically significant but other studies confirm that minor mineralisation of organic N may occur in plant-based digestate following incorporation into soil.

II-1 The total N amount in clover biomass did not increase when the biomass was harvested compared with being left in the field as green manure. This is in contrast to some other studies but the different results may depend on nutrient status in the soil (sandy soil here, conflicting results from studies performed on clay soils).

II-2 A clover-grass ley had a smaller residual N effect on a following beetroot crop when the biomass was harvested three times compared with all biomass being left in the field as green manure. The difference in the beetroot year corresponded to 80 kg N ha^{-1} of inorganic N equivalents as a mean for beetroot fertilised and not fertilised with digestate.

II-3 A clover grass ley harvested twice had better residual effect than if harvested three times. The difference in the beetroot year corresponded to 28 kg N ha^{-1} of inorganic N equivalents as a mean for beetroot fertilised and not fertilised with digestate.

II-4 When the third and final cut of a harvested ley was omitted, the residual N effect on the following crop was lower than when all cuts of a green manure were left in the field. The difference in the beetroot year corresponded to 52 kg N ha of inorganic N equivalents as a mean for beetroot fertilised and not fertilised with digestate.

III-1 Harvesting the ley and the beet foliage for biodigestion and returning the digestate as fertiliser increased the N supply to beet and cereals in the crop sequence This corresponded to a net effect of +56 kg inorganic N equivalents for the simulated three-year crop sequence with ley, barley and beetroot, assuming a unit of three hectares. The increase in inorganic equivalents with ley harvested twice was +26 kg and with three cuts it was +85 kg, but the difference was not statistically significant. The positive system N effect was negated when the effluent was used in small amounts to beetroot following harvested ley.

III-2 If the whole net increase in inorganic N equivalents was used to fertilise beetroot following barley, it corresponded to a 12 and

34% increase in marketable beetroot yield for the 2H-ley and 3H-ley systems, respectively, above the level of 17 Mg ha^{-1} in beetroot following GrM ley. The yield increase refers to field parts not affected by tramlines for spreading of digestate in the growing stand. For the 2H-ley system, all digestate may be optimally supplied before sowing the beetroot crop.

III-3 Unused N_{min} (0-90 cm depth) at beetroot harvest indicated that the risk of leaching in BG systems is lower than in GrM-systems. The amount of mineral N was 88 and 61 kg N_{min} ha^{-1} left after unmanured beets following GrM ley, and high-manured beets following barley, respectively. However, as N_{min} in late autumn after barley following harvested ley was not measured, the leaching risk at a crop rotation level could not be determined.

III-4 The nitrate content in harvested roots was not higher in beetroot fertilised with digestate to the level available from the three-year crop sequence than with green manure only.

IV-1 The BG system could have been better optimised by using adjusted N targets for split N application. The results indicated that the N target before sowing should be 70 kg ha^{-1} and that the soil layer should be less deep than 30 cm deep. Following this N target before sowing would presumably have improved the simulated system N effect of the crop sequence with harvested ley, beetroot and barley in both years. In 2003 it would presumably also have improved the system effects for the crop sequence with harvested ley, barley and beetroot.

IV-2 With transition to a biogas management system, nutrients other than N proved growth-limiting. This was most obvious for K but other nutrients may also have limited growth, especially when a small amount of digestate was supplied. Therefore dividing the digestate between different crops in the crop rotation on a sandy soil with low buffering capacity may reduce the beneficial system N effect, unless nutrients other than N are added to the crop following harvested ley.

5 Final remarks, practical implications and future perspectives

5.1 Biogas systems for organic farming

During recent years, farm-based biogas production has been recognised for its advantages as a renewable energy source. As with many other innovations, it can be used in an ecologically sound or less sound manner.

In Germany, farm-based biogas plants often focus on maize as feedstuff. Herrmann & Taube (2006) identified potential conflicts between energy maize production and EU cross-compliance standards, in particular with regard to the Fertiliser Directive and the obligation to maintain all agricultural land in good agricultural and ecological condition. Furthermore, they had reservations about an increase in energy maize production from the perspective of nature conservation and concluded that application of suitable models might facilitate assessment of energy maize production at field, farm and regional levels.

In the Netherlands, Grebrezgabher *et al.* (2010) describe how biogas plants open opportunities for maintaining or increasing a high concentration of animal production. In a case study, a green power biogas plant established by 50 swine farmers, the residues are separated into a solid fraction and a liquid fraction via pressing (Gebrezgabher *et al.*, 2010). The solid fraction contains parts with a high concentration of nutrients (N, P and K content 9.3, 19.2 and 5.9 kg ton^{-1}, respectively, which can pay (at least from a short-term economic view) for long transportation. From the liquid fraction a 'green fertiliser' with a NPK content of 6.8, 0.6 and 11.5 kg ton^{-1} is produced by ultrafiltration followed by reverse osmosis. This 'green fertiliser' has a high N and K concentration compared with farm manure or the wet digestate, and is expected to be subject to less regulations than farm manure. Other researchers have suggested that the solid fraction from biogas production could be used as solid fuel after drying (Kratzeisen *et al.*, 2010).

The above examples illustrate use of farm-based biogas production that is far from the ideal for organic agriculture. There is a need for dialogue about the kinds of farm-based biogas production that are desirable in organic farming systems.

5.2 Future work

It is not plausible that a farmer would build a reactor only in order to improve the nutrient management system on the farm, but it may be one interesting link in the chain. When optimising a crop rotation and nutrient management system for organic stockless farms that have changed from a GrM system to a BG system, consideration must be given to the complex interrelationships between soil type, climate, local market possibilities (for gas, food, organic products to digest, *etc.*), economic crops, cultivation practices, pests, weed situation *etc.* As a researcher it would be easy to enumerate a number of reductionistic research questions that need to be answered. However, farmers, like all managers, need to use their intuition and local knowledge. Traditional research and development methods can only give fragments of answers. As a biogas digester has many similarities with a cow, many good enough solutions may already be available, *e.g.* from mixed farms with milk or beef production and ley in their crop rotation.

Some issues that may need further research are:

- System effects on P and S in GrM systems compared with BG systems.
- Optimal positions for application of the solid and liquid phases of digestate from two-stage biogas digestion in a self-supporting organic crop rotation.
- Further studies of differences between pre-crops in N uptake efficiency when digestate is supplied based on the same target value.
- Impacts of the fertiliser effect of digestate depending on digestion technology.

Whatever research is done concerning the fertilisation effects of plant-based digestate, directly or for system effects, it is desirable to document NH_4-N/total N and C/N_{org} ratios, amount of VFA and lactate, pH in digestate and biogas production related to VS or DM fed. Information about type of process, organic loading rate, retention time, kind of storage and time for storage after the main biogas step is also helpful in determining the situations for which the results are valid.

Aeroponics studies to optimise nutrient proportions for beetroot can easily be continued as they are performed under controlled conditions in climate chambers. The first priority should be to establish optimal proportions of P and Mn.

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