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Nitrogen availability of biogas residues

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Dedicated to

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Table of contents

List of Tables

List of Figures

List of Abbreviations

^{15}N	labelled nitrogen
AANU	additional apparent nitrogen utilization
ANOVA	analysis of variance
ANU	apparent nitrogen utlization
BGR	biogas residue
C	carbon
C/N	carbon/nitrogen ratio
$CaCl_2$	calcium chloride
CAL	calcium acetate lactate
CEC	cation exchange capacity
C_{org}	organic carbon
C_t	total carbon
DF	degree of freedom
DM	dry matter
H_2O	water
ICP	Inductively Coupled Plasma Emission Spectrometer
K	potassium
KCl	potassium chloride
LBR	liquid biogas residue
LSD	least significant difference
Mg	magnesium
MS	mass spectrometer
N	nitrogen
NH_4-N	ammonium nitrogen
N_{min}	mineralized nitrogen

NO_3-N	nitrate nitrogen
N_{org}	organic nitrogen
N_t	total nitrogen
OM	organic matter
P	phosphorus
$p \leq 0.01$	1% probability level
$p \leq 0.05$	5% probability level
r^2	coefficient of determination
SBR	solid biogas residue

1. Introduction

1.1 Biogas production as a renewable energy resource

Few countries are responsible for most of the world's total greenhouse CO_2 gas emission in 2009 (BMU, 2010). The United States emit 25% of the world total, followed by China with 15%. Germany is ranged at the sixth position with 4% of the world total CO_2 gas emission. According to the Kyoto protocol Article 3, the countries agreed to reduce anthropogenic carbon dioxide equivalent emissions of the greenhouse gases by at least 5 per cent below the 1990 levels in the period 2008 to 2012. To reach this aim a number of measures are recommended in Article 2 such as the enhancement of energy efficiency, protection and enhancement of sinks and reservoirs of greenhouse gases, promotion of sustainable forest management and agricultural practices, development and increased use of new and renewable forms of energy. Therefore among other measures Germany's current policy strives to increase the contribution of renewable energy resources to substitute fossil energy resources in order to decrease CO_2 emissions but also to become less dependent on imports of fossil fuel.

A substantial proportion of around 70% of the total renewable energy resources is provided by biomass transformation (biogenic solid fuels, biogenic liquid and gaseous fuels, biogenic portion of waste, biogas, sewage gas, land fill gas and bio fuels). For electricity generation biogas is ranked fourth after wind energy, hydropower, and biogenic solid fuels. It represents 11% of the renewable energy resources in Germany in 2009. For heat generation the proportion for biogas in renewable resources accounted for 8%, and the production of biogas provided 2.4% of Germany's total energy consumption (BMU, 2010).

With the aim to further increase energy formation from organic wastes and biomass production biogas is financially supported by the German government. Generally in Germany, most biogas digesters are fed with animal wastes as a basic substrate and energy crops as a co-digestate at mesophilic temperatures (Weiland, 2000). Due to the limited availability of organic wastes, the co-digestion of agricultural crops was increased during the last years (Weiland, 2000), with crops such as maize forage, sweet sorghum, ryegrass, clover and barley being the most frequently used. Analysis of the biogas evaluation shows that maize and grass silage are the most used co-substrates in biogas plants (Weiland, 2006). Eighty percent of all biogas plants used simultaneous fermentation of manure and maize silage and 50% of them used grass silage for co-fermentation. More than 30 different energy–rich organic wastes from agriculture as well as food- and agro-industry were used, and often treated simultaneously with manure and energy crops (Weiland, 2006). In addition, the digestion of energy crops as a single substrate in a mono-fermentation becomes also more important.

1.2 N availability of unseparated biogas residues

1.2.1 Comparison of unseparated biogas residues with other organic fertilizers

Residues of the anaerobic digestion process are valuable sources of plant nutrients (N, P, K, S, etc.) which have to be recycled in crop production, and particularly for nitrogen the degradability is a decisive factor for nitrogen availability. In general, organic fertilizers are derived from different raw materials and that is why they vary in their nutrient composition. No clear recommendations exist for the application of organic fertilizers and since they differ in their nutrient composition as well as the rate of nutrient release, farmers tend to apply either too little or too much of them. Considerable portions of total nutrients particularly N and P in the organic fertilizers are organically bound. Chadwick et al. (2000) found that the plant availability of manure N is influenced by manure composition, hence the N availability from organic fertilizers is difficult to predict. Gutser et al. (2005) concluded that in the year of application, plants would take up less than 50% of N applied as organic fertilizers compared to 50-80% of N applied as mineral fertilizers. It is generally assumed that in the year of organic fertilizer application, their contents in mineral nitrogen and in organic substance are associated with the fractions of N which are available for crop uptake (Olesen et al., 2004; Gutser et al., 2005).

Organic fertilizers contain mineral N in most cases ammoniacal N, which is a readily plant-available form of N, ranging from very little to 90% of the total N. For example, NH_4-N proportion in total N is 80 to 90% in urine, 40 to 60% in animal slurry, 5 to 20% in solid manure, and up to 15% in bio-composts or horn meal (Gutser et al., 2005). Organic N may release ammoniacal N through mineralization. On the other hand, organic C in the fertilizer may reduce the amount of plant-available N through immobilization. After application of organic fertilizers the ammonium is available to plant, but part of it is immobilized in soil during microbial decomposition of organic compounds in the cattle slurry (Sørensen and Jensen, 1995). The organic N must be mineralized before it becomes available to the plant. However, the amount of plant available N from slurry compared to NH_4-N is depending on the soil type, way of application and time after application.

The N released from the organic sources is difficult to estimate. Computer programs that calculate manure application rates commonly presume that 100% of the NH_4^+-N and some additional percentage of up to 50% of the organic N becomes available for plant uptake during the growing season immediately following the organic fertilizers application (Thompson et al., 1997).

1.2.2 Effect of organic fertilizers' C:N ratio on N mineralization

The C:N ratio of organic substance is used as a potential predictor for the N release. Cabrera et al. (2005) summarized factors that control the net N mineralization of organic residues and concluded from studies by Withmore (1996) that values of C:N ratio of 20 to 40 in the organic material represent the break-even point between net N mineralization and net N immobilization. In some cases, this break-even point was found to be near C to N ratio of 15 (Gilmour, 1998). Another study (Seneviratne, 2000) found that plant residues with C:N ratio up to 27 lead to N mineralization and ratios exceeding 27 cause N immobilization. Qian and Schoenau (2002) studied the N release from different solid manures differing in C/N_{org} ratio and indicate that net N mineralization was related to the $C:N_{org}$ ratio of the manures and that the highest N uptake was observed from poultry and hog manures with the highest organic N contents and the lowest $C:N_{org}$ ratios of 7.6 and 6.6, respectively. Similar results are reported by Gutser et al. (2005) who noticed that from organic fertilizers with $C:N_{org}$ ratios below 6-7 a high N release can be excepted while organic fertilizers with a high C:N ratio lead to immobilization. From these studies it can be concluded that the net N mineralization can be enhanced by the application of organic sources with high quality, i.e. low C:N ratio.

Organic residues with similar C:N ratio may mineralize different amounts of N due to differences in their composition that are not explained by the C:N ratio. Several studies have indentified the groups of the compounds present in organic residues which are possibly involved in net mineralization or immobilization such as proteins, lignin, soluble carbohydrates, hemicelluloses and cellulose. Wood et al. (2010)

evaluated 87 different poultry wastes in an incubation experiment and found a negative correlation between N mineralization and lignin content and a weak but positive correlation with hemicellulose content. No correlation was observed between N mineralization and cellulose content. Another study by Jensen et al. (2005) used 76 samples of plant materials to evaluate the C and N mineralization based on mg N g^{-1} of added C and showed a negative correlation between cellulose C and hemicellulose C content and N mineralization. Nourbakhsh. (2006) observed that after 46 weeks of incubation a significant negative correlation between the lignin content (r=-0.54*) or lignin/N (r=-0.68**) and N mineralization and a highly positive correlation between the N content (r=0.96***) and N mineralization from different plant materials existed.

1.2.3 Characteristics of the unseparated biogas residues compared with animal slurry

Biogas residues are often described to resemble animal slurry with respect to their composition, particularly in the value of NH_4-N: total N (N_t) which is around 50% in cattle slurry and between 60 to 70% in pig slurry. Raw undigested animal slurry in comparison to residues from anaerobic digestion have lower pH value, NH_4^+-N contents and the ratio of NH_4-N:N_t. However C:N ratio, total C content and organic dry matter content in anaerobic digestion residues are lower than that in undigested cattle slurry (Gutser et al.,1987; Asmus et al., 1988; Möller et al., 2008). Gutser et al. (1987) used 2-4 months stored cattle slurry and anaerobically digested cattle slurry and found that cattle slurry had a pH value of 7.2, C:N ratio of 9.6, a dry matter content of 8.5 % and an NH_4/N_t ratio of 58 %, while the anaerobically digested cattle slurry had a pH value of 7.7, C:N ratio of 5.9, a dry matter content of 7% and an NH_4/N_t ratio of 65%.

With regard to the N availability from biogas residues the knowledge is rather limited and inconsistent. In some field experiments by Ross et al. (1989) there was no effect of biogas residues on crop yield compared to the unfertilized control, only the plant N content was found to be higher after the application of biogas residues. Studies by Messner and Amberger (1987) based on equal amounts of applied NH_4^+-N showed a slightly lower N removal from anaerobically fermented compared to untreated cattle and pig slurry. Möller et al. (2008) reported that in spite of the higher NH_4^+-N content in liquid digested slurry, N uptake from this material resembled that from liquid undigested slurry. In contrast, other studies found that N uptake from biogas slurry exceeded that from unfermented slurry by about 10 to 20% (Gutser et al., 1987; Asmus et al., 1988). De Boer (2008) compared a mineral fertilizer, undigested pig slurry and pig slurry co-digested with different industrial wastes (such as yeast, starch or dairy products) and found that the apparent N recovery of three cuts of ryegrass from co-digested pig slurry was rather comparable to that from the mineral fertilizer and higher than that from undigested raw slurry. With respect to plant residues, application of solid and liquid residues from biogas digestion of field crop residues gave higher N uptake within the crop rotation compared to a system without the digestion of crop residues (Stinner et al., 2008). Recently, Gunnarsson et al. (2010) concluded from a 180-day pot experiment based on equal amounts of mineral N and with subsequent harvests of ryegrass that the N use efficiency and N recovery were comparable between a biogas effluent and a mineral fertilizer. They further concluded the organic N fraction to be rather recalcitrant because within this period the estimated net mineralization of 12% of the organic N was assessed to be not substantial.

1.2.4 Biogas residues from different substrates

Raw materials used as substrates for biogas production do not only consist of animal excrements but are mostly mixtures with agricultural crops in widely varying proportions or are 100% plant based. Digested crops may also cover a wide range of chemical composition according to plant species and maturity (Kaiser, 2007). For example in different maize samples the crude protein contents ranged from 7.1 to 9.1% (1.14 to 1.46% N) and neutral detergent fiber (NDF) contents, representing cellulose, hemicellulose and lignin contents ranged from 38 to 62% in DM. For ryegrass samples N contents between 1.6 to 5.0% and NDF contents from 38 to 60% were observed. The effect of different agricultural crops used as raw materials for anaerobic fermentation on the N availability of biogas residues need more attention.

1.3 The role of soils in N availability from organic fertilizers

Arable soils are widely varying in physical, chemical and microbiological properties. Depending on these factors the degradability of added organic fertilizers including biogas residues may differ.

Ammonium as well as nitrate-N; i.e., mineral N present in organic fertilizers may be subject to immobilization in building bodies of micro-organisms i.e., into the microbial biomass. If such immobilized N is not released during the season, N availability for plants will be reduced (Paul and Beauchamp, 1994). Immobilized N may remain in the soil organic matter in forms which are recalcitrant to decomposition (Sørensen, 2004). The increase in the rate of N transformation does not necessarily affect the net rate of N mineralization or N immobilization. Mineral N is continuously released from the soil organic matter pool or organic fertilizers (N mineralization), predicting the N mineralization from organic N is difficult because it is affected by several factors. Because mineralization is a biological process, it only occurs when soil conditions are suitable for biological activity.

1.3.1 Factors affecting N mineralization in soils

1.3.1.1 Temperature and water content

Temperature and soil water content play a very important role in the decomposition of organic fertilizers and the uptake of nutrients by plants. Without application of organic fertilizers, optimum decomposition of soil organic matter by soil microorganisms occurs at a temperature of around 25 ^{o}C. Knoepp and Swank (2002) examined the effect of temperature at levels of up to 25°C and of soil moisture contents of 0.20 up to 0.45 g H_2O g^{-1}dry soil on soil N mineralization and found high N mineralization rates at temperatures of 22 and 25°C. The lowest values for N mineralization were obtained at a temperature of 5°C. With respect to soil water contents an interaction between soil temperature and soil moisture was observed. An increase in N mineralization occurred increasing soil water contents up to 0.45 g H_2O g^{-1} soil when the temperature was 22 to 25°C. At low temperatures of up to 10°C, and with soil moisture at about 0.30 to 0.45 g H_2O g^{-1} either no or even a negative effect on N mineralization was observed in soils. Zaman and Chang (2004) measured net N mineralization from soils of an agroforestry system and reported that after 30-day of incubation, soil net N mineralization occurred at 40°C only. At 25°C and 5°C there was no net N mineralization. N net mineralization decreased from 100% field capacity (FC) to 75% FC and from 75°C FC to 50°C FC.

1.3.1.2 Soil texture

Soil texture or type may affect the N release from soil organic pools in unfertilized soils. Egelkraut et al. (2000) studied the effect of the N mineralizable in different soils, and noticed that the highest N mineralization occurred in the soil with a medium clay content (14 %) which was however also high in the content of organic N. From the soils with comparable but lower organic N contents the lowest N mineralization rate was found for the soil with the highest clay content, while soils high in sand content could mineralize more nitrogen. Sørensen and Jensen (1995b) found that N mineralization in unfertilized soils which differed in their texture was lower in the soil amended with 75% sand than in a soil amended with 50% of sand which indicates that the soil N accumulation decreased with increasing sand content. Clay soils have generally more micropores than sandy soils. Hassink et al. (1993) found that the relative increase in N mineralization after fine sieving was correlated to the percentage of soil pore space occupied by pores with diameters <0.2 μm in clay soils ($r^2 = 0.81$). But the correlation with any other pore size class was poor. Fine sieving enlarges pores and organic matter situated in small pores may become accessible to microorganisms after sieving. Hassink et al. (1994) suggested that physical protection plays a more important role in clay soils with relatively more micropores than in sandy soils with fewer micropores thereby conditions in the soil should be conducive to mineralization. Since a sandy soil has larger macro pores it is able to release water (well drained) and allows air to occupy space. As a result of good aeration in a sandy soil, microorganisms are able to breath. Therefore, the breakdown of organic fertilizers to release nutrients (mineralization) is fast. In a wet soil (clay soil) microorganisms are unable to get oxygen due to micro-pores which are occupied by water under anaerobic conditions and, therefore, mineralization is poor or slow. Bosstta and Ågren (1997) reviewed soil texture effects on organic matter and concluded that soil N mineralization was positively correlated with clay content. In a pot experiment Sørensen and Jensen (1995b) studied the effect of soil texture on N uptake of ryegrass for 6 months, and found that the N uptake increased by increasing clay content (loamy soil > sandy loam soil > sand soil). Thomsen et al. (2001) showed that increasing soil clay content from about 10-40% leads to decreases in the net ^{15}N mineralization of ryegrass. Moreover the addition of silt to the soil did not affect net N mineralization.

1.3.1.3 Soil organic matter content

Basically soils varying in their soil texture also differ in their properties such as pH, organic matter and N_t content (Hassink, 1993; Egelkraut et al., 2000; Thomsen et al., 2001; Griffin et al., 2002). In a pot experiment, Legg and Stanford (1967) examined the N uptake from 12 soils differing in their organic matter content and C/N ratio for 8 weeks and noticed that soils higher in total carbon content (1.9-3.9%) and total nitrogen content (0.15-0.34%) showed a higher N uptake compared to soils with lower C_t (0.53-1.58%) and N_t (0.041 - 0.083%) contents. Another study by Egelkraut et al. (2000) investigated N mineralization in four unfertilized soils differing in their texture and organic N content and found that N mineralization was higher in soils which contained a higher C_{org} and N_{org} content (1.41 and 0.077%, respectively) while it was lower from the other soils which had lower C_{org} (0.26-0.44 %) and N_{org} (0.017-0.031%) contents. Sørensen and Jensen (1995b) found that the soils which were higher in a total N and total C content of 0.16 and 1.7%, respectively, resulted in a higher N uptake by ryegrass than the other two soils which were lower in their total N and total C content (0.11-0.14% N and 1.3-1.4% C). Li et al. (2003) studied the N mineralization rate in an incubation experiment with soils differing in their N_t, C_{org} and pH, and found that N mineralization increased with increasing soil N contents from 0.14 - 0.26% of N_t. Herlihy (1979) found that the N mineralization potential was higher in a soil with a high N content compared to the soil that was lower in N content. Reddy (1982) reported that organic soils very high in total N contents (2.1 - 3.7% in 0 – 30 cm soil depth) released high amounts of mineral N from 230- 870 kg mineral N ha^{-1} y^{-1}.

1.3.2 Effect of organic fertilizers on N mineralization in soils

Organic fertilizers may have a different behaviour when used in different soils. A study by Egelkraut et al. (2000) tested organic fertilizers of cotton leaf residues, cotton stem residues and compost in different soils in an incubation experiment for 179 days. They observed that the net N mineralization from cotton leaf residues ranged from 25 to 39% of the applied N dependent on the soil they were applied to, but there was no clear relation to soil texture or soil organic matter content. However, the differences in immobilization of applied cotton stem residues were ascribed to differences in soil texture and it was concluded that the soils high in sand immobilized less N compared with soils high in silt and clay. Sørensen and Jensen (1995a) studied net N mineralization from fresh and anaerobically stored sheep manure and observed a higher net N mineralization from both manures in the soil which contained a mixture of 75% soil: 25% sand, compared with pure soil (100% soil) and the soil containing a mixture of 50% soil: 50% sand.

A few reports deal with the interaction of incorporated organic fertilizers and soil OM. For mineral fertilizers Hart et al. (1986) found a larger positive additional N interaction for $(^{15}NH_4)_2SO_4$ in a soil with higher C_{org} content compared to a soil that was lower in C_{org}. When ryegrass was grown in 21 different grassland soils fertilized with ^{15}N labelled ammonium nitrate, it was found that yield without and with fertilizer was related to the total OM of the soils (r = 0.68***, r = 0.69***), but was not significantly related to their clay or sand content (Whitehead, 1984). Correlation coefficients between N use efficiency and different soil properties of sand, silt and clay content; and organic matter were not significant.

N mineralization can also be strongly affected by the way of fertilizer application into the soil. In most cases, surface applied slurry has a weaker N fertilization effect than does slurry incorporated into the soil (Sørensen and Amato 2002; Coelho et al., 2006). NH_3 volatilization reduces the recovery of ammoniacal N from surface-applied slurry. Another possible factor, especially in dry conditions is the adsorption of ammoniacal slurry N to the very top of the soil, where it is unavailable to the roots. In a study by Mooleki et al. (2002) it was observed that injection of liquid swine manure into the soil resulted in greater enhancement of available N, higher grain yield, and better nitrogen use efficiency than broadcast application. Thus the most efficient way to minimize N losses from applications of manure is incorporation into the soil. Recently Meade et al. (2011) compared the N uptake efficiency (NUE) from inorganic fertilizer and liquid pig manure, and found that it was higher from inorganic fertilizer in both 2 years studied compared to the liquid pig manure, thereby the NUE was higher from inorganic fertilizer and was between 58 to 73%, while for the liquid pig manure it was between 33 to 36%. Most of the studies investigated the N availability from organic fertilizers i.e., manure, poultry manures but the information about the N availability from biogas residue application in different soils is still rare.

1.4 Separation of biogas residues and other organic fertilizers

German livestock farms are producing a large quantity of manure from poultry, pig and cattle husbandry (BML, 1995). Animal manure contains solid and liquid components, the solid fraction being characterized by high concentrations of organic matter and total N and P in comparison to the liquid fraction (Møller et al., 2007; Bauer et al., 2009). Therefore the slurry separation is an efficient way to recover and improve the nutrient efficiency. Separation could be done in untreated slurry and biogas residues of fermented material (Møller et al., 2007). Møller et al. (2000) stated that slurry separation into solid and liquid fractions can decrease the demand of storage capacity and reduce transport costs. Hence, the liquid fraction can be transported over low distances whereas the solid phase can be transported over long distances to the arable land.

1.4.1 NH_3 losses from unseparated and separated organic fertilizers during storage and after application to soil

In most cases, solid and liquid fractions after separation are usually stored in uncovered tanks which increase the susceptibility of ammonia loss. Oenema et al. (2001) concluded that from unseparated organic fertilizers, total loss of gaseous N from animal housing and slurry storage ranged from about 2 to 15% of total N in the cattle slurry and 17 to 29% of total N in the pig slurry. It may be assumed that separation and storage in uncovered tanks could still increase these losses. Large losses of up to 50% of total initial N were reported in poultry manure (Oenema et al., 2001). The liquid fraction is characterized by low dry matter content which leads to a rapid infiltration into the soil thus reducing ammonia volatilization as reported by Vandré et al. (1997) who tested NH_3-N emission from separated and unseparated slurry after different application methods, i.e., surface and injection application. They reported that, surface fertilizer application (separated and unseparated slurry) in April and May led to higher NH_3-N losses compared to injection application. Their unseparated slurry was characterized by a higher dry matter content of 71 g/kg but after separation it dropped to 44 g/kg. Therefore, the higher NH_3-N loss was observed from the unseparated slurry compared to the separated one even after injection. They concluded that dry conditions increased NH_3-N loss which led to decreasing N uptake by the plant. Chantigny et al. (2007) studied ammonia emissions from raw liquid swine manure (LSM), digested LSM and the liquid fraction after the separation of the raw swine manure after annual application for 3 years. They found that the LSMs differed in their DM content (45.8, 25.9 and 16.5 kg DM/m^3 for raw, separated and digested, respectively). They concluded that the highest ammonia emission was observed after the application of raw LSM (22 kg NH_3-N/ha) followed by the separated (17.8 kg NH_3-N/ha) and the digested LSM (17.7 kg NH_3-N/ha). Bauer et al. (2009) concluded that the solid fraction after separation could be used as a fertilizer before seeding of the plants whereas the liquid fraction could be used as a rapidly available fertilizer in the vegetation period.

1.4.2 Separation efficiency

Bauer et al. (2009) reported that the efficiency of separation depends on the dry matter content (DM) of the fermentation residue. Therefore the higher the dry matter content is, the higher the proportion of solid phase after separation. They reported a negative correlation between dry matter content in the fermentation residue and dry matter content in the liquid phase after separation with $r^2 = 0.78$. Also the relationship between dry matter content in the unseparated slurry and the proportion of weight transferred to the solid phase after the separation was studied by Møller et al. (2002) and found to be positively correlated with R^2 of 0.76.

1.4.3 Characteristics of solid and liquid fractions of biogas residues and other manures

Peters et al. (2011) stated that there are different techniques of animal manure and slurry separation. The techniques included sedimentation, centrifugation, drainage and pressurized filtration. They found that distribution of nutrients i.e., N, C and P varied depending on the separation technique and particle size fraction. For all techniques of separation from both pig and cattle slurry more than 50 up to 90% of C was found in the solid fraction. Similar results were obtained for P. In contrast the proportion of total N in the solid fraction was lower with about 15 up to 60 %. They noticed that within the solid fraction organic nitrogen and phosphorus were mainly associated with the smaller particles (25-250 µm), whereas organic C was mainly associated within larger particles >250 µm. Additionally, they studied different kinds of separation (mechanical and chemical separation), and found that the chemical separation technique resulted in higher C:N_{org} and P proportions in the solid fraction than in the liquid fraction. Whereas, the mechanical separation technique resulted in higher C, N_{org} and P proportions in the liquid fraction than the solid fraction. Sørensen and Thomsen (2005) stated that separation could lead to a more efficient distribution of nutrients. Therefore, after separation the N_t was higher in the solid fraction compared to the unseparated manure but decreased slightly in the liquid fraction. There was a decrease in NH_4-N content in the solid fraction compared to the unseparated manure, while in the liquid fraction the NH_4-N content did not change compared to the unseparated manure. Møller et al. (2007) carried out a study to compare the characteristics of manure before and after separation. They showed that the solid fraction after the separation of pig manure, digested pig manure and dairy cattle manure was markedly higher in total N and total P in comparison to unseparated manure, while the contents of NH_4-N and K remained rather unchanged. On the other hand, the corresponding liquid fractions were characterized by very low total P contents and lower total N contents compared the unseparated manures. The NH_4-N and K contents of the liquid fraction resembled those in the unseparated manure. The most important differences between the liquid and solid fractions were the higher contents of total N and P in the solid fraction compared to the liquid fractions, whereas the NH_4-N and K contents were similar in both fractions. Møller et al. (2002) reported that total N in the solid fraction was 1.7 to 3.54 times higher than in the untreated manure. Møller et al. (2007) explained the higher N_t content in the solid fraction compared with the unseparated biogas fermentation residue by the fact that the mechanical separation transforms more organic N to the solid phase, contrarily, the dissolved NH_4-N remains in the liquid phase. They concluded that solid and liquid separation of digestate allows maintaining in the liquid fraction a higher percentage of total N in form of ammonium, readily available to crops, and in the solid fraction the slow release organic nitrogen will remain. Therefore, fertilizer value is improved by separation because nutrient composition reflects crops demand.

1.4.4 Nitrogen availability of separated solid and liquid biogas residues

Chantigny et al. (2007) studied N uptake of timothy grass from inorganic fertilizer, raw swine slurry, digested swine slurry and the liquid fraction of separated swine slurry using two different soils, a loamy sand and sandy loam. They reported that after 3 years of annual fertilizer application, the N uptake was higher from the fertilized treatments compared to the control. Additionally, N uptake was higher from both digested and separated slurries than from the raw swine slurry. The order of N uptake in both soils was, mineral > digested > separated > raw manure.

Chantigny et al. (2008) conducted a field experiment to study the yield of corn and N availability from an inorganic fertilizer, from raw liquid swine manure (LSM) and its separated liquid fraction and from digested manure (LSM) using two different soils (a clay soil and a loam soil). They found that grain DM and grain N uptake were higher from the different fertilizers compared to the control, while grain DM and grain N uptake was similar for all LSMs and the mineral fertilizer. Laboski et al. (2010) tested the potential available N (PAN) from different dairy manure, including raw, anaerobically digested before and after liquid-solid separation, non-digested separated liquids and solids, and composted bedded pack manures. Manure was incubated for 112 d; generally, the digested separated liquid had greater PAN (52.2%) of N_t than the raw liquid one (33.4%). However, the PAN for the digested liquid before separation (43.5%) was identical to the raw or digested separated liquid. On the other side, the digested separated solid manure had the lowest PAN with a negative value of -14.6%, which indicated N immobilization from this manure. The authors also studied the correlation between PAN and the composition of different manures. They found that PAN from the manures was significantly correlated to the organic fertilizers N_t and NH_4^+-N (r = 0.81* and 0.78*, respectively). Negative correlations were found between PAN and the content of dry matter, acid detergent fibre (ADF) and neutral detergent fibre (NDF) which accentuates that the higher concentration of lignin, cellulose and hemicellulose in manure leads to both greater microbial activity and demand of N therefore, reducing the potential available N from manure. In addition a two-term regression model was developed to predict PAN from parameters of manure composition. High correlations were obtained for the parameters ADF:N_t + C_t:N_{org} (R^2=0.913), NDF:N_t + C_t:N_{org} (R^2=0.915) and NH_4^+-N:N_t + N_{org} (R^2=0.875). It was concluded that manure separation was effective on N utilization with or without anaerobic digestion. While liquid and solid separation are effective in reproducing a liquid fraction with lower dry matter and higher NH_4-N content as percent in N_t compared to the raw manure, which leads to increased the N utilization from the liquid fraction. On the contrary, the separation of manure creates opposite properties for the solid fraction compared to the liquid fraction and reduces the N utilization of the solid fraction.

1.5 Objectives of the present study

The objectives of the present study are (i) to describe biogas residues which differ in substrates used for fermentation (plant residues or mixtures of plant residues and animal wastes), (ii) to determine the effect of these different biogas residues on N availability after one application as well as after repeated applications, (iii) to evaluate the role of different soils on N utilization of these biogas residues, (iv) to characterize the solid and liquid fractions obtained from physical separation of biogas residues after co-fermentation of different substrates, and to determine the effect on N availability.

To reach these objectives, as a test system two pot experiments were conducted each lasting about one year using perennial ryegrass as a model plant in subsequent harvests and with repeated fertilizer application. Several different biogas residues unseparated and separated and differing in chemical composition were tested with different soils to investigate their N availability.

2. Materials and methods

2.1. Experimental conditions

Two pot experiments with perennial ryegrass were conducted at the research station of the Institute of Plant Nutrition, Technische Universität München, Freising, Germany (48.4°N, 11.7°E). Experiment 1 was designed with five soils from different sites under greenhouse conditions from November 2007 to October 2008 (309 days). Experiment 2 was conducted from January 2010 to March 2011 (414 days). When natural day length was shorter than 12 hours (between October and March) additional light was applied (photon flux density 550 µmol photon m^{-2} s^{-1}) to extend the light period to 12 hours and to improve photosynthetic irradiation. In the first experiment air temperature was kept above 18º C/15°C (day/night). Monthly mean air temperature in Freising from November 2007 to October 2008 is given in Tab. 1 (Bavarian Research Centre for Agriculture). In summer the greenhouse walls were kept open for ventilation. Daily mean temperature from April 2008 to October 2008 was 8.0°C (April 2008) to 17.6°C (July 2008). For the second experiment the temperature was determined inside the greenhouse during the eleven months of plant growth (Tab. 2).

Table 1 Monthly mean air temperature measured 2 m above soil surface during the first experiment from November 2007 to October 2008

Years	**Months**	**Temperature** °C		
		Average	**Min**	**Max**
2007	**November**	1.6	-8.1	10.3
	December	-0.1	-10.4	13.3
2008	**January**	1.9	-5.7	12.6
	February	2.6	-9.6	19.3
	March	4.1	-8.1	19.3
	April	8	-5.2	23
	May	14.5	1.7	30.2
	June	17.3	4.1	31.8
	July	17.6	7.8	31.3
	August	17.3	5.7	32.2
	September	11.9	-0.2	28.2
	October	8.6	-1.8	20.9

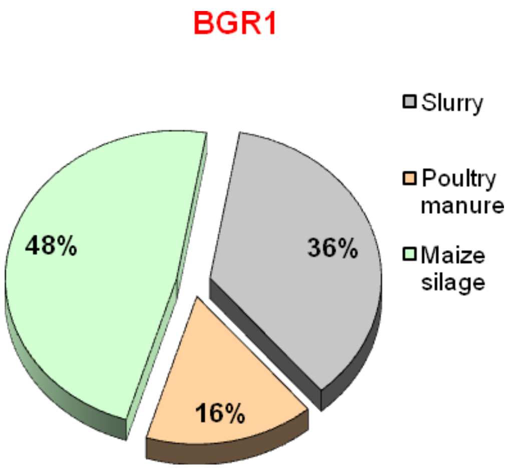
Table 2 Monthly mean temperature measured in the greenhouse during the second experiment from April 2010 to February 2011

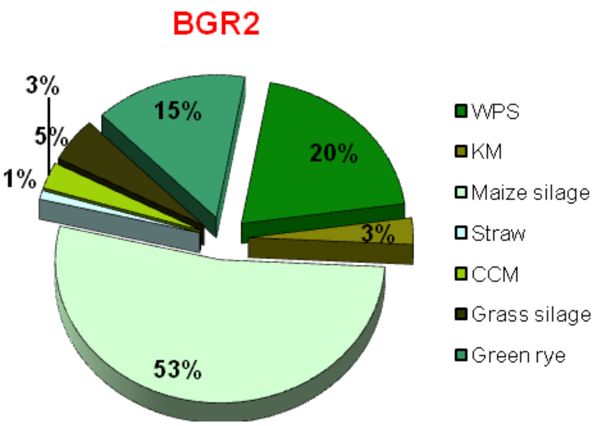
Years	**Months**	**Temperature** °C		
		Average	**Min**	**Max**
2010	**April**	25.7	20.5	30.8
	May	20.4	11.9	40.6
	June	24.7	21	30.7
	July	21.3	13.9	35.3
	August	20.1	12.4	34.8
	September	16.6	9.4	28.2
	October	16.4	7.8	25.2
	November	15.9	11.2	26.3
	December	14.1	10.5	26.9
2011	**January**	14.6	10.6	28.1
	February	15.2	10.6	28.9

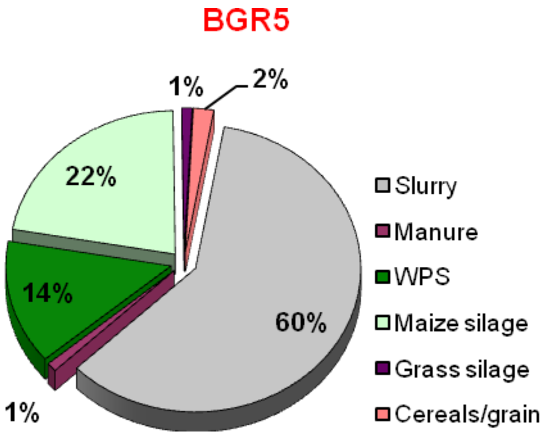
2.2 Fertilizers and soils used in the experiments

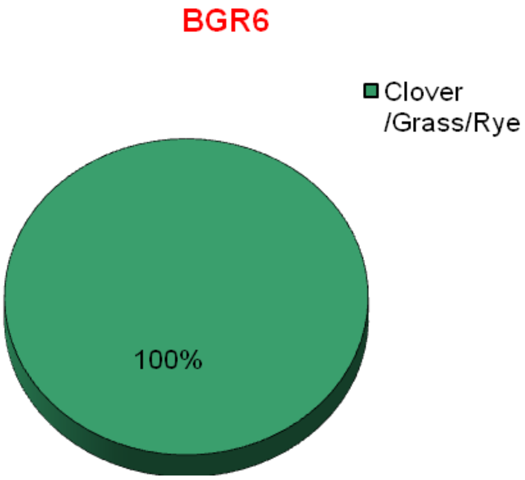
2.2.1 First experiment

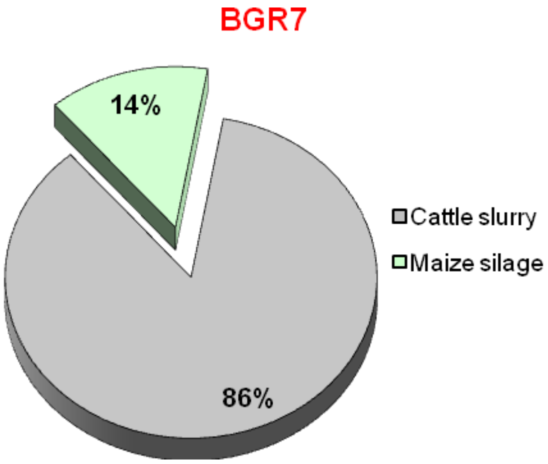
In the first experiment, seven different biogas residues were used coded (BGR1, BGR2, BGR5, BGR6, BGR7, BGR8 and BGR11) and one undigested cattle slurry coded CS. The biogas residues were collected from different biogas digesters. The biogas residues including the information on substrate composition were kindly provided by the Bavarian Research Centre for Agriculture from a research project. The composition of the substrates digested in the biogas plants differed in their composition (Fig. 1), some being exclusively plant based (BGR2, 6 and 8), others consisted of a mixture of plant material and animal excrement (pig or cattle slurry, poultry manure) (BGR 1, 5, 7 and 11). BGR7 was derived from cattle slurry (CS) used in the experiment co-digested with 14% maize silage.

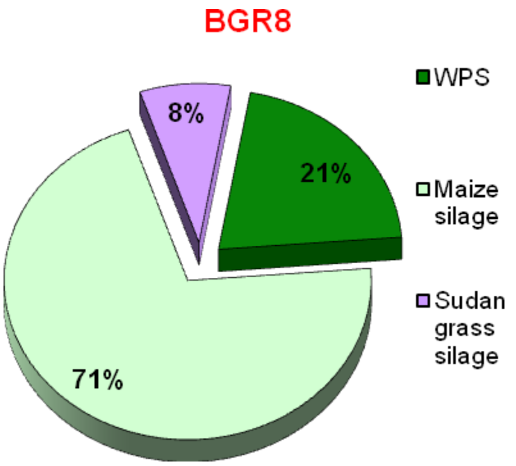

BGR1
Slurry
Poultry manure
Maize silage
48%
36%
16%


BGR2
WPS
KM
Maize silage
Straw
CCM
Grass silage
Green rye
3%
5%
1%
15%
20%
3%
53%


BGR5
Slurry
Manure
WPS
Maize silage
Grass silage
Cereals/grain
1%
2%
22%
14%
60%
1%


BGR6
Clover /Grass/Rye
100%


BGR7
Cattle slurry
Maize silage
14%
86%


BGR8
WPS
Maize silage
Sudan grass silage
8%
21%
71%

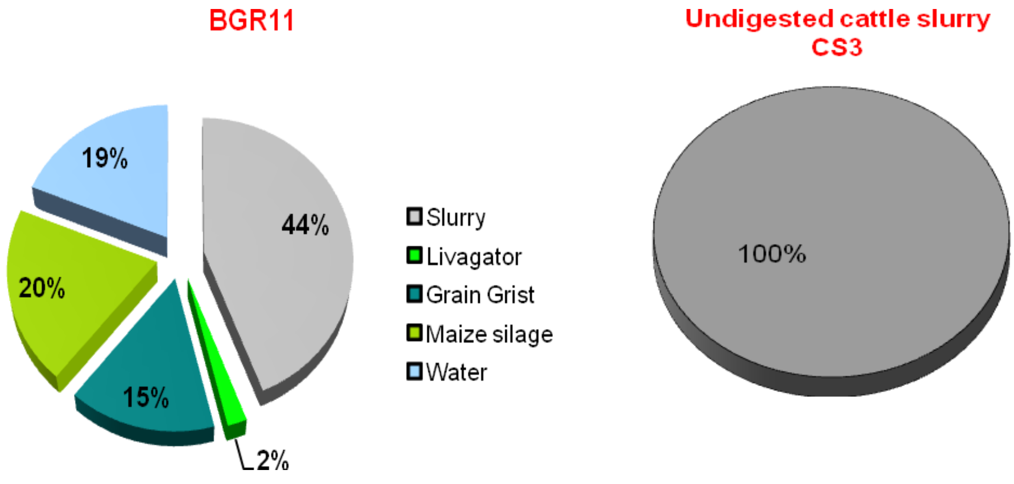


Figure 1 Composition of substrates used for the fermentation process to obtain biogas residues for the first experiment

The organic fertilizers were each tested in five soils (Tab. 3). Topsoils were collected from arable land near Freising from the locations Marktschwaben (soil 1), Dürnast (soil 2), Thalhausen (soil 3), Mintraching (soil 4) and Grünseiboldsdorf (soil 5). The soils were air-dried and sieved at 10 mm. The physico-chemical characteristics of the soils are given in Tab. 3.

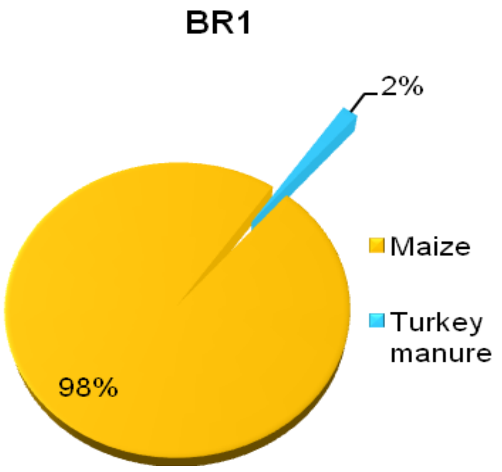
Table 3 Characteristics of the soils used in the first experiment

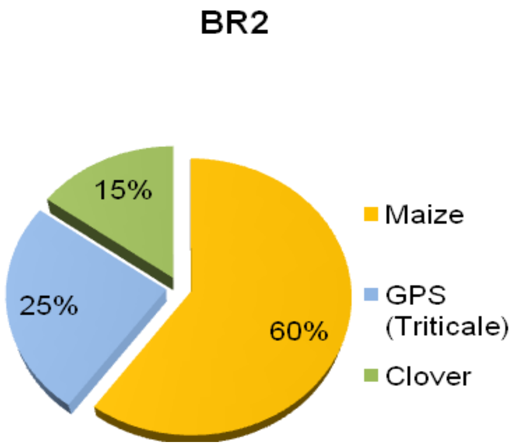
Soil name	Clay	Silt	Sand	pH	N_t	C_t	P^1	K^1	N_{min}
	(--------------	%----------------	)	$CaCl_2$	g $100g^{-1}$		mg kg^{-1}		mg pot^{-1}
Soil 1	19	73	8	6	0.21	1.9	66	192	53
Soil 2	26	57	17	6.5	0.15	1.2	83	150	57
Soil 3	6	8	86	5.3	0.06	0.41	35	66	11
Soil 4	35	26	31	7.7	0.45	4.4	288	540	288
Soil 5	21	60	19	6.6	0.20	1.7	118	282	55

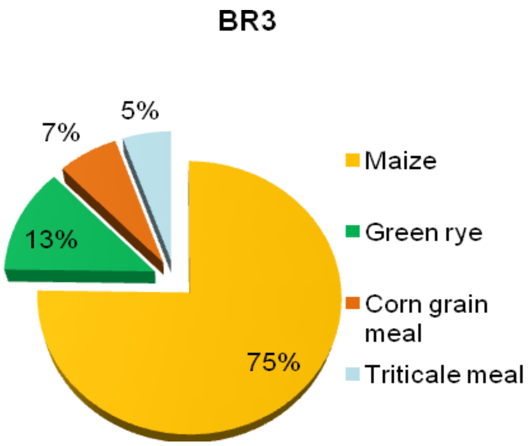
[1] soluble in Ca-acetate/Ca-lactate

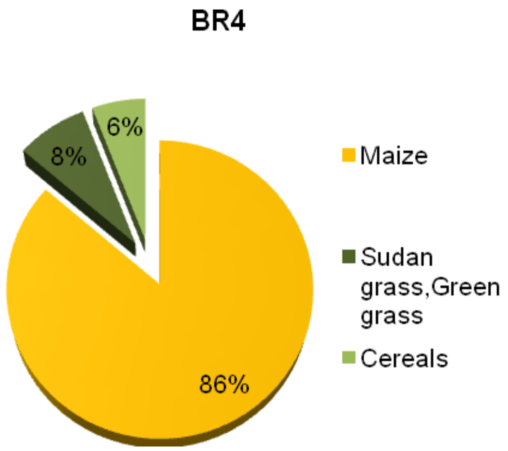
2.2.2 Second experiment

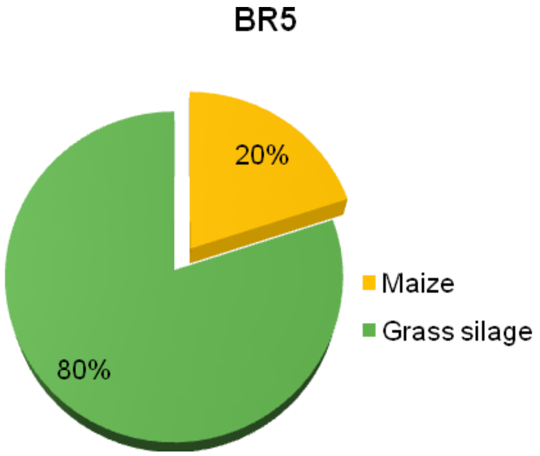
In the second experiment, biogas residues from 7 different biogas plants were separated each into a liquid and a solid fraction to obtain 14 different biogas residues (Fig. 2). In the 7 biogas digesters different substrates were used for fermentation. The composition of the substrates is shown in Fig. 2. All substrates were exclusively plant based except BGR1 and BGR7 consisting from a mixture between plant material and animal excrement, where BGR1 contained 2% turkey manure and BGR7 contained 67% poultry manure. Generally the biogas residues contained different amounts of maize silage ranging from 20% in BGR5 and 98% in BGR1.

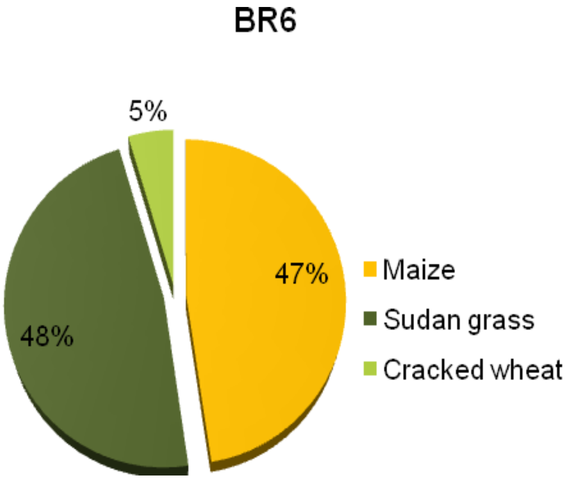

BR1
2%
Maize
Turkey manure
98%


BR2
15%
25%
60%
Maize
GPS (Triticale)
Clover


BR3
5%
7%
13%
75%
Maize
Green rye
Corn grain meal
Triticale meal


BR4
6%
8%
86%
Maize
Sudan grass,Green grass
Cereals


BR5
20%
80%
Maize
Grass silage


BR6
5%
47%
48%
Maize
Sudan grass
Cracked wheat

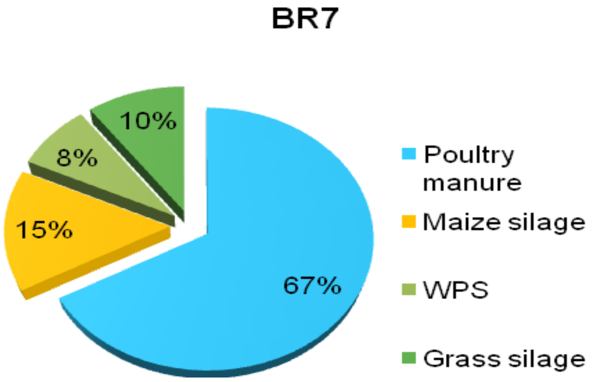


Figure 2 Composition of different substrates used for the fermentation process to obtain biogas residues for the second experiment

The biogas residues were studied in two different soils. Topsoils were collected from two different sites Mühlfeld and Dürnast near Freising in southern Germany and treated similarly like the soils in the first experiment. For the characteristics of the soils see Tab. 4.

Table 4 Characteristics of the soils used in the second experiment

Soil	Clay	Silt	Sand	pH	N_t	C_t	P^1	K^1
	(--------------	%--------------	)	$CaCl_2$	g kg^{-1}		mg kg^{-1}	
Mühlfeld	13	21	66	5.7	0.09	0.80	66	192
Dürnast	57	26	17	6.5	0.13	1.09	83	150

[1] extractable in Ca-acetate/Ca-lactate

2.3. Experimental design

Pots used in the experiments (24.5 cm length, 20.0 cm width and 24.0 cm height) received 10 kg of air dried soil. Perennial ryegrass seeds (*Lolium perenne*. L. cv. Belcampo used in the first experiment and *Lolium perenne*. L. cv. Ivanna used in the second experiment) were sown at a rate of 2.0 g/pot in two rows. Soils were regularly watered with distilled water to achieve 70% of the maximum water-holding capacity (WHC) during the experiments.

Biogas residues and cattle slurry were applied to the pots in 3 rows each located besides and between the plant rows. To avoid NH_3 losses the organic fertilizers were incorporated into the soil by slit application and immediately covered with soil. As control treatments pots with mineral fertilization (NH_4NO_3) and without fertilization were prepared. All fertilizer applications with biogas residues or cattle slurry were based on 300 mg NH_4^+-N per pot and on 300 mg N as NH_4NO_3 for the mineral treatment. Fertilizer application was done five times in both experiments, i.e. once at the beginning of each growth cycle.

For the **first experiment** altogether nine cuts of ryegrass were taken (grass was cut 3 cm above the surface of the soil). Cuts No. 1, 2 and 3 represent the first growth cycle (Σ of 1, 2 and 3 cuts), and the cuts 4 and 5 represent the second growth cycle (Σ of 4 and 5 cuts), and the cuts 8 and 9 represent the fifth growth cycle (Σ of 8 and 9 cuts). Cut 1 was taken in mid-December 2007 and cut 9 was taken in mid-October 2008. For equal experimental conditions one cut was removed prior to the first fertilization to take up the mineral N present at the beginning of the experiment. Dates for fertilizer application and cutting of ryegrass in the first experiment are given in Fig. 3. Because of the optimal soil P (> 44 mg P kg^{-1}) and K (> 120 mg K kg^{-1}) contents (see Tab. 3) only Mg (200 mg pot^{-1}) and S (260 mg pot^{-1}) were applied as basic fertilization. At the end of the experiment shoots were tested for P, K, Mg and S concentrations and were found to be in the optimal range.

The pots were placed in the greenhouse in a randomized block design (four replicates per treatment). At the end of the experiment soil samples (4 pooled cores per pot from top to bottom) were taken immediately after the last cut to be analyzed for total N, total carbon and mineral N. Mineral N was found to be less than 84 mg pot $^{-1}$ in Marktschwaben soil and 80 mg pot^{-1} in Dürnast soil, and is therefore not considered for further data interpretation.

For the **second experiment** also including five growth cycles (Fig. 4) the grass was cut 10 times during the experiment, i.e. twice within each growth cycle (stubble height 3 cm from the soil surface). Cut 1 was taken in middle of March 2010 and the last cut was taken in the first week of March 2011.

For an equal supply of K, P, Mg and S among the treatments and for balancing K, P, Mg and S applied by the biogas residues control and mineral treatments were supplied with K_2HPO_4 at rate of 500 mg K pot^{-1} corresponding to 200 mg P pot^{-1} and with $MgSO_4$ at rate of 50 mg Mg pot^{-1} and 67 mg S pot^{-1}. Moreover liquid biogas residues treatments were supplied with $CaHPO_4$ at a rate of 200 mg P pot^{-1} and with $MgSO_4$ at a rate of 50 mg Mg pot^{-1} and 67 mg S pot^{-1}.

The experiment included additional mineral treatments for the collection of roots and stubbles after each growth cycle. The pots were placed in the greenhouse in a randomized block design (three replicates per treatment). At the end of the experiment a soil sample from each pot was taken immediately after the last cut. The soil was mixed thoroughly, then sieved to 2 mm, ground and analyzed for N_t and C_t.

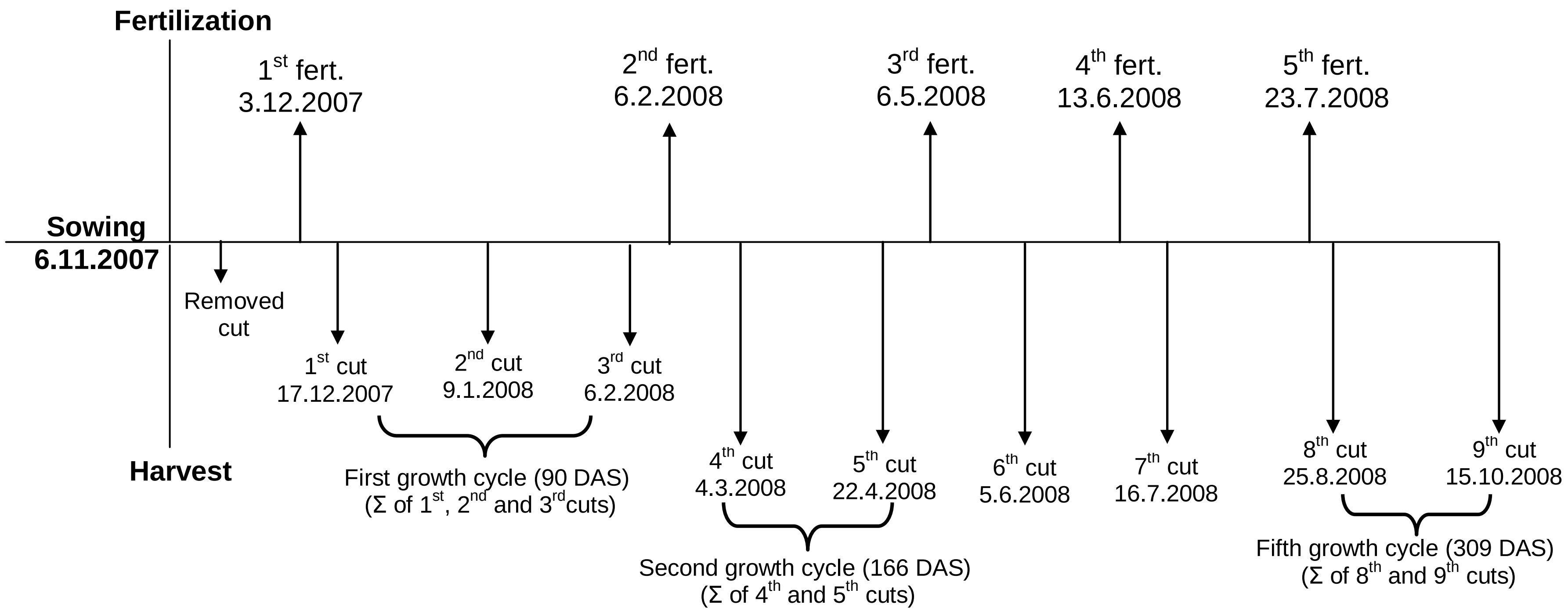


Figure 3 Time schedule of fertilization and cutting during each growth cycle of ryegrass in the first experiment

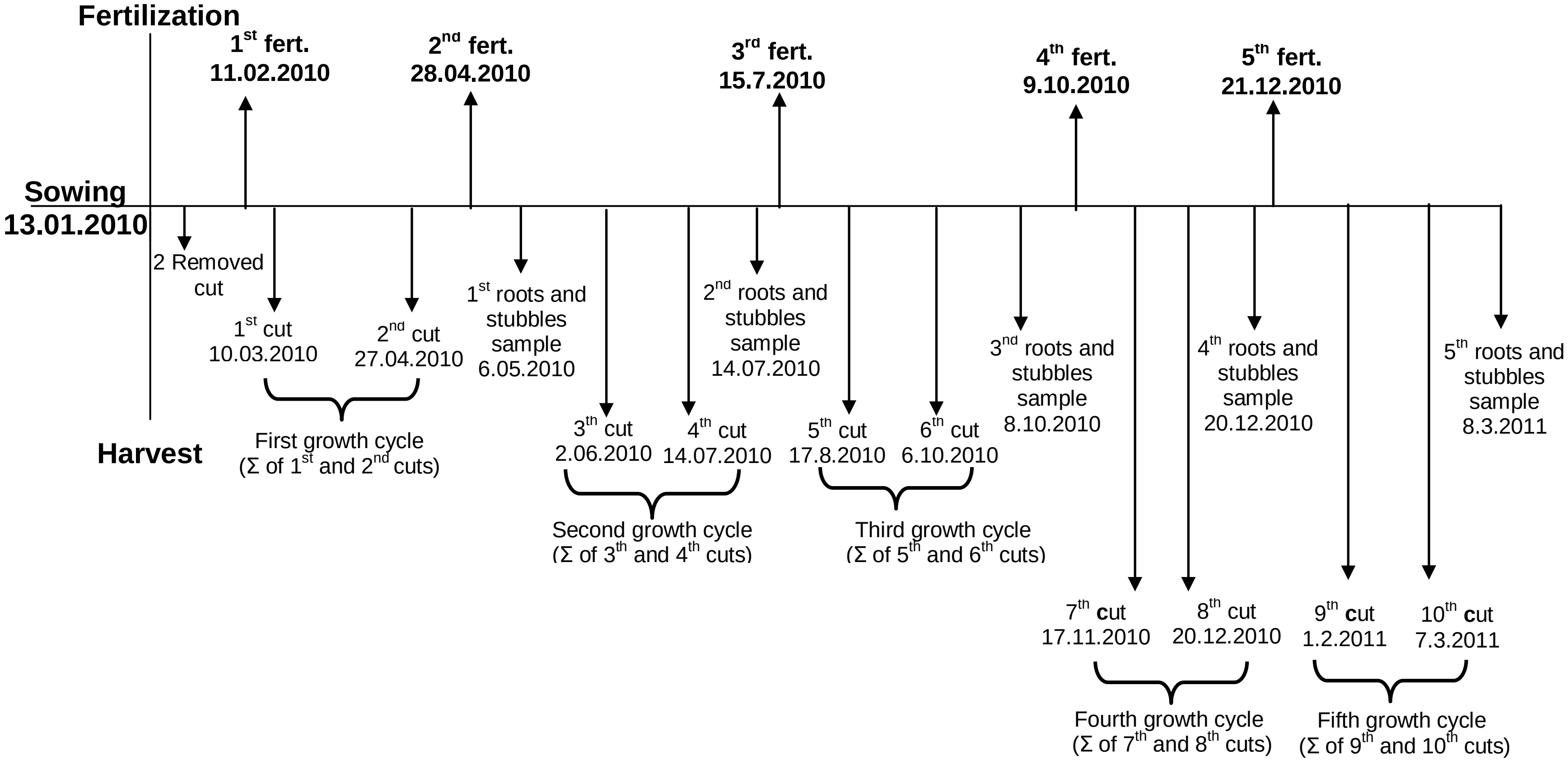


Figure 4 Time schedule of fertilization and cutting during each growth cycle of ryegrass in the second experiment

2.4 Analytical methods

2.4.1 Soil analysis

Soil texture was determined by wet-sieving for the sand fraction and by the pipette method for the silt and clay fractions *Normenausschuss Wasserwesen* (2002). The pH value was measured in 0.01 mol L^{-1} $CaCl_2$ solution (Hoffmann, 1997). C_t and N_t were analyzed following the Dumas procedure (combustion method combined with an isotopic ratio mass spectrometer (Anca SL 20-20 Crewe Europe Scientific UK)). Plant available P and K in soil were measured after extracting with 0.1 mol L^{-1} Ca acetate + 0.1 mol L^{-1} Ca lactate + 0.3 mol L^{-1} acetic acid at pH 4.1 with soil-to-extracting ratio of 1:20, shaking for 2 h (Schüller, 1969). For the soil mineral N content NO_3-N was extracted with 0.0125 mol L^{-1} $CaCl_2$ (1:2) and NH_4-N with 1 mol L^{-1} KCl (1:5). Nitrate was measured by HPLC according to Vilsmeier (1984) and ammonium according to Mulvaney (1996).

2.4.2 Plant analysis

For each cut fresh and dry matter yield were determined. Root and stubble dry matter yield were determined after each growth cycle. Dry matter yield was obtained after drying for 24 hours at 105 °C. Total N was determined after grinding of dried material by elemental analysis according to the Dumas method (combustion method combined with an isotopic ratio mass spectrometer (Anca SL 20-20 Europe Scientific, Crewe, UK)).

Plant analysis for P, K, Mg and S was conducted after wet digestion with HNO_3 and H_2O_2 in closed vessels in a microwave oven. The nutrient concentrations were measured with ICP (Liberty, Varian, Mulgrave, Australia).

2.4.3 Analysis of biogas residues

Total N and NH_4^+-N in the biogas residues were determined in fresh subsamples using the Kjeldahl technique (Vapodest 12, Gerhardt, Germany) prior to and after the digestion with H_2SO_4 conc. N_{org} content was calculated as the difference between N_t and NH_4^+-N. Dry matter was determined after drying at 105 °C. Total carbon was determined in freeze dried subsamples according to Dumas combustion method combined with an isotopic ratio mass spectrometer (Anca SL 20-20 Europe Scientific, Crewe, UK). Carbonate content was measured following the Scheibler procedure

(Schlichting et al., 1995) according to DIN 18129. C_{org} was calculated as the difference between C_t and CO_3-C.

2.5 Calculations and statistical analysis

The apparent nitrogen utilization (ANU), which refers to the mineral N component of the fertilizers (NH_4^+-N in biogas residues and slurry), was calculated as:

ANU = $(PN_F – PN_O)/N_F$
Additional N offtake = $(PN_F – PN_O)$

where
PN_F = the amount of N taken up by the plant shoots in the fertilized treatment,
PN_O = the amount of N taken up by the plant shoots in the unfertilized treatment, and
N_F = the amount of mineral N applied (i.e., as NH_4-N in the organic fertilizers).

The additional apparent nitrogen utilization of organic fertilizers (AANU) was calculated as:

AANU = $(ANU_{org} – ANU_{min}) / ANU_{min}$ *100
where ANU_{org} and ANU_{min} = the apparent nitrogen utilization of the organic fertilizer (biogas residues and cattle slurry) and mineral treatments, respectively.

Soil net mineralization rate was calculated as:
Soil net N mineralization rate, % = N offtake of an unfertilized cut/ soil N_t *100.
This rate was calculated for the last cut and the soil N_t content after the experiment and expressed per day. For comparison with data from the literature it was expressed as % per year.
PASW version 17.0 (PASW Inc., Chicago, IL) and Microsoft Excel 2007 were used for statistical analysis. The results of the pot experiments were subjected to one- or two-way analyses of variance with the significance of the means tested with a Tukey, HSD-test at $P \leq 0.05$. Correlation analyses were conducted to determine the relationship between C_{org}/N_{org} in biogas residues or soil N content after the experiment and N offtake.

3. Results

3.1 Chemical properties of unseparated biogas residues and cattle slurry

The biogas residues obtained from the biogas digesters after the fermentation of different raw materials showed distinct differences in the chemical composition (Tab. 5). Compared to CS with 12.1%, the dry matter content of the biogas residues were similar or lower ranging from 5.2 to 12.5%. The NH_4^+-N contents were comparable (BGR5 and 7) or markedly higher (0.49 to 0.51% in BGR11 and 1) than in CS with 0.22%. The total N contents ranged from 0.36 to 0.75%. Part of the total C (ranging from 2.0 to 4.74%) was detected as CO_3-C (0.20 to 0.48%). As a result from both varying C_{org} and N_{org} contents the C_{org}/N_{org} ratio ranged from 8.6 (BGR1) to 13.4 (BGR7) in the biogas residues which is lower than in CS. Both the P and K contents varied by the factor of about 3 in all organic fertilizers (data not shown).

Table 5 Chemical composition of the biogas residues obtained from different raw materials and cattle slurry

Treatments	**Dry matter**	**pH**	**N_t**	**NH_4-N**	**C_t**	**CO_3-C**	**C_{org}**	**N_{org}**	**C_{org}/N_{org}**	**NH_4-N/N_t**
			% in FM							
BGR 1	6.3	8.4	0.75	0.51	2.27	0.20	2.07	0.24	8.6	0.68
BGR 2	6.8	8.2	0.49	0.29	2.89	0.32	2.57	0.20	12.9	0.60
BGR 5	5.7	8.1	0.42	0.25	2.16	0.28	1.88	0.17	11.1	0.60
BGR 6	12.5	8.3	0.72	0.37	4.74	0.26	4.48	0.34	13.2	0.52
BGR 7	7.4	8.2	0.36	0.2	2.49	0.34	2.15	0.16	13.4	0.55
BGR 8	5.2	8.2	0.48	0.27	2.00	0.23	1.77	0.21	8.4	0.57
BGR 11	6.1	8.3	0.7	0.49	2.45	0.48	1.97	0.22	9.0	0.69
CS	12.1	7.9	0.44	0.22	4.41	0.14	4.27	0.23	18.6	0.49

% N_{org} % = N $_{total}$ % – NH_4-N%; C_{org} % = C $_{total}$ % – CO_3-C%

3.2 Yield and N availability of seven different unseparated biogas residues and cattle slurry as tested in two soils

3.2.1. Shoot dry matter yield

The biogas residues and cattle slurry application increased the shoot dry matter yield of ryegrass over the five growth cycles in comparison with the control. In Marktschwaben soil, the increase in dry matter yield in the first growth cycle ranged from 16.1 g/pot (BGR2) to 18.4 g/pot (BGR8), while in Dürnast soil it ranged from 13.1 g/pot (CS) to 15.6 g/pot (BGR1). In the different treatments, including control, shoot dry matter yield was high in the second and fifth growth cycle in comparison to the first growth cycle; this is clear in both soils (Fig. 5).

In the first and the second growth cycle, the fertilized treatments were significantly higher in the dry matter yield than the unfertilized treatment (control), but there was no significant difference observed between the biogas residues and the mineral fertilizer in both soils.

In the fifth growth cycle, the organic fertilizers treatments (CS, BGR5, 7 and BGR8) in the Marktschwaben soil were significantly higher in the dry matter yield than the mineral fertilizer. In the fifth growth cycle, the organic fertilizers treatments in Dürnast soil were significantly higher in the dry matter yield than the mineral treatment, except with BGR2 was significantly lower than the mineral treatment.

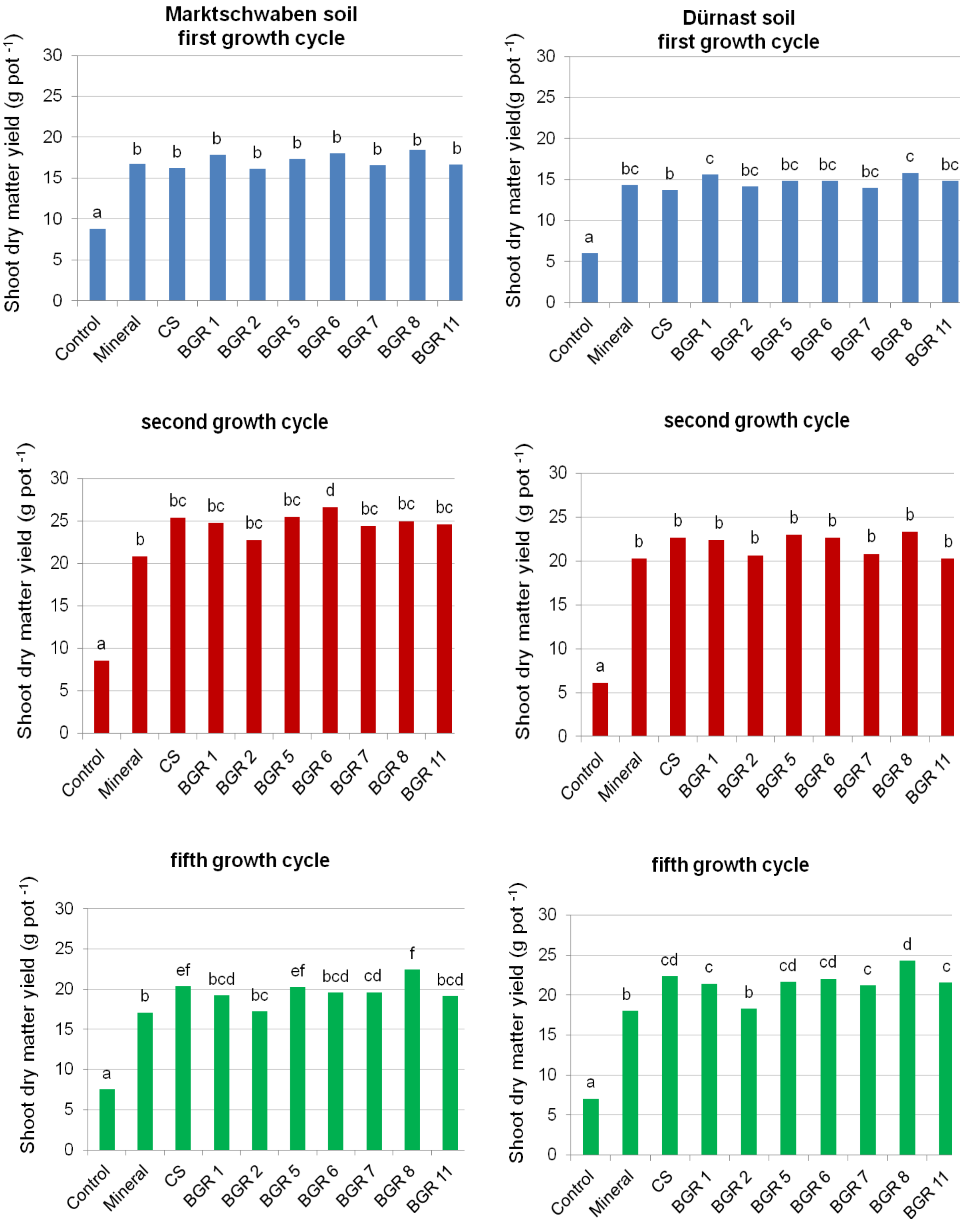


Figure 5 Shoot dry matter yield of different biogas residues and cattle slurry collected in the growth cycles 1, 2 and 5 in two different soils. Different letters indicate statistically significant differences between the fertilizers ($p \leq 0.05$)

3.2.3 Shoot N content

The whole biogas residues and cattle slurry had a large effect on the shoot nitrogen content of the ryegrass, however it reached up to 4.5 and 4.0% in the first and fourth cut (fertilized cuts), respectively in both soils (Fig. 6). Shoot N content was generally higher in the fertilized cuts (first and fourth) than the unfertilized cuts (second, third and fifth) and this is clear with the first and the second growth cycles in both soils (short term fertilizers application). Shoot N content of the fertilized treatments in the unfertilized cuts was decreased to half in the second and third cuts compared to the first cut.

After five times of fertilizer application (long term of fertilizers application) this was not the case with the eight cut after the fifth fertilizer application, where the grass N content was lower in the eight cut compared to the ninth cut. Also after the fifth fertilizer application (eighth cut), shoot N content was decreased to half in comparison to the shoot N content in the first and second fertilizer application (first and fourth cuts).

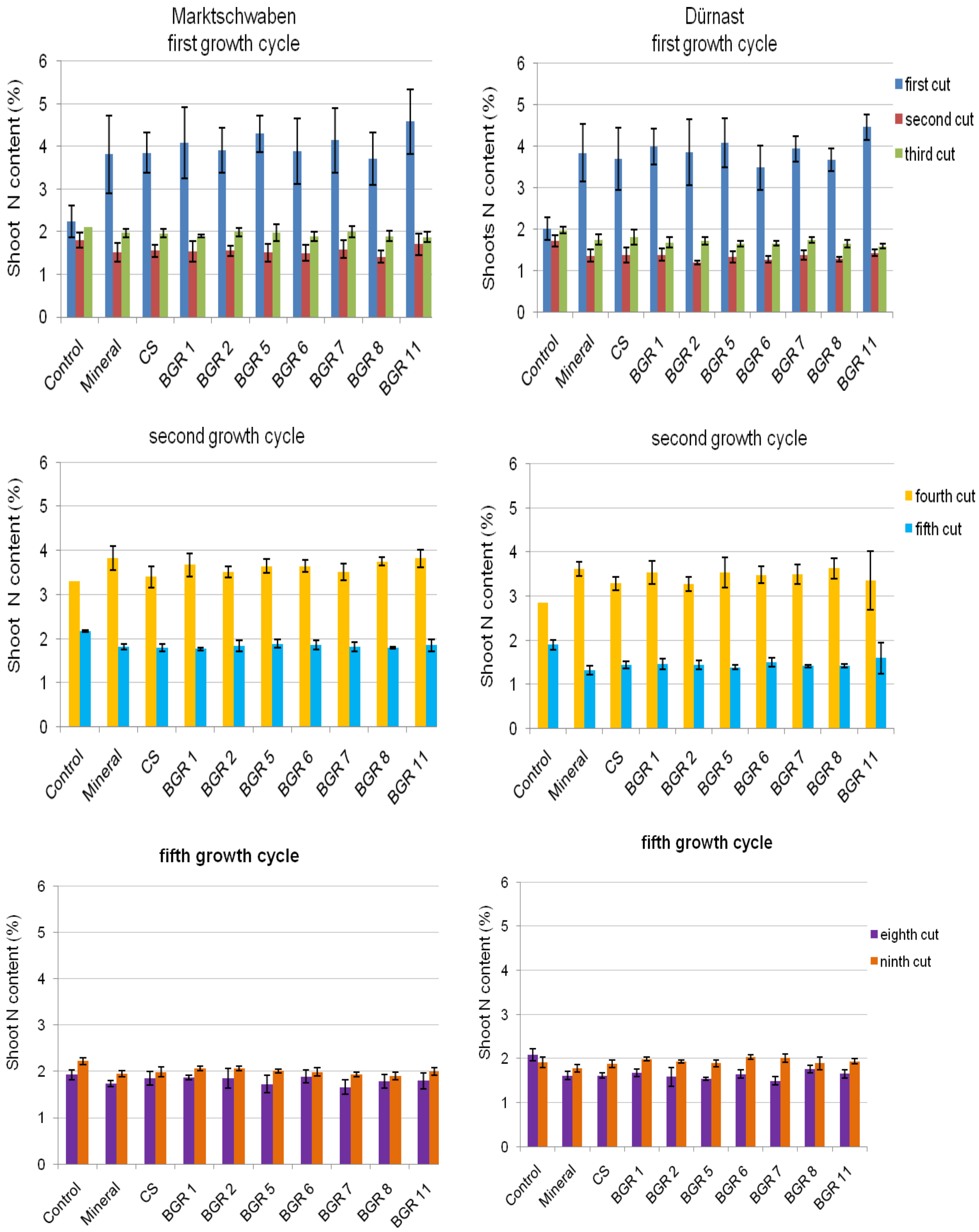


Figure 6 Shoot N content of different biogas residues and cattle slurry collected in the growth cycles 1, 2 and 5. in two different soils. Error bars represent standard deviation

3.2.4 Shoot N offtake

Shoot N offtake from all organic fertilizers, biogas residues and cattle slurry, was generally comparable or higher than that from the mineral fertilizer in both soils (Fig. 7). However, there were significant differences in the N offtake between the different organic fertilizers.

In the first growth cycle the N offtake from all biogas residues except BGR2 was significantly higher compared to cattle slurry in the soil Marktschwaben. In soil Dürnast only BGR1, 5, 8 and 11 yielded a higher N offtake compared to CS. Hence an increase in the N offtake from cattle slurry co-fermented with maize (BGR7) compared to undigested cattle slurry was found only in the soil Marktschwaben.

In the second growth cycle both the differences among the organic fertilizers and between the organic fertilizers and the mineral treatment were higher in the soil Marktschwaben than in the soil Dürnast. In the soil Marktschwaben all biogas residues except BGR2 and BGR 7 resulted in a significantly higher N offtake to mineral N.

In the fifth growth cycle the N offtake from all organic fertilizers except BGR2 significantly exceeded that from mineral N in both soils. In this growth cycle a significantly higher N offtake was obtained from the undigested cattle slurry compared to BGR7.

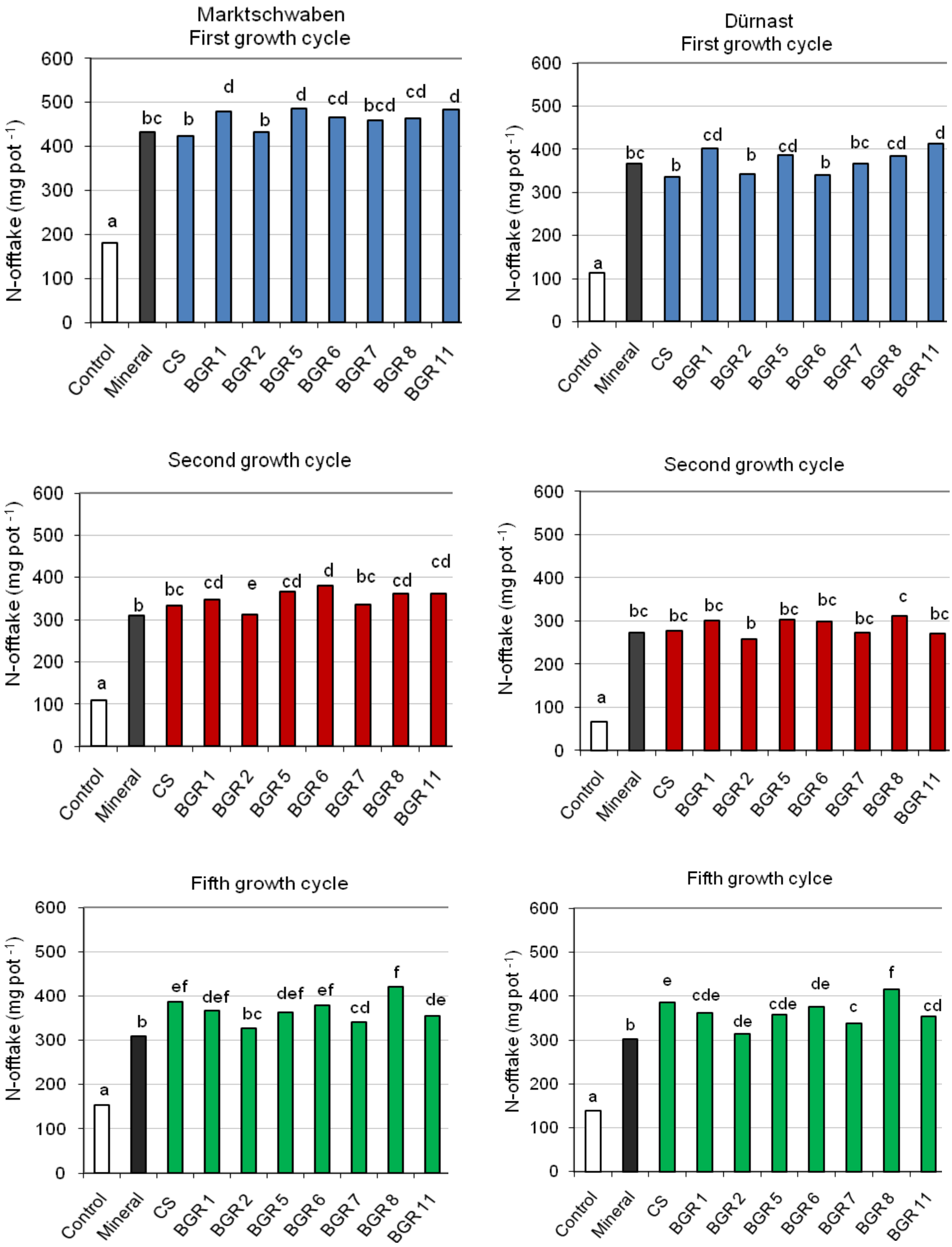


Figure 7 Shoot N offtake of different biogas residues and cattle slurry collected in growth cycles 1, 2 and 5 in two different soils. Different letters indicate statistically significant differences between the fertilizers ($p \leq 0.05$)

3.2.5 Apparent N utilization

The apparent N utilization (ANU) calculated on the basis of applied NH_4-N for the organic fertilizers and on NH_4NO_3 for the mineral N ranged between about 80% (mineral, CS and BGR2) up to about 100% (BGR1, 5 and 11 in soil Marktschwaben and BGR 11 in soil Dürnast) in the first growth cycle (Fig. 8).

In the second and fifth growth cycle ANU differed between about 70% (mineral treatment and BGR2) to 80 - 90% for most of the biogas residues or between about 50-60% (mineral treatment and BGR2) up to about 90% (i.e. BGR8) respectively. In consequence with some exceptions ANU seemed to decrease from the first to the fifth growth cycle.

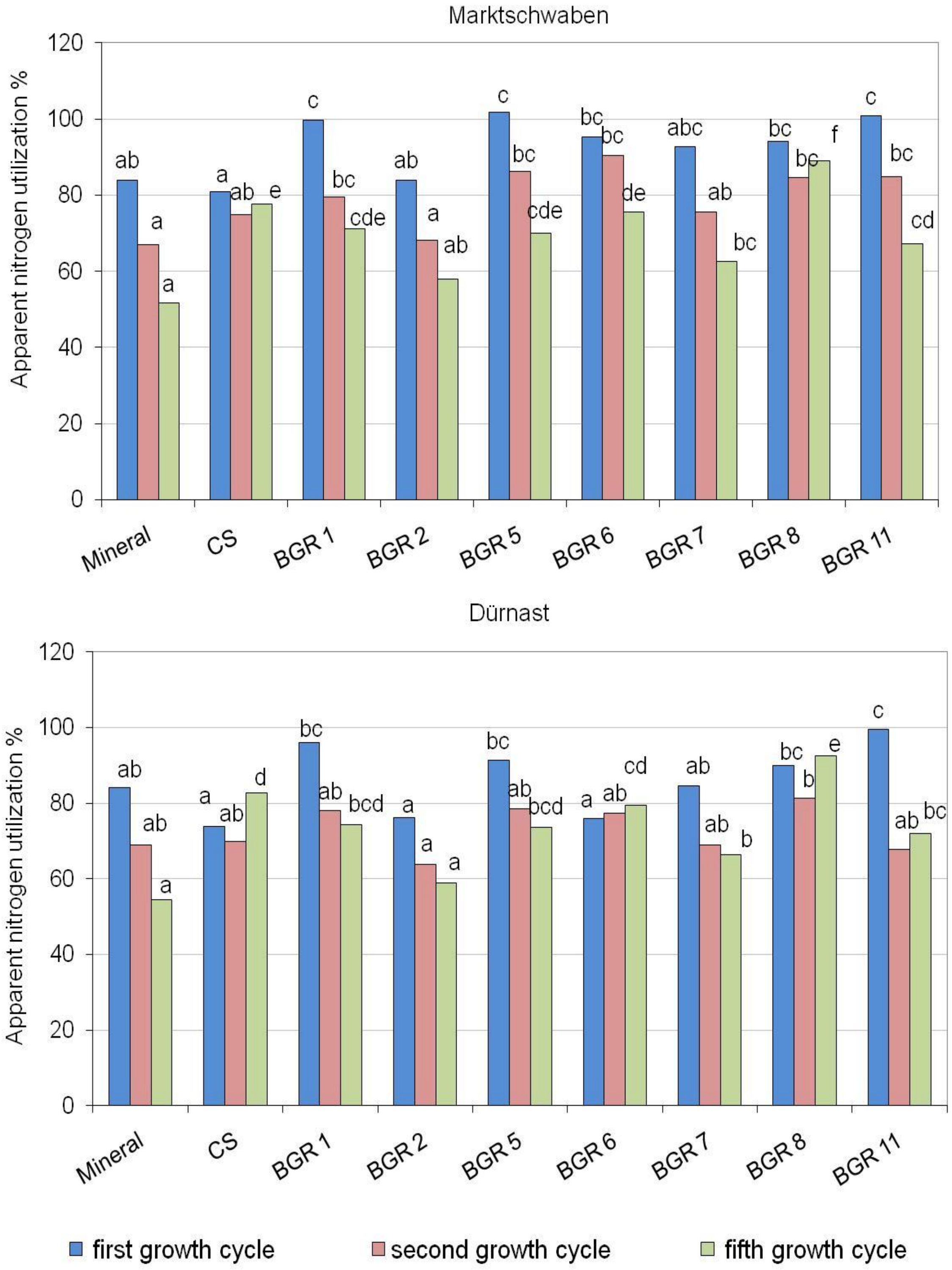


Figure 8 Apparent N utilization of biogas residues and cattle slurry collected in growth cycles 1, 2 and 5 in the two different soils. *Different letters indicate statistically significant differences between the fertilizers of one growth cycle ($p \leq 0.05$)*

3.2.6. Additional apparent nitrogen utilization

Because for the mineral fertilizer ANU decreased across the growth cycles, the ANU of the organic fertilizers was related to that of the mineral fertilizer in each respective growth cycle. Such a relationship compensates for any differences in plant growth caused by, for example, by different climatic conditions during the growing season and enables the evaluation of the possible changes in the ANU of the organic fertilizers as a consequence of repeated fertilizer application. The additional ANU, therefore, is a sensitive measure for the additional net nitrogen release from the organic fraction of the fertilizers that becomes available to the plant in addition to that from the applied ammonium (Fig 9).

In the first growth cycle, up to 20% more N (e.g. for BGR11) was taken up by the rye grass leaves from the organic fertilizers in both soils in addition to that from the supplied mineral N. However, no additional N utilization was found for BGR2 and CS, and for BGR2, 6, 7 and CS in the Marktschwaben and Dürnast soils, respectively. AANU increased significantly by the repeated application of the organic fertilizers, ranging from up to 20% in the first growth cycle (one application) to up to 70% in the fifth growth cycle (five fertilizer applications). The greatest increase in AANU from the first to the fifth growth cycles was observed for BGR8, CS and BGR6. By contrast, both the final AANU in the fifth growth cycle as well as the increase compared to the value in the first growth cycle was relatively low for BGR11, 7 and 2.

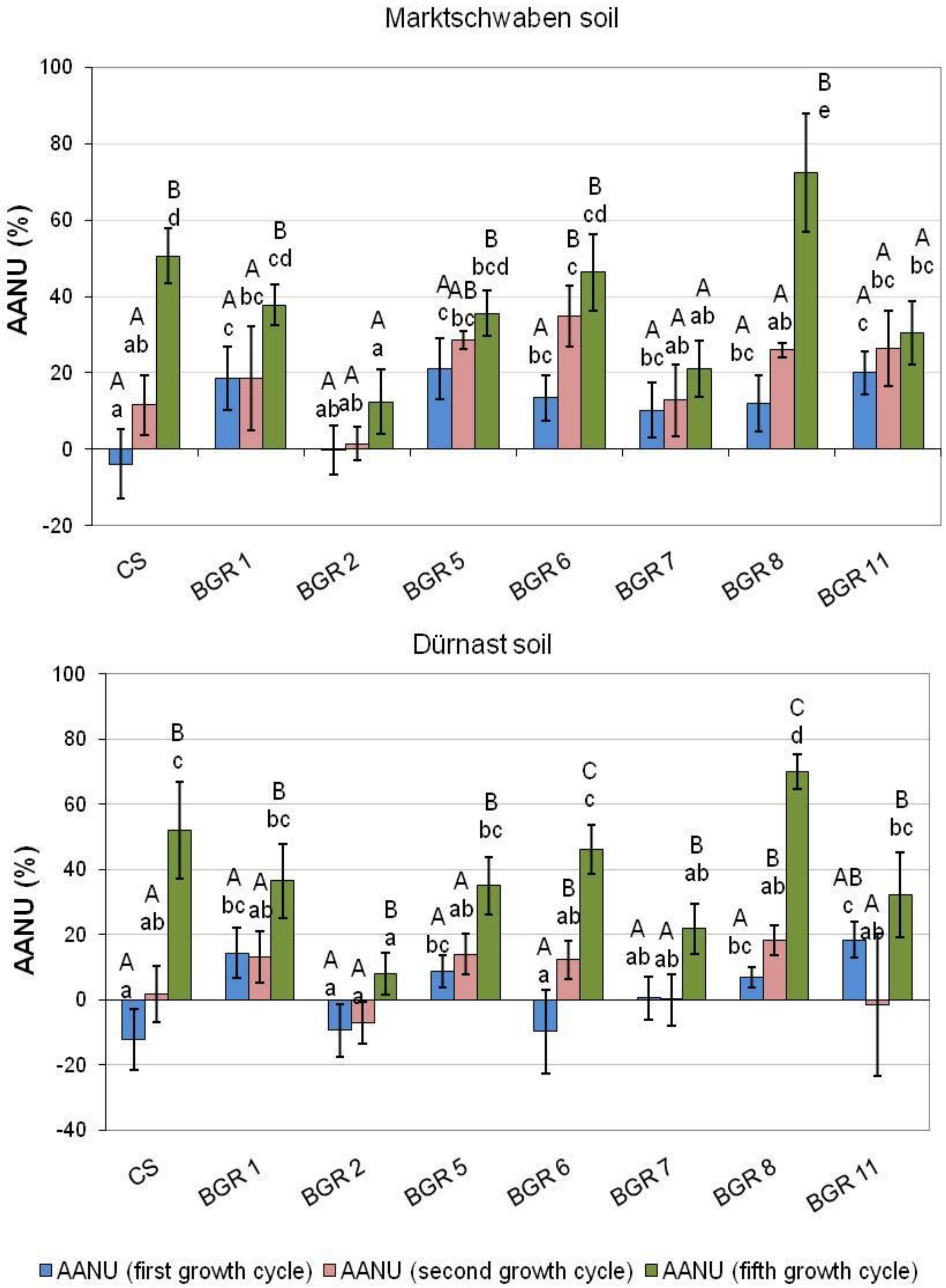


Figure 9 Additional apparent N utilization of the organic fertilizers collected in growth cycles 1, 2 and 5 in two different soils. Different lower case letters indicate statistically significant differences between the fertilizers within one growth cycle ($p \leq 0.05$). Different capital letters indicate statistically significant differences between the first, second and fifth growth cycle for one organic fertilizer ($p \leq 0.05$)

3.2.7 Soil nitrogen and carbon contents

The five-fold repeated application of fertilizer across the duration of the experiment changed soil N_t and C_t contents differently depending on the fertilizer applied (Tab. 6). In both soils, repeated application of BGR 6 and 8 resulted in significantly higher N_t and C_t soil accumulation compared to BGR 1 and 11; the value for the latter not being significantly different from that for the mineral fertilizer application. High soil N_t and C_t content were also observed for CS, with values being comparable to those with BGR 6 and 8. BGR 2, 5 and 7 comprise an intermediate group characterized by moderate N_t and C_t accumulation although the soil C_t content of BGR 7 was in the range of BGR 6 and 8. Generally, the soil levels of N_t and C_t were lower in the Dürnast soil compared to the Marktschwaben soil.

Differences in the final soil N_t contents were correlated with the different amounts of organic N input from the organic fertilizers (r = 0.81* ($p \le 0.05$) and r = 0.93** ($p \le 0.01$) in the Marktschwaben and Dürnast soils, respectively). No significant relationship was observed between the organic C input and the final soil C_t content.

Table 6 Soil contents of total nitrogen and total carbon at the end of the experiment

Treatments	**Marktschwaben soil**		**Dürnast soil**	
	^1N_t%	**^2C_t%**	**^3N_t%**	**^4C_t%**
Control	0.19^{a}	1.84^{a}	0.13^{a}	1.17^{a}
Mineral	0.20^{ab}	1.90^{ab}	0.14^{ab}	1.24^{a}
CS	0.21^{cd}	2.13^{d}	0.18^{e}	1.63^{f}
BGR1	0.20^{bc}	1.97^{bc}	0.15^{bc}	1.31^{abc}
BGR2	0.21^{cd}	2.05^{cd}	0.16^{cd}	1.44^{bcd}
BGR5	0.21^{cd}	2.05^{cd}	0.16^{cd}	1.42^{bcd}
BGR6	0.22^{d}	2.13^{d}	0.17^{de}	1.48^{def}
BGR7	0.21^{cd}	2.10^{d}	0.16^{cd}	1.53^{ef}
BGR8	0.22^{d}	2.13^{d}	0.17^{de}	1.54^{ef}
BGR11	0.20^{bc}	1.96^{bc}	0.15^{bc}	1.30^{ab}

Content before fertilizer application: ^1N_t = 0.20, ^2C_t = 1.90, ^3N_t = 0.14, ^4C_t = 1.19. Different letters indicate statistically significant differences between the fertilizers for either N_t or C_t for a given soil ($p \le 0.05$)

3.2.8 Correlations between shoot N offtake and C and N in organic fertilizers and soils

With the aim to evaluate relations between the plant N offtake and properties of the organic fertilizers correlations were calculated with the C_{org}:N_{org} of the organic fertilizers. The N offtake of the ryegrass showed a significant negative correlation to the C_{org}:N_{org} of the organic fertilizers after the first growth cycle in both soils (Tab. 7). However, for the second and fifth growth cycle i.e. after the second and the fifth fertilizer application in none of the soils any significant correlation was found between N offtake and C_{org}:N_{org} of the organic fertilizers. It seems that digestion of the cattle slurry increased the N uptake (which is higher in C_{org}: N_{org}) and was conducive to more mineralization compared to BGR7 and consequently gave a higher N offtake (Fig. 10).

Table 7 Correlations between shoot N offtake in the 1st, 2nd and 5th growth cycle and C_{org}: N_{org} ratios of the organic fertilizers

Soil	Growth cycle	r
Marktschwaben soil	**first**	**-0.781***
	second	**-0.410 NS**
	fifth	**-0.105 NS**
Dürnast soil	**first**	**-0.833****
	second	**-0.424 NS**
	fifth	**-0.071 NS**

Significance levels: *(5%); ** (1%); NS (not significant)

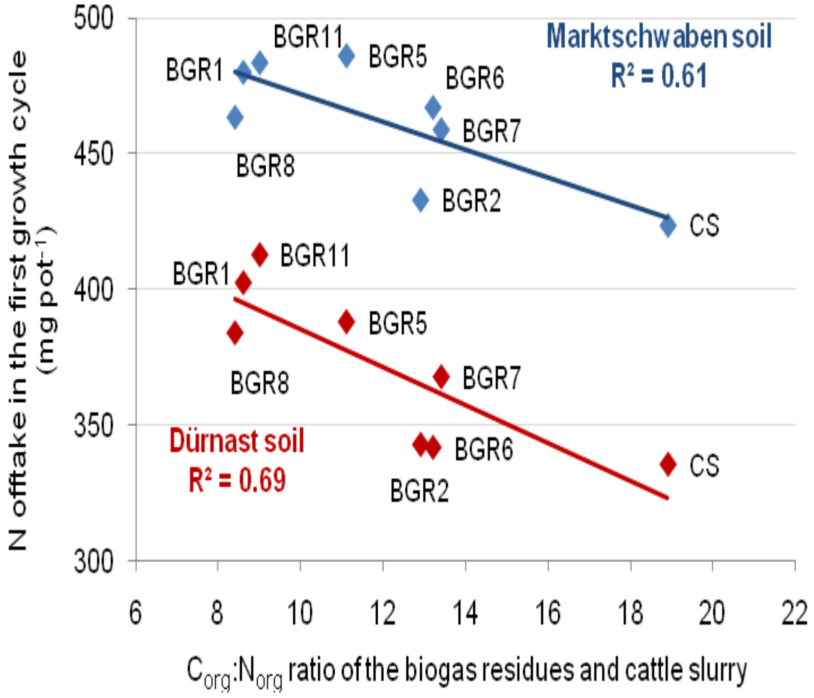


Figure 10 Relationship between C_{org}:N_{org} of unseparated biogas residues and cattle slurry and the N offtake in the first growth cycle

After five repeated applications, however shoot N offtake from the fertilizers was significantly correlated to the soil total N content at the end of the experiment (Tab. 8). This was observed for the ryegrass N offtake of the whole fifth growth cycle but was most prominent in the last, unfertilized cut where correlation coefficients of 0.83** (Marktschwaben soil) and 0.80** (Dürnast soil) between the N offtake of ryegrass and soil total N content were found.

Table 8 Correlation coefficients between soil N contents at the end of the experiment and shoot N offtake (last unfertilized biomass harvest and fifth growth cycle)

N content in soil	Shoot N offtake (fifth growth cycle)	Shoot N offtake (last cut)
Marktschwaben	0.72*	0.83**
Dürnast	0.70*	0.80**

Significance level: * (5%) and ** (1%)

3.3 Yield and N availability of ryegrass from three unseparated biogas residues as tested in five soils

3.3.1 Nitrogen offtake of unfertilized soils

The N offtake in plants from unfertilized soils can be used as indicator to describe the N mineralization of organic nitrogen in soils. The N offtake of unfertilized soils ranged between about 40 to 340 mg N/pot and differed between the soils (Fig. 11). In all growth cycles the highest N offtake was observed in soil 4 followed by soil 5, 1 and 2. Soil 3 showed the lowest N offtake. The N offtake from the different soils was an indication of the soil total N content which was highest in soil 4 and lowest in soil 3 see Tab. 3.

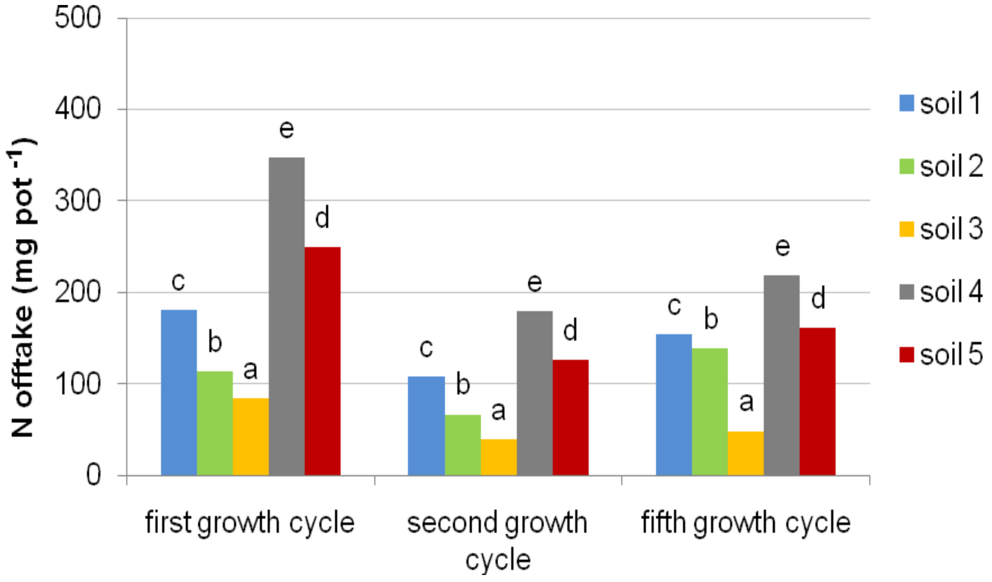


Figure 11 N offtake of unfertilized treatments in five different soils collected in the 1st, 2nd and 5th growth cycles. Different letters indicate statistically significant differences between the soils within one growth cycle ($p \leq 0.05$)

3.3.2 Shoot dry matter yield

Application of N either as mineral fertilizer or biogas residues, in the different soils increased the ryegrass dry matter yield (Fig. 12). In the first growth cycle (after one fertilizer application), dry matter yield of the fertilized treatments was significantly higher compared to the unfertilized treatments. In the first growth cycle, there was no significant difference in the dry matter yield between the biogas residues and the mineral treatment in soil 2 and soil 3, but in soil 1, 4 and 5 only BGR8 was significantly higher compared to the inorganic treatment.

In the second and the fifth growth cycle, ryegrass dry matter yield of the different biogas residue treatments were significantly greater compared with the inorganic treatment for soil 1 and 2. However in soil 3 the yield from either BGR6 or BGR8 was significantly higher than from the mineral fertilizer. For soil 5, only BGR8 resulted in a significantly higher yield than from the inorganic fertilizer treatment.

3.3.3 Shoot N content

Treatments which received fertilizers showed considerably higher N content compared to the unfertilized control, particularly after the first applications of fertilizers. The shoot N concentration was higher in both cuts after the fertilization (first and fourth cuts) compared to the unfertilized cuts (second, third and fifth cut) but this is not true for the eighth cut (Fig. 13).

After the fifth application of fertilizers, the eighth cut had a lower N content than the ninth cut. In addition, for the eighth cut the N content dropped to half after the fifth fertilizer application compared to the first and fourth cuts (fertilized cuts). Different soils had similar effects on N content in all growth cycles.

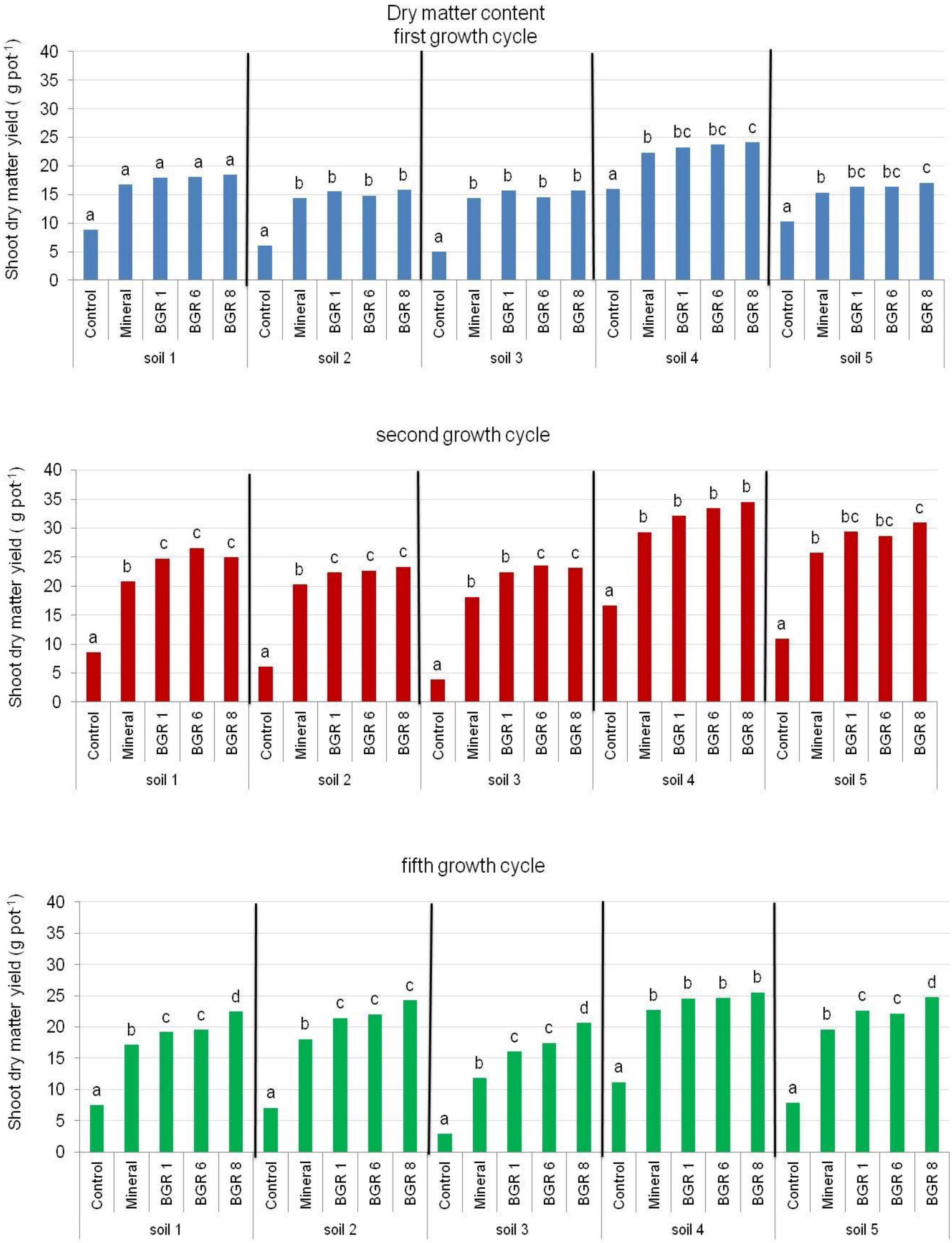


Figure 12 Shoot dry matter content of different biogas residues collected in growth cycles 1, 2 and 5 in five different soils. Different letters indicate statistically significant differences between the fertilizers ($p \leq 0.05$)

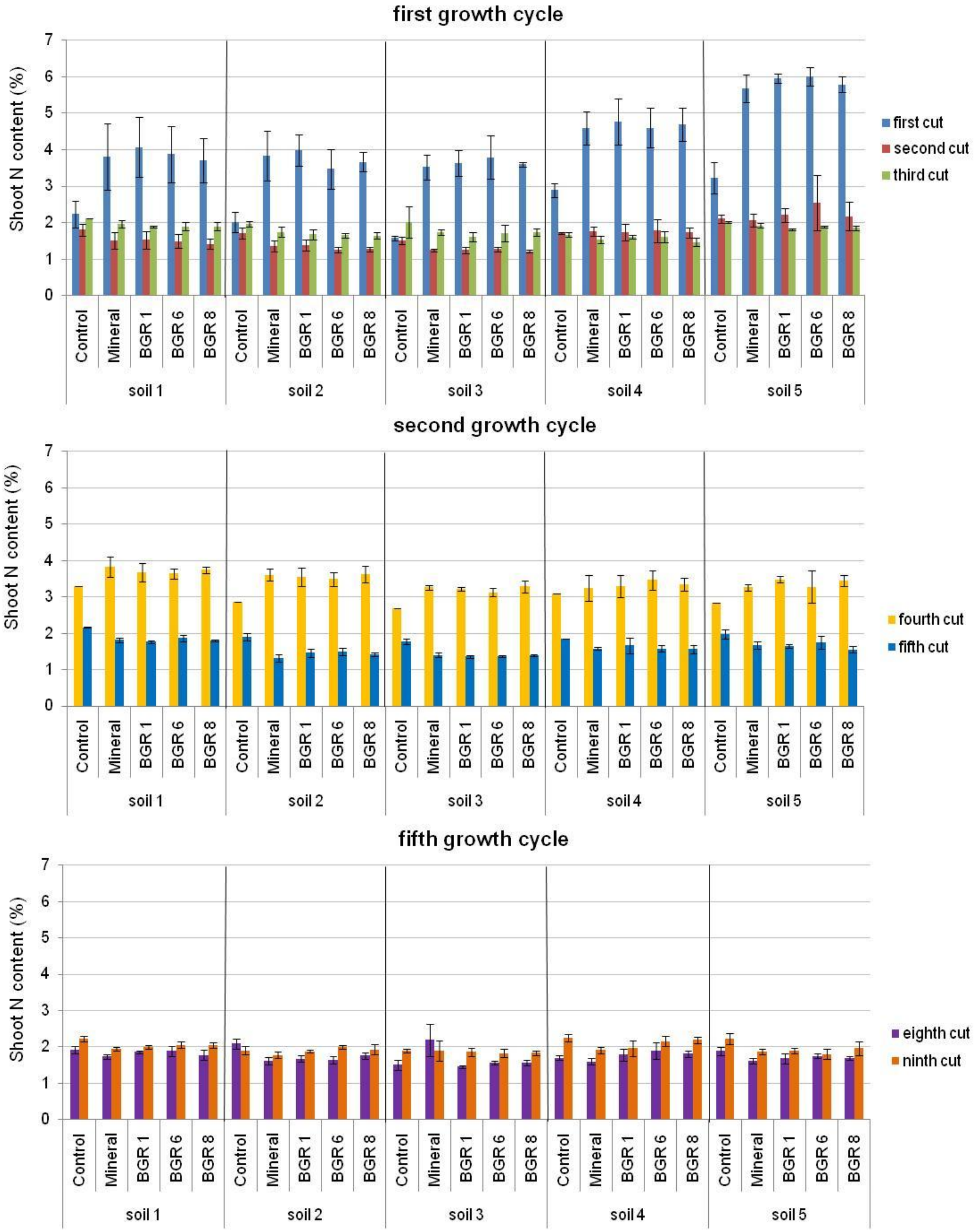


Figure 13 Shoot N content of different biogas residues collected in the growth cycles 1, 2 and 5 in five different soils. Error bars represent standard deviation

3.3.4 Shoot addtional N offtake

Statistical analysis of the effects of different soils and mineral or organic fertilizers applied (Tab. 9) showed that for all growth cycles there were significant effects for both the soil-factor and the fertilizer-factor on the N offtake of ryegrass but there were no significant interactions between soil and fertilizer.

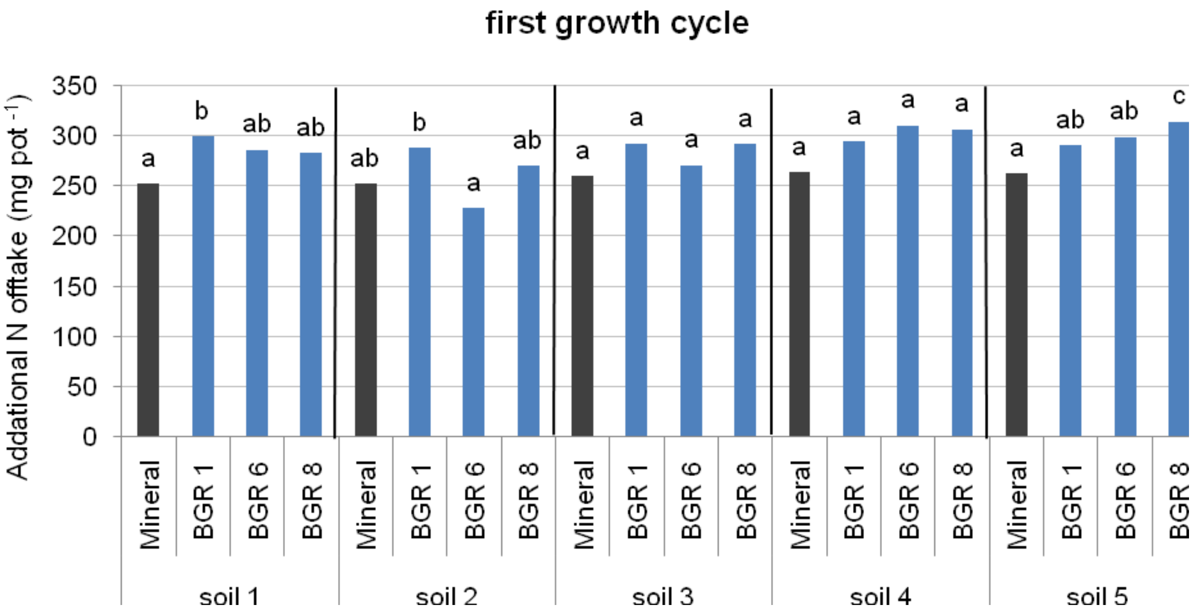
The effect of the soil-factor, averaged over all fertilizers was inconsistent and varied from one growth cycle to another; i.e. the lowest addtional N offtake in the first growth cycle was observed in soil 2 whereas in the second growth cycle this was true for soil 4; and in the fifth growth cycle it was true for soil 1 (Fig. 14).

In each growth cycle the additional N offtake from the biogas residues, averaged over all soils, was significantly higher than that from the mineral fertilizer and this effect seemed to increase with time (see also Tab. 9). While in the first and second growth cycle no differences appeared between the biogas residues, in the fifth growth cycle the additional N offtake increased significantly from biogas residue 1 to 6 and 8.

Table 9 F-statistics of two-way ANOVA of the effects of soils (1, 2, 3, 4 and 5) and fertilizer sources (mineral, BGR1, BGR6 and BGR8)

ANOVA main effects+ interactions	1st growth cycle			2nd growth cycle			5th growth cycle		
	DF	F value	Significance	DF	F value	Significance	DF	F value	significance
Soil	4	4.98	0.002*	4	8.31	0.000***	4	2.58	0.046*
Fertilizer	3	9.28	0.000***	3	30.45	0.000***	3	104.0	0.000***
Soil × Fertilizer	12	1.48	0.157 NS	12	1.05	0.420 NS	12	1.548	.132 NS

Significant level: * $P \leq 0.05$. * $P \leq 0.001$. * $P \leq 0.0001$



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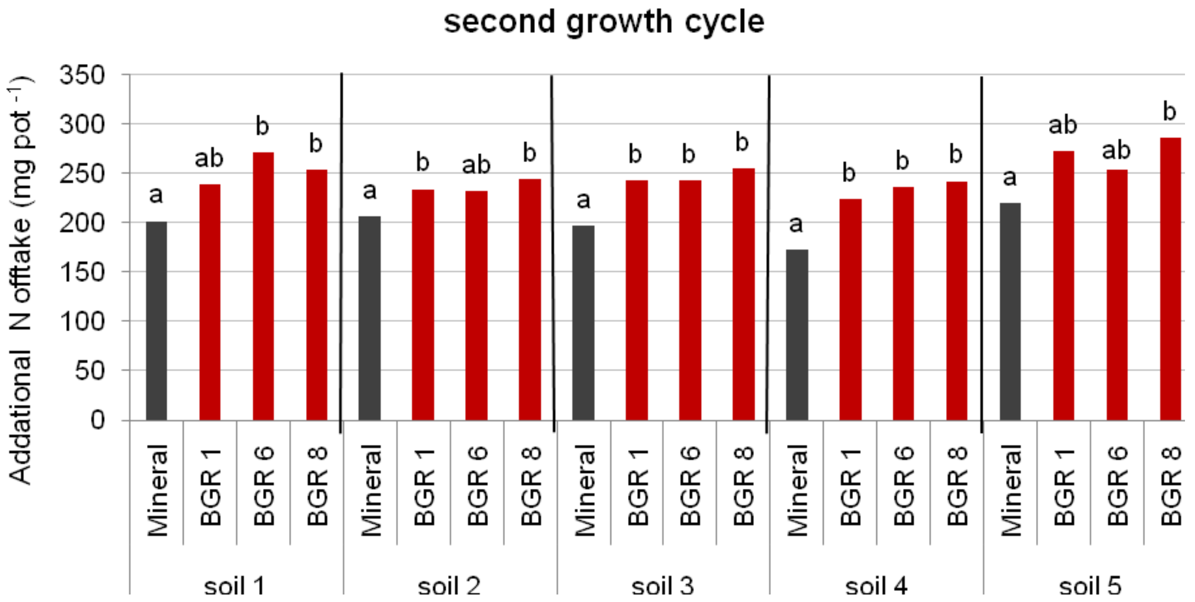

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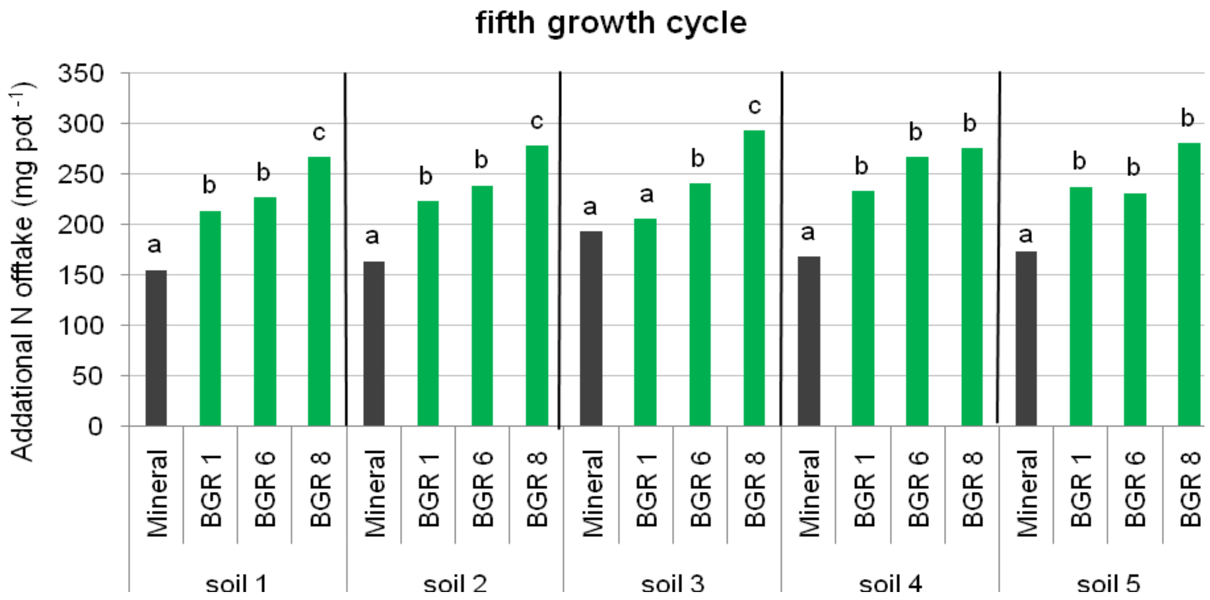


Main factor soil	**A**	**AB**	**AB**	**B**	**AB**			
Main factor fertilizers					**A**	**B**	**C**	**D**

Figure 14 Shoot addtional N offtake of biogas residues collected in the growth cycles 1, 2 and 5 in five different soils. Shoot N offtake in unfertilized treatments was subtracted for each soil respectively. Different lower case letters indicate statistically significant differences between the fertilizers within one soil ($p \geq 0.05$)

3.3.5 Additional apparent N utilization

In order to evaluate the biogas residues for the amount of N that was plant available additionally to the applied ammonium the additional N offtake was calculated as the difference in N offtake between the organic fertilizers and the mineral fertilizer for each soil related to the N offtake of the mineral fertilizer. This procedure also allows comparing different soils and growth cycles irrespective of different levels of N offtake achieved from the mineral fertilizer.

In the first growth cycle the additional apparent N utilization (AANU) for all soils and fertilizers accounted for an average value of 12%. In the second growth cycle the AANU increased to 24 % and further to 46% in the fifth growth cycle (Fig. 15).

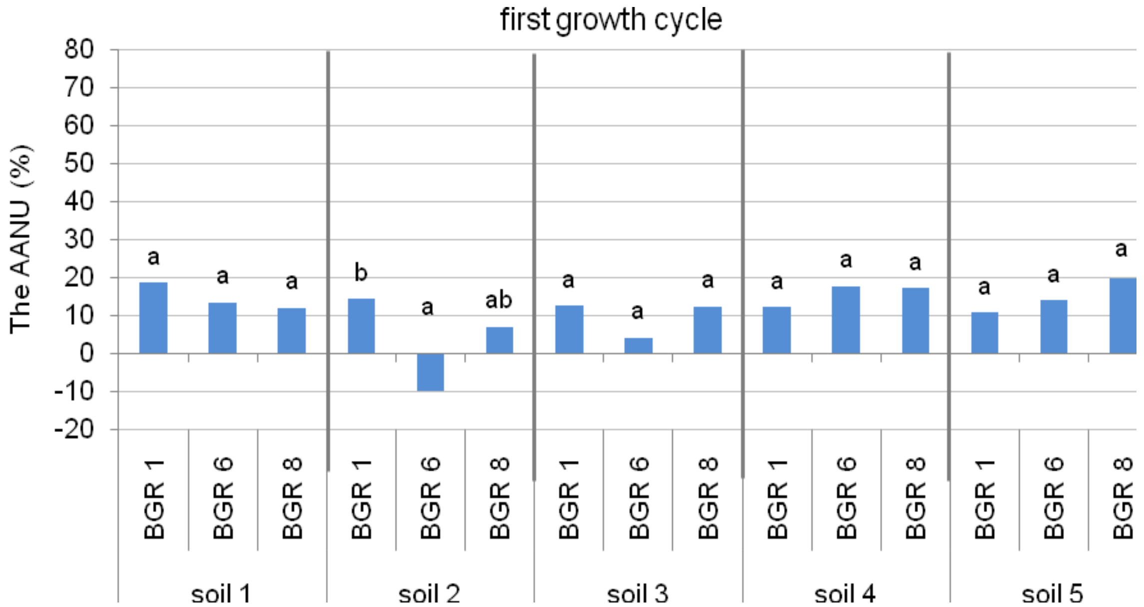
Statistical analysis of the effects of the soils and fertilizers in each growth cycle showed that the effect of soil on the AANU was significant (Tab. 10) whereas the effect of the fertilizers was significant only in the fifth growth cycle. No significant interaction was found between the two factors.

The effect of soil, averaged over all fertilizers in the first and second growth cycle showed that the highest additional apparent N utilization occurred in soil 4 and the lowest AANU was in soil 2 (Fig. 15). In the fifth growth cycle however, the AANU in soil 3 was significantly lower compared to the other soils. Averaged over all soils there was no effect of the fertilizers on additional N offtake in the first and second growth cycle. However in the fifth growth cycle the AANU was significantly higher and with BGR 1 it ranged from 7% in soil 3 to 39% in soil 4, with BGR 6 it ranged from 24% in soil 3 to 59% in soil 4 and with BGR8 it ranged from 52% in soil 3 to 72% from soil 1. These differences between the fertilizers reflect the different amount of organic N input as a consequence of the fertilizer application based on equal amounts of ammonium-N. With one exception i.e., BGR 1 in soil 3 AANU of all fertilizers in all soils increased from the first to the fifth growth cycle.

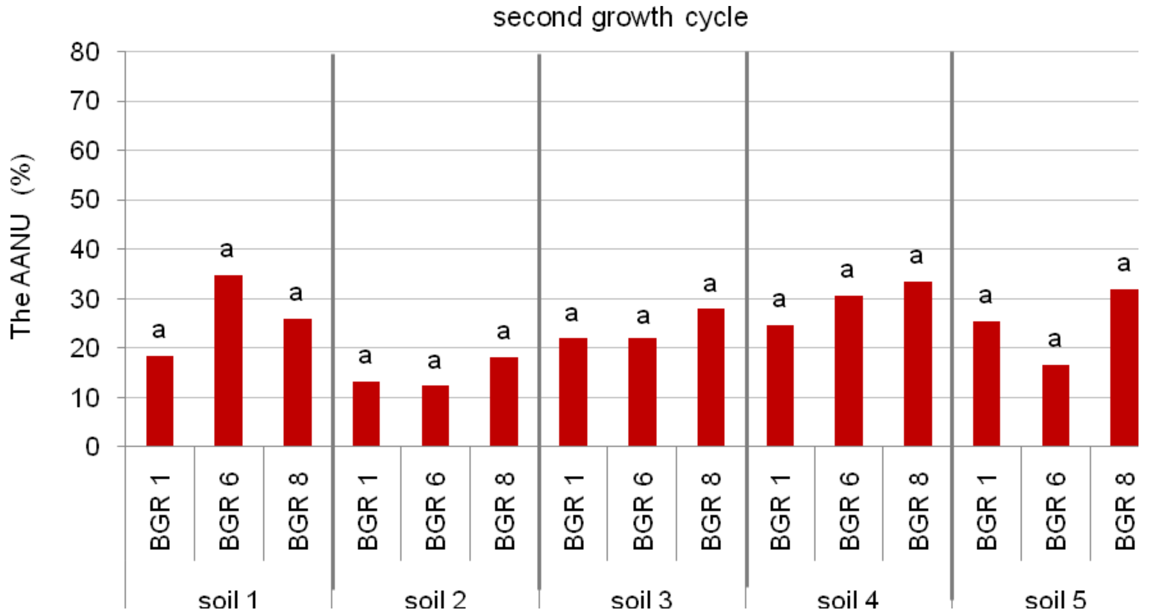
Table 10 F-statistics of two-way ANOVA of the effects of soils (1, 2, 3, 4 and 5) and fertilizer sources (BGR1, BGR6 and BGR8)

ANOVA	1st growth cycle			2nd growth cycle			5th growth cycle		
main effects+ interactions	DF	F value	significance	DF	F value	Significance	DF	F value	Significance
Soils	4	3.14	0.023*	4	3.37	0.017*	4	9.69	0.000***
Fertilizer	2	2.31	0.111NS	2	2.06	0.139 NS	2	39.48	0.000***
Soil × Fertilizer	8	1.69	0.127NS	8	0.98	0.464 NS	8	1.16	0.341 NS

Significant level: * *P* ≤ 0.05. * *P* ≤ 0.001. * *P* ≤ 0.0001



Main factor soil	**AB**	**A**	**AB**	**B**	**AB**		
Main factor fertilizers					**a**	**a**	**a**



Main factor soil	**AB**	**A**	**AB**	**B**	**AB**		
Main factor fertilizers					**a**	**a**	**a**

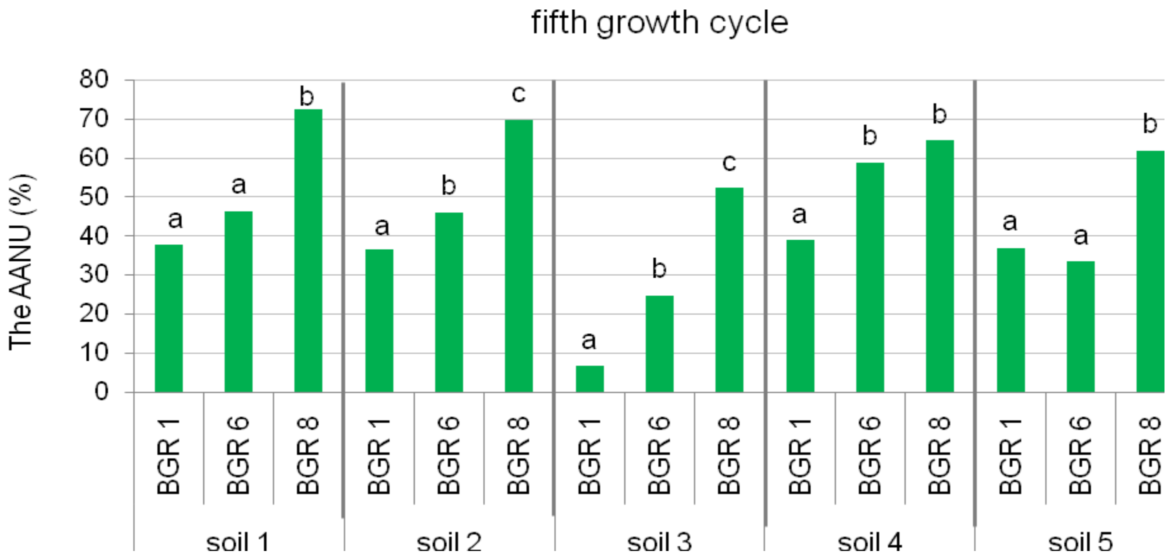


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Figure 15 Additional apparent N utilization of biogas residues collected in the growth cycle 1, 2 and 5 in five soils different. ANOVA results present the effect of fertilizers, soils and their interaction on the apparent N utilization. Different lower case letters indicate statistically significant differences between the fertilizers within one growth cycle ($p \leq 0.05$). Different capital letters indicate statistically significant differences between the first, second and fifth growth cycle for one organic fertilizer ($p \leq 0.05$)

3.3.6 Soil organic matter content

After five repeated fertilizer applications soil total N contents of the fertilized treatments increased as compared to the unfertilized control (Tab. 11). While the application of NH_4NO_3 as a mineral fertilizer had only a small effect, the application of the biogas residues significantly increased soil total nitrogen content.

Soil total N accumulation was higher after the application of the biogas residues with BGR6 and BGR8 compared with BGR1. This is due to the different amounts of organic N applied which were 2.6 or 2.9 times higher, respectively for BGR 6 and BGR8 compared with BGR1.

Table 11 Soil N_t content (g kg^{-1}) at the end of the experiment

Treatments	soil 1	soil 2	soil 3	soil 4	soil 5
Control	1.90^{a}	1.33^{a}	0.52^{a}	4.29^{a}	1.81^{a}
Mineral	1.95^{ab}	1.40^{ab}	0.53^{ab}	4.33^{a}	1.86^{a}
BGR1	2.05^{bc}	1.50^{b}	0.68^{b}	4.46^{ab}	1.95^{b}
BGR6	2.18^{c}	1.68^{c}	0.79^{c}	4.62^{b}	2.06^{c}
BGR8	2.18^{c}	1.70^{c}	0.78^{c}	4.41^{ab}	2.08^{c}

Different letters indicate statistically significant differences between the fertilizers ($p \leq 0.05$)

3.3.7 Net N mineralization rate from different soils

After five repeated fertilizer applications the rate of net N mineralization from soil total nitrogen (extrapolated to one year) was calculated for the ninth cut which is the unfertilized cut this was done to assess differences in the N release affected by soils or fertilizers. N mineralization rate in unfertilized soils ranged from 2 to 4 % of soil total N (Fig. 16). Compared with the unfertilized soils mineralization rate after application of biogas residues was significantly greater in all soils except in soil 4. The N mineralization rate of soil N after biogas effluent application ranged between 3 to 7 % of the soil total N with the highest mineralization rate in the sandy soil 3 followed by the silty soils 5, 1 and 2 and the lowest rate occurred in the organic clayey soil 4. No significant differences in N mineralization rate appeared between the different biogas residues.

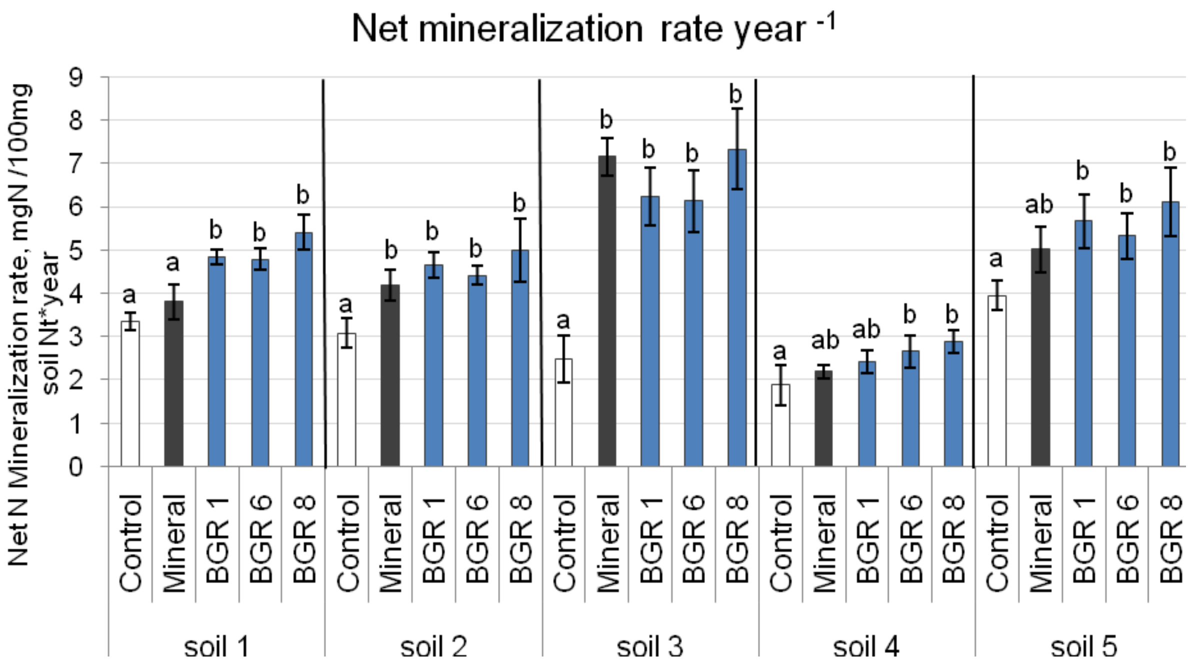


Figure 16 Net N mineralization rate of fertilizers collected in the growth cycles 1, 2 and 5 in five different soils. Different letters indicate statistically significant differences between the fertilizers ($p \le 0.05$)

3.4 Chemical properties of solid and liquid biogas residues after separation

After separation of the biogas residues dry matter content was highly enriched in the solid fraction compared to the liquid fraction (Tab. 12). Thus the liquid biogas residues (LBRs) and the solid biogas residues (SBRs) dry matter content in the SBRs ranged from 22.4 to 30.3% in fresh matter, the dry matter content in the LBRs ranged from 5.4 to 10.2% in FM. For most of the residues the pH value of the SBRs (pH range 7.7 to 8.8) was comparable or markedly higher than that of the liquid fraction which had pH values from 7.7 to 8.2. The NH_4-N content ranged from 0.27% to 0.47% in the LBRs and from 0.20% to 0.62% in the SBRs. In five of the seven residues the NH_4-N content tended to be higher in the LBRs, in the other two residues 3 and 7, the NH_4-N content of the SBRs was higher than that of the LBRs. Regarding total N there was no consistent difference between LBRs and SBRs. For example, in residues 1, 3 and 7 total N in the SBRs exceeded that of the LBRs whereas in residue 2 and 4 total N was higher in the LBRs. For all liquid and solid biogas residues total N was between 0.51 and 1.08% of the fresh matter. The content in organic nitrogen ranged from 0.04 to 0.46% in fresh matter and was higher in the SBRs except for residues 2 and 4 where the organic N content was lower in the SBRs compared to the LBRs. The SBRs were characterized by higher organic carbon contents and higher C:N ratios compared to the liquid fraction. The C_{org}:N_{org} ratio ranged from 6.9 to 9.8 for the LBRs and from 16.8 to 30.3 for the SBRs. The extraordinary higher C_{org}:N_{org} ratio of LBR7 (41.5) derived from its exceptionally low organic N content. Both P and K contents varied between the SBRs and the LBRs. Phosphorus content in the LBRs was between 0.06-0.22% and in the SBRs was between 0.13-1.20% being higher in the SBRs than in the LBRs. Potassium content in LBRs was between 0.45- 0.91% and in the SBRS was between 0.36- 0.78 (data not shown), with rather higher contents in the LBRs in all cases. The biogas residues were reanalyzed for total N and NH_4-N prior to the fourth fertilizer application (Tab. 13). Most of the biogas residues changed only slightly in NH_4-N or total N contents. However, based on this analysis SBR6 showed very low NH_4-N contents, most probably because of high NH_3 losses during storage. Therefore, a new sample of SBR6 from the same biogas digester was collected to replace the former.

Table 12 Chemical composition of liquid and solid biogas residues obtained from different raw materials (% in FM) used for the first, second and third growth cycles

Biogas residues	pH	DM	N_t	N_{org}	NH_4-N	C_t	C_{org}	C_t/N_{org}	C_t/N_t	C_{org}/N_{org}	NH_4-N/N_t
LBR 1	7.7	6.32	0.56	0.26	0.30	2.5	2.3	9.4	4.4	8.6	0.53
LBR 2	7.7	9.11	0.76	0.42	0.34	3.5	3.2	8.3	4.6	7.6	0.44
LBR 3	8.1	5.39	0.54	0.23	0.31	2.0	1.6	8.6	3.7	6.9	0.57
LBR 4	8	7.63	0.63	0.35	0.28	3.0	2.7	8.6	4.8	7.8	0.44
LBR 5	7.8	10.15	0.76	0.35	0.41	3.7	3.5	10.6	4.9	9.8	0.53
LBR 6	7.8	6.14	0.53	0.27	0.27	2.3	2.2	8.9	4.4	8.2	0.50
LBR 7	8.2	7.57	0.51	0.04	0.47	2.3	1.8	53.6	4.5	41.5	0.92
SBR 1	7.7	26.63	0.58	0.35	0.23	10.3	10.2	29.6	17.9	29.4	0.40
SBR 2	8.8	23.55	0.63	0.36	0.27	9.8	9.7	27.1	15.6	26.7	0.43
SBR 3	8.8	23.64	0.76	0.40	0.36	9.5	9.5	24.1	12.6	23.9	0.48
SBR 4	8.4	24.65	0.58	0.34	0.24	10.4	10.3	30.6	17.8	30.3	0.42
SBR 5	8.8	30.3	0.75	0.42	0.33	11.6	11.4	27.9	15.6	27.4	0.44
SBR 6	8.8	22.43	0.53	0.33	0.20	9.4	9.3	28.8	17.8	28.5	0.38
SBR 7	7.8	29.85	1.08	0.46	0.62	8.3	7.7	18.2	7.7	16.8	0.57

Table 13 Chemical composition of liquid and solid biogas residues obtained from different raw materials used for the fourth and fifth growth cycles

Biogas residues*	N_t	N_{org}	NH_4-N	C_t/N_{org}	C_t/N_t	C_{org}/N_{org}	NH_4-N/N_t
LBR 1	0.57	0.25	0.31	9.8	4.4	9.0	0.55
LBR 2	0.75	0.41	0.34	8.5	4.6	7.8	0.45
LBR 3	0.54	0.22	0.32	9.0	3.7	7.2	0.59
LBR 4	0.63	0.35	0.29	8.7	4.8	7.9	0.45
LBR 5	0.76	0.34	0.42	11.1	4.9	10.2	0.55
LBR 6	0.53	0.26	0.27	8.9	4.4	8.2	0.50
LBR 7	0.56	0.21	0.35	10.8	4.1	8.3	0.62
SBR 1	0.56	0.37	0.19	27.7	18.4	27.5	0.34
SBR 2	0.64	0.33	0.32	30.3	15.3	29.9	0.50
SBR 3	0.77	0.37	0.40	25.7	12.4	25.4	0.52
SBR 4	0.57	0.32	0.25	32.4	18.0	32.1	0.44
SBR 5	0.74	0.37	0.37	31.3	15.7	30.7	0.50
SBR 6	0.78	0.48	0.30	21.8	13.3	21.8	0.39
SBR 7	0.79	0.46	0.33	18.3	10.6	16.9	0.42

*New analysis was done before the fourth and fifth fertilization and a new source of SBR6 was applied

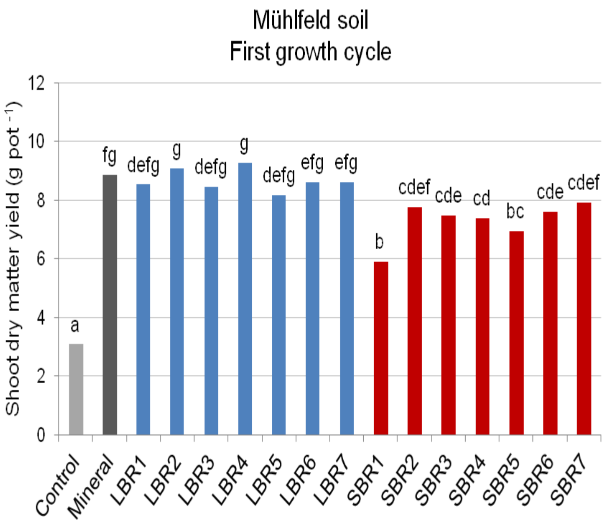
3.5 Yield and N availability of ryegrass from liquid and solid biogas residues after separation as tested in two soils

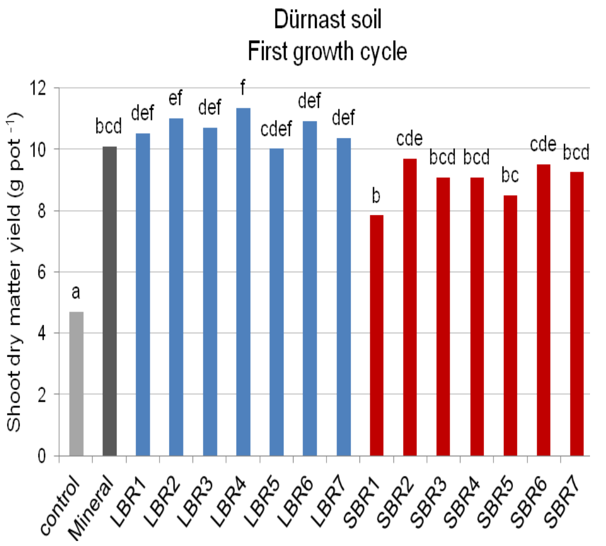
3.5.1 Shoot dry matter yield

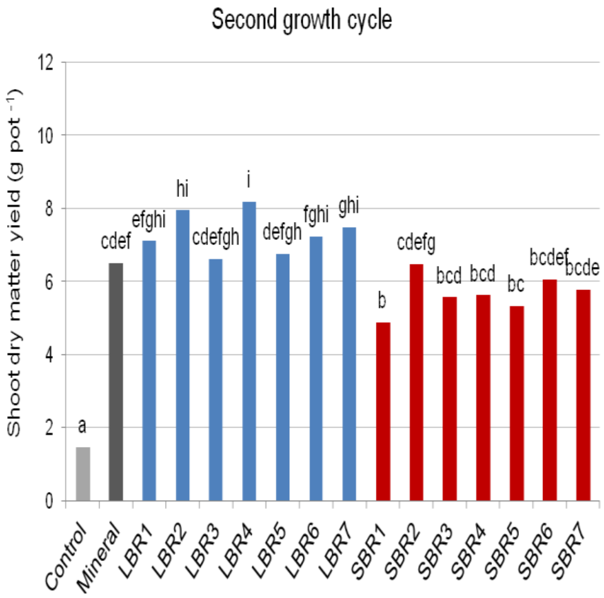
Ryegrass dry matter yield increased after the application of the different biogas residues (Fig. 17). In the first growth cycle, yield of the mineral fertilizer treatment and LBRs were rather similar. Yield of the SBRs treatments were significantly lower than those of the mineral fertilizer treatment in the Mühlfeld soil only. However yield of the SBRs treatments were comparable to those of the mineral fertilizer in the Dürnast soil. In the second growth cycle, the ryegrass yield was comparable between the mineral treatment and the LBRs treatments in both soils except for LBR2, 4 and 7 which were significantly higher than that of the mineral treatment in the Mühlfeld soil. Yield of the SBRs was similar to the yield of the mineral treatment. Only SBR1 was significantly lower than that of the mineral treatment. In the Dürnast soil, yield was similar in both the mineral and LBR treatments, but was significantly lower for the SBR treatments than for the mineral treatment. In the third growth cycle, yield from LBR 4, 5, 6 and 7 was significantly higher than the mineral one in the Mühlfeld soil. In the Dürnast soil all LBRs resulted in higher yield than given by the mineral treatment. Yield from the SBRs was comparable to of the mineral treatment in both soils. In the fourth growth cycle, the ryegrass yield of the LBR treatments was comparable or higher than that of the mineral treatment. However yield of the SBRs was comparable to that of the mineral treatment. After five repeated fertilizer application the highest yield was obtained from the LBR treatments in both soils, while yield of the SBR treatments was similar to that of the mineral treatment.

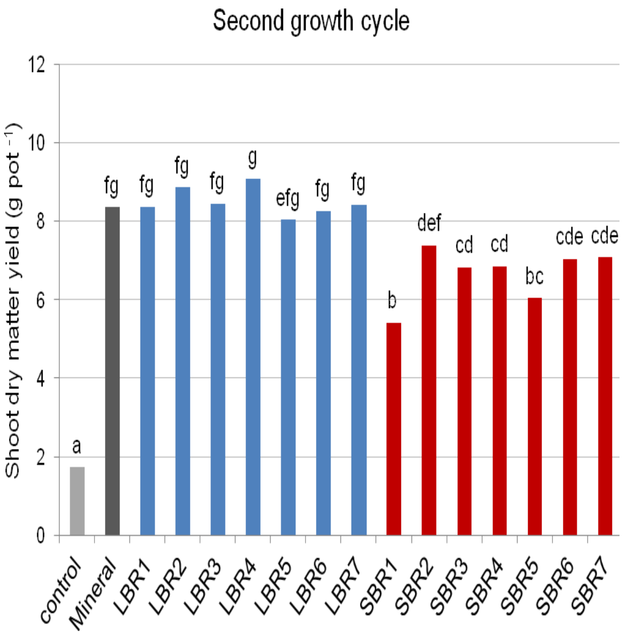
3.5.2 Shoot N content

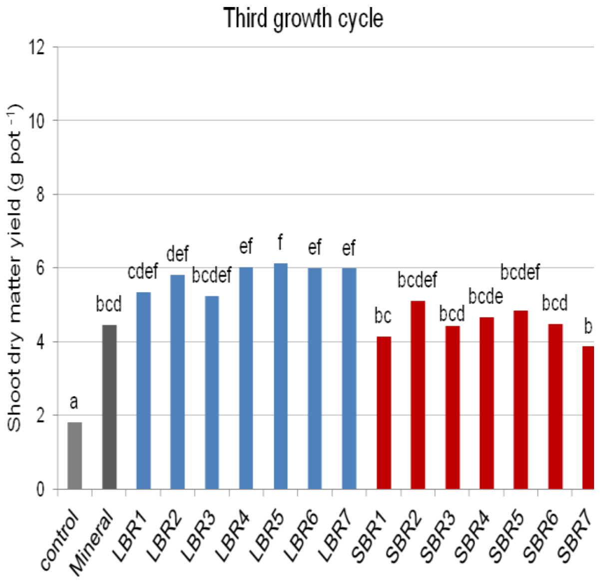
The application of LBRs, SBRs and mineral fertilizers increased the shoot N content (Fig. 18). It is obvious that the fertilized cut (cuts 1, 3, 5 and 7) contained higher N content than the unfertilized ones (cuts 2, 4, 6 and 8). After the last fertilizer application, the N content was similar from cut 8 and 9. However the highest shoot N content occurred in cuts 5 and 7 with N contents of 3.7% and 3.0%, respectively, in the Mühlfeld soil. and with 2.9% and 2.3%, respectively, in the Dürnast soil. The lowest N content occurred in the cut 3 being 2% in the Mühlfeld soil and 1.8 in the Dürnast soil.

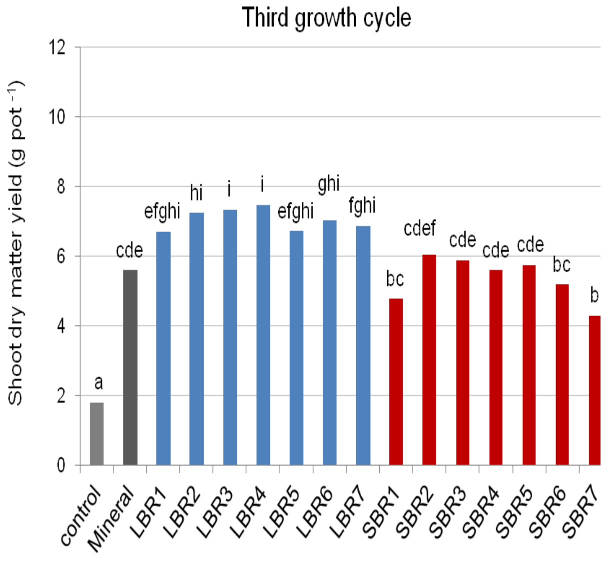

Mühlfeld soil
First growth cycle
Shoot dry matter yield (g pot $^{-1}$)
0
2
4
6
8
10
12
a
fg
defg
g
defg
g
defg
efg
efg
b
cdef
cde
cd
bc
cde
cdef
Control
Mineral
LBR1
LBR2
LBR3
LBR4
LBR5
LBR6
LBR7
SBR1
SBR2
SBR3
SBR4
SBR5
SBR6
SBR7


Dürnast soil
First growth cycle
Shoot dry matter yield (g pot $^{-1}$)
0
2
4
6
8
10
12
a
bcd
def
ef
def
f
cdef
def
def
b
cde
bcd
bcd
bc
cde
bcd
control
Mineral
LBR1
LBR2
LBR3
LBR4
LBR5
LBR6
LBR7
SBR1
SBR2
SBR3
SBR4
SBR5
SBR6
SBR7


Second growth cycle
Shoot dry matter yield (g pot $^{-1}$)
0
2
4
6
8
10
12
a
cdef
efghi
hi
cdefgh
i
defgh
fghi
ghi
b
cdefg
bcd
bcd
bc
bcdef
bcde
Control
Mineral
LBR1
LBR2
LBR3
LBR4
LBR5
LBR6
LBR7
SBR1
SBR2
SBR3
SBR4
SBR5
SBR6
SBR7


Second growth cycle
Shoot dry matter yield (g pot $^{-1}$)
0
2
4
6
8
10
12
a
fg
fg
fg
fg
g
efg
fg
fg
b
def
cd
cd
bc
cde
cde
control
Mineral
LBR1
LBR2
LBR3
LBR4
LBR5
LBR6
LBR7
SBR1
SBR2
SBR3
SBR4
SBR5
SBR6
SBR7


Third growth cycle
Shoot dry matter yield (g pot $^{-1}$)
0
2
4
6
8
10
12
a
bcd
cdef
def
bcdef
ef
f
ef
ef
bc
bcdef
bcd
bcde
bcdef
bcd
b
control
Mineral
LBR1
LBR2
LBR3
LBR4
LBR5
LBR6
LBR7
SBR1
SBR2
SBR3
SBR4
SBR5
SBR6
SBR7


Third growth cycle
Shoot dry matter yield (g pot $^{-1}$)
0
2
4
6
8
10
12
a
cde
efghi
hi
i
i
efghi
ghi
fghi
bc
cdef
cde
cde
cde
bc
b
control
Mineral
LBR1
LBR2
LBR3
LBR4
LBR5
LBR6
LBR7
SBR1
SBR2
SBR3
SBR4
SBR5
SBR6
SBR7

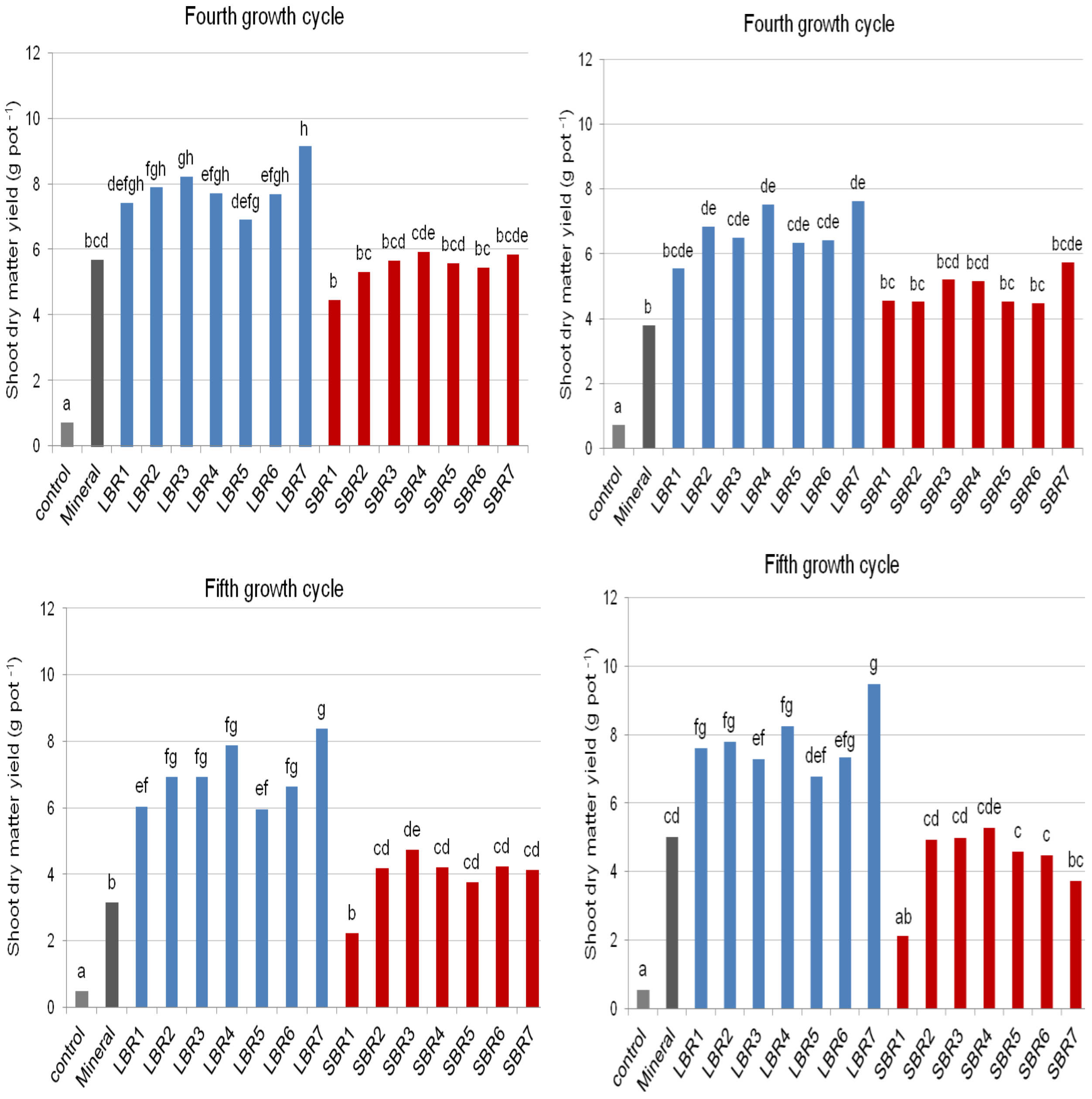
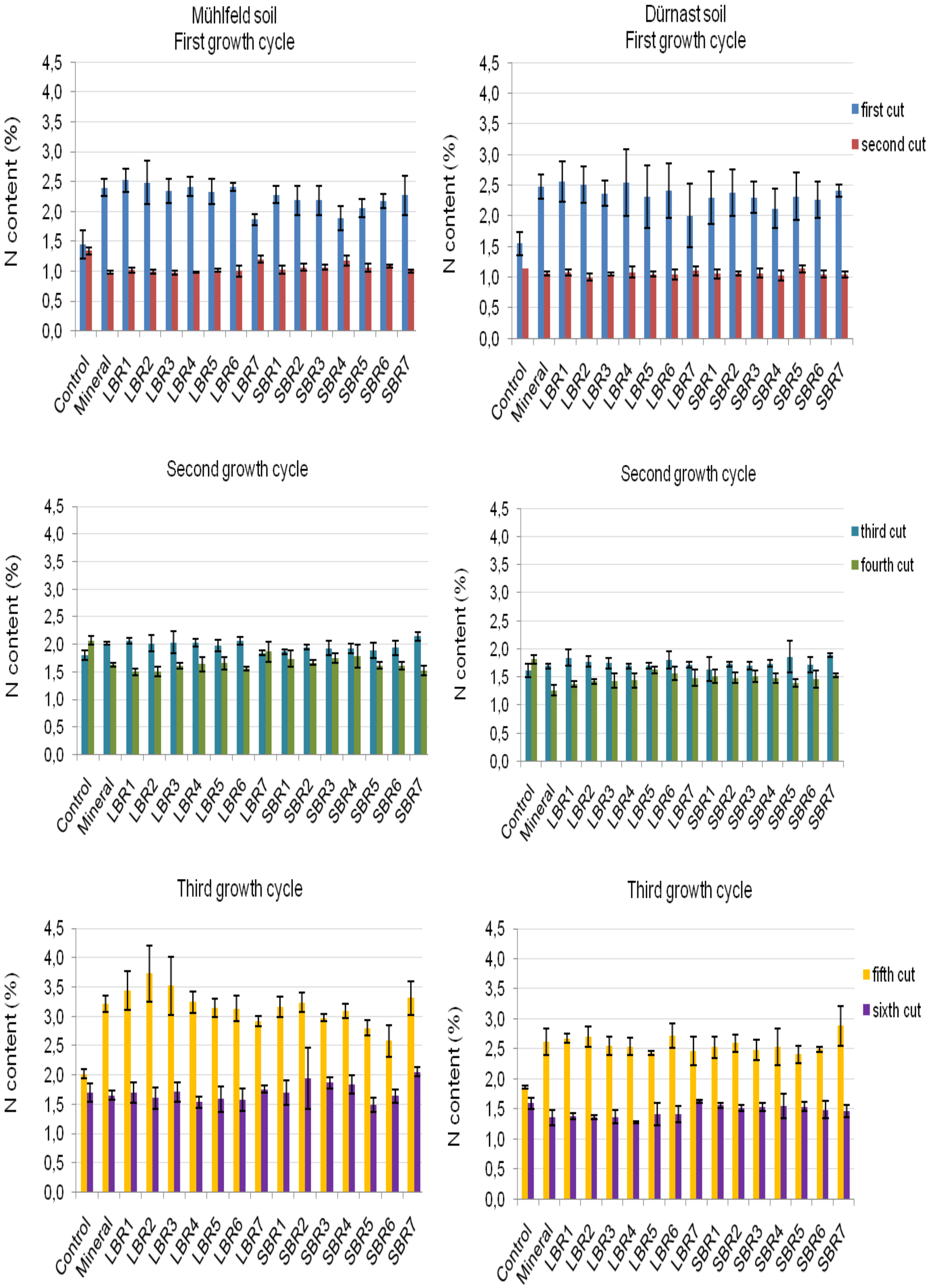


Figure 17 Shoot dry matter yield of liquid and solid biogas residues collected in five growth cycles in two different soils. Different letters indicate statistically significant differences between fertilizers ($p \leq 0.05$)


Mühlfeld soil
First growth cycle
Dürnast soil
First growth cycle
N content (%)
4,5
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
first cut
second cut
Second growth cycle
Second growth cycle
third cut
fourth cut
Third growth cycle
Third growth cycle
fifth cut
sixth cut
Control
Mineral
LBR1
LBR2
LBR3
LBR4
LBR5
LBR6
LBR7
SBR1
SBR2
SBR3
SBR4
SBR5
SBR6
SBR7

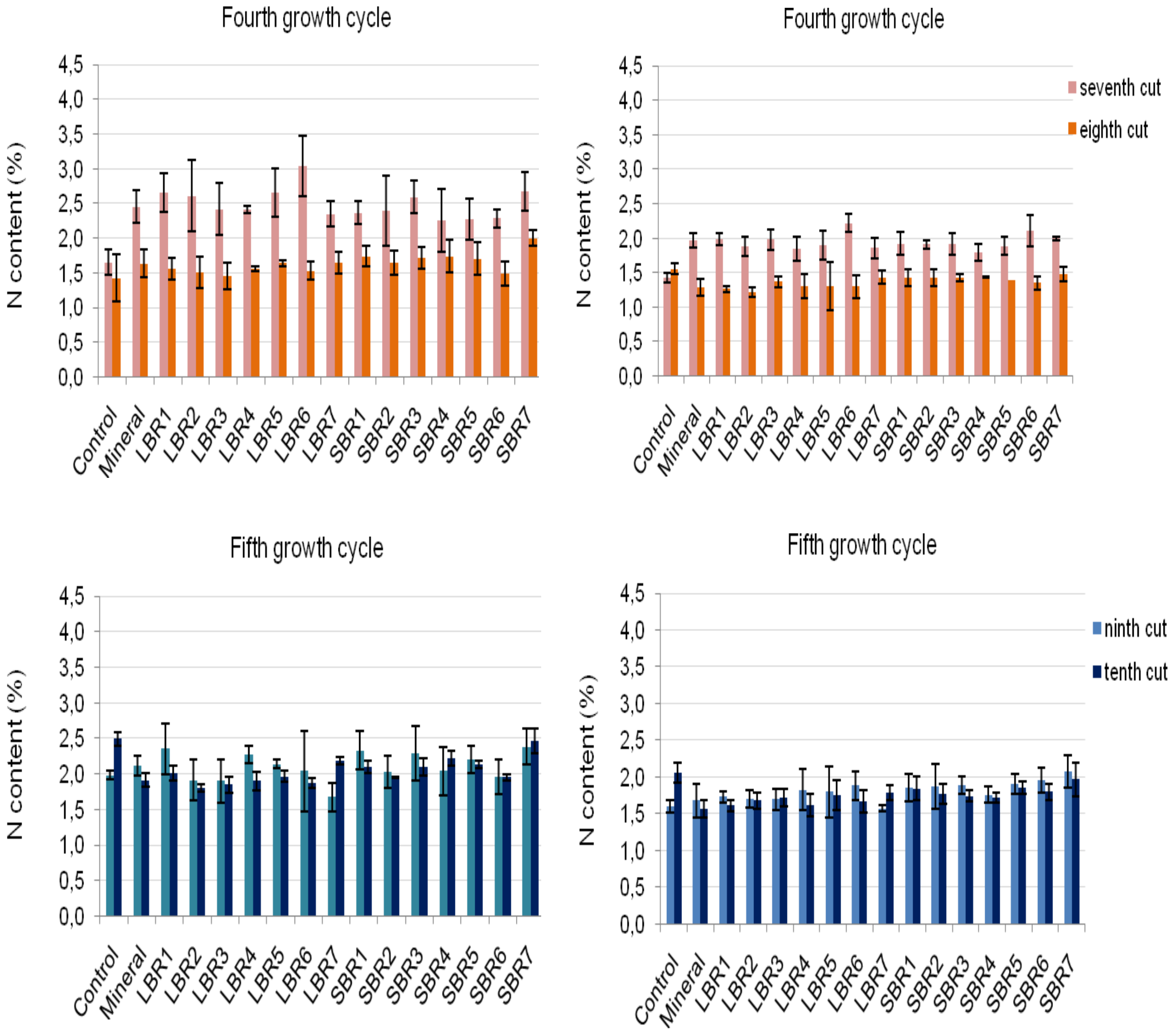
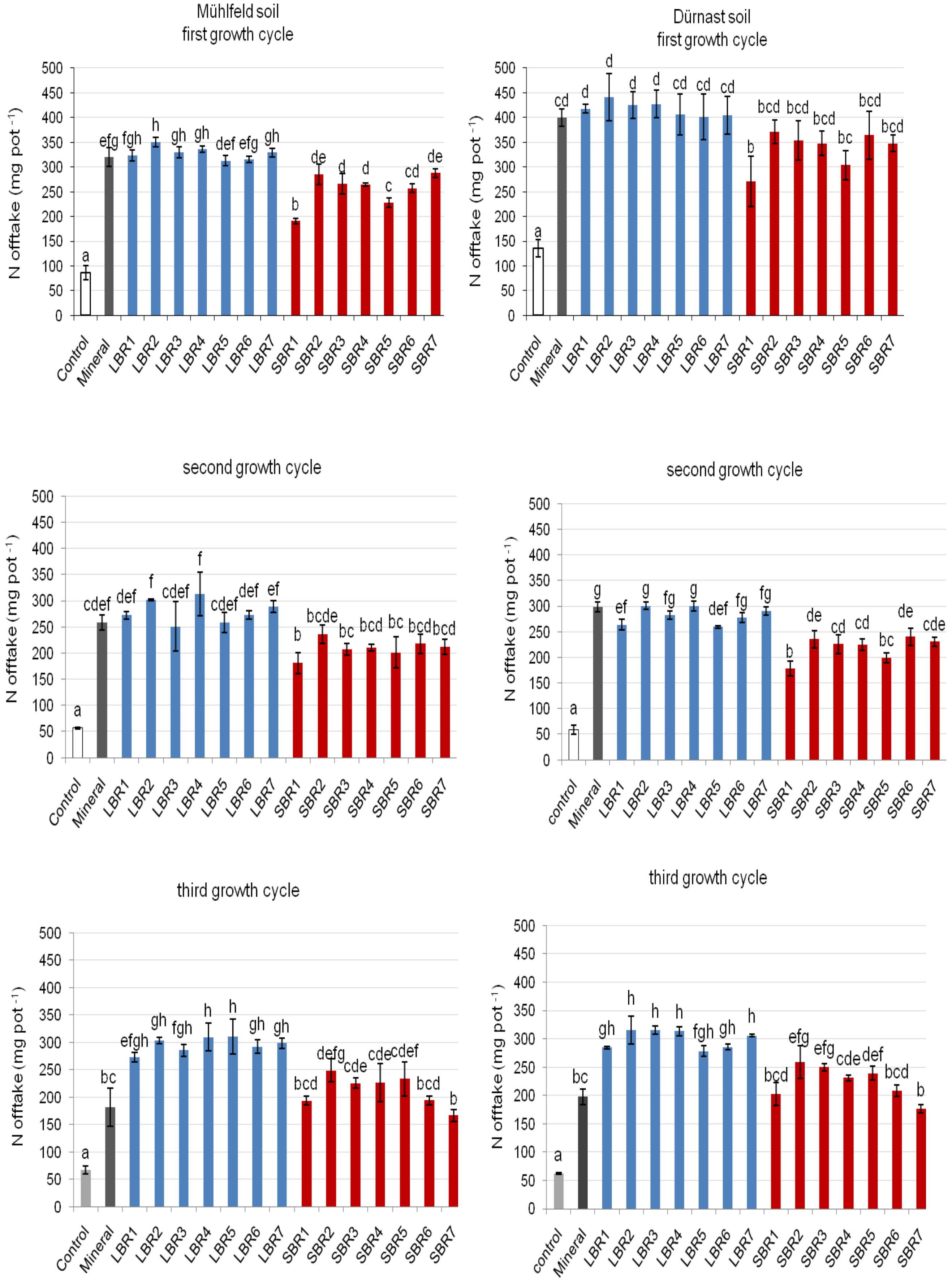


Figure 18 N content of liquid and solid biogas residues collected in five growth cycles in two different soils. Error bars represent standard deviation

3.5.3 Shoot N offtake

Over all the five growth cycles the ryegrass N offtake in the two soils was generally higher after application of liquid biogas residues (LBRs) compared to solid biogas residues (SBRs) (Fig. 19). In the first growth cycle after one fertilizer application and in both soils, shoot N offtake of LBRs was similar to that from the mineral treatment, except LBR2 in Mühlfeld soil of the shoot N offtake which was significantly higher than the mineral one. The N offtake for the SBRs was similar or lower compared with the mineral treatment. In the second growth cycle the N offtake from the LBRs was comparable to that of the mineral treatment in the Mühlfeld soil, but comparable or lower than the mineral treatment in the Dürnast soil. The SBRs treatments released less N compared with the mineral treatment in the Dürnast soil. However N release from SBRs in Mühlfeld soil was comparable to the mineral treatment, only SBR1 released less N compared to the mineral treatment in this soil. In the third growth cycle the N offtake from all LBRs was significantly higher than from the mineral treatment in both soils. The N offtake from SBRs was comparable or higher than that of the mineral treatment in both soils. The N offtake of the SBR2 was higher than that of the mineral treatment in the Mühlfeld soil; however N offtake from SBR2, 3 and 5 were higher than from the mineral treatment in the Dürnast soil. In the fourth growth cycle the N offtake values of all LBRs and SBRs treatments were similar to the N offtake of the mineral treatment. Only N offtake from LBR7 was higher than from the mineral treatment in both soils. After five fertilizer applications (fifth growth cycle), the N offtake for all LBRs treatments was higher than for the mineral treatment in both soils, only LBR5 was comparable to the mineral treatment in the Dürnast soil. Most of the SBRs provided N offtake similarly to that of the mineral treatment in both soils but the N offtake of SBR1 was markedly lower than that of the mineral treatment.

Generally, the ryegrass shoot N offtake showed that liquid biogas residues provide available N at least similar to that of the mineral fertilizer in all growth cycles and in both soils. N offtake of the various SBRs was in most cases not only significantly lower than that of LBRs, but also sometimes lower compared with that of the mineral fertilizer particularly after the first two fertilizer applications. In the course of the five fertilizer applications it became more obvious that N availability from the SBRs was lower than that from the LBRs.


Mühlfeld soil
first growth cycle
Dürnast soil
first growth cycle
second growth cycle
second growth cycle
third growth cycle
third growth cycle
N offtake (mg pot -1)
0
50
100
150
200
250
300
350
400
450
500
Control
control
Mineral
LBR1
LBR2
LBR3
LBR4
LBR5
LBR6
LBR7
SBR1
SBR2
SBR3
SBR4
SBR5
SBR6
SBR7
a efg fgh h gh gh def efg gh b de d d c cd de
a cd d d d d cd cd cd b bcd bcd bcd bc bcd bcd
a cdef def f cdef f cdef def ef b bcde bc bcd bc bcd bcd
a g ef g fg g def fg fg b de cd cd bc de cde
a bc efgh gh fgh h h gh gh bcd defg cde cde cdef bcd b
a bc gh h h h fgh gh h bcd efg efg cde def bcd b

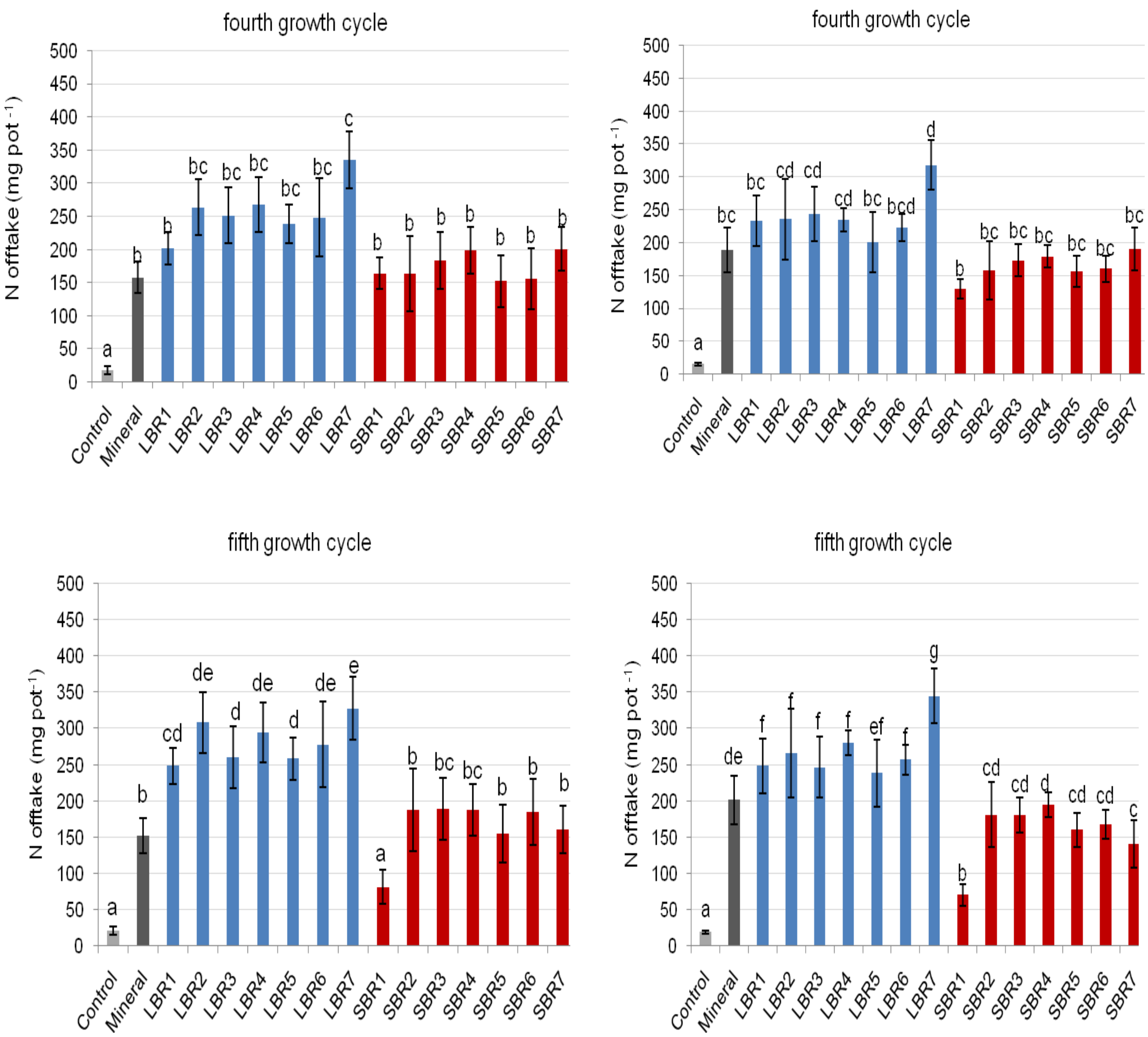


Figure 19 Shoot N offtake of liquid and solid biogas residues collected in five growth cycles in two different soils. Different letters indicate statistically significant differences between fertilizers ($p \leq 0.05$)

3.5.4 Apparent N utilization

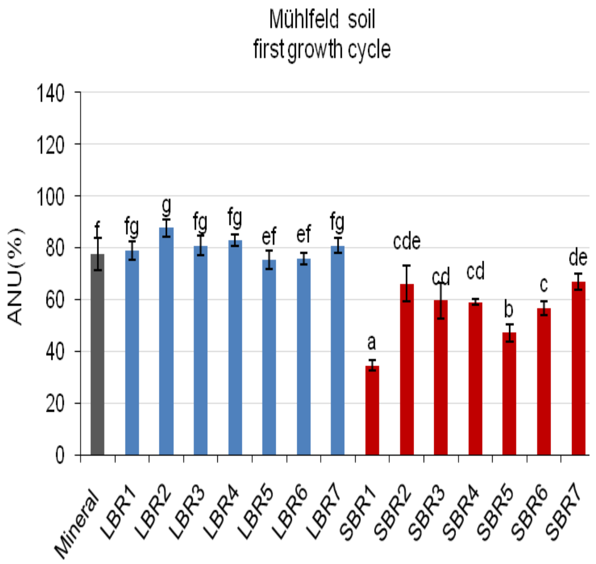
In the five growth cycles the ANU of the mineral fertilizer ranged between 40% and 80% in Mühlfeld soil and between 60% and more than 80% in Dürnast soil (Fig. 20). In the first growth cycle, the ANU in Mühlfeld soil from the liquid biogas residues was between 80-85%, in contrast to only 45-68% from the solid biogas residues. However in Dürnast soil, the ANU from LBRs ranged from 85-100%, but from SBRs it ranged only from 55 to 80%. From both soils it is clear that LBR2 had the highest ANU i.e., an increase of 10% compared to the mineral treatment. By contrast, SBR1 had the lowest ANU which shows a reduction of 43% compared to the ANU of the mineral fertilizer.

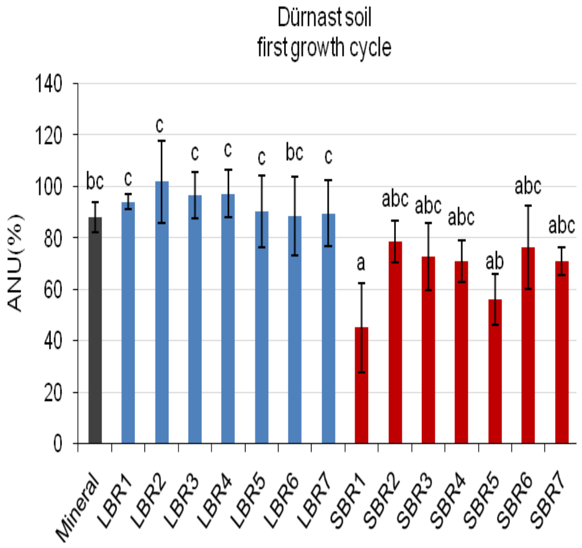
In the second and the third growth cycle, the ANU of the liquid biogas residues ranged between 65 – 85% in both soils, and the ANU from the solid biogas residues was between 40 up to 60%.

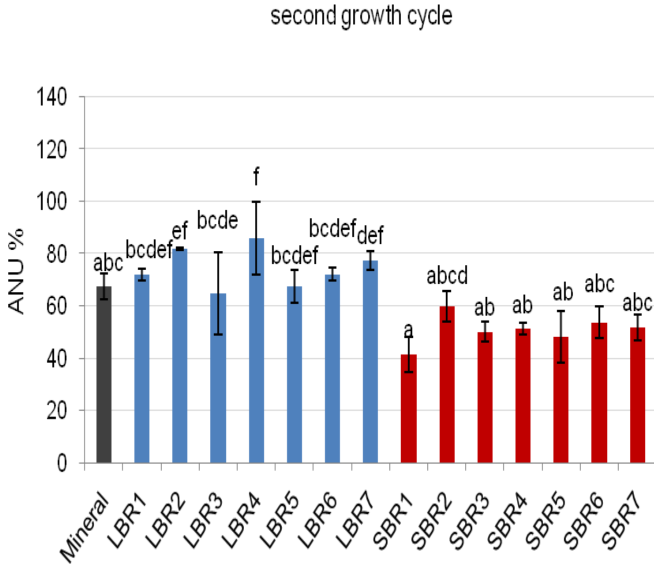
In the fourth growth cycle, the highest ANU was observed from LBR7 (up to 100%) in both soils. On the other hand, the lowest ANUs were observed from SBR5 and 6 in Mühlfeld soil, while in Dürnast soil the lowest ANU with only 40% was found for SBR1.

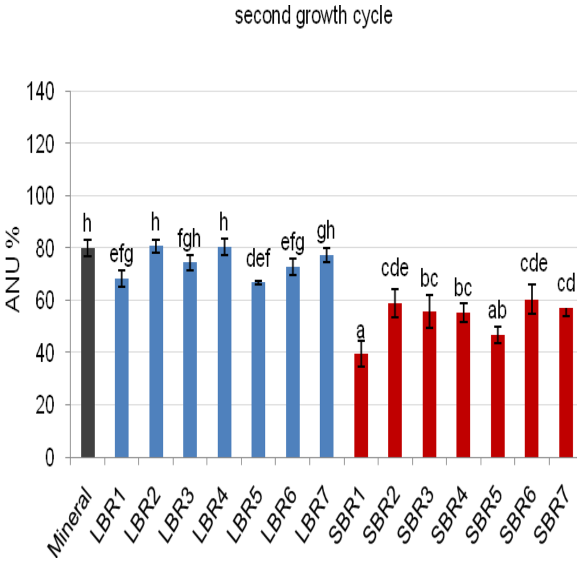
In the fifth growth cycle, LBR7 reached the highest ANU with up to 110% in both soils. For the SBRs the ANU from SBR2, 3 and 4 was similar but the lowest ANU of 18-20% was observed for SBR1. It is interesting to notice that the ANU from SBRs, except SBR 1 was comparable to that of the mineral treatment in this growth cycle.

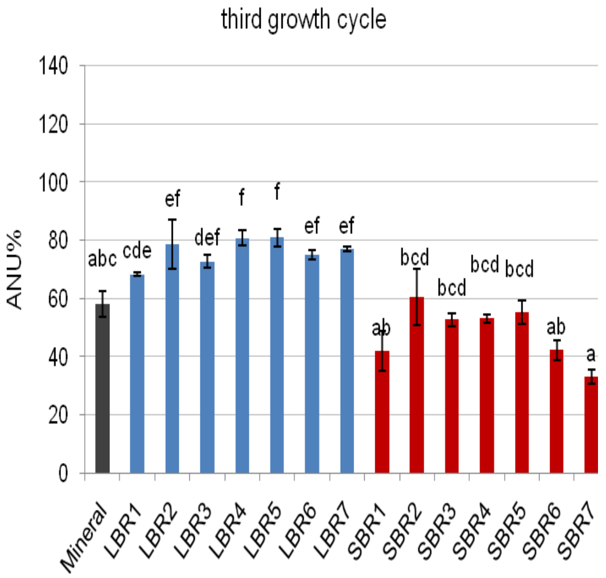
Generally from the first to the fifth application the ANU of the liquid biogas residues seemed to increase when compared to the mineral fertilizer. Similarly however on a distinctly lower level, the ANU of the solid biogas residues seemed to increase in the course of five repeated fertilizer applications with most SBRs reaching the ANU level of the mineral fertilizer in the fifth growth cycle.

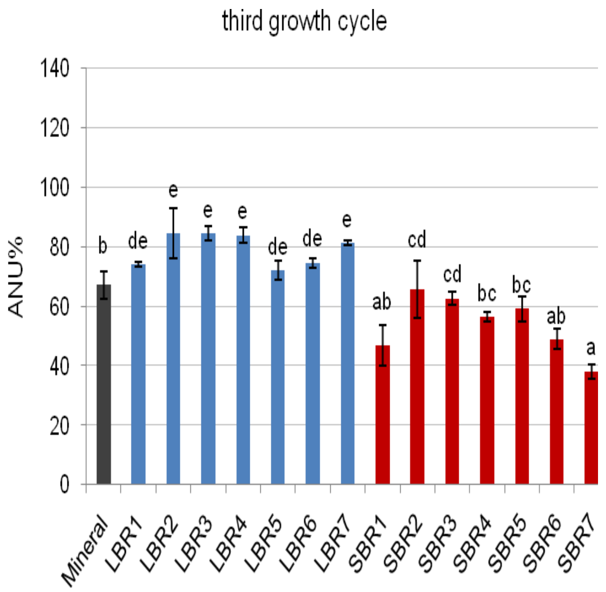

Mühlfeld soil
first growth cycle
ANU(%)
0
20
40
60
80
100
120
140
f
fg
g
fg
fg
ef
ef
fg
a
cde
cd
cd
b
c
de
Mineral
LBR1
LBR2
LBR3
LBR4
LBR5
LBR6
LBR7
SBR1
SBR2
SBR3
SBR4
SBR5
SBR6
SBR7


Dürnast soil
first growth cycle
ANU(%)
0
20
40
60
80
100
120
140
bc
c
c
c
c
c
bc
c
a
abc
abc
abc
ab
abc
abc
Mineral
LBR1
LBR2
LBR3
LBR4
LBR5
LBR6
LBR7
SBR1
SBR2
SBR3
SBR4
SBR5
SBR6
SBR7


second growth cycle
ANU %
0
20
40
60
80
100
120
140
abc
bcdef
ef
bcde
f
bcdef
bcdef
def
a
abcd
ab
ab
ab
abc
abc
Mineral
LBR1
LBR2
LBR3
LBR4
LBR5
LBR6
LBR7
SBR1
SBR2
SBR3
SBR4
SBR5
SBR6
SBR7


second growth cycle
ANU %
0
20
40
60
80
100
120
140
h
efg
h
fgh
h
def
efg
gh
a
cde
bc
bc
ab
cde
cd
Mineral
LBR1
LBR2
LBR3
LBR4
LBR5
LBR6
LBR7
SBR1
SBR2
SBR3
SBR4
SBR5
SBR6
SBR7


third growth cycle
ANU%
0
20
40
60
80
100
120
140
abc
cde
ef
def
f
f
ef
ef
ab
bcd
bcd
bcd
bcd
ab
a
Mineral
LBR1
LBR2
LBR3
LBR4
LBR5
LBR6
LBR7
SBR1
SBR2
SBR3
SBR4
SBR5
SBR6
SBR7


third growth cycle
ANU%
0
20
40
60
80
100
120
140
b
de
e
e
e
de
de
e
ab
cd
cd
bc
bc
ab
a
Mineral
LBR1
LBR2
LBR3
LBR4
LBR5
LBR6
LBR7
SBR1
SBR2
SBR3
SBR4
SBR5
SBR6
SBR7

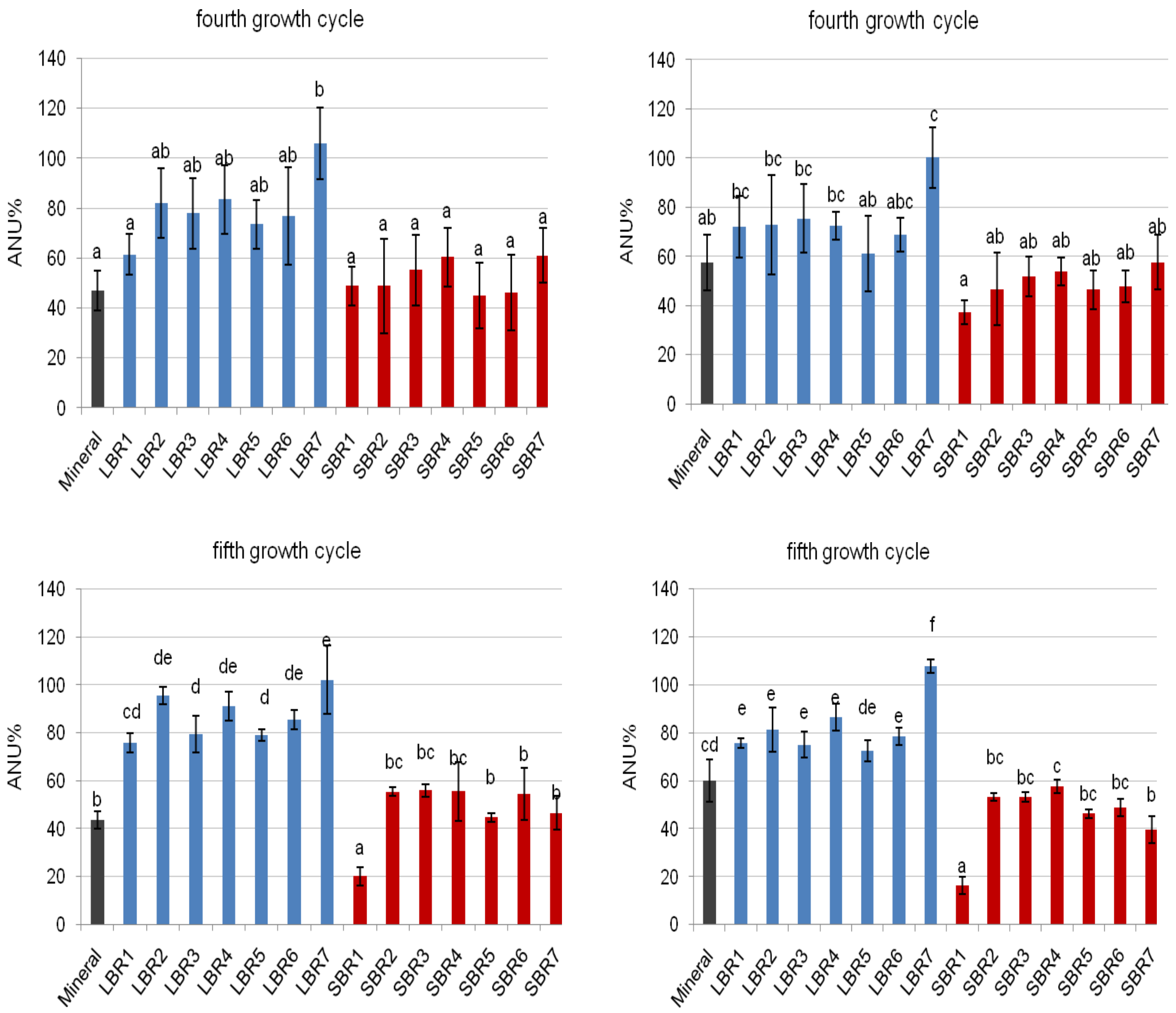


Figure 20 Shoot N utilization of liquid and solid biogas residues collected in five growth cycles in two different soils. Different letters indicate statistically significant differences between the fertilizers ($p \leq 0.05$)

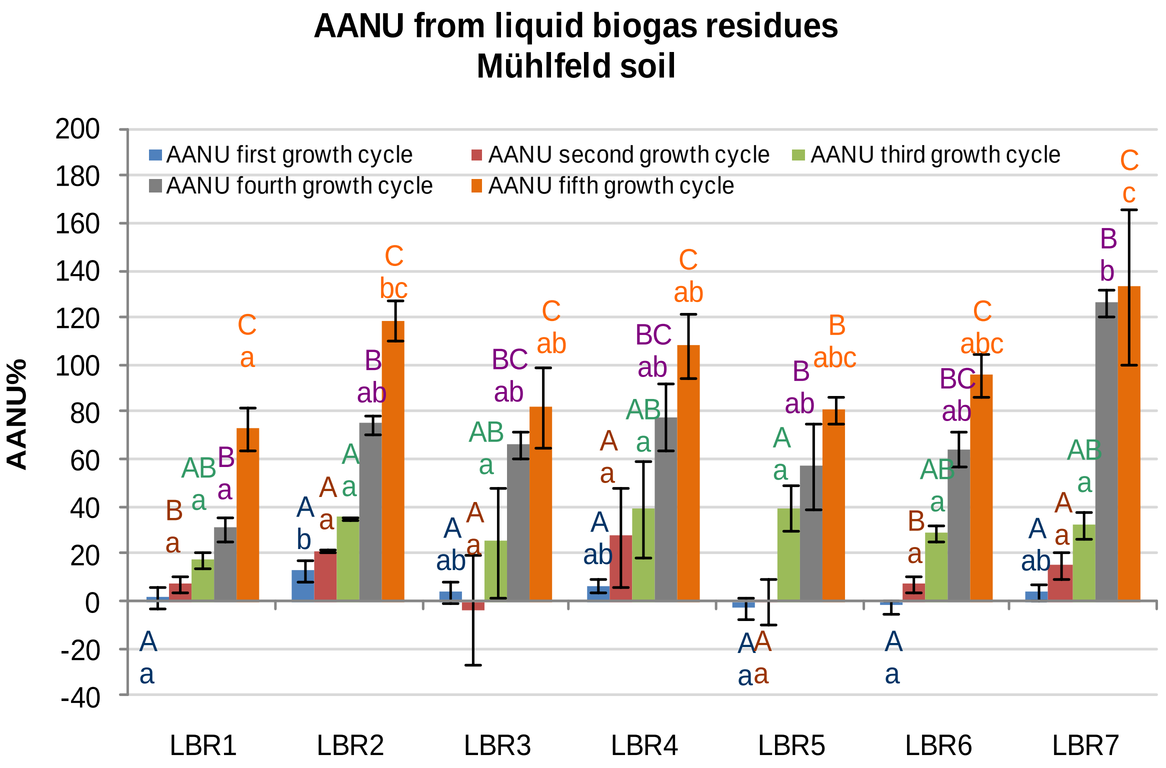
3.5.5 Additional apparent nitrogen utilization

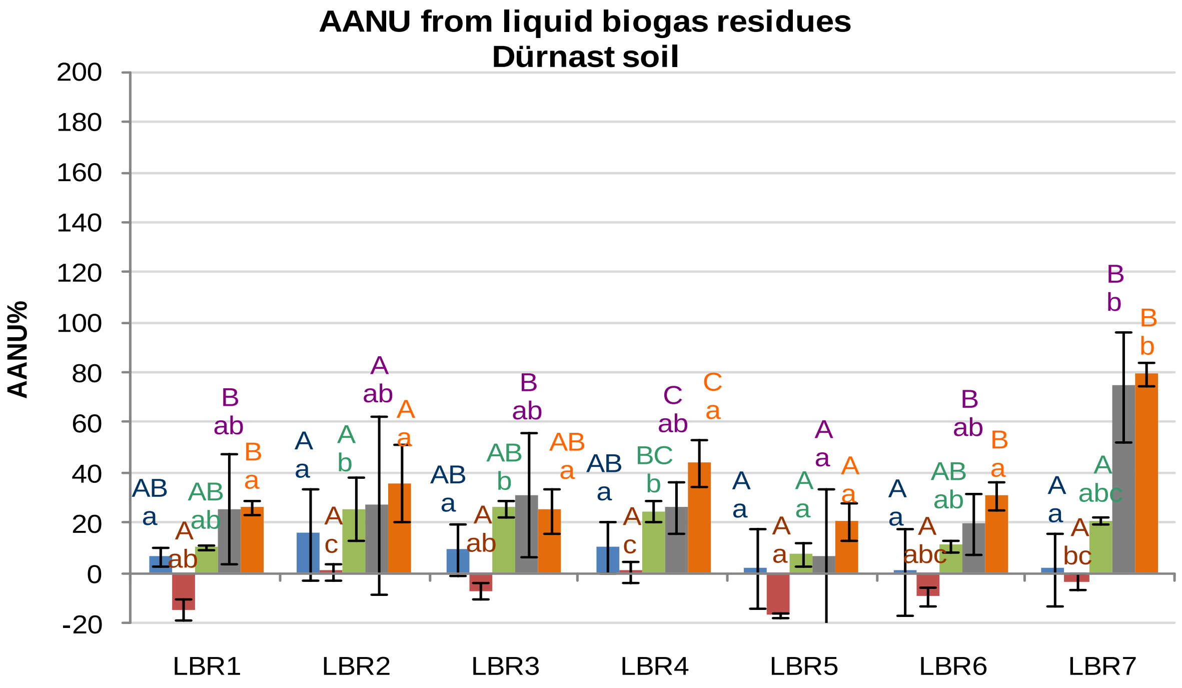
The additional apparent nitrogen utilization (AANU) for all LBRs was increasing significantly in the course of the five fertilizer applications (Fig. 21). This was most obvious in the Mühlfeld soil, while in the Dürnast soil the increase in AANU was more heterogenous for the different LBRs treatments. The AANU for the LBRs in the first growth cycle ranged between -3 to 13% in the Mühlfeld soil, in the fifth growth cycle it reached 70 to 130%. In this cycle the highest AANU occurred with the LBR 2 and 7. Lowest AANU occurred in the LBRs 1, 3 and 5.

In the Dürnast soil the highest AANU after five fertilizer applications was also found with LBR7, and the AANU of all other LBRs was significantly lower.

Generally, the increase in the AANU was lower in the Dürnast soil than in the Mühlfeld soil. In contrast to the LBRs the pattern of the AANU for the SBRs is characterized by mostly negative values indicating an immobilization of the mineral nitrogen in the SBRs treatments. The AANU for the SBRs also increased from the first to the fifth growth cycle in most SBRs in the Mühlfeld soil up to positive value of about 20%. After five repeated fertilizers applications low and negative values for AANU were observed in SBR1.

In the Dürnast soil no positive values for the AANU could be found. Even a tendency to an increasing AANU by repeated fertilizer applications was rather missing, with the exception of SBR4. In both soils SBR1 showed the lowest and continuously negative values of AANU indicating severe immobilization of the mineral fertilizer nitrogen even after repeated applications.


AANU from liquid biogas residues
Mühlfeld soil
AANU%
AANU first growth cycle
AANU second growth cycle
AANU third growth cycle
AANU fourth growth cycle
AANU fifth growth cycle
200
180
160
140
120
100
80
60
40
20
0
-20
-40
LBR1
LBR2
LBR3
LBR4
LBR5
LBR6
LBR7


AANU from liquid biogas residues
Dürnast soil
AANU%
200
180
160
140
120
100
80
60
40
20
0
-20
LBR1
LBR2
LBR3
LBR4
LBR5
LBR6
LBR7

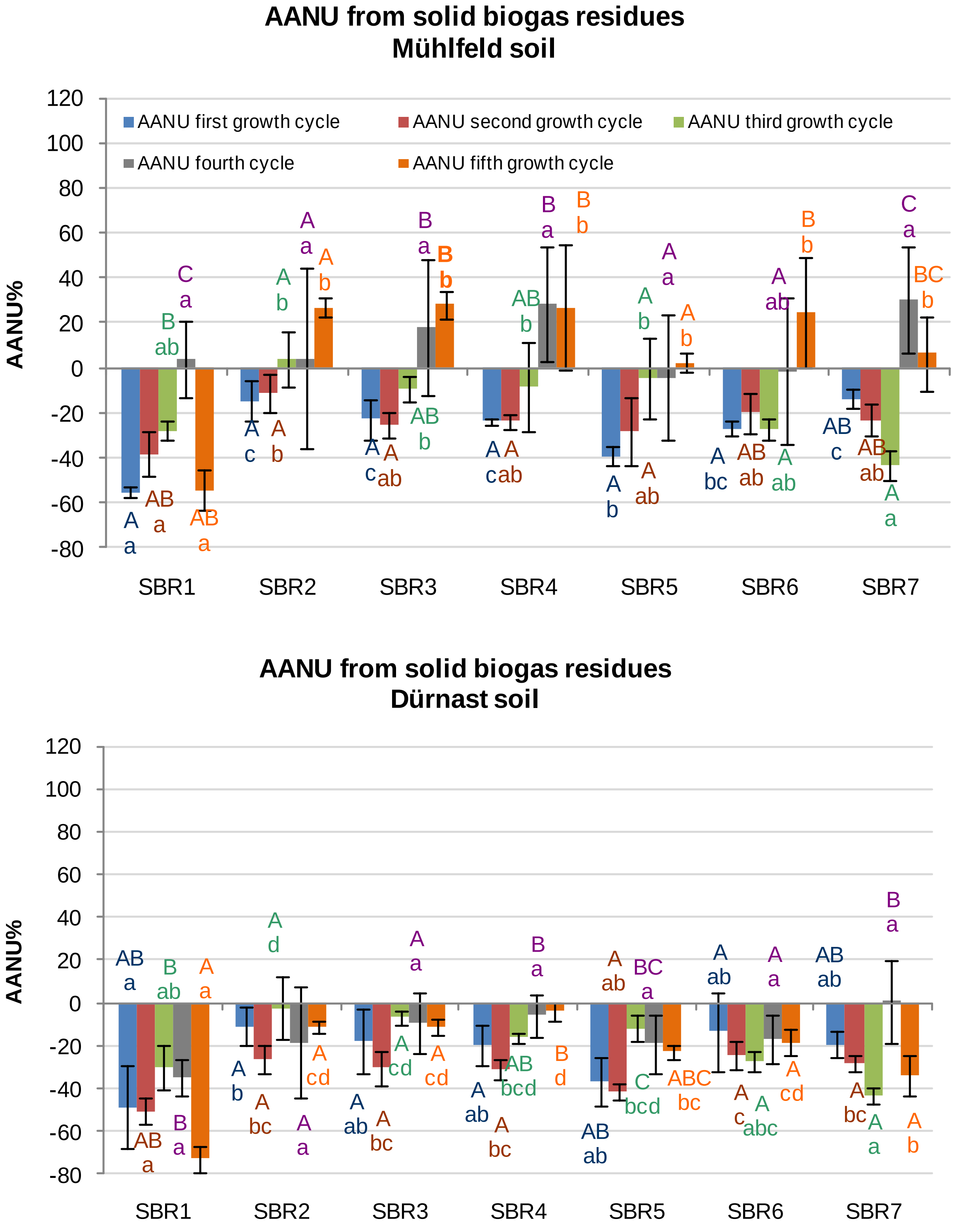


Figure 21 Additional apparent N utilization of liquid and solid biogas residues collected in five growth cycles in two different soils. Different letters indicate statistically significant differences between the fertilizers ($p \leq 0.05$)

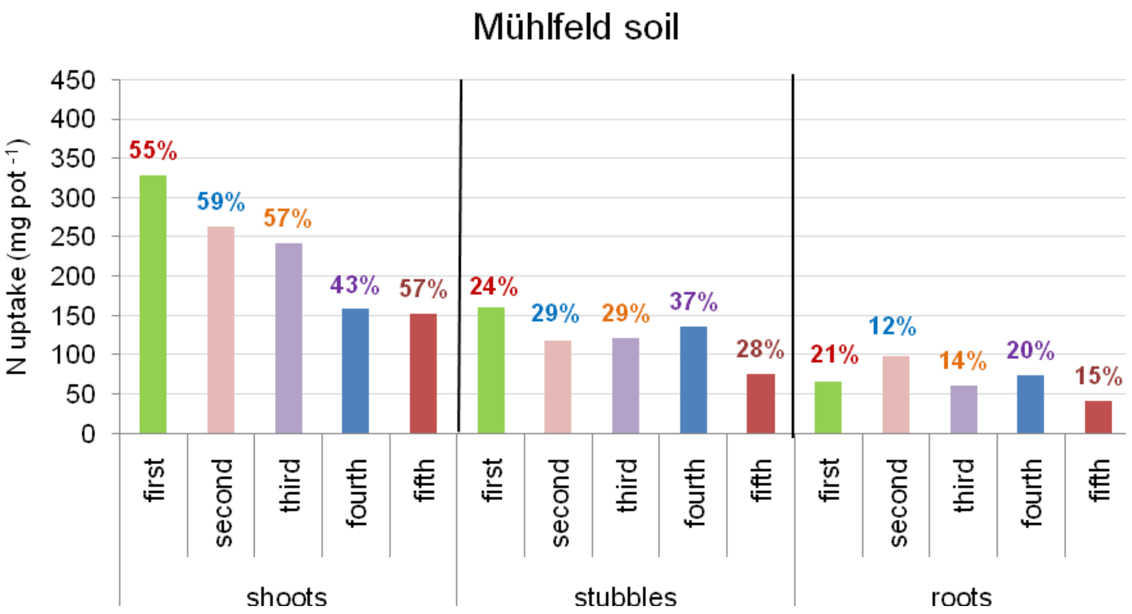
3.5.6 N uptake (shoot, stubble and root) from selected treatments

3.5.6.1 N uptake from mineral treatments during five growth cycles

The N uptake from the mineral treatment generally was higher in shoots followed by stubbles and the roots which showed the lowest N uptake in both soils (Fig. 22). In both soils and from the different growth cycles, shoot N uptake ranged from 43 to 60 % of the total N uptake, stubble N uptake ranged from 14 to 37% and root N uptake ranged from 12 to 27% of the total N uptake. Therefore in general, in both soils shoots took up about 55% from the total N uptake while in the stubbles 24% and in the roots about 21% of the total N uptake was found. Although the absolute N uptake in mg/pot decreased from the first to the fifth growth cycle, the proportion of shoots and stubbles did not significantly change with time.

3.5.6.2 N uptake in the last growth cycle

The fertilized treatments had a greater plant N uptake (shoots, roots, stubbles) than the unfertilized treatment (Fig. 23). At the end of the experiment the highest N uptake was found in the shoots of LBR1 (61% for the Dürnast and 64% for the Mühlfeld soil of the total N uptake), followed by the mineral treatment (59% for the Dürnast and 57% for the Mühlfeld soil) and finally by SBR1 (29% for the Dürnast and 36% for the Mühlfeld soil) which indicates a higher N immobilization from the solid compared to the liquid biogas residues (Fig. 23). The highest amount of N was accumulated in the roots of SBR1 (45% of all N uptake for the Mühlfeld soil and 52% of total N uptake for the Dürnast soil). In general, the amount of N in roots at the final growth cycle tended to be higher in the LBR1 and SBR1 than in the mineral fertilizer treatment. With the application of SBR1 the absolute N uptake of the roots as well as the proportion of root N in total N uptake exceeded that of the shoots.



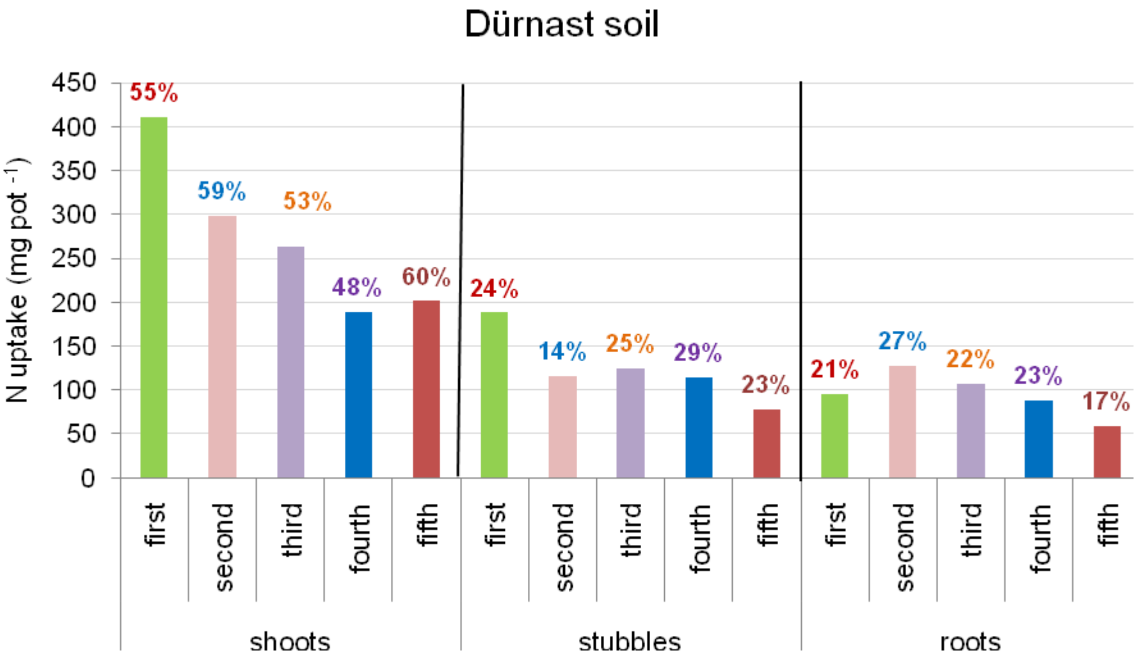
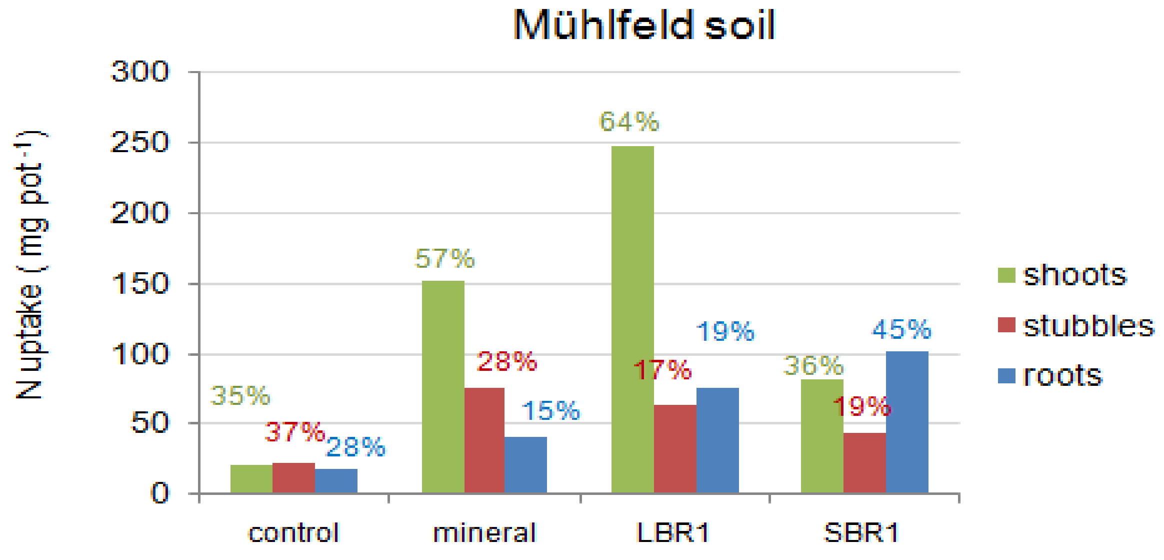


Figure 22 Partitioning of N uptake in shoots (all biomass harvests in each growth cycle), stubbles and roots from the mineral treatment during five growth cycles for two soils. Numbers above the columns represent the relative distribution of N in shoots, stubbles and roots in each growth cycle (sum of N in shoots + stubbles + roots = 100%)



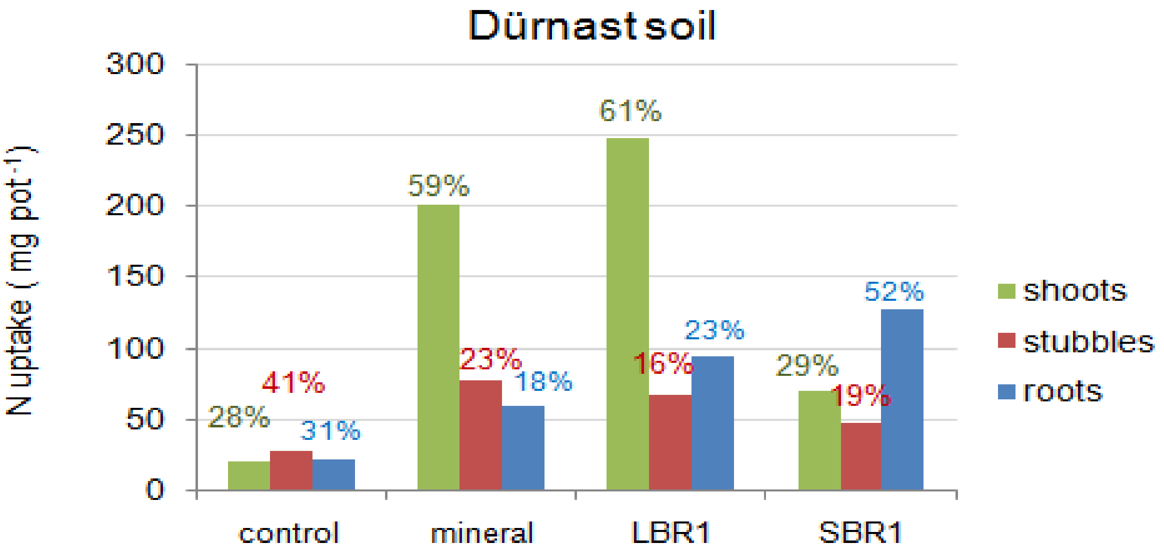


Figure 23 **Partitioning of N uptake in shoots (all cuts in each growth cycle), stubbles and roots from the control, mineral fertilizer, liquid biogas residue 1 and solid biogas residue 1 in the last growth cycle for two soils. Numbers above the columns represent the relative distribution of N in shoots, stubbles and roots in each growth cycle (sum of N in shoots + stubbles + roots = 100%)**

3.5.7 Relationship between N offtake of the liquid and the solid biogas residues and their C_{org}:N_{org} ratio

The C:N ratio can be a good indicator for the N mineralization from organic fertilizers. Tab. 14 shows the correlation between the N offtake and the C_{org}:N_{org} ratio for all liquid and solid biogas residues. In both soils the negative correlation between the C_{org}:N_{org} ratio and the N offtake of the ryegrass ranging from -0.76 to 0.93 was highly significant (*$p \leq 0.01$)* and valid from the first to the fifth growth cycle. However analyzing single data points makes clear that when the data set is subdivided into liquid (C_{org}:N_{org} 6.9 to 9.8) and solid (C_{org}:N_{org} 16.8 to 30.3) biogas residues this correlation is lower for LBRs or almost missing for SBRs (Fig. 24).

Table 14 Correlation coefficients between N offtake of the five growth cycles and C_{org}:N_{org} ratio of the solid and the liquid biogas residues

N uptake	C_{org}:N_{org}	
	Mühlfeld soil	**Dürnast soil**
1st growth cycle	-0.883**	-0.850**
2nd growth cycle	-0.843**	-0.847**
3th growth cycle	-0.759**	-0.756**
4th growth cycle	-0.886**	-0.931**
5th growth cycle	-0.851**	-0.810**

Significance level: * (5%) and ** (1%)

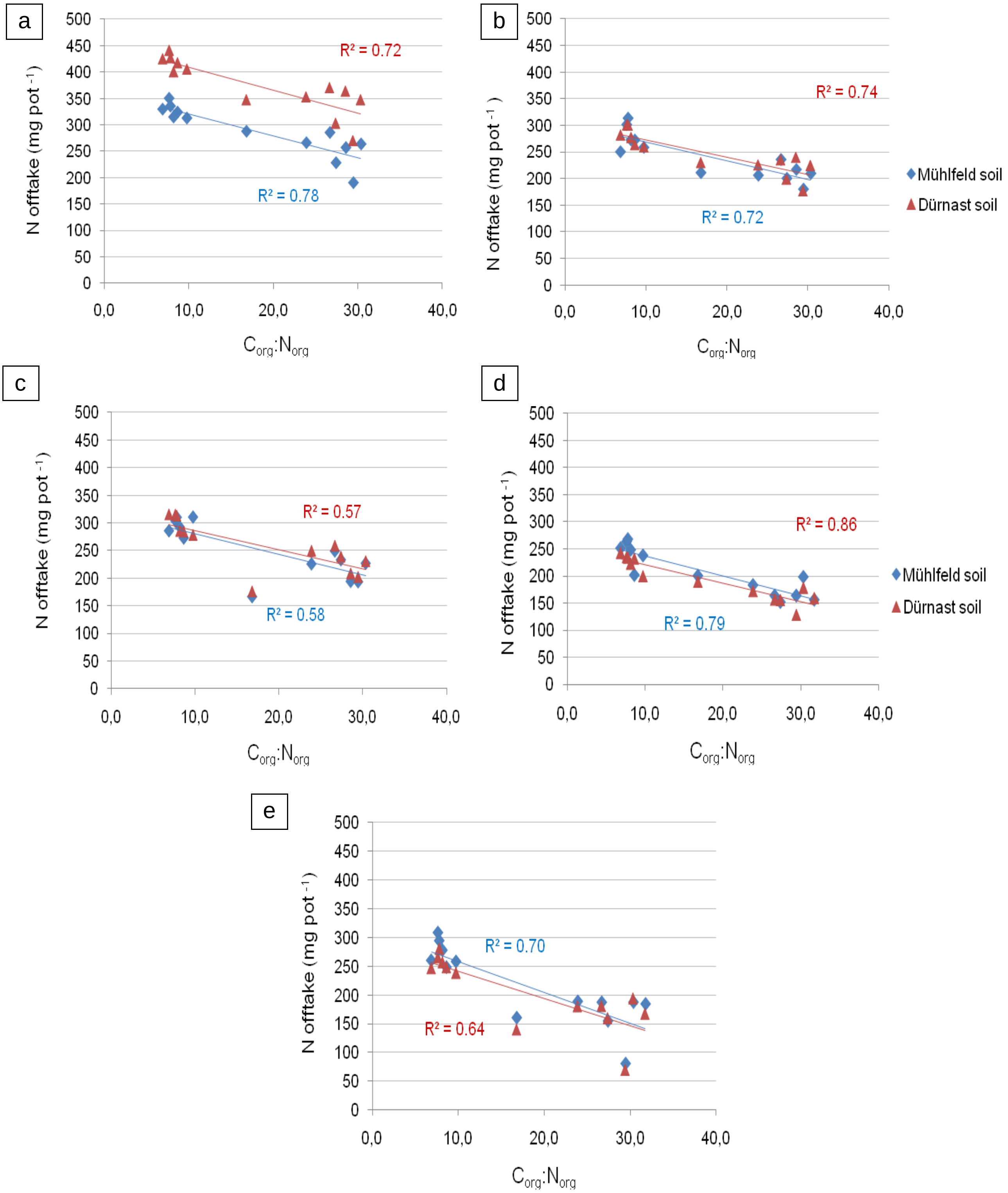


Figure 24 Relationship between N offtake in the five growth cycles and C_{org}:N_{org} ratio of solid and liquid biogas residues. The letters a,b,c,d,e represent the growth cycles from the first to the fifth growth cycle

3.5.8 Relationship between N offtake of liquid and solid biogas residues and the amounts of N_{org} and C_{org} applied in five growth cycles

As the fertilizer application was based on equal amounts of NH_4-N, different amounts of organic N and also organic C were applied (Tab. 15). When comparing LBRs and SBRs the organic carbon application from SBRs exceeded that from LBRs by far whereas the application of organic nitrogen was not much differing between LBRs and SBRs. With some exceptions e.g., a significant positive correlation coefficient of 0.969** in the second growth cycle in Mühlfeld soil, there was no significant correlation between the amount of N_{org} or C_{org} applied and the N offtake of the ryegrass in the five growth cycles although correlation coefficients were mostly negative for SBRs and positive for LBRs.

Table 15 Correlation coefficients between N offtake of five growth cycles and the amounts of C_{org} and N_{org} applied as solid and liquid biogas residues in two soils

Growth cycle	Mühlfeld soil				Dürnast soil			
	Liquid		Solid		Liquid		Solid	
	N_{org}	C_{org}	N_{org}	C_{org}	N_{org}	C_{org}	N_{org}	C_{org}
Applied amounts/ one application (g/pot)	0.22-0.37	1.6-3.5	0.22-0.48	7.7-11.1	0.22-0.37	1.6-3.5	0.22-0.48	7.7-11.1
First growth cycle	0.628 **NS**	0.145 **NS**	-0.516 **NS**	-0.647 **NS**	0.509 **NS**	0.132 **NS**	-0.183 **NS**	-0.229 **NS**
Second growth cycle	**0.969****	0.407 **NS**	-0.097 **NS**	-0.318 **NS**	0.774 **NS**	0.338 **NS**	-0.211 **NS**	-0.259 **NS**
Third growth cycle	0.579 **NS**	0.735 **NS**	0.310 **NS**	0.659 **NS**	0.421 **NS**	0.183 **NS**	-0.270 **NS**	-0.267 **NS**
Fourth growth cycle	0.542 **NS**	-0.603 **NS**	-0.627 **NS**	-0.574 **NS**	0.165 **NS**	-0.603 **NS**	-0.696 **NS**	-0.574 **NS**
Fifth growth cycle	**0.346***	0.379 **NS**	-0.166 **NS**	-0.174 **NS**	**0.884***	0.105 **NS**	-0.229 **NS**	0.030 **NS**

Significance levels: *(5%); ** (1%); NS (not significant)

3.5.9 Soil total N and total C at the end of the experiment

At the end of the experiment i.e., after the last cut and after five repeated applications of different LBRs and SBRs, soil total nitrogen and total carbon contents were determined (Tab. 16). The N contents in the soils changed compared to the start of the experiment in all organic fertilizer treatments. Compared to the mineral fertilizer soil N contents after the application of LBRs did not change significantly in both soils (except LBR2 in Mühlfeld soil). In contrast the application of most of the SBRs significantly increased soil N content compared with the mineral fertilizer treatment (except SBR 3 in both soils and SBR2 in the Dürnast soil). Similar results were obtained for the soil C_t contents. The application of LBRs did not significantly change soil C_t contents compared to the mineral treatment in both soils. By contrast soil C_t contents in both soils were significantly increased after five repeated applications of SBRs (except SBR3 in the Mühlfeld soil).

Soils C:N ratio significantly increased by the application of SBRs compared to the application of the mineral treatment in both soils except for SBR7, whereas the soil C:N ratio remained largely unchanged after the applications of LBRs. In addition comparing LBRs to the corresponding SBRs the soil C:N ratio was found to be higher with SBRs except for SBR3 and SBR7 in the Mühlfeld soil and for SBR 2 and SBR7 in the Dürnast soil.

Table 16 Soil contents of total nitrogen and total carbon at the end of the experiment

Treatments	Mühlfeld soil			Dürnast soil		
	$^{1}N_t$%	$^{2}C_t$%	C:N ratio	$^{3}N_t$%	$^{4}C_t$%	C:N ratio
Control	0.087^{a}	0.80^{a}	9.14^{ab}	0.134^{a}	1.205^{a}	8.99^{a}
Mineral	0.093^{ab}	0.828^{ab}	8.90^{a}	0.136^{ab}	1.253^{ab}	9.22^{ab}
LBR1	0.106^{bcde}	0.991^{abcd}	9.34^{ab}	0.153^{bcd}	1.414^{abcde}	9.25^{ab}
LBR2	0.109^{cdef}	1.022^{bcde}	9.37^{ab}	0.152^{abc}	1.422^{bcde}	9.38^{abc}
LBR3	0.100^{abc}	0.947^{abc}	9.85^{ab}	0.151^{abc}	1.399^{abcde}	9.25^{ab}
LBR4	0.106^{bcde}	0.995^{abcd}	9.41^{ab}	0.156^{bcd}	1.428^{bcdef}	9.15^{ab}
LBR5	0.103^{abc}	0.956^{abc}	9.28^{ab}	0.153^{bcd}	1.452^{bcdef}	9.50^{bcde}
LBR6	0.105^{bcde}	0.992^{abcd}	9.43^{ab}	0.151^{abc}	1.424^{bcde}	9.43^{abcd}
LBR7	0.101^{abc}	0.919^{abc}	9.08^{ab}	0.147^{abc}	1.336^{abc}	9.09^{ab}
SBR1	0.125^{f}	1.399^{g}	11.21^{e}	0.160^{cd}	1.641^{fg}	10.27^{f}
SBR2	0.115^{cdef}	1.215^{efg}	10.56^{de}	0.155^{bcd}	1.526^{cdef}	9.85^{cdef}
SBR3	0.103^{bcd}	1.015^{bcde}	9.86^{bcd}	0.154^{bcd}	1.524^{cdef}	9.92^{def}
SBR4	0.114^{cdef}	1.182^{defg}	10.36^{cde}	0.162^{cd}	1.609^{defg}	9.96^{ef}
SBR5	0.119^{ef}	1.267^{fg}	10.62^{de}	0.167^{d}	1.676^{g}	10.02^{f}
SBR6	0.117^{def}	1.256^{efg}	10.75^{e}	0.159^{cd}	1.626^{efg}	10.20^{f}
SBR7	0.110^{cdef}	1.052^{cdef}	9.56^{abc}	0.157^{cd}	1.485^{cdefg}	9.43^{abcd}

Before the experiment ^{1}Nt=0.09, $^{2}C_t$=0.80, $^{3}N_t$= 0.13 and $^{4}C_t$=1.09

Different letters indicate statistically significant differences between the fertilizers ($p \leq 0.05$)

3.5.10 Relationship between N offtake of the liquid and the solid biogas residues and soil N_t at the end of the experiment

Correlation analysis between N offtake in the last cut and soil N_t at the end of the experiment was done to study the long term effect of five repeated applications of LBRs and SBRs (Fig. 25). A weak positive correlation between N offtake of the last cut and soil N_t at the end of the experiment was found only in the liquid biogas residues in Mühlfeld soil. If LBR7 that is characterized by a very low N_{org} is excluded from the correlation analysis the correlation coefficient increases from 0.396 NS to 0.857*. A weak negative correlation between N offtake of the last cut and soil N_t content at the end of the experiment was found for the solid biogas residues in both soils. In contrast, a significant correlation between the N offtake of the last cut and soil N_t at the end of the experiment was observed in both soils when both liquid and solid biogas residues were included (r =-0.665** in the Mühlfeld soil and r =-0.623* in the Dürnast soil).

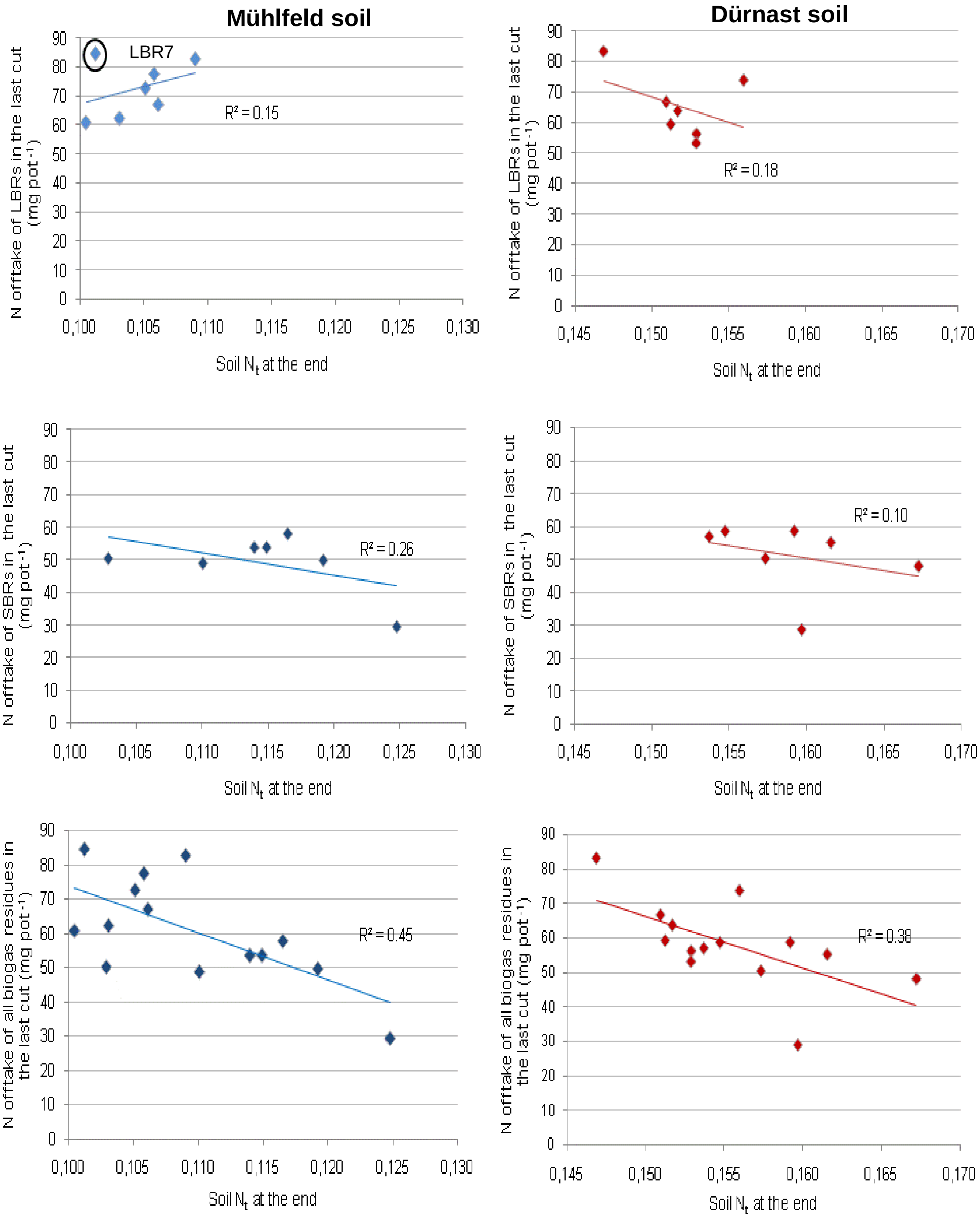


Figure 25 Relationship between ryegrass N offtake in the last cut and soil N_t at the end of the experiment after the applications of liquid and solid biogas residues in two soils

4. Discussion

4.1 N availability from different unseparated biogas residues

The present study investigates the “short term” effects of a single fertilizer application of different biogas residues on the N offtake of ryegrass, as well as the “long term” effects after five repeated applications (one application at the start of each of five growth cycles being based on equal amounts of ammonium).

After the application of organic fertilizers the ammonium will become available to plants, but part of it is immobilized in soil during microbial decomposition of organic compounds in the cattle slurry (Sørensen and Jensen 1995a). In contrast after the first growth cycle, the shoot N offtake of ryegrass from biogas residues and the undigested CS was comparable to or higher than that from the inorganic N fertilizer of ammonium nitrate (Fig. 7). Minimizing or avoiding ammonia volatilization by immediate incorporation of the organic fertilizers and nitrate leaching by using closed pots most likely accounts for this observation given that Matsunaka et al. (2006) showed that more N had to be applied as anaerobically digested CS compared to mineral fertilizer to achieve an equal N uptake because of considerable N losses through nitrate leaching and ammonia volatilization from organic fertilizers. However, our results are congruent with those of Gunnarsson et al. (2010), who found that N uptake of ryegrass foliage and stubbles from the application of a biogas residue in a pot experiment was equal to that from an inorganic fertilizer following one application (i.e., after one growth cycle). Similar results were also obtained by de Boer (2008), who compared the apparent N utilization from calcium ammonium nitrate with that from pig slurry co-digested with different co-products. The higher N release from organic fertilizers was particularly shown in the 2nd and 5th growth cycles (Fig. 9). This indicates that the superiority of organic sources over the mineral source was more marked with time. It must be pointed out that N application of the organic sources as well as the mineral source was done on the basis of their content of NH_4^+-N**.** Since the organic sources have additional N in the form of organic N as compared to the mineral source, organic sources may provide more N to plants upon its mineralization. Kirchmann and Lundvall (1993) reported that animal slurries provide efficient slow release from N sources. Accumulation of N in the soil was reported by Flowers and Arnold (1983) as a result of using organic N for fertilization.

The apparent N utilization (ANU) from all fertilizers decreased with time (Fig. 8), and the highest utilization occurred in the first growth cycle and the lowest was in the fifth growth cycle. An increasing proportion of non-harvested stubbles may at least partly account for this. In the first growth cycle of the present study, there was no significant difference between digested cattle slurry with 14% of maize silage and undigested cattle slurry (Fig. 7). Rubaek et al. (2001) found that digested slurry resulted in a higher N uptake than given by undigested slurry, and attributed this to the higher ammonia volatilization from the latter source.

The present study showed higher N offtake in the fifth growth cycle from CS than from BGRs (BGR 2,7 and 8 in Marktschwaben soil and BGR7 and 11 in Dürnast soil). This may be due to the higher C:N ratio of CS. Gutser et al. (2005) reported that the residual effect of N increased with a decreasing short term N release from organic fertilizers. Further soil microorganisms may have increased in the slurry treatment immobilizing N in their bodies. Such biomass N would need some years to mineralize again for crops (Sørensen and Amato 2002). These results agree with results by Martyniuk et al. (2002) who concluded that the long term fertilizer application for 17 years increased soil fertility more than mineral fertilizers. These results are similar to those found by Rubaek et al (2001) who observed more microbial immobilization of N in raw slurry than in digested slurry. On the other hand, results obtained by Loria et al., (2007) showed no difference after a 3-year experiment between raw and digested slurry.

4.2 Effect of unseparated biogas residues' C:N ratios on N mineralization

The C:N ratio can be a reliable predictor for the N mineralization to distinguish nitrogen-rich from nitrogen-poor materials. Thus the C:N ratio can be a useful tool for the N availability. The N offtake of ryegrass after the first growth cycle was significantly related to the composition of the biogas residues or the CS (Fig 10). In both soils tested, a significant negative correlation was found between the C_{org}:N_{org} ratio of the organic fertilizers ranging from 8.6 to 18.6 and the N offtake of ryegrass. Similar negative correlations between N uptake and the C_{org}:N_{org} or C:N ratio of organic fertilizers were also reported in other studies (Gutser et al. 1987; Chadwick et al. 2000; Sørensen et al. 2003; de Boer 2008). Chadwick et al. (2000) found a highly significant negative correlation between C:N_{org} of organic manures and N mineralization during the first 30 days of an incubation experiment, but such correlation was not significant after 45 days of incubation. Such a relation was not found with the second and the fifth growth cycles in the present study (Tab. 7). Van Kessel and Reeves (2002) studied correlations between N mineralization and manures composition after a 56-day incubation experiment and obtained a highly significant correlation between organic N mineralization and $C:N_t$ ratios of manures.

When co-fermented slurry is compared to undigested raw slurry, it was often found that the C/N ratio decreased through the process of anaerobic digestion (Gutser et al. 1987; Asmus et al. 1988; de Boer 2008; Möller et al. 2008), thereby enabling increased plant N uptake. Similarly, in the present experiment, CS co-fermented with 14% maize silage was characterized by a lower C_{org}/N_{org} ratio compared to undigested CS. However, the C_{org}/N_{org} ratio for the former is still relatively high compared with the other tested biogas residues. This fact may be the reason why no significant increase in either the N offtake or in the apparent N utilization was observed in the first and second growth cycle between undigested and co-fermented CS.

Although the biogas residues differed considerably in their ammonium N, N_{org} and C_{org} contents, no consistent relationship existed between the composition of the biogas residues and the different fermentation substrates used for the digestion (i.e., whether the residues were either purely plant based or derived from co-fermentation with animal excrements). Residues obtained from co-fermentation (BGR 1, 7 and 11) contained both high and low C_{org}/N_{org} ratios or NH_4-N contents, with the chemical composition of the 100% plant based residues being similar to those from co-fermentation. It remains unclear, however, whether the N availability to ryegrass from the biogas residues is related to the composition of the fermentation substrates used for digestion. Although the lowest N offtake in both soils was obtained from the application of a purely plant based residue (BGR2) and the highest N offtake from one derived from co-fermentation (BGR11), the N offtake from another purely plant based residue (BGR8) was not significantly different from the co-fermentation residues BGR1 and 11.

4.3 N utilization from different unseparated biogas residues

The apparent N utilization (ANU) after the first growth cycle ranged from about 80% for the mineral fertilizer to about 100% for some biogas residues (e.g., BGR11). This additional ANU of up to 20% compared to NH_4NO_3 indicates a significant mineralization of organic N even after a single fertilizer application (Fig 8). No statistically significant net N immobilization was found for either biogas residues or for CS. This latter result seems to contrast with observations by Sorensen and Jensen (1995a) and Flowers and Arnold (1983), who both report a net N immobilization of 24 to 40% compared to ammonium sulfate when cattle or pig slurry were mixed into the soil. However, such high net N immobilization can be explained by the comparatively high C/N_{org} ratio of the CS used in their experiment (about 30 compared to 18.6 here).

The additional apparent N utilization (AANU) from some of the organic fertilizers as compared to the mineral fertilizer increased significantly after repeated fertilizer application (Fig 9). Such increasing N releases after regular application of organic fertilizers with high proportions of organic N has been reviewed by Gutser et al. (2005). Organic N from organic fertilizers acts mainly via the soil N pool because of the slow release characteristics of the organically bound N. Residual effects expressed as an increase in plant N uptake from N_{org} after long-term application of animal slurry are well described (Martynuik et al. 2002; Schröder et al. 2005; Angers et al. 2010) and are often associated with an observed increase in soil N_t.

4.4 Soil N accumulation after five repeated applications of different unseparated biogas residues

Mineral N content in the soil at the end of the experiment reflects the balance between the processes of addition, transformation and loss in the soil. Removal of N from soil occurs mainly by N uptake by the plants. The N remaining in the soil which received a mineral N source would be expected to be less than that which received an organic source. Most of the N remains in the soil at the end of the experiment adding to the long term pool of soil organic N (Morvan et al. 1997).

In the present study, both an accumulation of soil N_t and an increased additional ANU were observed after five fertilizer applications over the course of nearly one year. This result may be due to the intensive soil-fertilization-crop system used as a model here, which seems to accelerate such processes.

Moreover, because all fertilizer applications in this experiment were based on an equal amount of ammonium, the amount of N_{org} applied differed considerably among the organic fertilizers resulting in different N accumulations in the soil at the end of the experiment (Tab. 6). Consequently, N offtake by ryegrass in the fifth growth cycle and particularly that of the last biomass sampling, which received no direct fertilizer application, were closely related to the soil N content. In contrast to the effect observed after the first growth cycle, no correlation between N offtake and the C_{org}/N_{org} ratio of the organic fertilizers was found after repeated fertilizer application, possibly indicating that agricultural soils that are regularly supplied with organic fertilizers might react less to different qualities of organic fertilizers over the long term. Dick (1992) noted a positive relationship between soil organic matter content and soil microbial biomass, and concluded that any practice that increases organic matter in soil increases the biological activity in the soil. Organic and inorganic input of N, when combined with appropriate management, increases the amount of residue returning to the soil, and thus increases the soil biological activity. Concerning the C content, the highest was that within the cattle slurry treatment. The importance of N-mineralization in determining N uptake from manure is dependent on the amount of organic N initially present as well as the potential for mineralization (Douglas and Magdoff 1991). Many animal manures, particularly poultry manure and slurry, contain considerable proportion of their N in soluble inorganic forms (Beauchamp 1983) with as much as up to 80% of the total N. However in some cases a considerable proportion may be lost by volatilization from such sources (Beauchamp 1986).

4.5 N mineralization from different soils

4.5.1 Effect of soil type and organic matter content on N release from unfertilized and fertilized soils

The ammonium present in organic fertilizers may be immobilized into the microbial biomass. If the immobilized N is not released for plants during the season its availability will be decreased (Paul and Beauchamp 1994). Immobilized N may remain in the soil organic matter in forms recalcitrant to decomposition (Sørensen 2004). However the increase in the rate of N transformation does not necessarily affect the net rate of N mineralization or immobilization. Mineral N is continuously released and mineralized from the soil organic matter pool or organic fertilizers but predicting the N mineralization from organic N is difficult because it is affected by several factors such as soil temperature, soil water content, soil texture and soil organic matter content. Because mineralization is a biological process, it only occurs when soil conditions are suitable for biological activity. Soil type can affect the dynamic processes of soil organic matter build up as well as the dynamics of N mineralization. Moreover, N transformation depends on soil type and organic matter content. Therefore the N offtake in soil supplied with organic fertilization as compared with that in soils not supplied gives a relative indicator to interpret the decomposition of the organic fraction in soil. Hence in the present study the N offtake by the control soils (without fertilizer application) was greatest in soil 4 (organic soil). Soils 1, 2 and 5 (silt loam soils) mineralized intermediate amounts of N and soil 3 (sandy soil) mineralized the least N (Fig. 14). The N offtake of plants from unfertilized soils can be used as an indicator to describe the N mineralization of organic nitrogen in soils. The N offtake in unfertilized soils which did not receive fertilizer ranged from 40 to 340 mg N/pot and varied among the soils (Fig. 11). In all growth cycles the highest N offtake was observed in the organic soil followed by silt loam soils. The sandy soil showed the lowest N offtake when unfertilized. The N offtake from different soils was associated with the soil total N content which was highest in the organic soil and lowest in the sandy soil. These results agree with those reported by Egelkraut et al. (2000) who found that in the untreated soils, the sandy loam and loamy sand soils high in clay content showed a high N uptake as compared with the sandy soil. Accordingly, the highest N offtake would be in the organic soil which contained 0.45 g N_t/kg soil and the lowest N offtake was in the sandy soil which contained 0.15 g N_t/kg soil. Thomsen et al. (2001) showed that increasing the soil clay content from 10% to 40% leads to a decrease in the net ^{15}N mineralization of the ryegrass.

The additional apparent nitrogen utilization (AANU) can predict the increasing net N mineralization of the BGRs (Fig. 15). Results show no effect obtained from different soil types on the N mineralization from the biogas residues. These results disagree with results obtained by Amato and Ladd (1992); Hassink (1992); Gordillo and Carbrere (1997) who studied the N mineralization in different soils and found that N mineralization was related to sand content. They explained these results due to soil aeration being suitable for the microbial activates.

Sørensen and Jensen (1995b) found that N uptake by grass in unfertilized soils was increased by increasing the soil clay content. They attributed this to the fine soil particles causing reduction of decomposition of organic residues through a protection mechanism. Bossta and Ågren (1997) reviewed that N mineralization from the soil was related to clay content. Hassink et al. (1994); Sørensen and Jensen (1995); Griffin et al. (2002) reported that the decomposition rate of organic residues in the soil is lower in fine-textured soils than in coarse-textured soils.

The N release in our present study from the five unfertilized soils is obviously related to its total N and C, in all growth cycles. These results agree with those obtained by Vellinga and André (1999) who studied soil nitrogen supply from peat, clay and sand soils and observed a higher soil nitrogen supply from the peat soil than from the clay or the sand soil. They attributed the high soil nitrogen supply from peat soil to its high organic matter compared with the clay soil or the sand soil. On the other hand Whitehead (1984) did not observe such relationship between the N mineralization in soil and its organic matter content. The present study shows that the additional N offtake from the fertilizers differed between soils in each growth cycle but that it was not related to soil organic matter content or soil texture. This result disagrees with the results of Legg and Stanford (1967) who examined the N uptake from 12 soils differing in their organic matter content and C/N ratio for 8 weeks and reported that soils which were higher in total carbon content (1.9-3.9%) and total N (0.15-0.34%) showed a higher N uptake compared to soils with lower total C (0.53-1.58%) and total N (0.041-0.083%) contents.

4.5.2 Effect of organic fertilizers on N mineralization in different soils

For all growth cycles, there were significant effects of both soils and fertilizers on the N offtake of ryegrass but there were no significant interactions between soils and fertilizers (Table 9). This result agrees with the result obtained by Chantigny et al. (2008) who found a significant effect of different organic fertilizers on N uptake in the plant grown in loam and clay soils.

The effect of the soil, averaged over all fertilizers was inconsistent and changed from one growth cycle to the other. The lowest additional N offtake in the first growth cycle was observed in soil 2 (silt loam) whereas in the second growth cycle this was true for soil 4 (organic soil) and in the fifth growth cycle it occurred in soil 1 (silt loam) (Fig. 14).

In each growth cycle, the average additional N offtake from the biogas residues fertilizers, was significantly higher than from the mineral fertilizer and seemed to increase with time. In the first and second growth cycles no differences appeared between the biogas sources. In the fifth growth cycle the N offtake was significantly greater from biogas residue BGR1 to BGR6 and BGR8. The N mineralization from the three BGRs (BGR1, 6 and 8) differed. BGR 6 and 8 gave higher shoot additional N offtake particularly in the fifth growth cycle. This reflects the higher organic N applied with BGR6 and BGR8 (22 and 25 g N_{org}/pot) compared to BGR1 (6 g N_{org}/pot). Based on an equal amount of N applied N mineralization will depend on the N content of the material. For example, Egelkraut et al. (2000) reported a higher net mineralization rate from cotton leaves of 28.3 g N_{org}/kg compared to cotton stems of 15.4 g N_{org}/kg. Sørensen and Jensen (1995b) found that N mineralization was higher from an-aerobically stored sheep manure of 33.6 g N_{org}/kg than from fresh manure of 31.7 g N_{org}/kg. Whitemore and Groot (1997) found that the N availability was strongly dependent upon the amount of N added in the crop residues since spinach with C:N ratio of 6 released more N compared with other plant residues having C:N ratios of 13 to 15 and concluded that sandy soils mineralize N earlier than the silt loam soils. From the present study it is concluded that the soil and fertilizers represented important factors for N mineralization but no interaction was obtained between soil and fertilizers.

4.5.3. Soil N accumulation at the end of the experiment from different soils

After five repeated fertilizer applications soil total N contents of fertilized treatments were higher as compared with the unfertilized treatment (Tab. 11). While application of NH_4NO_3 as a mineral fertilizer had little effect, application of biogas residues had greater effect. Soil total N accumulation was higher after the application of BGR 6 and 8 compared with BGR 1. This is associated with the difference in amounts of organic N applied which were 2.6 or 2.9 times higher for BGR 6 and 8, respectively, compared with BGR 1. At the end of the experiment, the soils varied in N accumulation. The N accumulation in the soil seemed to be associated more to the silt content which was high in soil 1 (of 73% silt) followed by soil 5 (of 21% of silt) and was lowest in soil 3 (of 8% silt). This result agrees with the finding by Hassink et al. (1994). Higher N accumulation was obtained in soils treated with organic fertilizers than in those treated with mineral fertilizers. Sørensen and Jensen (1995b) attributed the higher N mineralization in manure-treated soils compared to the mineral fertilizer treated ones to the mineralizable organic N in manure. Sørensen and Amato (2008) found that the N accumulation of barley crops from the injection of pig slurry in the first year was comparable from a loamy sand soil having 1.4 g total N/kg soil and a sandy loam soil having 1.6 g total N/kg. They attributed that to the lower clay fixation of N in the loamy sand soil due to its lower clay content. They concluded that soils of high clay content have a higher capacity for physical protection. Herlihy (1979) found that N accumulation in the soil depends on soil texture; higher N accumulation was obtained in a loam soil and lower accumulation occurred in a loamy sand soil.

4.5.4 Net mineralization of soil organic matter from different soils

Results show that the highest net N mineralization rate/year occurred in the soil 3 (sandy soil) and the lowest was in soil 4 (clay soil). Net N mineralization of soil organic matter was found to be more rapid in sandy soils than in clayey soils (Sørensen 1981; Hassink et al. 1993). Giardina et al. (2001) found in a short term litter decomposition study lasting 2 years that soil N transformation was little related to soil clay content. Soil aeration would enhance microbial and microfauna populations present in this soil as well as cause a physical protection of the soil organic matter (Hassink et al.1993; Hassink et al.1994). Barker and Pilbeam (2007) reported that the turnover of humus N could be about 1 to 3% of the soil N depending on the type of the soil, climate and other factors.

4.6 Separated biogas residues (liquid and solid)

4.6.1 N availability from liquid and solid biogas residues

Liquid biogas residues applied to the ryegrass increased the shoot N offtake from the first until the fifth growth cycle compared to that of the solid biogas residues (Fig. 18). This result is in agreement with findings reported by Manfredini et al. (2010) who found more plant available nitrogen from the liquid digested pig manure compared to that from the solid digested one from the 10th until the 75th day of the incubation period.

The liquid biogas residues which were used in the present study were characterized by a low C:N ratio (6.9 to 9.8). Persson and Kirchmann (1994) concluded that the decomposing process in arable soils are favoured by a C:N ratio of about 10 and by suitable aeration, sufficient nutrient status and suitable pH. Gutser et al. (2005) noted that a high N release can be expected from organic fertilizers with a $C:N_{org}$ ratio below 6 to 7 while organic fertilizers with a high C:N ratio lead to immobilization. Qian and Schoenau (2002) studied the N release from different solid manures differing in their C/N_{org} ratio and found that the net N mineralization was associated with a low C:N_{org} leading to a high N uptake from the animal manures with high organic N contents and the low C:N ratios of about 7.6 to 6.6. In the present study the N offtake was comparable between the liquid biogas residues and mineral fertilizer in the first growth cycle. These results are similar to those found by Chantigny et al. (2008) who reported that the N offtake by plants after one fertilizer application from separated liquid swine manure was similar to that from mineral fertilizer, while the N offtake by plants from solid biogas residues was less compared to the mineral treatment. However (using labelled N) Sørensen and Thomsen (2005) showed a higher recovery of N from the mineral (55%) than from separated liquid pig slurry (51%) after one fertilizer application, while the recovery of N from solid separated pig slurry was 26.5%. After 3 years recovery of N dropped to 3.5% for separated solid pig slurry followed by 2.3% for liquid separated pig slurry and 1.7% for the mineral fertilizer.

In the present study the long term effect was studied after four and five applications. After five applications the N offtake from the liquid biogas residues exceeded that from the mineral fertilizer by a factor of about 2, indicating that about all the organic N applied in addition to the fertilizers' ammonium might have become plant available (approx. the NH_4-N/N_t ratio is 50% see Tab. 12 and 13). These results agree with the results reported by Chantigny et al. (2008) who found that after annual applications for three years based on total N, the N export in grains was similar from the liquid swine manures as compared to the mineral fertilizer. They attributed this to the intermediate incorporation of the digested and liquid swine manure into the soil resulting in a decreased NH_3 volatilization. Chantigny et al. (2007) studied the N uptake of imothy grass from inorganic fertilizer, raw swine slurry, digested swine slurry and the liquid fraction of separated swine slurry using two different soils, a loam and sandy loam. They reported that after 3 years of annual fertilizer application, the N uptake was higher from the fertilized treatments and that N uptake was higher from the digested as well as from the separated slurry than from the raw swine slurry. Moreover, N uptake in the loam was higher compared to the sandy loam, a fact that is in agreement with the results obtained here as well.

To achieve comparable N offtake from mineral and solid biogas residues five repeated applications were needed.

In the five growth cycles of the present study the apparent N utilization (ANU) of the mineral fertilizer ranged from 40% to 80% in the Mühlfeld soil and between 60% to more than 80% in the Dürnast soil (Figure 20). In the first growth cycle, the ANU in the Mühlfeld soil from the liquid biogas residues was between 80 and 85% but it increased up to 100% in the fifth growth cycle from both soils. Meade et al (2011) found that based on total N the nitrogen uptake efficiency (NUE) of an inorganic N fertilizer was significantly higher (67%) compared with that of the liquid pig manure (33%) after two fertilizer applications during two years. Sørensen and Amato (2002) attributed the lower pig manure NUE to the immobilization of NH_4-N during microbial decomposition of the organic matter in the manure after application to the soil. They also reported that a higher proportion of applied N remained in the soil system. Sørensen and Jensen (1996) found that the N recovery from a sandy loam soil was 62% from urine and 78% from urea, but was between 51 and 53% from urine and urea fertilizers in a sandy soil after 6 months from grass sowing, with high N mineralization in the sandy soil.

Additional apparent nitrogen utilization (AANU) for all liquid biogas residues (LBRs) increased significantly along the course of the five fertilizer applications (Fig. 21). The increase was most obvious in the Mühlfeld soil, while in the Dürnast soil the increase was lower. The AANU from the LBRs in the first growth cycle ranged between -3 to 13% in the Mühlfeld soil and reached 70 up to 130% in the fifth growth cycle. Higher N uptake occurred in the Mühlfeld soil than in the Dürnast soil, and this reflects the difference between the properties of the two soils. This denotes a difference in cycling and turnover of organic nitrogen through the process of immobilization–mineralization, soil aeration and the suitable water status enhance the microbial activity (Hassink et al., 1993). The AANU from the solid biogas residues was up to -42% in both soils. These results agree with results by Laboski et al. (2010) who found that plant available nitrogen after applying digested separated liquid cattle manure was 52.2%, but from the solid digested separated manure it was -14.6%. They stated that the negative values of available nitrogen from separated solid manures indicate high N immobilization. In the present study the N availability increased over time especially from liquid as well as solid biogas residues. The current results are also in good agreement with those by Manfredini et al (2010) who found that after 80 days of incubation available N was higher from digested liquid pig manure (60%) than that from digested solid pig manure (-22%). Results are also in agreement with those by Pereira et al. (2010) who found that mineralization /immobilization efficiency from the liquid fraction and the solid fraction after the separation was -9.7 and -35.6 mg N/kg, respectively, after 93 days of incubation in a silt loam soil.

High correlations between N offtake and C:N ratio were observed in both soils from the first to the fifth growth cycle. In the first growth cycle in the Mühlfeld soil a highly significant negative correlation (r = -0.883**) occurred, a similarly significant one (r = -0.850**) was found in the Dürnast soil (Tab. 14). Therefore N mineralization from both liquid and solid residues was related to the C:N ratio with the lowest one of 6.9 for a liquid residue and the highest one of 30.5 for a solid residue. Solid biogas residues had higher C:N ratio and lower N mineralization, while liquid had lower C:N ratio and higher N mineralization (Fig. 24). The C:N ratio may not be an adequate parameter for mineralizable N. In the third and the fourth growth cycles for the liquid biogas residues there was a significant correlation with r = - 0.862* in the third growth cycle and with r = - 0.910* in the fourth growth cycle. This result conflicts with the result found by Qian and Schoenau (2002) who studied the relationship between N uptake and available N supply over time from 11 solid manures of different C:N ratios (6 to 21). They found that the relationship increased over time (67days) in a loam and a sandy loam soils. For the group of the solid biogas residues alone, the C_{org}:N_{org} ratios of 16.8 up to 30.3 were not sufficient to explain the N mineralization from the solid biogas residues used in the present experiment. The C_{org}:N_{org} ratio of the liquid biogas residues of 6.9 to 9.8 is also not adequate to explaine the N mineralization from the liquid biogas residues. Thus the C_{org}:N_{org} ratio differs between the liquid and the solid biogas residues, but not among the liquid biogas residues or among the solid biogas residues (Fig. 24). Organic residues with similar C_{org}:N_{org} ratio may mineralize different amounts of N due to the differences in their composition that are not explained by the C_{org}:N_{org} ratio. Wood et al. (2010) evaluated 87 different poultry wastes in an incubation experiment and found a significant negative correlation between N mineralization and lignin content and a positive correlation between N mineralization and hemicellulose content. Nourbakhsh (2006) observed that after 46 weeks of incubation using different plant materials there was a highly significant positive correlation between the N content and N mineralization.

Cabrera et al. (2005) summarizing factors that control the net N mineralization of organic residues concluded from studies by Withmore (1996) that a range in C:N ratios of 20 to 40 in the organic material represents the break-even point between net N mineralization and net N immobilization. In some cases, this break-even point was found to be near a C:N ratio of 15 (Gilmour, 1998). It was concluded by Laboski et al. (2010) that manure separation was effective for N utilization with or without anaerobic digestion.

4.6.2 Accumulation of N in shoot, stubble and root at the biomass harvest

The fertilizer treatments increased the N uptake by roots. Gunnarsson et al. (2010) mentioned that N fertilization led to decreased organic matter as well as decreased root respiration. The amount of N in roots at the final cut tended to be higher in the solid biogas residues than the liquid one and the mineral fertilizer (Fig. 23). These results are in agreement with the results by Gunnarsson et al. (2010) who found that N recovery in ryegrass roots after 172 days tended to be higher with biogas effluents than with inorganic sources. They noted the direct impact on C and N status. Sørensen and Thomsen (2005) found that N recovery by the barley stubbles + roots after 3 years application was higher from the separated solid pig slurry (0.5%) followed by the separated liquid one (0.3%) and the lowest was obtained from the mineral fertilizer (0.2%).

4.6.3 Soil total N after ryegrass harvest at the end of the experiment

Soil total N after the last harvest of the ryegrass was significantly greater in fertilized treatments of different types of biogas residues (solid and liquid fraction) applied during one year compared to the unfertilized treatment. A high accumulation of N in the soil after the harvest from the solid biogas residues compared to the liquid biogas residues was observed (Tab. 16). Gunnarsson et al. (2010) reported an increase in soil mineral nitrogen after 172 days after the application of biogas effluent compared to the mineral treatment. They concluded that based on an equal amount of N applied the soil delivery capacity increased when the N supply source was changed from inorganic fertilizer to biogas residues. Sørensen and Thomsen et al. (2005) found a high N accumulation in the soil 0-20 cm top layer after three years of applying separated solid pig slurry compared to the separated liquid pig slurry and mineral fertilizer. The present results also agree with the results reported by Chantingny et al. (2007) who found higher N accumulation after 3 years applying separated liquid swine manure compared to mineral fertilizers, and more N accumulated in a loam soil than a sandy loam soil. Higher N accumulation in the soil at the end of the experiment occurred in the Dürnast soil (silt loam) than in the Mühlfeld (sandy soil). This result also agrees with those found by Qian and Schoenau (2001) who reported higher available N supply in a clay loam soil than in a sandy loam soil, and that N mineralization in coarse textured soils was more rapid than in the fine textured soil (Hassink et al.1994).

Bertora et al. (2008) reported a higher N accumulation in the soil after 85 days of incubation was obtained from urea and the liquid fraction of pig slurry than from the solid fraction of pig slurry (138 mg NO_3-N/kg soil). They concluded that the amount of soil nitrate was strongly affected by the type of the slurry.

5. Conclusions

The unseparated biogas residues derived from different biogas plants fed with different substrates for fermentation varied extremely in their chemical composition. A similar high variability in the chemical composition was observed for liquid as well as solid biogas residues derived from different digesters after separation. Further research will be needed to elucidate the possible relationship between the composition of biogas residues and the composition of substrates as well as the efficiency of the fermentation process.

Based on the same amount of NH_4^+-N application, the unseparated biogas residues and the separated liquid biogas residues provided N at least corresponding to their NH_4-N contents particularly after one time fertilizer application. However, the separated solid biogas residues provided similar or less N than it would be expected from their NH_4-N contents. After one fertilizer application the C_{org}:N_{org} ratio of unseparated and separated biogas residues, including both the liquid and solid fractions, was a decisive factor for the short term N availability to ryegrass. Whereas the C_{org}:N_{org} ratio revealed to an unsatisfactory parameter for the short term N availability from liquid biogas residues or solid biogas residues alone. After repeated applications, a positive correlation between soil N_t content and plant N offtake from unseparated biogas residues indicated that the accumulation of N_{org} from the organic fertilizers leads an increased release of N from the soil N pool. In contrast, the increased soil N_t after the repeated application of solid biogas residues did not result in an increased soil N mineralization whereas the soil C:N ratio was increased instead. The increased additional apparent N utilization (AANU) from the first growth cycle to the fifth growth cycle found for all unseparated biogas residues and all separated liquid fractions, indicated a considerable N mineralization from the organic N of these fertilizers. However, the negative AANU from the solid biogas residues demonstrates their potential for N immobilization. Consequently, it can be suggested that the continuous application of biogas residues leads to an increase in soil organic matter and soil total N which can possibly be mineralized by time. Such increased N mineralization will increase soil fertility and plant available N but also the risk of N losses by denitrification and nitrate leaching. In addition, for an efficient use of biogas residues application techniques, such as effective incorporation by injection, needs to be improved in order to avoid N losses by NH_3 volatilization.

6. Summary

The residues of the anaerobic digestion process contain valuable nutrients (N, P, K, S, etc.) which need to be recycled in crop production in order to fulfil the aims of a sustainable and environmentally efficient biogas production. However, biogas residues are obtained from different substrates used for digestion, being either exclusively plant based or derived from co-fermentation with animal excrements, a fact which may lead to differences in their composition and possibly also in nutrient availability. In addition physical separation of the biogas residues into a solid and a liquid fraction may change their composition which may affect the nutrient efficiency from both of them.

The objectives of this study were to

- characterize biogas residues either unseparated or separated into liquid and solid phases from the fermentation of different raw materials with respect to their N and C content,
- investigate short and long term effects of the application of these biogas residues on the N availability and N utilization by ryegrass
- evaluate the role of different soils on the N utilization of these biogas residues.

To reach these aims, as a test system two pot experiments each lasting for about one year were conducted with perennial ryegrass as a model plant in being regularly harvested. Short and long term effects on N offtake were investigated after single and repeated fertilizer application. In the first experiment, seven biogas residues obtained from the digestion of different raw materials and one undigested cattle slurry were incorporated into the soil based on an equal amount of NH_4-N (300 mg NH_4-N/pot*fertilization). In order to investigate the effect of soils on ryegrass N utilization, a number of soils with different soil texture (two silt loam soils, one clay loam soil, one sandy soil and one organic soil) were used. In the second experiment, biogas residues from seven different biogas plants separated each into a liquid and a solid fraction were used. The different biogas residues (liquid and solid fractions) were applied to two soils (one sandy and one silt loam soil) based on an equal amount of NH_4-N (300 mg NH_4-N/pot*fertilization). In both experiments, the grass was grown in five growth cycles each starting with a fertilizer application. Within each growth cycle the grass was cut two up to three times.

The following results were obtained:

(1) Based on an equal amount of NH_4-N applied, the N offtake of ryegrass from the unseparated biogas residues and cattle slurry was comparable or higher than that from NH_4NO_3. However, already after one fertilizer application there were significant differences in the N offtake of up to about 20% between the biogas residues. In the 1st growth cycle the N offtake of ryegrass was significantly related to the C_{org}/N_{org} ratio of the organic fertilizers. The additional crop N offtake from the organic fertilizers as compared to NH_4NO_3 increased from the 1st to the 5th growth cycle. At the end of the experiment, after five subsequent fertilizer applications the N offtake of ryegrass was positively correlated to the soil organic matter content which increased in response to the different amounts of organic N applied.

(2) The influence of soil type was investigated with five soils and three different biogas residues. The N offtake from the unfertilized five soils in all growth cycles was affected strongly by the soil N_t content. Consequently after five repeated applications, the highest N offtake was found in soil 4 with the highest organic matter content (4.4 g N_t/kg soil), but the lowest was observed with soil 3 with the lowest organic matter content (0.6 g N_t/kg soil). The additional nitrogen offtake from the fertilizers differed between soils in each growth cycle but with no consistent relationship to soil organic matter content or soil texture being observed. Moreover there was no interaction between soils and fertilizers. Soil net N mineralization rate of the accumulated organic matter at the end of the experiment did not differ between biogas residues.

(3) Based on the same amount of NH_4^+-N, after one fertilizer application shoot N offtake from the LBRs was comparable to the inorganic fertilizer in both soils. In contrast shoot N offtake from SBRs was comparable or lower than that from the inorganic fertilizer in both soils. Including LBRs and SBRs there was a significant negative correlation between the $C_{org}:N_{org}$ ratio of the biogas residues and the N offtake of ryegrass after one as well as after the following four successive fertilizer applications. However, within the respective groups of LBRs or SBRs the $C_{org}:N_{org}$ ratio was not a reliable parameter to predict ryegrass N offtake.

The additional apparent N utilization (AANU) describing the N release from the organic N fraction of the organic fertilizers increased with time from the first to the fifth growth cycle for all biogas residues.

However, while the positive values for the AANU of the LBRs indicated a significant mineralization of fertilizer organic N, the AANU of the SBRs were mostly negative, suggesting a considerable N immobilization of the applied ammonium.

After five repeated fertilizer applications, soil N_t and C_t contents increased after the application of SBRs but not of LBRs. However, this increase in soil organic matter content did not result in any increase in plant available N.

It is concluded that unseparated biogas residues from both 100% plant based substrates and from co-fermentation with animal excrements provide N at least corresponding to their ammonium content and that after a first fertilizer application the C_{org}:N_{org} ratio of the biogas residues was a crucial factor for the N availability. After repeated i.e., long term application the organic N accumulated in the soil leads to an increased release of N. Similarly, the application of the liquid fraction of separated biogas residues will provide N at least corresponding to their ammonium content with an increased release of N form the organic fraction after repeated application. In contrast, depending on the soil and the type of biogas residue part of the ammonium applied from the solid fraction after separation may be unavailable to plants. Moreover, in the case of solid biogas residues N accumulated in soil seems to be rather recalcitrant to mineralization.

7. Zusammenfassung

Rückstände aus der Anaerobvergärung enthalten wertvolle Mineralstoffe (z.B. N, P, K, S etc.), deren Rückführung in die landwirtschaftliche Produktion erforderlich ist, um das Ziel einer nachhaltigen und umweltfreundlichen Biogasproduktion zu erreichen. Biogasgärreste entstehen jedoch aus Vergärung einer Vielzahl unterschiedlicher Substrate, die ausschließlich aus pflanzlichen Rohstoffen wie auch aus Mischungen von pflanzlichen Rohstoffen mit tierischen Ausscheidungen stammen können. Es ist zu vermuten, dass dies zu Unterschieden sowohl in der Zusammensetzung der Gärreste als auch in deren Nährstoffwirkung führt. Zusätzliche Veränderungen in der Zusammensetzung können sich durch die mechanische Separierung in eine flüssige und eine feste Fraktion (Dünnseparat und Feststoff) ergeben, was ebenfalls die Nährstoffwirkung beeinflussen könnte.
Ziel der vorliegenden Arbeit ist es daher

- Rückstände aus der Vergärung von unterschiedlichen Substraten hinsichtlich ihrer N und C Gehalte zu charakterisieren, wobei nicht separierte Gärreste sowie Dünnseparate und Feststoffe nach der Separierung berücksichtigt wurden,
- die kurz- und langfristige Wirkung des Einsatzes dieser Gärreste auf die N Verfügbarkeit und die N Ausnutzung durch die Pflanze zu untersuchen und
- die Bedeutung verschiedener Böden für die N Ausnutzung aus diesen Gärresten zu bewerten

Als Modellsystem hierfür wurden zwei Gefäßversuche von jeweils etwa einem Jahr Dauer durchgeführt, in denen Weidelgras regelmäßig gedüngt und geschnitten wurde. Dieses System erlaubte eine Untersuchung der kurzfristigen Wirkung nach einmaliger Düngung wie auch der langfristigen Wirkung nach wiederholter Düngung. Im ersten Experiment wurden sieben Gärreste aus der Vergärung unterschiedlicher Substrate und eine unvergorene Rindergülle eingesetzt. Gedüngt wurde auf der Basis des enthaltenen Ammoniums (300 mg NH_4-N pro 12-l-Gefäß und Düngung) in Form einer Bandapplikation und sofortiger Einarbeitung. Der Einfluss des Bodens auf die N Verwertung wurde mit fünf Böden unterschiedlicher Textur bzw. unterschiedlichen Gehalts an organischer Substanz (2 schluffige Lehme, 1 toniger Lehm, 1 Sandboden, 1 organischer Boden) untersucht. Im zweiten Experiment wurden Gärreste aus sieben verschiedenen Biogasanlagen eingesetzt, die jeweils in Dünnseparat und Feststoff separiert worden waren. Diese 14 Gärreste wurden jeweils in zwei Böden (1 sandiger Boden und 1 schluffiger Lehm) ebenfalls auf der Basis von 300 mg NH_4-N pro 12-l-Gefäß und Düngung nach sofortiger Einarbeitung untersucht. Als Vergleich diente jeweils eine Mineraldüngung mit 300 mg N als NH_4NO_3 und eine Kontrolle ohne N Düngung. In beiden Experimenten wurde das Weidelgras in fünf Wachstumszyklen mit jeweils ein bis drei Aufwüchsen kultiviert, wobei die Düngung zu Beginn jedes dieser Wachstumszyklen erfolgte.

Folgende Ergebnisse wurden erzielt:

(1) Auf der Basis des gleichen Angebots an Ammonium- bzw. Mineral-N war die N Aufnahme von Weidelgras aus den nicht separierten Gärresten und der Rindergülle vergleichbar oder höher als die N Aufnahme nach Düngung mit NH_4NO_3. Bereits nach der ersten Düngung bestanden jedoch auch Unterschiede in der N Aufnahme von bis zu 20% zwischen den Biogasgärresten. Die N Aufnahme nach dem ersten Wachstumszyklus war signifikant negativ mit dem C_{org}:N_{org} Verhältnis der organischen Dünger korreliert. Die über die N Aufnahme aus NH_4NO_3 hinausgehende zusätzliche N Aufnahme aus den organischen Düngern stieg vom ersten zum fünften Wachstumszyklus an. Am Ende des Versuchs nach fünf aufeinander folgenden Düngungen zeigte sich eine signifikante positive Korrelation zwischen der N Aufnahme des letzten Aufwuchses und dem im Boden akkumulierten organischen Stickstoff, der wiederum in enger Beziehung zur unterschiedlichen Menge an appliziertem organischem N stand.

(2) Der Einfluss der Bodenart wurde mit fünf Böden und mit drei unterschiedlichen Biogasgärresten untersucht. Die N Aufnahme aller Wachstumszyklen aus den ungedüngten Böden war durch deren unterschiedliche Gehalte an Gesamt-N geprägt. Demzufolge war die N Aufnahme in dem organischen Boden mit 4,4 g N_t/kg Boden am höchsten, während der Sandboden mit einem Gehalt von 0,6 g N_t/kg Boden die geringste N Aufnahme zeigte. Die N Aufnahme aus den drei Düngern unterschied sich zwischen den Böden in jedem Wachstumszyklus, zeigte aber keine konsistente Beziehung zur Bodenart oder dessen Gehalt an organischer Substanz. Außerdem bestand keine signifikante Wechselwirkung zwischen Düngern und Böden. Die Nettomineralisationsrate des über die Dünger im Boden akkumulierten organischen Stickstoffs zeigte keine Unterschiede zwischen den Gärresten.

(3) Auf der Basis des gleichen Angebots an Ammonium- bzw. Mineral-N war die N Aufnahme nach der ersten Düngung mit Dünnseparaten in beiden Böden genauso hoch wie nach der Zufuhr von Mineraldünger. Im Unterschied dazu war die N Aufnahme nach der Düngung mit Feststoffen eher niedriger als mit Mineraldünger. Unter Einbeziehung sowohl der Dünnseparate wie auch der Feststoffe bestand eine signifikant negative Beziehung zwischen der N Aufnahme von Weidelgras und dem C_{org}:N_{org} Verhältnis in den Gärresten. Dies galt für die erste wie auch für alle vier folgenden Düngungen. Innerhalb der jeweiligen Gruppe der Dünnseparate bzw. der Feststoffe bestand jedoch keine Beziehung zwischen N Aufnahme und C_{org}:N_{org} Verhältnis im Gärrest.

Die zusätzliche scheinbare N Ausnutzung (additional apparent N utilization; AANU), die die N Freisetzung aus der organischen Fraktion des Gärrestes beschreibt, zeigte von der ersten bis zur fünften Düngung einen deutlichen Anstieg. Während diese zusätzliche scheinbare N Ausnutzung durchwegs für die Dünnseparate erhöht war, zeigten die negativen Werte für die Feststoffe eine mehr oder weniger deutliche Immobilisierung des gedüngten Ammoniums nach der Anwendung dieser Fraktion.

Nach fünf aufeinander folgenden Düngungen mit Feststoffen konnte eine Erhöhung der N_t und C_t Gehalte im Boden beobachtet werden, während nach der Düngung mit Dünnseparaten die Gehalte an N_t und C_t im Boden auf dem Niveau der Mineraldüngung blieben. Der Anstieg der Boden N_t Gehalte nach Feststoffdüngung führte jedoch nicht zu einer Zunahme an pflanzenverfügbarem N.

Aus den vorliegenden Untersuchungen kann geschlussfolgert werden, dass nicht separierte Biogasgärreste unabhängig davon, ob sie ausschließlich aus pflanzlichen Substraten oder aus der Co-Fermentation mit Wirtschaftsdüngern stammen, mindestens eine dem Ammoniumgehalt entsprechende Menge an pflanzenverfügbarem N bereitstellen. Dabei steht die N Freisetzung nach der ersten Anwendung mit dem C_{org}:N_{org} Verhältnis der Gärreste in Beziehung. Nach wiederholter, d.h. Langzeitanwendung führt der durch die erhöhte Zufuhr an organischem Stickstoff gestiegene Gehalt an organischem N im Boden zu einer Erhöhung der N Freisetzung. Ähnliches zeigt sich nach der Anwendung von Dünnseparaten, deren N Verfügbarkeit nach einmaliger Düngung ihrem Gehalt an Ammonium-N entspricht und die mit zunehmender Häufigkeit der Anwendung steigende Mengen an N aus dem organischen Anteil freisetzen. Im Gegensatz dazu ist, abhängig vom Boden und der Art des Gärrestes, nach der Düngung von Feststoffen ein mehr oder weniger großer Teil des gedüngten Ammoniums nicht mehr pflanzenverfügbar d.h. wird immobilisiert. Zudem scheint der durch die Feststoffe im Boden akkumulierte organische N weniger gut mineralisierbar zu sein.

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