Biodigestion of Plant Material Can Improve Nitrogen Use Efficiency in a Red Beet Crop Sequence

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Abstract. Nitrogen (N) tied up in or lost from decomposing biomass decreases the residual N effects of green manure and of other crop residues. During anaerobic degradation in a biogas digester (biodigestion), N mineralization takes place under conditions in which losses can be kept to a minimum. Therefore, biodigestion of green manure biomass and beet foliage was tested to generate readily available N and compared with a direct green manure fertilization system. The effluent was applied as fertilizer in field experiments on a sandy soil as a tool for improving N supply for an organic farming system. Data from the field experiments were used for simulating the amount of net inorganic N equivalents (inorganic N equivalents from effluent plus inorganic N equivalents from pre-crops) in three crop sequences: A) green manure ley, red beets, winter rye; B) harvested ley, red beets, winter rye; and C) harvested ley, spring barley, red beets in which (B) and (C) represented biogas nutrient management systems and (A) a green manure system. When all available effluent from biogas production from 1 ha of grass–clover ley with two or three harvests (2H-ley or 3H-ley) and one hectare of beet foliage was used as a fertilizer for red beets (*Beta vulgaris* var. *conditiva* Alef) after barley (*Hordeum vulgare* L.), the yield of marketable red beets increased by 5.7 Mg·ha⁻¹ (33%) with effluent from 2H-ley and beet foliage and 9.1 Mg·ha⁻¹ (53%) with effluent from 3H-ley and beet foliage compared with red beets grown without effluent fertilization after a green manure ley. When total dry matter production was taken into account, the advantage for the BG systems with 2H- and 3H-ley was 15% and 28%, respectively. The nitrate concentration in the red beets was not higher with effluent supplied at this level than with green manure as the only N source. The simulated amount of net inorganic N equivalents was 128 kg N for the whole of crop sequence (C) with 1 ha of each crop and where effluent supply to red beets was based on digested biomass in ley and beet tops. The corresponding amount of net inorganic N equivalents for the green manure crop sequence (A), in which no effluent was supplied, was 73 kg N. Unused soil mineral N (0- to 90-cm depth) at red beet harvest indicated that the risk of leaching in BG systems was lower than in GrM systems (88, 76, and 61 kg N_{min}/ha left after unmanured beets after GrM-ley, low manured beets after 3H-ley and high manured beets after barley, respectively). Effluent fertilization of red beets directly after 2H- and 3H-ley gave unexpectedly low yield responses compared with red beets after barley. The reasons may be the result of nutritional imbalance of other nutrients than N or may be plant pathological in nature. The conclusion is that a nutrient management system with biodigestion can increase net inorganic N equivalents and reduce risk for N leaching, but inappropriate use of the effluent, i.e., at an unsuitable point in the crop rotation, may negate the benefits.

three times in the summer of the GrM year and incorporated into the soil the next spring. When growing an N-demanding crop such as red beets for food processing, a certified organic fertilizer containing some inorganic N is often applied immediately before planting the crop. Thus, the farms are then not N self-sufficient.

Leaving the plant material of a cut green manure crop on the field involves a risk of N losses (Janzen and McGinn, 1991; Torstensson et al., 2006). It has been suggested that the N use efficiency of GrM can be improved by using the biomass as raw material in an anaerobic biodigester for biogas production and returning the residues as fertilizer instead of leaving the ley biomass in the field for its direct green manure effect (Svensson, 2005). Whether a biogas nutrient management system (BG system) with its N supply originating from biodigested plant material has a better first-year N fertilization effect than a green manure nutrient management system with its N supply originating from grass–clover green manure ley (GrM system) would depend on many factors. We reasoned that important factors would be 1) the effect of removing, instead of leaving, the cut grass–clover biomass on growth, N uptake, N fixation, and chemical composition, especially C:N ratio of the different ley species; 2) the amount of N and the chemical composition of other crop residues such as red beet foliage that can be used in biogas production; 3) the net mineralization of N from crop residues; 4) the efficiency of microorganisms in the biodigester to degrade carbon and thereby simultaneously mineralize organic plant N to ammonium N (NH₄-N); 5) the ability of the crop to use effluent N; and 6) the ability of the crop to use N mineralized from crop residues of green manure, harvested grass–clover ley, and from other crop residues. The total impact of these parameters would determine the difference between a GrM system and a BG system. Some of the parameters in factor (1), (3) and (6) have already been identified using data from the present investigation as reported by Gunnarsson et al. (2008): (1) a harvested grass–clover ley produced a smaller amount of total aboveground biomass and N than a cut green manure ley, but the clover proportion was unaffected by the treatment; (3) and (6): both apparent net N mineralization and N uptake by crop was less in a red beet crop after harvested ley than after GrM-ley (Gunnarsson et al., 2008). Loges et al. (2000) also found that N₂ fixation by leguminous plants can be increased if the ley is harvested instead of being used as a green manure. For a silty loam with 25% to 30% clay, Stinner et al. (2008) showed an increase both in total N uptake and estimated biological N fixation of a harvested grass–clover ley compared with a GrM-ley. From the same experiments as those reported by Stinner et al. (2008), Möller (2009) reported an impaired N balance (in this case a larger surplus calculated as N with N₂ fixation minus output in sold products) in a BG system compared with a GrM system. The humus balance was kept positive although the

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Green manure (GrM) crops are important for improving or maintaining soil fertility in organic farming. Nitrogen fixing species are essential for organic farming systems intended to be self-sufficient with respect to N. A typical practice in organic farms without livestock in southern Sweden is cultivation of a GrM crop consisting of a mixture of white clover, red clover, and grass undersown in a cereal crop in the year preceding the GrM year. The ley biomass is normally cut two or

surplus was lower in the BG system than in the GrM system. Soil mineral N in fall was lower in the BG system than in the GrM system (Möller and Stinner, 2009). We only found two other works about nutrient system effects of biogas digestion of plants, both on soils with rather high clay contents (Báth and Elfstrand, 2008; Ross et al., 1989).

In the present investigation, effluent from biogas production (biodigester effluent) was used as a fertilizer in the production of red beets. The impact on red beet yield and quality and the net inorganic N equivalents (inorganic N equivalents from effluent plus inorganic N equivalents from previous crops) were studied as part of a crop rotation with and without biodigestion of crop residues on a sandy soil in southern Sweden. The aim of this study was to test the hypothesis that harvesting the ley and beet foliage for biodigestion and returning the biodigester effluent as fertilizer would improve the N supply to beets and cereals in the crop sequence and increase the marketable red beet yield without increasing the content of unused plant-available N in soil after red beet harvest and without jeopardizing red beet quality in terms of excessive nitrate (NO_3^-) concentration.

Materials and Methods

Field trials

A 2-year field trial was carried out in 2002–2003 and repeated in 2003–2004 in an organic cultivation system on the Lilla Böslid experimental farm (lat. $56^\circ 60'$ N, long. $12^\circ 54'$ E) in southern Sweden. The field experiments included a pre-crop year with either spring barley (*Hordeum vulgare* L. cultivar Baronesse) with undersown perennial ryegrass (*Lolium perenne*) or a first-year ley crop harvested twice (2H-ley), three times (3H-ley), or not harvested but grown for its green manure effect (GrM-ley) and a subsequent crop of red beet (*Beta vulgaris* var. *conditiva* Alef. cultivar Boro) with different fertilization regimes. The red beet crop was used as a model crop because it has a relatively high N requirement.

Pre-crop year (2002 and 2003). In 2001, the ley of the first experiment was undersown in unfertilized spring barley after weed harrowing in the two- to three-leaf stage of the barley. In 2002, the ley of the second experiment was sown in the beginning of July without barley after a short fallow period with a mechanical treatment to control couch-grass (*Agropyron repens*). The seed mixture (22 $\text{kg}\cdot\text{ha}^{-1}$) consisted of 20% red clover (*Trifolium pratense* L.), 10% white clover (*Trifolium repens* L.), and 70% perennial ryegrass (*Lolium perenne* L.) based on weight. In the pre-crop year, the biomass in 2H- and 3H-leys was harvested with a flail forage harvester in the beginning of June (Week 23), late July (Week 30), and, for the 3H-ley, also in the beginning of October (Week 41) (same weeks in 2002 and 2003). The biomass in the GrM-ley and the 2H-ley was left untouched from late July until soil tillage in the spring, including ploughing. Apart from the third harvest, the 2H-ley and the 3H-ley underwent exactly the same treatment. The GrM-ley was cut with a Votex Maxxum 4/340 (Votex b.v., The Netherlands) on the same dates in June and July as the 2H-ley and 3H-leys. The machine chopped the plant material into small pieces and distributed it evenly on the soil surface. The mean ratio of clover in ley biomass dry matter (DM) in the pre-crop years was 60%, of which 80% to 90% was white clover (further details presented by Gunnarsson et al., 2008). Grain and straw of spring barley were harvested at maturity. No fertilizer was supplied in the pre-crop year or in the year preceding the pre-crop year. Soil tillage after barley was performed at the same time as after the ley pre-crops.

Red beet year (2003 and 2004). The red beet crop was fertilized with effluent from biodigested plant material according to four differently chosen mineral N target values (denominated 1, 2, 3, or 4 N-t) or without addition of effluent (0 EF) but with Basal (AB Hansson & Möhring) and Kali vinasse (Table 1). The intention with Treatments IV and VIII to XI (Table 1), with increasing N supply to

red beets with a spring barley pre-crop, was to determine an N response equation for the actual field and year. The target values for soil mineral N ($\text{NH}_4\text{-N} + \text{NO}_3\text{-N} = \text{N}_{\text{min}}$) plus effluent $\text{NH}_4\text{-N}$ were estimated for three application times using the growth and fertilization model N-ABLE (Greenwood, 2001). Target values were chosen so that a red beet yield of 15 to 20 $\text{Mg}\cdot\text{ha}^{-1}$ could be expected in the treatments with the lowest effluent supply and with barley as a pre-crop (Treatment VIII and IX) and 50 $\text{Mg}\cdot\text{ha}^{-1}$ at the highest level (Treatment XI). The 50- $\text{Mg}\cdot\text{ha}^{-1}$ level was chosen according to earlier experience of the potential yield. The target values after leys were the same as for Treatment VIII (with the 1 N-t fertilizing regime) in 2003 and as for Treatment IX (with the 2 N-t fertilizing regime) in 2004 and are therefore denominated Low N-t. For calculating the need of effluent $\text{NH}_4\text{-N}$ supply at the first application (before drilling), N_{min} in the 0- to 30-cm layer was used, whereas N_{min} in the 0- to 60-cm layer was considered for the second and third applications. The effluent was applied before drilling with a band spreader and incorporated into the soil within 1 h of spreading by harrowing and by interrow cultivating after the applications in June and July. Additional effluent application to reach the N target values was only needed in July in the three highest regimes after barley in 2003 and in the two highest N target regimes in 2004. The highest effluent rate added in a single application was 73 $\text{m}^3\cdot\text{ha}^{-1}$. This rate percolated quickly into the sandy soil and did not cause problems with surface runoff. Nutrient content in Kali vinasse, used in treatments without effluent application, was (%): 33 potassium (K), 1 organic N (N_{org}), 0 $\text{NH}_4\text{-N}$, 0.002 phosphorus (P), 0.7 calcium (Ca), 0.6 magnesium (Mg), and 20 sulfur (S). Basal (crushed rock salt) contained 38% sodium (Na) as sodium chloride (NaCl). Because the P balance in the preceding crop rotations was positive and soil P status was good, no extra P was added.

Table 1. Pre-crops and nutrient supply in the different fertilization regimes in the field trials during the second experimental year (red beet year).^z

		Fertilization regime during the red beet yr				Abbreviation
		Nutrient supply with fertilizers (kg·ha ⁻¹)				Pre-crop/fertilization regime ^y
Treatment	Pre-crop (during the first experimental yr)	Na	K	NH ₄ -N	Total N	
<i>Fertilization: Kali vinasse and Basal</i>						
I	Ley, used as green manure (control)	40	100	0	2	GrM–ley/0 EF
II	Ley, harvested 2 times	40	200	0	6	2H-ley/0 EF
III	Ley, harvested 3 times	40	200	0	6	3H-ley/0 EF
IV	Spring barley with undersown ryegrass	40	150	0	3	Barley/0 EF
<i>Fertilization with effluent from biogas production using crop materials</i>						
V	Ley, used as green manure	20	84	26	61	GrM–ley/Low N-t
VI	Ley, harvested 2 times	41	174	54	127	2H-ley/Low N-t
VII	Ley, harvested 3 times	50	210	65	153	3H-ley/Low N-t
VIII	Spring barley with undersown ryegrass	33	139	43	101	Barley/1 N-t
IX	Spring barley with undersown ryegrass	81	339	105	250	Barley/2 N-t
X	Spring barley with undersown ryegrass	120	504	156	364	Barley/3 N-t
XI	Spring barley with undersown ryegrass	155	653	202	473	Barley/4 N-t

^zMean for 2003 and 2004. Ley = grass-clover.

^y0 EF = without addition of effluent; 1, 2, 3, and 4 N-t = fertilization to red beets determined according to increasing N targets for soil mineral N plus effluent $\text{NH}_4\text{-N}$ in which 1 N-t is the treatment with the lowest N targets and 4 N-t with the highest. Low N-t is used for grass-clover leys in which the N target was the same as in Treatment VIII in 2003 and as in Treatment IX in 2004.

Na = sodium; K = potassium; N = nitrogen.

The red beet seed was planted at 40 seeds per meter with rows spaced of 50 cm. This allowed a final plant density of ≈ 550 thousand plants per hectare, which is normally sufficient for maximum root yield. In 2003, May was wetter and the summer warmer than in 2004 (Table 2), which resulted in later drilling and earlier harvesting in 2003 than in 2004. Average dates in 2003 and 2004 for the main cultivation activities during the red beet years were: 8 and 23 Apr., respectively, for incorporation of the ley with a heavy disc harrow (≈ 10 -cm soil depth), 19 May and 29 Apr. for ploughing (≈ 20 -cm depth), 27 and 12 May for fertilization before drilling (effluent or Kali vinasse and Besal; see Table 1), 31 and 18 May for drilling, 3 July and 21 June for the second and 31 and 28 July for the third effluent fertilizations (if needed according to target values), and 3 and 21 Sept. for harvesting.

Experimental design, soil, and weather. The experiments were performed using a randomized complete block design with four replicates. Plot size was 14 m $\times$ 6 m with 4-m distance between plots in the longitudinal direction. The experiments in 2002–2003 and 2003–2004 were located on adjoining fields with the same crop rotation. The topsoils, according to samples from the 30-cm soil layer, were sandy with on average 89% sand, 7% silt, 4% clay, 1.6% carbon (C), C/N = 9, and pH (H₂O) = 6.4. In the subsoil (30 to 90 cm), the clay content was on average less than 2% and total C was 0.4%. Plant-available P, K, Mg, and Ca in the topsoil with barley pre-crops was 12, 7, 9, and 76 mg/100 g air-dried soil, respectively, according to analyses with the ammonium acetate lactate method (Egnér et al., 1960). Water-soluble boron was 0.3 mg·kg⁻¹. The fields had been under organic production since 1995 with a 6-year crop rotation comprising spring barley with under-sown grass-clover, green manure ley followed by potatoes or red beets; thus, potatoes or red beets were grown every sixth year. During the period with organic farming, farmyard manure had been applied twice in the crop rotation. For more details about plant-available nutrients see, Gunnarsson et al. (2008).

Temperature, precipitation, and irrigation are shown in Table 2. In July 2003 and in July and Aug. 2004, the precipitation was much above the 30-year mean. According to Brewster and Sutherland (1993), the lowest temperature for red beet growth is 5.6 °C. With that as the base temperature, above which temperature is counted, the accumulated number of day-degrees from sowing to

harvest was 1077 in 2003 (96 d) and 1175 in 2004 (127 d).

Biodigestion and effluent production

The biodigestion was performed with a one-step process at 35 °C in a batch-fed stirred 80-m³ anaerobic biodigester. The biodigester tank was fed with beet leaves (approximately one-third of added DM) and grass-clover from a first-year ley. Because more plant material was needed, for feeding the 80-m³ reactor, than could be produced in the experiment plots, plant material was obtained from organically grown crops from other fields and stored as silage before being fed into the biodigester. On average, N concentration in the ley and beet foliage used for feeding the biodigester was the same as in the plant material harvested in the field experiment. In the fall of 2002, when the biodigester was started, it was inoculated with sludge from another anaerobic biodigester using beet foliage. Volatile fatty acids (VFA) increase N immobilization in the effluent when added to soil (Kirchmann and Lundvall, 1993). In the final effluent, used as fertilizer, the VFA levels were below the detection limit (0.01 g·L⁻¹) in both years, and pH averaged 7.4. Biogas production was stable with an estimated methane (CH₄) yield, measured at atmospheric pressure, of 0.21 m³·kg⁻¹ volatile solids (VS) in 2003 and 0.25 m³·kg⁻¹ VS in 2004. The stirring equipment was less efficient in the first year, which was probably the main reason for the lower methane production and also for a lower NH₄-N:total N ratio: 0.38 (± 0.06) in 2003 and 0.46 (± 0.01) in 2004 ($\pm$ SD; n = 3). C:N_{org} ratio was 9 (± 1) in 2003 and 11 (± 1) in 2004. As a mean for 2 years and three sampling times per year, the DM content in the effluent was 6%. The nutrient concentrations in the effluent (kg·Mg⁻¹ fresh weight) were: 1.3 NH₄-N, 1.9 N_{org}, (i.e., 3.2 total N), 0.4 P, 4.2 K, 1.0 Na, 0.4 Mg, 0.2 S, 0.141 iron, 0.006 manganese, 0.005 zinc, 0.004 boron, and 0.001 copper. The chloride content was 1.5 kg·Mg⁻¹ fresh weight. The density of the effluent was 1 kg·L⁻¹.

Sampling and analyses

In the first experimental year, a flail forage harvester with a balance was used to measure yields of harvested ley from 21 m² per plot. The material was analyzed for DM and Kjeldahl-N (Kjeltec Auto 1035 Analyser; Tecator AB, Höganäs, Sweden). The N analyses were performed on one sample per harvest, treatment, and block.

Mineral N in the 0- to 30- and 30- to 60-cm soil layers was determined on four occasions in the second experimental year: before drilling, in June (5 to 7 d before the second fertilization with effluent), in July (5 to 7 d before the third fertilization with effluent), and immediately after beet harvest. On the first and last sampling occasions, the 60- to 90-cm soil layer was also sampled. Before drilling, N_{min} was determined by block and pre-crop history and on the other sampling occasions plotwise. Each soil sample consisted of 10 soil cores mixed together. Extraction was made in 2 M KCl (w:v 1:3; 3 h shaking at room temperature) and the extracts were analyzed for ammonium N and nitrate N by flow injection analysis. Sample handling followed the international standard ISO 14 256. The N_{min} was used as the basis for N target fertilization (more data presented in Gunnarsson et al., 2008). Before drilling the red beet crop, separate samples from the 0- to 25-cm soil layer from each treatment, thus with each pre-crop, were also taken and then analyzed for plant-available P, K, Mg, and Ca according to the ammonium acetate lactate method (Egnér et al., 1960). Plant-available K was used to decide the application rate of Kali vinasse.

Red beets were harvested by hand within four 5-m red beet row sections per plot. Harvested beets were shredded in a food processor, and beets and leaves were dried at 70 °C and analyzed for total N content according to Dumas on a LECO FP-428 (LECO Corporation, St. Joseph, MI). All yield and nutrient determinations were performed on beets from rows that were unaffected by wheel traffic during drilling, effluent spreading, or other machine operations.

Beet nitrate concentrations were determined by high-performance liquid chromatography. For this, the fresh biomass of unpeeled red beets from the 40- to 60-mm diameter (ϕ) fraction was used with the root tip and red beet top removed. This determination was made on red beets from all treatments with barley as the pre-crop (IV, VIII to XI) and from the treatments with GrM-ley and 3H-ley as a pre-crop without the addition of effluent to the red beets (I and III).

Samples for determination of volatile fatty acids and lactate in the effluent from the biodigester were analyzed as described by Björnsson et al. (2000). Nutrient content was measured before each application, i.e., three times a year. Total N and NH₄-N in the effluent were determined on fresh subsamples using the Kjeldahl technique (Kjeltec Auto 1035 Analyser; Tecator AB). Dry matter was determined after drying at 105 °C. Micro-nutrients and P, K, Ca, Mg, Na, and S were determined on dried subsamples with inductively coupled plasma-optical emission spectroscopy (ICP-OES) after digestion according to NMKL (1998). Water-soluble chlorine was determined with ICP-OES. Carbon was determined according to Dumas on a LECO FP-428 (LECO Corporation, St. Joseph, MI). Carbon determination was made after drying the samples at 50 °C until the residual water content reached $\approx 10\%$. Nitrate was not

Table 2. Weather data for the experimental period and 30-year mean values (1961–1990, for Halmstad 10 km north of the experimental site; Alexandersson and Eggertsson Karlström, 2001).^z

Period	Avg temp (°C)				Precipitation + irrigation (mm)			
	2002	2003	2004	30-yr mean	2002	2003	2004	30-yr mean
January to April	4	1	2	1	210	152	184	170
May	13	11	11	11	81	98	55 + 10	42
June	16	16	13	15	135	53	85	61
July	18	18	15	16	104	121 + 14	156	71
August	20	17	18	16	82	84 + 35	121 + 20	86
September (until harvest)	—	10	14	—	—	0	69	—

^zIrrigation was only used on the red beet crop.

determined in the effluent because it was kept under anaerobic conditions until the application day.

Simulated crop sequences and calculation of nitrogen supply and efficiency in the systems

In a BG nutrient management system, compared with a GrM system, the pre-crop effect of ley and red beets is reduced as a result of removal of plant materials from the fields (Gunnarsson et al., 2008), but increased flexibility in the system is gained as a result of the effluent (Möller et al., 2008a; Stinner et al., 2008). A fair judgment of system effects needs to take at least 3 years into account. Therefore, despite the 2-year field experiments, we 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) **red beets**; 3) winter rye.
BG system, Sequence B: Year 1) **harvested ley**; 2) **red beets**; 3) winter rye.
BG system, Sequence C: Year 1) **harvested ley**; 2) **spring barley**; 3) **red beets**.

The aim was to simulate how both beets and cereals were supplied with N produced in the crop sequences as a result of pre-crop effects. The crop sequence simulation was made with the following prerequisites and assumptions:

1. The experiments did not include measurements in the year after the red beets. To account for the increased N fertilization effect of red beet foliage compared with cereals ($N_{R\text{-foliage}}$), we estimated it for relevant climate and soil conditions (see further under Eq. 2). We assumed winter rye to be the most probable cereal after red beets because it can be drilled in September, is suitable for the sandy soil, and may benefit from mineralized N from red beet foliage and unused $N_{\min}$ after red beets. The winter rye was assumed to be unfertilized and to have the same residual effect as barley.
2. Crop sequence C was meant to illustrate the flexibility in choosing a crop sequence in an organic BG system with the opportunity to move N from the ley through the biodigester to a crop not directly after ley. However, the experiments did not include any treatment with spring barley after harvested ley like in crop sequence C. Therefore, we assumed that the increased N fertilization effect of ley compared with cereals ($N_{R\text{-ley}}$) was the same for spring barley as for red beets. The barley was assumed to be unfertilized and followed by perennial ryegrass as an undersown catch crop ploughed under in spring.

For comparing the GrM and BG systems with respect to the crop Sequence A, B, and

C, parameters characterizing N supply to crops and N efficiency are introduced subsequently.

When comparing different composted or anaerobically digested organic fertilizers with each other or with mineral fertilizers, the sum of inorganic N in the fertilizer is generally the best overall predictor of effects on yield, total N yield, and apparently bio-available N (e.g., Svensson et al., 2004). Consequently, in this work, N_{EF} (Eq. 1), net inorganic N equivalents (Eq. 2), and the apparent recovery fraction of $NH_4\text{-N}$ in applied effluent (Eq. 3) all refer to the ammonium N fraction of the effluent fertilizer, i.e., to an inorganic N equivalent.

N_{EF} . The amount of $NH_4\text{-N}$ available with the effluent within the BG system (N_{EF}), assuming 1 ha of ley and 1 ha of red beets, was calculated as:

$$N_{EF} = (N_{H\text{-ley}} + N_{H\text{-foliage}}) \times (NH_4N:Total\ N) \quad [1]$$

where $N_{H\text{-ley}}$ is the amount of total N from harvested ley available for supply to the biodigester. Data for the 2H-ley and 3H-ley crop during the pre-crop year were used. Because biomass in harvested ley was measured on the flail forage harvester, no correction factor was used to obtain the harvestable fraction; $N_{H\text{-foliage}}$ is the amount of total N from harvested red beet foliage available for supply to the biodigester in the BG system. The mean of red beet foliage data from Treatments VIII, IX, and X was used as an approximation for red beets grown with the amount of plant-available N present in a BG system. Red beet foliage was harvested by hand in the experiments. Therefore, for the amount of N in harvestable red beet foliage, a reduction factor of 0.66 was applied to compensate for the fact that only $\approx 66\%$ can be harvested in practice by current machinery; $NH_4N:total\ N$ is the ratio of $NH_4\text{-N}$ to total N in the effluent from biodigested plant material used in the experiment.

Net inorganic nitrogen equivalents. Net inorganic N equivalents refer to the amount of inorganic N from effluent plus inorganic N from pre-crops, the unit being kilograms (assuming 1 ha of red beets and 1 ha of ley in the crop sequence). Thus, the net inorganic N equivalents in crop Sequence A in the GrM system and Sequences B and C within the BG system were calculated as:

$$\text{Net inorganic N equivalents} = N_{EF} + N_{R\text{-ley}} + N_{R\text{-foliage}} \quad [2]$$

where N_{EF} is obtained from Eq. 1 and was thus 0 in the GrM system crop sequence; $N_{R\text{-ley}}$ was calculated from the amount of effluent $NH_4\text{-N}$ required for a red beet yield with barley as a pre-crop that corresponded to the marketable yield of red beets after ley. The equations for response curves presented in Figure 1 for red beets after barley (Treatments IV and VIII to XI) were used; $N_{R\text{-foliage}}$ was calculated on the basis of N and C in red beet foliage left in the field. The amount of mineralized N was calculated according to

Eq. 3 in Gunnarsson et al. (2008) on basis of the amounts of organic C and N in the plant material considered to remain in the soil after decomposition for this using the constant 0.26 for the humification coefficient (compare with Kolenbrander, 1974) and 10 for C/ N_{org} in humus. Finally, we assumed that 50% of the mineralized N corresponded to the first-year N fertilization effect, which is somewhat less than what can be estimated from sugar beet tops harvested in late October (Thomsen and Christensen, 1998). According to this, e.g., 20% of all N in the foliage in crop rotation A was considered $N_{R\text{-foliage}}$. This N effect of red beet foliage is at the higher end of what can be anticipated for the region and assumes forage handling that minimizes the risk of losses and drilling rye in early September as a subsequent crop (review by Lindén, 2008, used as the basis for fertilization recommendations by the Swedish Board of Agriculture). When $N_{R\text{-foliage}}$ was calculated for the BG systems, the mean of red beet foliage data from Treatments VIII, IX, and X was used (data from Table 3 after taking account of the fact that 66% of the foliage was removed for biogas production).

The barley pre-crop was used as a zero level for the fertilization effect related to pre-crop inorganic N equivalents and was therefore not included in the equation for net inorganic N equivalents. The net inorganic N equivalents were only calculated for the systems that were self-sufficient in N. Data from Treatment V (GrM-ley/Low N-t) were therefore not used, because that treatment represents an import-dependent system, thus with different system boundaries than the other treatments.

Recovery fraction of $NH_4\text{-N}$ in effluent applied to red beets. Apparent recovery fraction of ammonium N in effluent was calculated as:

$$NH_4\text{-N recovery fraction} = (BN_F + N_{\min F} - BN_0 - N_{\min 0})/N_F \quad [3]$$

where BN_F is the amount of N taken up by the red beet crop, excluding fibrous roots, in the effluent-fertilized treatment in question; BN_0 is the amount of N taken up by the red beet crop, excluding fibrous roots, in the treatment with the same pre-crop but without addition of effluent; $N_{\min F}$ is $N_{\min}$ in the 0- to 90-cm layer at red beet harvest in the effluent-fertilized treatment in question.

$N_{\min 0}$ is $N_{\min}$ in the 0- to 90-cm layer at red beet harvest in the treatment with the same pre-crop but without addition of effluent; N_F is the amount of effluent $NH_4\text{-N}$ applied. 'Apparent' is used because part of the recovered N may be derived from organic N (N_{org}) in the effluent that has been mineralized after supplied to the soil.

Residual nitrogen of ley. Residual N of ley is the increase of N in red beet plants and as $N_{\min}$ (0 to 60 cm) in the year after ley compared with barley as the pre-crop and was calculated as:

Fig. 1. Red beet yield response to nitrogen (N) supply in which N supply = $\text{NH}_4\text{-N}$ with effluent + residual N of ley in which residual N of ley is increase of N uptake in plants and $N_{\min}$ at harvest in red beets after ley compared with in red beets after barley (see Eq. 4). Dotted lines: all data included; solid lines: only data from red beets after barley included in the analysis. N supply = $\text{NH}_4\text{-N}$ with effluent + residual N of ley in which residual N of ley is increase of N uptake in plants and $N_{\min}$ at harvest in red beets after ley compared with in red beets after barley (see Eq. 4). Eqs. 2003 (linear response): barley pre-crops: $Y = 10.491 + 0.136X$ ($R^2_{\text{adj}} 0.66$); all pre-crops: $Y = 10.025 + 0.145$ ($R^2_{\text{adj}} 0.74$). Eqs. 2004 (monomolecular response): barley pre-crops: $Y = 27.461 \{1 - e^{[-0.02426(X + 7.6)]}\}$ ($R^2_{\text{adj}} 0.60$); all pre-crops: $Y = 29.008 \{1 - e^{[-0.01041(X + 22.5)]}\}$ ($R^2_{\text{adj}} 0.50$). P values for regressions < 0.00001.

Residual N of ley = (apparent soil N in treatment with ley as pre-crop) – (apparent soil N in treatment with barley as pre-crop) [4]

Apparent soil N included recovered N in total red beet plants, except fibrous roots, and $N_{\min}$ 0 to 60. The way of calculating Apparent soil N is further described in Gunnarsson et al. (2008).

Sum of all nitrogen. The Sum of all N is the total amount of N in crop residues of ley and red beets plus harvested N ($N_{\text{H-ley}}$ and $N_{\text{H-foliage}}$) in the actual nutrient management system. For the harvested ley, the data on N in crop residues are based on the amounts left in October the pre-crop year. For green manure ley also, N cut and left on the ground in June and July in the pre-crop year is included. N in roots of ley is estimated as aboveground biomass N content $\times 0.33$ (compare with Hansson and Pettersson, 1989; Pettersson et al., 1986).

Statistics

Analysis of variance was performed on the data from the time of red beet harvesting, including $N_{\min}$. (General Linear Model in Minitab 15; Minitab Inc.). Blocks and years were used as replicates. Analyses of $N_{\min}$ and ratio $N_{\min}$:root yield data were transformed to logarithmic scales to satisfy the assumptions in the model. All data were tested with Dunnett's two-sided test for differences to Treatment I (GrM/0 EF), because that treatment represented a control. Pairwise differences between the treatments were also evaluated with Tukey's test for least significant difference at the $P < 0.05$ level. When the word "tendency"

is used, it refers to results from a statistical analysis in which $0.05 \leq P < 0.10$.

Data for $N_{\text{R-ley}}$, net inorganic N equivalents, and ratio for net inorganic N equivalents to the Sum of all N were statistically analyzed with a single-factorial model with all five combinations (GrM system, Sequence A; BG system, Sequence B or C and with two or three ley harvests) as treatments. In the single-factorial model, Dunnett's test was used for evaluating differences to the GrM system as the control. Dunnett's test was used when the P value for treatments in the model was < 0.10. Data for the BG systems were also statistically analyzed with a two-factorial model with harvests (two or three) and crop sequence (B or C) as factors. Because a difference in $N_{\text{R-ley}}$, net inorganic N equivalents, and the ratio net inorganic N equivalents to Sum of all N between crop Sequences B and C was found in the two-factorial analysis ($P < 0.05$, < 0.10, and < 0.05, respectively), contrasts were made (Montgomery, 1997, p. 90–91) with crop Sequence B or C compared with the GrM system (Sequence A). Because the hypothesis was that the BG system would have lower $N_{\text{R-ley}}$ and higher net inorganic N equivalents and ratio net inorganic N equivalents to the Sum of all N, one-sided tests were used for the contrasts. In all other cases, two-sided tests were used.

Unless otherwise stated, there was no significant interaction between treatments and years in the single-factorial analyses or between the factors in the two-factorial analyses.

Linear fits were made and analyzed statistically in Minitab 15 or Origin 8 (Origin-Lab), and for curvilinear fits, Origin 8 was used. For red beet yield versus N supply (Fig. 1), linear, linear plateau, monomolecular, and quadratic responses were tested. The equa-

tions presented were the fits with the highest adjusted R^2 . Linear plateau model was fitted to trial data as described by Uhte (1990). From the curve fit with $N_{\min}$ per unit marketable red beet versus root yield, the levels of the residuals was tested by analysis of variance for differences between ley pre-crops and barley.

Results and Discussion

Nitrogen to biodigester and $\text{NH}_4\text{-N}$ in effluent (N_{EF}). Mean total DM yield of harvested ley material in the two experimental periods was 6.1 and 9.0 $\text{Mg} \cdot \text{ha}^{-1}$ in 2H-ley and 3H-ley, respectively, containing 140 and 218 kg N/ha (Table 4; $N_{\text{H-ley}}$). Harvestable red beet foliage contained 1.5 Mg DM and 34 kg N/ha ($N_{\text{H-foliage}}$) with small differences between the years. Together, these gave a theoretical input to the biodigester of 174 and 252 kg N from the BG system with 2H and 3H-ley, respectively, based on 1 ha of ley and 1 ha of red beets (= totally 2 ha). This N input to the biodigester assumed that there were no N losses in the silage process, because the harvested ley material was stored as silage before digestion in the biodigester. With an airtight silage seal, as used in this case, the N losses to the air should have been small or negligible, and silage effluent can be captured in the biodigester.

According to the Bushwell equation (Bushwell and Mueller, 1952), a high degree of methane production from the carbon in a certain N-containing organic material will give a higher ratio of mineralized N in the residues than if the methane production from the same material is low (Khanal, 2008, p. 38) although the relationship cannot be linear as a result of different N concentrations in

Table 3. Yield of red beets and foliage as means for 2003 and 2004; biomass fresh weight (FW), dry weight (DW), nitrogen content (N), nitrate (NO_3^-) concentration in fresh roots with diameter (ϕ) 40 to 60 mm, ratio for soil mineral N (N_{min}) 0 to 90 cm (at harvest) to marketable red beet yield (ϕ 30 to 75 mm).^z

Treatment	Precrop ^w	N supply incl. res. N of ley ^v (kg·ha ⁻¹)		Storage root					Foliage			Total plant	
		Fertilizing regime ^v		Marketable FW (Mg·ha ⁻¹)	Total FW (Mg·ha ⁻¹)	DW (Mg·ha ⁻¹)	N (kg·ha ⁻¹)	NO_3^- (mg·kg ⁻¹)	Ratio N_{min} : yield (kg·Mg ⁻¹)	DW (Mg·ha ⁻¹)	N (kg·ha ⁻¹)	DW	N
IV	Barley	0 EF	0	8.7 a	12.2 a	2.15 a	27.8 a	41 a	20.6 a	1.54 a	27 a	a	a
II	2H-ley	0 EF	18	10.8 a	13.6 a	2.38 a	33.1 ab		26.6 a	1.78 ab	32 ab	abc	a
III	3H-ley	0 EF	22	12.4 ab	15.3 abc	2.45 a	34.4 ab	106 a	15.2 ab	2.01 ab	37 ab	abc	ab
VIII	Barley	1 N-t	43	13.5 abc	16.5 abc	2.77 ab	36.3 ab	84 a	5.7 abc	1.65 ab	34 ab	abc	ab
I (control)	GrM-ley	0 EF	50	17.1 abcd	20.0 abcd	3.26 abc	44.8 ab	203 a	17.6 ab	2.36 abc	46 abc	abcd	ab
VII	3H-ley	Low N-t	53	11.6 a	14.3 ab	2.36 a	31.4 ab		10.7 ab	1.63 ab	31 ab	ab	a
VI	2H-ley	Low N-t	88	18.4 abcd	21.3 abcd	3.46 abc	49.6 ab		4.6 abc	2.16 abc	50 abc	abcd	ab
V	GrM-ley	Low N-t	99	23.8 bcd	26.4 bcde	4.05 abc	62.9 bc		5.6 abc	2.78 bc	58 bc	bcde	bc
IX	Barley	2 N-t	105	25.1 cde	28.0 cde	4.62 bc	56.1 abc	250 a	2.8 bc*	2.42 abc	49 abc	cde	abc
X	Barley	3 N-t	156	28.4 de*	32.2 cde*	4.94 c (t)	84.2 c**	666 b**	2.6 bc*	2.90 c (t)	72 c*	de (t)	c**
XI	Barley	4 N-t	202	32.7 e***	37.3 cde***	5.84 c***	89.3 c***	563 b*	1.9 c**	2.83 c**	67 c	e**	c**
Year													
	2003			21.3 B	23.6 B	3.84 B	66.4 B	413 B	3.4 B	2.07	49	B	B
	2004			15.7 A	19.5 A	3.12 A	33.6 A	134 A	17.3 A	2.30	42	A	A

^aRows sorted according to nitrogen (N) supply (including residual N of ley). Asterisks show significant difference to the control according to Dunnett's test in a one-way analysis: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, $t =$ tendency: $P < 0.1$. Values for treatments within columns followed by different lower-case letters are statistically different at $P < 0.05$ according to Tukey's test. Values for years within columns followed by different upper-case letters are statistically different at $P < 0.05$. Values for total plant is the sum of storage root and foliage.

^vN supply = $\text{NH}_4\text{-N}$ with effluent + residual N of ley in which residual N of ley is increase of N uptake in plants and N_{min} at harvest in red beets after ley compared with in red beets following barley (see Eq. 4).

^wFor nitrate, there was a significant interaction between treatment and year, mainly caused by smaller differences in 2003.

^x2H-ley and 3H-ley = ley for biogas production harvested two or three times; GrM-ley = green manure ley.

^y0 EF = without addition of effluent, 1, 2, 3, and 4 N-t = fertilization to red beets according to increasing N-targets in which 1 N-t is the treatment with the lowest N target and 4 N-t with the highest. Low N-t is used for grass-clover leys where the N-target was the same as in Treatment VIII in 2003 and as in Treatment IX in 2004.

FW = fresh weight; DW = dry weight.

different plant material fractions (Jensen et al., 2005). The $\text{NH}_4\text{-N}$:total N ratio in our effluent in 2003 was 0.38 with methane production of $0.21 \text{ m}^3\cdot\text{kg}^{-1}$ VS. The relatively long fibers in ley biomass demand efficient stirring in large-scale biodigestors. In 2004, when the stirring equipment in the biodigester was improved, the $\text{NH}_4\text{-N}$:total N ratio increased to 0.46 and methane production to $0.25 \text{ m}^3\cdot\text{kg}^{-1}$ VS. In a further development of the biogas technique in 2005 to 2008, the $\text{NH}_4\text{-N}$:total N ratio in effluent from the same biodigester became as high as 0.5 to 0.7 with a mean of 0.6 (results not shown). This suggests that the low $\text{NH}_4\text{-N}$:total N ratio and methane production in 2003 was caused by insufficient stirring in our relatively large biodigester. Ratios of 0.64 to 0.87 for $\text{NH}_4\text{-N}$:total N in effluent from biodigested grass-clover or lucerne have been reported from laboratory scale digestors (8 to 16 L) (Nordberg et al., 2007; Zauner and Kuntzel, 1986) and 0.57 for a biodigester with 10 to 15 m^3 active volume (Bath and Elfstrand, 2008).

Because the short-term N fertilization effect of N_{org} from residues of biodigested plant material is small or insignificant (Gunnarsson et al., 2010), the $\text{NH}_4\text{-N}$:total N ratio is of great importance for the net inorganic N equivalents. With the actual $\text{NH}_4\text{-N}$:total N ratio in the effluent from the biodigester, the total amount of $\text{NH}_4\text{-N}$ that could be used as effluent fertilizer was 73 kg (digested from 1 ha of ley and 1 ha of red beet foliage) for the BG system type with 2H-ley and 106 kg with 3H-ley as a 2-year mean. However, because the low $\text{NH}_4\text{-N}$:total N ratio in 2003 caused by insufficient stirring, we performed the calculations for N_{EF} using the ratio 0.46 for $\text{NH}_4\text{-N}$:total N for both years. The 2-year means became then 80 and 116 kg $\text{NH}_4\text{-N}$, respectively.

Nitrogen supply effects on yield of and nitrogen uptake by red beets. Red beets not fertilized with effluent in Treatments II to IV yielded $\approx 10 \text{ Mg}$ marketable roots per ha, but after GrM-ley (Treatment I), the yield was $17 \text{ Mg}\cdot\text{ha}^{-1}$ (Table 3; not significantly different). For a yield of $25 \text{ Mg}\cdot\text{ha}^{-1}$, an N supply of $\approx 100 \text{ kg } \text{NH}_4\text{-N}/\text{ha}$ was needed and, as an average for the two experimental periods, a yield of $33 \text{ Mg}\cdot\text{ha}^{-1}$ was achieved at $200 \text{ kg } \text{NH}_4\text{-N}/\text{ha}$. The 2-year mean yield increase/kg N supplied with application of $100 \text{ kg N}/\text{ha}$ and $200 \text{ kg N}/\text{ha}$ was 143 and 119 kg marketable red beets/ha, respectively (all pre-crops included, monomolecular model, $R^2_{\text{adj}} = 0.59$, $n = 88$). In general, the ranking between treatments on the basis of root yield followed the size of N supply. However, root and foliage biomass and N content of red beets in Treatment VII (3H-ley/Low N-t) were lower than expected from N supply.

The marketable root yield ($24 \text{ Mg}\cdot\text{ha}^{-1}$) in Treatment V with GrM-ley and added effluent (low N-t) was at the same level or somewhat higher than normal for organic red beet production in the region during these years. Yield levels and responses for N were both at the levels reported in previous studies on red beets and N fertilization with mineral N.

Table 4. Mean of two experimental periods for nitrogen (N) ($\text{kg}\cdot\text{ha}^{-1}$) from grass–clover ley and red beet foliage in the crop sequences representing systems with and without biogas production.^z

Nutrient management system ^y	N in ley and red beet foliage ($\text{kg}\cdot\text{ha}^{-1}$)				
	Total N in crop residues			Total N in effluent	
	Ley, aboveground	Roots (estimated ^x)	Red beet foliage	N _{H-ley}	N _{H-foliage}
GrM system	282	94	46	0	0
BG system, 2H-ley	70	79	17	140	34
BG system, 3H-ley	13	87	17	218	34
SEM ^w	7	6	2	2	3
					Sum of all N Σ
					422
					340
					369
					22

^zData for total N in ley crop residues refer to N in crop residues left in October in the pre-crop year and for the green manure ley (GrM-ley) also including N that was cut and left on the ground in June and July (presented previously in Gunnarsson et al., 2008). Data for total N in effluent refer to amount of total N available for supply to the biodigester, assuming no N losses, in which the index H in N_{H-ley} and N_{H-foliage} stands for harvested as the columns show the amount of N in the harvested part of ley biomass and beet foliage in the BG nutrient management systems.

^yGrM system = green manure system; BG system = biogas system; 2H-ley and 3H-ley = ley for biogas production harvested two or three times.

^xN aboveground at harvest or cutting times in June, July, and October $\times 0.33$ (compare with Hansson and Pettersson, 1987).

^wSEM = standard error of mean.

However, local conditions always determine the response to applied N fertilizer. Takácsné Hájos et al. (1997) in Hungary reported optimum N fertilization to irrigated red beets as low as 70 kg N/ha with a yield of 27 Mg·ha⁻¹ and with a mean increase of 43 kg of marketable roots per kg added N at that fertilization level. Greenwood et al. (1980) in the United Kingdom reported a higher response for N to red beets with an optimum of 245 kg N/ha at a yield of 62 Mg·ha⁻¹ and a mean increase of 133 kg of marketable roots per kg added N at the level of 245 kg·ha⁻¹. Differences in response between the years in the present investigation can mainly be understood by later planting date in 2003, a larger amount of N_{min} at planting (48 kg N/ha in 2003 and 31 kg N/ha in 2004 with barley pre-crop, data not shown), higher level of soil N mineralization after planting in 2004 [in the middle of June when the most intensive growth period started in both years, the apparent available N [$N_{\text{min}}(0-60\text{ cm}) + N$ in plant] was 78 kg in 2003 and 91 kg N/ha in 2004, in the unfertilized treatment with barley as pre-crop, data not shown}, and low potential yield in 2004 resulting from rainy weather in July and August.

The ratio of marketable yield ($\varnothing$ 30 to 75 mm) to total yield showed a monomolecular relationship to total root yield with R^2_{adj} of 0.76 and an asymptote at 93% marketable yield at the highest yield levels (data not shown). In most plots with a yield level above 20 Mg·ha⁻¹, 90% of the yield was in the marketable fraction. Marketable red beet yield increased linearly with increasing N supply in 2003 and gave a monomolecular response in 2004 (Fig. 1). Because N in the red beet plants with spring barley as a pre-crop, and corresponding N_{min} at harvest, was used as a zero level when calculating the residual N effect of ley (Eq. 4), N supply corresponded to NH₄-N supply with effluent where the pre-crop was barley. With the figures 80 and 116 kg·ha⁻¹ of N from N_{EF} (as estimated previously to be available from 2H-ley and 3H-ley crop sequences, respectively) entered in the yield response equations for red beets

after barley (Fig. 1), the mean yield, for both years, of marketable red beets after spring barley was calculated to be 22.8 Mg·ha⁻¹ for a BG system with crop rotation C (harvested ley – spring barley – red beets) and 2H-ley and 26.2 Mg·ha⁻¹ with 3H-ley. This corresponds to a yield increase of 33% and 53%, respectively, compared with the 17.1 ton red beets per ha in the control Treatment I (GrM/0 EF; Table 3), i.e., compared with GrM preceding red beets as in crop rotation A. Yield increase was 29% and 44%, respectively, if dry weight in the total root yield was taken into account instead of marketable fresh weight yield. When total DM production inclusive of foliage was considered, the advantage for BG systems with 2H- and 3H-ley was only 15% and 28% as beets after GrM-ley gave a larger portion of biomass in foliage than the effluent fertilized beets after barley. Other field studies with a cropping system approach report positive yield effects (10% yield increase; Stinner et al., 2008) or non-significant yield influences (Báth and Elfstrand, 2008; Ross et al., 1989) in systems using effluent from biodigested plant material as fertilizers to non-leguminous crops. The differences may be the result of experimental sites with different preconditions for N losses from green manure resulting from leaching or denitrification, different application strategies, or different levels of net mineralization of N in native soil organic matter. Growth-limiting factors other than N may also explain the absence of a yield increase, like in two of the cited references. Moreover, differences in ammonia losses after application of the effluents resulting from spreading technique could affect N use efficiency (Möller and Stinner, 2009).

Quality effects. Treatments X and XI (barley/3 N-t and barley/4 N-t) resulted in higher NO₃⁻ concentrations in red beets than in the other treatments where this was measured (Table 3). There was a significant linear increase in NO₃⁻ in red beets (Y, mg NO₃⁻/kg) with increasing N supply (kg N/ha) with the equation $Y = 66 + 2.5 \times N$ supply ($P < 0.001$; $R^2 = 0.31$). Applying this equation to N_{EF} on

a BG system with 3H-ley and crop Sequence C (red beets after barley, effluent based on 1 ha 3H-ley and 1 ha beet foliage for each hectare of red beets) and assuming that all the effluent is supplied to the red beets gives a beet NO₃⁻ concentration less than 400 mg·kg⁻¹.

Red beet is a vegetable with among the highest nitrate concentrations. Contents above 2500 mg NO₃⁻/kg are common and maximum levels in commercial red beets range from 3000 to 4500 mg NO₃⁻/kg in countries with limits for red beets (Santamaria, 2006). In our experiments, the levels were higher in 2003 than in 2004 and the highest concentration was 1070 mg·kg⁻¹, which was found in 2004 in Treatment X. Thus, the beet nitrate contents in the current experiments were low compared with normally accepted values for food to adults. Although recent reports show that the blood pressure-lowering effect of red beet juice can be explained by a high NO₃⁻ content (Webb et al., 2008), experts generally agree that high levels of nitrate in vegetables should be avoided (Santamaria, 2006). However, our results indicate that with the moderate N supply expected on organic farms that are N self-sufficient, the beet NO₃⁻ levels should not constitute a health problem.

The beet DW:fresh weight (FW) ratio was between 0.15 and 0.17 with a significant negative correlation with increasing cumulative supply of residual N from ley pre-crops plus total NH₄-N added with effluent (data not shown). However, R^2_{adj} was only 0.21. No treatment differed significantly from the control treatment as regarding the DW:FW ratio.

Nitrogen fertilization effect of residues of red beet foliage and ley left in the field ($N_{R\text{-foliage}}$ and $N_{R\text{-ley}}$). As a 2-year mean, $N_{R\text{-ley}}$ increased in the order 3H-ley < 2H-ley < GrM-ley (Table 5), which was expected according to the apparent net mineralization presented by Gunnarsson et al. (2008). The $N_{R\text{-ley}}$ was smaller in crop Sequence B than in C. (To interpret this, we remind of that the concept $N_{R\text{-ley}}$ and the concept residual N of ley used in this article are not equivalent; see section “Materials and Methods.”) In crop Sequence B, $N_{R\text{-ley}}$ was even negative as compared with the N conditions with spring barley as a pre-crop, constituting the zero value of $N_{R\text{-ley}}$. For example, in 2004, in Treatments VII (3H-ley pre-crop) and IX (barley pre-crop), the same target values were used: 75 kg N/ha before planting, 170 kg N/ha in June, and 80 kg N/ha in July. The amount of NH₄-N supplied with effluent was 92 kg·ha⁻¹ in Treatment VII and 106 kg·ha⁻¹ in Treatment IX. Still the root yield in Treatment VII was only 11.6 t·ha⁻¹, but in Treatment IX, it was 25.9 t·ha⁻¹. To continue with that example, the yield in Treatment VII corresponded to an N supply of 15 kg N/ha using the equation in Figure 1 for 2004 with barley as the pre-crop. However, because the actual supply was 92 kg NH₄-N, the $N_{R\text{-ley}}$ in this case becomes -77 kg NH₄-N (i.e., 15 minus 92). This indicates that fertilizing the red beet crop with biogas effluent up to a certain N target value, with harvested ley as a pre-crop, is less efficient than using the

Table 5. Nitrogen (N) fertilization effect of ley residues left in the field (N_{R-ley}), net inorganic N equivalents as influenced by all crops in each crop sequence, and N use efficiency (expressed as the ratio for net inorganic N equivalents relative to the Sum of all N) derived from grass–clover ley and red beet foliage (see Table 4) in the nutrient management systems with and without biogas production.²

Nutrient management system	Ley harvests	Crop sequence	N_{R-ley} (kg·ha ⁻¹)	Net inorganic N equivalents ^y	Ratio of net inorganic N equivalents to Sum of all N
Analysis with one factorial model					
GrM (control)	—	A (Ley, red beets, winter rye)	64	73	0.19
BG	2	B (Ley, red beets, winter rye)	-2	89	0.26
BG	3	B (Ley, red beets, winter rye)	-52***	58	0.16
BG	2	C (Ley, barley, red beets)	22	99	0.29
BG	3	C (Ley, barley, red beets)	27	158 t	0.43 t
Analyses for contrasts to GrM (control), one-sided test ^x					
BG	Mean	B (Ley, red beets, winter rye)	-27*	74	0.21
BG	Mean	C (Ley, barley, red beets)	24***	129*	0.36*
Analysis, two factorial model, <i>P</i>			<i>P</i>		
Harvests in biogas ley: 2 or 3			0.27	0.62	0.78
Crop rotation in biogas systems: B or C			<0.05	<0.10	<0.05

²The zero value for N_{R-ley} refers to the conditions with spring barley as pre-crop. Means for both experimental periods. GrM = green manure system; BG = biogas system. The net inorganic N equivalents are expressed in kilograms from 1 ha red beets and 1 ha of ley. Asterisks show significant differences to the control according to Dunnett's two-sided test in a one-factorial model or, for mean of the ley harvest regimes, according to contrasts to the control: * $P < 0.05$; *** $P < 0.001$. *t* = tendency: $P < 0.1$.

^yAlso including $N_{R-foliage}$, i.e., net fertilization effect of red beet foliage residues left in the field.

^xOne-sided test in accordance with the hypotheses.

effluent on red beets after barley. This could only be explained to a very limited extent by starting on a higher level of the response curve when adding effluent to red beets after ley than after barley. The main factor causing low N_{R-ley} in the BG system with crop Sequence B in general and within the BG system with 3H-ley and crop Sequence B in particular was the low yield response to effluent supply to red beets after harvested ley (Treatments VI and VII in Table 3 and N_{R-ley} rotation B in Table 5). Factors other than N may have limited the growth of red beets after harvested ley. Large amounts of nutrients are removed with the harvested ley and the relatively low levels of effluent in the harvested ley treatments may not have compensated fully for harvested nutrients. Hence, nutrients other than N may have been limiting, both as regarding N mineralization (compare with Campino, 1982) and red beet growth. Crop residues from *Trifolium* spp., other plants, and organic waste can have conducive effects on the root pathogens *Pythium* spp. and *Rhizoctonia solani*, both of which harm red beet plants in early stages (Bonanomi et al., 2007). To our knowledge, biogas effluent has not been studied in this regard.

$N_{R-foliage}$ was calculated to be 9 and 4 kg N/ha for GrM and BG systems, respectively, with small differences between the two trials (data not shown). The differences between the GrM and BG systems were statistically significant. In absolute values, however, those differences were much smaller than the differences in N_{R-ley} (Table 5).

Net inorganic nitrogen equivalents. In the BG system with crop sequence C (2H or 3H-ley – barley – red beets), Net inorganic N equivalents were on average 56 kg higher than in the GrM crop sequence A (green manure ley – red beets – winter rye), whereas

the BG system with crop sequence B (2H or 3H-ley – red beets – winter rye) did not differ from the GrM crop sequence (Table 5). The amount of net inorganic N equivalents did not differ significantly between the BG system with 2H-ley and that with 3H-ley (Table 5).

In crop Sequence A (green manure ley – red beets – winter rye), the red beets were supplied with N as a result of the residual effect of the green manure ley, the effect depending on the synchrony between the rate of N mineralization and N uptake by the beets. Because beet foliage was left in the field, its mineralized N may have been exposed to N losses by volatilization and leaching during the fall and winter, although the rye crop was assumed to be established as soon as possible after beet harvest to favor N uptake during fall and to reduce losses.

In crop Sequence B (2H or 3H-ley – red beets – winter rye), the N supply to red beets was affected negatively by the number of harvests of the ley and the resulting smaller amounts of crop residues in 3H-ley than in 2H-ley, but it was also affected positively by the corresponding increased amount of effluent N from the biodigester. Because red beet foliage was harvested here, less foliage was left on the ground than in Sequence A. This must have reduced the risk of leaching, but the N supply to rye was somewhat lower than in Sequence A.

In crop rotation C (2H or 3H-ley – barley – red beets), only the barley was likely to be affected by the residual effect of ley. The second-year effect of a 1-year ley is small (Granstedt and L-Baekström, 2000). Therefore, we did not calculate with a second-year effect of ley in any of the crop sequences. The beets were supplied with N from effluent. In Sequence C, the beet foliage was harvested, thus causing less N losses than in Sequence A (compare with Thomsen and Christensen, 1998).

In general, $N_{R-foliage}$ had little effect on the differences in net inorganic N equivalents between the crop sequences. Foliage N yield was moderate, in general 40 to 50 kg·ha⁻¹ (Table 3) and although the beet crop was assumed to be followed by winter rye, the residual effect of non-harvested beet foliage should generally have been small (compare with review by Lindén, 2008).

The amount of net inorganic N equivalents in the 2 years differed clearly, although there were no interactions between treatments and years. The BG system was more advantageous compared with the GrM system in 2003–2004 than it was in 2002–2003 (data not shown). In 2002–2003, the net inorganic N equivalents in the BG system with rotation C were 146 kg, compared with 116 kg in the GrM crop with sequence (A), whereas the corresponding net inorganic N equivalents in 2003–2004 were 110 and 30 kg, respectively. The main reasons for the greater advantage of the BG systems in 2003–2004 than in 2002–2003 were: 1) lower N yield of harvested ley in 2002 than in 2003; and 2) greater N_{R-ley} of GrM–ley in the first experimental period compared with N_{R-ley} of harvested ley. This may be the result of greater N leaching in 2004 than in 2003 probably causing more leaching of mineralized N from GrM–ley than from the other pre-crops. In an adjacent comparable field with lysimeters, the accumulated runoff for July, Aug., and Sept. 2004 was 55 mm in 2003 and 220 mm in 2004, and N leaching during these months amounted to 1 and 13 kg of N/ha for 2003 and 2004, respectively (G. Torstensson, personal communication).

For growers familiar with animal manure, the concept of the net inorganic N equivalents should give understandable information about the summarized differences in N supply to crops between the BG and GrM crop sequences. However, if transferring the expression net inorganic N equivalents used in this work to fertilizer value of commercial inorganic N fertilizers, e.g., NH_4NO_3 , account must be taken of the greater NH_3 emissions from the effluent, among other things. Because effluent was incorporated into the soil within 1 h of spreading in the present experiment, such losses would not have been large. Account should also be taken of possible mineralization of N_{org} in the effluent. With a mineralization rate corresponding to 12% of N_{org} in a 6-month period (compare with Gunnarsson et al., 2010), the net contribution of such mineralized N_{org} would be of the same magnitude as the NH_3 losses (compare with Sommer and Hutchings, 2001).

Our results refer to a sandy soil and a maritime, humid climate. A soil with higher clay content may give other results. The relation between GrM–ley and harvested ley may differ concerning production of biomass and N (probably an advantage for the BG system). Nitrogen leaching caused by GrM–ley may be lower with increasing clay content and less precipitation (an advantage for the GrM system). Denitrification may be higher in clayey soils (an advantage for the BG system). The net effect is difficult to forecast.

Ratio of net inorganic nitrogen equivalents to Sum of all N in ley and red beet foliage (nitrogen use efficiency). The ratio of net inorganic N equivalents to the Sum of all N in ley and red beet foliage was higher for the BG system with crop Sequence C than for the GrM system (Table 5). This indicates a better N use efficiency in the BG system. However, for the BG system with crop Sequence B, the ratio did not differ significantly from the GrM system.

Recovery fraction of $\text{NH}_4\text{-N}$ in effluent applied to red beets. The overall mean for the $\text{NH}_4\text{-N}$ recovery fraction (Eq. 3) was 0.73 with no statistically significant differences between treatments or years (results not shown). Gunnarsson et al. (2010) reported that 175 d after application of a similar effluent used as fertilizer to ryegrass in a pot experiment, the total N recovery (in plant, roots, and as unused $\text{N}_{\min}$) corresponded to 100% of the $\text{NH}_4\text{-N}$ plus 12% of N_{org} added with the effluent. The lower N recovery during cultivation of red beets in the present field study may be explained by a shorter growing period and a different crop and also by the effects of field conditions such as more pronounced ammonia volatilization and N leaching and by the fact that fibrous roots were not included when calculating N recovery. Denitrification was probably a less plausible reason, because a sandy soil does not offer a beneficial environment for denitrification (Włodarczyk et al., 2004). In a “healthy” biogas process, the VFA concentration in the effluent is relatively low, usually in the range corresponding to 0.05 to 0.25 g acetic acid/L (Sawyer et al., 2003, cited by Khanal, 2008, p. 58). VFA and lactate may cause N immobilization when fertilizing with farm manure that has not been processed in a biodigester or with biogas effluent that, as a result of an “unhealthy” anaerobic pro-

cess, has high levels of VFA (Kirchmann and Lundvall, 1993). In our experiment, immobilization should not have been severe as the concentrations of VFA and lactate were low. We applied some of the effluent in the growing season of the red beet crop and although soil tillage was performed within 1 h of effluent application, there may have been some ammonia losses.

Effects of $\text{N}_{\min}$ after red beet harvest. The amounts of $\text{N}_{\min}$ remaining unused at red beet harvest in the 0- to 90-cm soil layer were largest in treatment V (GrM/Low N-t) and smallest in treatment IV (Barley/0 EF; Fig. 2). All treatments with spring barley as a pre-crop and Treatment III with 3H-ley as a pre-crop and 0 EF had less $\text{N}_{\min}$ than the control (red beets after GrM-ley/0 EF). The ranking between treatments was similar when only $\text{N}_{\min}$ in the 0- to 60-cm layer was considered. There was no statistically significant correlation between $\text{N}_{\min}$ within 0 to 90 cm and the total N supply, i.e., supply with effluent and residual N from ley.

Red beet has been classified as a deep-rooted crop (Christiansen et al., 2006). However, in our experimental soil, with its low clay and C content in the subsoil, the roots cannot be expected to have reached the 60- to 90-cm soil layer (review by Heinonen, 1985, p. 51) and obviously could not use $\text{N}_{\min}$ that moved down to that layer, especially in 2004 (Fig. 2).

Significantly more unused $\text{N}_{\min}$ per unit marketable roots was found in the control treatment (I; GrM-Ley/0 EF) than in Treatments IX to XI with a barley pre-crop and the largest effluent supply (Table 3). The amount of $\text{N}_{\min}$ per unit marketable red beet showed an exponentially declining response to yield ($R^2_{\text{adj}} = 0.95$). However, the model residuals were positive in treatments with ley as a pre-crop and negative in treatments with barley

as a pre-crop ($P < 0.01$, all observations included; $P < 0.1$, only effluent-fertilized treatments included). The larger residuals with leys as a pre-crop indicate that the synchrony of N release from ley residues with N uptake by red beets was not optimal throughout the growing season.

The smaller amounts of unused $\text{N}_{\min}$ in the effluent-fertilized treatments with red beets after barley than with red beets in the control treatment (I) indicate that risk for leaching is lower in a BG system with crop Sequence C than in a GrM system with crop Sequence A. Furthermore, leaching of N in the treatment with GrM-ley as well as with 2H-ley may have been larger than with 3H-ley during the winter before the red beets, although the leys were incorporated with a disc harrow and ploughed late in spring. The risk of leaching after barley after harvested ley in crop Sequence C must also be taken into account for a full judgment of crop Sequence C compared with A. However, this may be avoided by an undersown catch crop in barley, as used in our experiment in the pre-crop year. Harvesting the cereal straw in BG systems for use in the biodigester may increase the risk of leaching in the first years, as a result of less N immobilization, but only to a small extent (Myrbeck et al., 2006). However, the long-term effects of harvesting the straw would lead to somewhat lesser $\text{N}_{\min}$ during fall and winter, indicating decreased amounts of mineralizable organic N in the soil compared with continuous incorporation of straw according to a 12-year study by Myrbeck et al. (2006). Moreover, applications of effluent may cause a minor increase in $\text{N}_{\min}$ in the fall after harvest as a result of a continued mineralization of N_{org} in the effluent after harvest of red beets (compare with Gunnarsson et al., 2010). Thus, an awareness

Fig. 2. Unused soil mineral nitrogen (N) ($\text{N}_{\min}$) at harvest of red beets as average for 2003 and 2004. Lower part of columns = 0- to 60-cm layer, the upper part = 60 to 90 cm. N supply = $\text{NH}_4\text{-N}$ with effluent + residual N of ley, in which residual N of ley corresponds to increase of N uptake in plants and $\text{N}_{\min}$ at harvest of red beets after ley compared with in red beets after barley (see Eq. 4). Abbreviations for pre-crops: 2H-ley and 3H-ley = ley for biogas production harvested two or three times. Abbreviations for fertilizing regimes: 0 EF = without addition of effluent, 1, 2, 3, and 4 N-t = fertilization to red beets according to increasing N-targets were 1 N-t is the treatment with the lowest N-target and 4 N-t with the highest. Low N-t is used for grass-clover leys in which the N-target was the same as in Treatment VIII in 2003 and as in Treatment IX in 2004. Asterisks show significant difference to the control, for $\text{N}_{\min}$ 0 to 90 cm according to Dunnett's test in a one-factorial analysis: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Different letters show statistically differences, for $\text{N}_{\min}$ 0 to 90 cm, at $P < 0.05$ according to Tukey's t test. P value refers to differences between years. Error bars show SE of the mean for treatments: the upper for the layer 0 to 90 cm ($n = 8$; 4 replicates and 2 years).

of how to prevent leaching may be the most important factor, although organic BG systems offer opportunities to reduce N leaching. This implies a need for fall-growing crops such as catch crops (Dabney et al., 2001; Möller et al., 2008b), e.g., after the barley and after the red beets in crop Sequence C. However, the need for recommendations for preventing leaching may be as relevant for BG systems as for GrM systems (Shepherd and Chambers, 2007).

In the long-term perspective, slow release of organic N from effluent applied regularly in the crop rotation will increase soil fertility and N mineralization and leaching. Ross et al. (1989) reported more N_{min} and increased N mineralization potential after 6 years of fertilization with effluent from biodigested crops than when only mineral fertilizer was used, although the total amount of applied N was similar. This suggests that the use efficiency of N from ley and red beet foliage will increase in the long term along with the risk of leaching as part of the N mineralization occurs also in the winter. However, Ross et al. (1989) compared a BG system with a mineral fertilizer system. The long-term effect on N mineralization in a BG system and a GM system may not differ.

We determined N_{min} immediately after harvest of red beets. For estimating the risk for N leaching, it would have been more advantageous to measure again in the first half of October (normally before N leaching starts) to see if fall mineralization from ley pre-crops had increased differences between effluent fertilized red beets after barley and red beets after ley pre-crops. However, dynamics for net N mineralization in the field experiment presented in Figure 1 in Gunnarsson et al. (2008) indicate that apparent net N mineralization of the ley pre-crops had already reached its peak in the end of June. This can be understood considering that the main biomass in the ley was white clover, which is known to give rise to fast N mineralization (Kirchmann and Marstorp, 1991).

Conclusions

The hypothesis that the net inorganic N equivalents and the marketable red beet yields in the BG crop sequences would increase without jeopardizing red beet quality compared with the GrM crop sequence tested was confirmed for the BG system with crop Sequence C, where red beets followed spring barley. However, for Sequence B, where red beets followed harvested ley, the amount of net inorganic N equivalents did not increase, probably as a result of factors other than N limiting plant growth. An explanation should be sought for the low yield response for effluent in red beets after harvested ley.

When effluent was applied to red beets after barley in the BG system, the amount of unused N_{min} at harvest was smaller than after red beets after green manure and not fertilized with effluent. This indicates a potential for reduced risk of N leaching in BG systems compared with GM systems, although the

risk may depend on several other factors, e.g., how the increased N fertilization effect is used in the whole crop rotation.

The need for efficient methanogenic activity and a high degree of digestion of potentially digestible C in the biodigester, and thereby a high $NH_4\text{-N}$:total N ratio, should not be underestimated when discussing biodigestion as a tool for improving the N effect of green manure ley and other crop materials.

The results reported here refer to red beet rows not damaged by wheel pressure during effluent spreading. Practical possibilities to spread the relatively large amount of effluent needed for red beets without damaging the soil or crop must be considered when introducing the BG technique on a farm scale.

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