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ORIGINAL ARTICLE

Nitrogen fertilizer replacement value of digestates from three green manures

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Abstract Green manure mixtures including legumes and forbs can help to increase N availability in organic arable systems. Anaerobic digestion of green manures may provide ammonium rich digestate, which can be redistributed as fertilizer. The aim of this study was to investigate the effect of plant species composition, cutting strategy and anaerobic digestion on the N fertilizer replacement value (NFRV) of different green manures. Digestates obtained from silages of pure stand lucerne (four cuts/year) and a mixture including lucerne, grass and forbs (two or four cuts/year) were used to fertilize winter wheat (surface banding) and spring barley (injection). In general, NFRV was 46–173% higher in spring barley than winter wheat, due to the different application method and timing, which reflect the common practices in Denmark. NFRV of digestates were 25–63% higher than the corresponding silages, with the largest increase with the most fibrous material (mixture at two cuts/year). Total N concentration (DM based) in the silages largely explained NFRV of the digestates. To obtain NFRV above 60%, total N concentration of silage should exceed 3.5 g 100 g^{-1} DM, achievable with silages from four-cut strategies. Silages of plant materials with different composition and N content may be similar in terms of biomethane production, but the fertilizer value of the digestates varies considerably depending on total N concentration.

Keywords Arable · Cutting frequency · Forage legumes · Multi-species mixtures · Organic farming · Slurry

Abbreviations

N	Nitrogen
DM	Dry matter
NFRV	Nitrogen fertilizer replacement value
Mix2	Mixture, two cuts
Mix4	Mixture, four cuts
Lu4	Lucerne, four cuts
CS	Cattle slurry
D	Digested
U	Untreated
NDF	Neutral detergent fiber

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Introduction

Nutrients and especially nitrogen (N) availability and weed infestations are two major constraints to organic production in stockless systems. The inclusion of forage legumes in the rotation is a strategic way to increase the robustness of the system, by providing N through biological fixation and by competing against perennial weeds (Melander et al. 2016). In stockless systems, forage biomass is a valuable green manure, and it is a common practice to leave the cuts on the field as mulch (Stinner et al. 2008). Mulching of green manure contributes to soil organic N, but it also increases the risk of N losses to the environment (Dahlin et al. 2011). Nadeem et al. (2012) reported that mulching significantly increased N_2O emissions during the green manure growing season, if compared to cuts removal. Moreover, leaving plant residues on the soil surface can potentially increase NH_3 emissions (de Ruijter et al. 2010). Even though part of the N in the mulch can be recycled to the regrowth (Dahlin et al. 2011), a significant increase in N leaching occurs during the green manure growing season (De Notaris et al. 2018). In a crop rotation perspective, mulching provides a high N supply in the first year following the green manure, but this can cause a low N use efficiency (Stinner et al. 2008). Moreover, N availability decreases with time after incorporation of the green manure biomass, mismatching N demand along the rotation, with negative consequences in terms of crop yields and N recovery efficiency (Brozyna et al. 2013). To overcome these problems, removal of green manure cuts from the field and redistribution of the biomass as “mobile green manure” is recognized as a valid alternative to mulching (Sørensen and Thorup-Kristensen 2011; Sørensen et al. 2013).

In order to obtain the maximum benefit for the system, the removed biomass can be used as a substrate for anaerobic digestion (Brozyna et al. 2013; Dahlin et al. 2011; Stinner et al. 2008). The outcome of anaerobic digestion of green manure cuts is the production of biogas and digestate, which can be returned to the crop rotation as strategic fertilizer. Digestates have a higher NH_4^+-N/total N ratio and a lower dry matter (DM) content than the undigested material, due to the transformation of the easily degradable carbon (C) into biogas, leading to lower C/N ratios (Arthurson 2009; Möller and Müller 2012). The high proportion of mineral N, the possibility to target N application and the improved synchronization between nutrient availability and crop demand can lead to increased N use efficiency and crop yields (Stinner et al. 2008). This, combined with the utilization of the produced biogas, can increase the environmental sustainability of organic arable systems (Knudsen et al. 2014).

In order to improve the economic sustainability of anaerobic digestion of green manure, the amount of harvested biomass should be maximized (Tersbøl and Malm 2013). Inclusion of forbs increases the aboveground productivity of grass–clover mixtures, thus increasing the energy yield (Cong et al. 2017). Additionally, multiple species mixtures can provide several ecosystem services, such as increased abundance of pollinators and biodiversity (Allan et al. 2013; Bluthgen and Klein 2011). In addition, pollinators also benefit from a reduced cutting frequency (Potts et al. 2009). Performing fewer cuts per year would also decrease management costs, with effects on the initial quality of the material but no significant reduction in the biogas yield (Wahid et al. 2015).

However, the initial quality of a specific material, and in particular its degradability and total N content, is directly affecting the NH_4^+-N and the DM content of the digestate obtained after anaerobic digestion (Möller and Müller 2012). NH_4^+-N content is considered to be the most important factor for the N fertilizer replacement value (NFRV) of an organic fertilizer (Jensen 2013). In addition, N availability from the organic fraction is influenced by the C/N ratio of the material (Jensen 2013), which can in turn be related to the DM/N ratio, as the C content on a DM basis is fairly stable (Sørensen et al. 2003; Wahid et al. 2015). Thus, the management of green manure biomass should take into consideration its effect on digestate NFRV.

The aim of this study was to determine the effect of plant species composition, cutting strategy and anaerobic digestion on NFRV of green manures, applied to winter wheat by surface banding and to spring barley by injection. In addition, we aimed to quantify the relation between total N concentration (on a DM basis) in the green manure and the NFRV of the corresponding digestates. This would provide a practical estimation of how the initial quality of the substrate will be reflected in the NFRV of mono-digested plant materials.

Materials and methods

Plant material used as substrates

Substrates for anaerobic digestion included plant material obtained from a field experiment established in 2014 at Foulumgaard (56°30′N, 9°34′E), Denmark. Three substrates were selected for anaerobic digestion: a mixture composed of perennial ryegrass (*Lolium perenne* L.), lucerne (*Medicago sativa* L.), chicory (*Cichorium intybus* L.), ribwort plantain (*Plantago lanceolata* L.) and caraway (*Carum carvi* L.) under two- and four-cut regimes (Mix2 and Mix4) and a monoculture of lucerne under four-cut regime (Lu4). In this way, the substrates varied based on their plant composition (Mix4 and Lu4) and the cutting strategy (Mix2 and Mix4). In early October 2015, the last annual cut was harvested at 0.07 m stubble height. The botanical composition of the mixture under the two-cut regime was 49% lucerne, 33% chicory, 9% plantain, 3% grass, 1% caraway and 5% unsown species on a dry matter basis, while under the four-cut regime the dry matter mass fractions were 34% lucerne, 23% chicory, 19% plantain, 12% grass, 1% caraway and 11% unsown species (Wahid et al. 2018). The plant material from the four replicates was mixed, chopped to approximately 0.05 m, vacuum packed and ensiled at room temperature for three months before anaerobic digestion. The silages obtained had different fiber compositions (Table 1). In particular, NDF (Neutral detergent fiber, which includes hemicellulose, cellulose and lignin) was the highest in Mix2 (51.9 g 100 g^{-1} DM) and the lowest in Lu4 (33.3 g 100 g^{-1} DM) (Wahid et al. 2018).

Anaerobic digestion and quality of the material

Three 20 L continuous stirred tank reactors (CSTR) with 15 L working volume were used for anaerobic mono-digestion of the substrates. During an initial stabilization phase, the reactors were fed with cattle manure. Thereafter, semi-continuous feeding was followed by continuous feeding with a mass ratio of 40% silage and 60% water for 65 days. The procedure consisted in adding 600 g of substrate after manually unloading a corresponding amount of digestate. A constant temperature of 52 °C and a hydraulic retention time (HRT) of 25 days were kept during the experiment, in accordance to the common practice in the Danish biogas sector. More details about the digestion process and biogas production can be found in Wahid et al. (2018). Subsamples of digestates from the reactors and silages were used for determination of DM, ammonium and total N content. Total N was determined by Kjeldahl analysis and ammonium-N by distillation (Sommer et al. 1992).

Field experiment

A field experiment was established in September 2015 at Foulumgaard (56°30′N, 9°34′E), Denmark, in order to test the NFRV of digestates obtained from the digestion of Lu4, Mix2 and Mix4 and the corresponding silages. The quality of silages, digestates and raw and digested cattle slurry are reported in Table 1. The soil is classified as a Typic Hapludult, according to the USDA Soil Taxonomy System with 7% clay, 10% silt, 81% sand and 1.7% C in the topsoil (0–0.02 m). Soil pH was 5.8 ($CaCl_2$), bicarbonate-extractable P (Olsen-P) 34 mg kg^{-1} DM soil and exchangeable K was 120 mg kg^{-1} DM soil.

The selected test crops were winter wheat and spring barley both following spring barley in an arable rotation. In September 2015 winter wheat was sown with a distance of 0.12 m between rows. In November 2015, after emergence of winter wheat, 52 PVC cylinders with an internal diameter of 0.3 m (area = 0.0707 m^2) and a length of 0.3 m were inserted leaving 0.05 m above ground. Each cylinder included two rows of wheat. In April 2016, after spring ploughing of barley stubble without catch crop, another 52 similar cylinders were inserted in bare soil to be hand-seeded with spring barley after fertilization.

In winter wheat cylinders, digestates (D) from Lu4, Mix2 and Mix4, untreated (U) and digested cattle slurry (CS) and five rates of liquid ammonium nitrate (0, 50, 100, 150, 200 kg N ha^{-1}) were applied, in April 2016, for a total of ten treatments. The three digestates and the cattle slurry had a similar pH, which was approximately 8. The organic materials were applied by surface-banding at a rate of 120 kg total N ha^{-1}. Bands were placed at the center of each cylinder between the two plant rows. The treatments were organized in a complete randomized block design, with four replicates each.

In spring barley cylinders, untreated silages and digestates from Lu4, Mix2 and Mix4, raw and digested cattle slurry were applied before sowing, at a rate of

Table 1 Initial quality of the materials used as fertilizer for winter wheat and spring barley

Material	DM (g 100 g^{-1} FM)	Hemicellulose (g 100 g^{-1} DM)	Cellulose (g 100 g^{-1} DM)	Lignin (g 100 g^{-1} DM)	Total N (g 100 g^{-1} DM)	NH_4-N (g 100 g^{-1} tot N)
Lucerne 4 cut, U (Lu4-U)	17.2	3.2	25.3	4.8	4.9	23
Lucerne 4 cut, D (Lu4-D)	3.7	–	–	–	8.9	73
Mixture 4 cut, U (Mix4-U)	15.1	9.6	26.7	5.7	3.6	9
Mixture 4 cut, D (Mix4-D)	2.8	–	–	–	7.9	59
Mixture 2 cut, U (Mix2-U)	21.8	9.2	33.8	8.9	2.6	14
Mixture 2 cut, D (Mix2-D)	5.9	–	–	–	4.1	54
Cattle slurry, U (CS-U)	6.4	–	–	–	4.5	59
Cattle slurry, D (CS-D)	4.8	–	–	–	6.2	67

DM dry matter, *FM* fresh matter, *NDF* neutral detergent fiber; *U* untreated, *D* digested

80 kg total N ha^{-1}. To simulate direct injection, the materials were placed in 0.01 m deep slits at the center of each cylinder in the direction of the rows. After fertilizer application, spring barley was hand-seeded (26 kernels per cylinder) in two rows with a distance of 0.12 m. Spring barley was seeded also around and in between the cylinders. By the end of April, ammonium nitrate in liquid form was applied at five rates (0, 30, 60, 90, 120 kg N ha^{-1}) to establish an N response curve. In this way, totally 13 treatments were established in barley, organized in a complete randomized block design in four replicates.

To avoid deficiency of other nutrients than N, phosphorus (P), potassium (K) and sulphur (S) were applied in liquid form at a rate of 25 kg P, 99 kg K, 15 kg S ha^{-1} to both wheat and barley. Furthermore, calcium (100 kg ha^{-1}), magnesium (49 kg ha^{-1}), manganese (2.3 kg ha^{-1}), zinc (0.26 kg ha^{-1}), boron (0.15 kg ha^{-1}), copper (0.14 kg ha^{-1}), molybdenum (0.06 kg ha^{-1}) and cobalt (0.1 kg ha^{-1}) were applied. Hand weeding was performed three–four times during the growing season.

Plant sampling, analyses and calculations

Total above-ground biomass of winter wheat and spring barley was harvested at maturity by hand in August 2016. Grain and straw were divided and dried (60 °C for 48 h) for DM determination. The samples were then finely milled for total N determination using the Dumas method (Hansen 1989).

Based on DM and N yield from the cylinders fertilized with mineral N, it was possible to fit N-fertilizer response curves (Schröder 2005), which identify the yield response to different levels of fertilization. Both winter wheat and spring barley DM and N yield showed linear responses to mineral fertilizer levels. Regression coefficients of the different N response curves were then used to calculate the NFRV of the applied materials, based on grain and total DM and N yield (Jensen 2013). In this way, the NFRV indicates the equivalent amount of mineral N (kg N ha^{-1}) that the applied material can replace, under those specific conditions, which was then expressed as a percentage of the amount of total applied N (120 kg N ha^{-1} in winter wheat, 80 kg N ha^{-1} in spring barley).

Statistical analysis

Statistical analysis and data exploration were performed using R (R Core Team 2016), following the protocol described by Zuur et al. (2010). After a visual investigation of the data, the effect of treatment (applied material) on DM and N yields, N concentration and NFRV was assessed using analysis of variance (ANOVA) tests, separately for winter wheat and spring barley. The assumptions of normality and homoscedasticity were verified with the Shapiro–Wilk test and visual examination of the residuals against fitted values. When the assumptions were not met, data were log transformed. Post-hoc comparisons were performed using the Tukey's HSD test, and allowed to identify differences between specific treatments.

NFRV based on total N yield can be used as an indicator of N utilization from the applied material

(Jensen 2013). Correlations between initial quality of the material and NFRV based on total N yield were assessed with Pearson correlation coefficients, using the cor.test function of the R Stats package.

For all statistical tests $\alpha = 0.05$.

Results

Climate

From October 2015 until August 2016, the minimum average monthly temperature was 0 °C in January 2016 and the maximum average monthly temperature was 16 °C in July 2016. The cumulative precipitation during the same period amounted to 776 mm, with a minimum of 25 mm in March 2016 and a maximum of 119 mm in November 2015. Figure 1 shows the daily average temperature and precipitation during the whole period. The average temperature for the whole period was 8.5 °C and for comparison, the average temperature during the same months in the previous 10 years (2004–2014) was 7.8 °C, while the average cumulative precipitation was 598 mm.

Winter wheat yield

There was a significant effect of treatment ($P < 0.001$) on winter wheat DM and N yield (Fig. 2). A post hoc comparison among treatments revealed that the cutting strategy had an effect on winter wheat yield (Mix4 higher than Mix2), as well as plant composition (Lu4 higher than Mix4). However, the effect of plant composition was significant just for total N yield.

Grain DM yield ranged from 3.6 to 5.1 Mg DM ha^{-1}, and it was the lowest in the treatment with application of digestate from Mix2 and the highest with digestate from Lu4. Total DM yield followed a similar pattern, and it ranged from 6.6 to 9.6 Mg DM ha^{-1}. Grain N yield ranged from 47 to 68 kg N ha^{-1} and total N yield ranged from 61 to 89 kg N ha^{-1}. Total N yield was significantly higher in Lu4-D than in Mix4-D, which was significantly higher than Mix2-D.

Nitrogen concentration in winter wheat grains and straw was not significantly affected by the treatment, with grain N concentration ranging from 1.28 to 1.40 g 100 g^{-1} DM (Table 2).

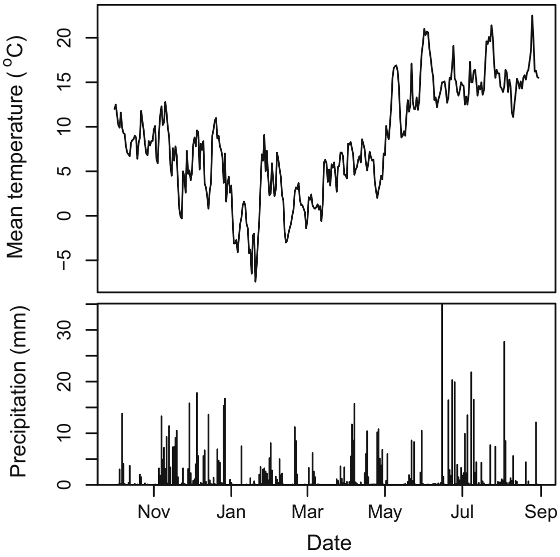


Fig. 1 Mean daily temperature and precipitation from October 2015 to August 2016 at the experimental site

Both DM and N yield showed a linear response to increasing levels of mineral N fertilization (Fig. 2).

Spring barley yield

The effect of treatment on spring barley DM and N yield was statistically significant ($P < 0.001$) (Fig. 3). A post hoc comparison among treatments revealed that spring barley yield was generally higher with digested than with untreated silages and it was influenced by plant composition and cutting strategy. The effect of the treatment factors varied among the different yield types.

Grain DM yield ranged from 3.0 to 5.4 Mg DM ha^{-1}, being the lowest in treatments with Mix2-U and the highest with Lu4-D. Anaerobic digestion of the plant material increased grain DM yield by an average of 23%. The effect of plant species composition was similar in digested and untreated silages (Lu4 17% higher than Mix4), while the effect of cutting strategy was more pronounced with untreated silages than with digested materials (Mix4-D 19% higher than Mix2-D, Mix4-U 30% higher than Mix2-U). Total DM yield followed a similar pattern, with some of the differences being more pronounced. Conversely, even though anaerobic digestion increased grain and total N yields, the effect was not significant. Grain N yield was the lowest in treatments

Fig. 2 Winter wheat N response curves to five mineral N levels (left) and winter wheat grain, total dry matter (DM), grain N and total N yields measured after surface application of digested (D) green manures and digested and untreated (U) cattle slurry (right). Error bars indicate standard errors (n = 4), and different letters indicate significant differences ($P < 0.05$) within the same yield type

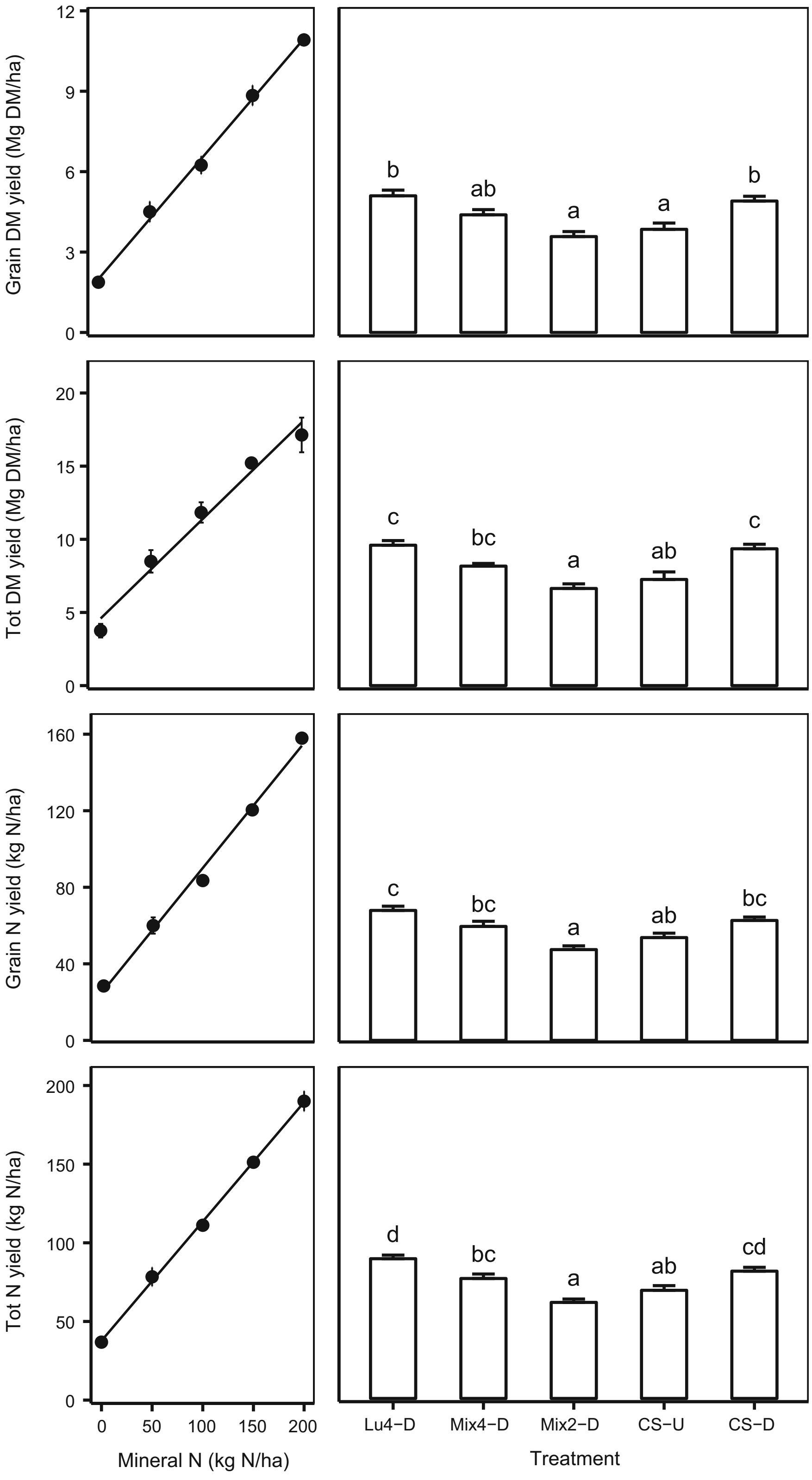


Table 2 Average N concentration (g 100 g^{-1} DM) in winter wheat and spring barley grain and straw (n = 4)

Treatment	Winter wheat		Spring barley	
	Grain N	Straw N	Grain N	Straw N
Lucerne 4 cut, U (Lu4-U)	–	–	1.28 a	0.68 a
Lucerne 4 cut, D (Lu4-D)	1.33 a	0.48 a	1.22 a	0.62 a
Mixture 4 cut, U (Mix4-U)	–	–	1.46 b	0.72 a
Mixture 4 cut, D (Mix4-D)	1.36 a	0.45 a	1.25 a	0.67 a
Mixture 2 cut, U (Mix2-U)	–	–	1.49 b	0.84 b
Mixture 2 cut, D (Mix2-D)	1.33 a	0.46 a	1.27 a	0.66 a
Cattle slurry, U (CS-U)	1.40 a	0.46 a	1.25 a	0.66 a
Cattle slurry, D (CS-D)	1.28 a	0.42 a	1.23 a	0.62 a

In each column, values followed by different letters are significantly different ($P < 0.05$)

U untreated, *D* digested

with Mix2-U and the highest with Lu4-D, and ranged from 45 to 66 kg N ha^{-1}. There was no significant difference based on plant composition, while cutting strategy significantly affected grain N yield after application of untreated silages (Mix4-U higher than Mix2-U). Total N yield followed a similar pattern. The non-significant effect of digestion on N yield can be explained by the generally increased grain N concentration after application of untreated silages, if compared to digested material (Table 2).

Similarly to winter wheat, spring barley DM and N yield increased linearly with increasing levels of mineral N fertilization (Fig. 3).

NFRV based on DM and N yield

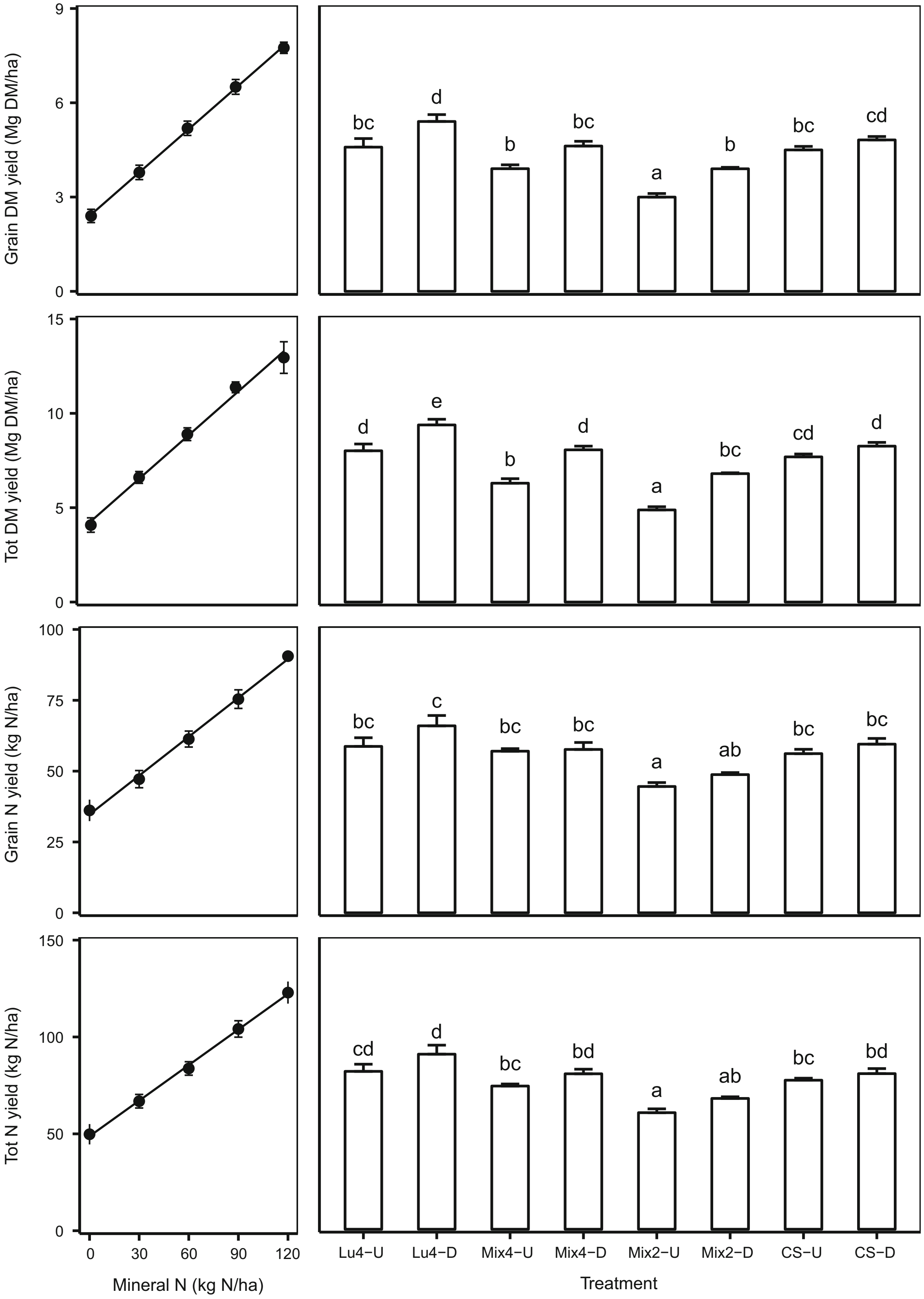
NFRV of the applied materials was generally higher for spring barley than for winter wheat (Figs. 4, 5). NFRV indicates the amount of mineral fertilizer N that can be replaced by 100 units of N in the applied organic material.

In winter wheat, the effect of treatment on NFRV was significant ($P < 0.001$). NFRV varied slightly according to the choice of reference (either DM or N), although the general trend was consistent. When based on grain DM yield, the average NFRV varied from 28% (Mix2-D) to 57% (Lu4-D), while when based on total N yield, it varied from 24% (Mix2-D) to 55% (Lu4-D). Lu4-D had a generally higher NFRV than Mix4-D, which had an average NFRV of 42% (effect of plant species composition), but the main difference was between Mix4-D and Mix2-D (effect of cutting strategy).

Also in spring barley, there was a significant effect of treatment on NFRV ($P < 0.001$), with Mix4-U having the lowest and Lu4-D the highest NFRV. When NFRV was based on grain and total DM yield, a post hoc comparison showed that treatments with digestates had significantly higher NFRV than the ones with the corresponding untreated silages. Also plant species composition and cutting strategy affected NFRV, with the differences being most pronounced when based on total DM. When based on grain DM yield, NFRV ranged from 16 to 83%. Most of the differences among treatments were leveled out when NFRV was calculated based on spring barley grain N yield, and in particular the effect of digestion and plant composition. In this case, NFRV ranged from 69% in Mix2-U to 81% in Lu4-D. This reflected the increase in barley grain N concentration after application of untreated silages (Table 2). When based on total N yield, which is an indicator of the overall N uptake, NFRV ranged from 24 to 86%. In this case, the effect of digestion and plant species composition was positive, but not statistically significant.

Correlation between NFRV (based on total N yield) and initial quality of the applied material

There was a significant linear correlation between NFRV and total N content (g 100 g^{-1} DM), in the applied materials for both winter wheat ($P < 0.05$) and spring barley ($P < 0.01$) (Fig. 5a). In both cases, it explained approximately 75% of the variation in NFRV. The slopes of the regression lines indicate that NFRV increased with increasing total N content to a similar rate in winter wheat and spring barley. NH_4^+-N content, expressed as percentage of total N, can be used as an indication of the N that is immediately available to the crop. Its correlation with NFRV was statistically significant for winter wheat ($P < 0.05$), with a coefficient of determination of approximately


Grain DM yield (Mg DM/ha)
Tot DM yield (Mg DM/ha)
Grain N yield (kg N/ha)
Tot N yield (kg N/ha)
0
3
6
9
0
5
10
15
0
25
50
75
100
0
50
100
150
bc
d
b
bc
a
b
bc
cd
d
e
b
d
a
bc
cd
d
bc
c
bc
bc
a
ab
bc
bc
cd
d
bc
bd
a
ab
bc
bd
0
30
60
90
120
Mineral N (kg N/ha)
Lu4−U
Lu4−D
Mix4−U
Mix4−D
Mix2−U
Mix2−D
CS−U
CS−D
Treatment

◀**Fig. 3** Spring barley N response curves to five mineral N levels (left) and spring barley grain, total dry matter (DM), grain N and total N yields measured after direct injection of digested (D) and untreated (U) green manures and cattle slurry (right). Error bars indicate standard errors (n = 4), and different letters indicate significant differences ($P < 0.05$) within the same yield type

90% (Fig. 5b). Conversely, the correlation between NH_4^+-N and NFRV in spring barley was not significant, indicating that N uptake varied irrespective of NH_4^+-N content.

For the digestates, the NFRV could be explained almost exclusively by the total N concentration (based on DM) in the silages (Fig. 6). In spring barley an increase in total N concentration of silages by 1 percentage point lead to a 20 percentage points increase in NFRV of the corresponding digestate. To obtain NFRV above 60%, total N concentration in silage should exceed 3.5 g 100 g^{-1}, which was achievable for the silages from 4-cut-strategies (Table 1). In winter wheat, an increase in total N concentration of silages by 1 percentage point lead to a 13 percentage point increase in NFRV of digested material, while a total N concentration of 3.5 g 100 g^{-1} in silage resulted in only 37% NFRV.

Discussion

Effect of plant species composition and cutting strategy

The initial quality of the green manure biomass was determined by plant species composition and cutting strategy. In particular, inclusion of forbs and the two cuts/year strategy led to an increase in DM and NDF and a decrease in N concentration, as also reported by Khalsa et al. (2014) and Wahid et al. (2015). The differences in the initial quality of green manure were reflected in the digestates, where N concentration and NH_4-N/total N proportion were the highest in Lu4 and the lowest in Mix2. This is in accordance with the idea that fiber content and initial N concentration (based on DM) will determine the proportion of NH_4-N/N in the digestate (Möller and Müller 2012).

NH_4-N/N proportion in the mobile manure is considered to be the main factor determining its fertilizer value (Jensen 2013). This could be confirmed in winter wheat, where NFRV was strongly correlated with NH_4^+-N/N (r^2 = 0.91), which varied with plant species composition and cutting strategy. In winter wheat, where digestates and slurry were applied to the surface, also DM content of the applied material played an important role in the determination of NFRV. Materials with higher DM have lower infiltration rates, thus higher risk of ammonia volatilization and a reduced N availability to crops (de Jonge et al. 2004; Webb et al. 2013). In the present study, Mix2-D, Mix4-D and CS-U had similar proportions of NH_4^+-N/N but different NFRV. This could be explained by the different DM content, whose effect should be interpreted in combination with the proportion of NH_4^+-N/N, which is generally negatively correlated with DM content (Jensen 2015). In this way, in winter wheat, plant species composition and cutting strategy affected the fertilizer value of the applied materials by influencing the proportion of NH_4^+-N/N and DM content, thus the readily available N and the infiltration rate.

In spring barley, where the untreated and digested materials were injected, the proportion of NH_4^+-N/N was non-significantly correlated to NFRV (based on total N yield). However, NFRV was significantly correlated to total N concentration, on a DM basis (r^2 = 0.73). This indicates that the crop could take up inorganic N after mineralization of the organic N, as also reported by Jensen (2015). The mineralization of organic N is mainly influenced by the C/N ratio of the material (Parton et al. 2007), which can be directly related to the DM/N ratio, thus to the N concentration (Sørensen et al. 2003). Materials with a lower N concentration (higher DM/N ratio) lead to a slower release of mineral N (Parton et al. 2007). In the present study, a later availability of mineral N from Mix4-U and Mix2-U was associated with a significant increase in grain N concentration, which is mainly determined by N assimilation during the grain filling phase (Cox et al. 1986). However, grain and total DM yield were negatively affected by the low initial N availability. Overall, in spring barley, plant species composition and cutting strategy affected the fertilizer value of the applied materials by influencing the total N concentration of the silages and the timing of N availability, with consequences on DM yield and grain N concentration.

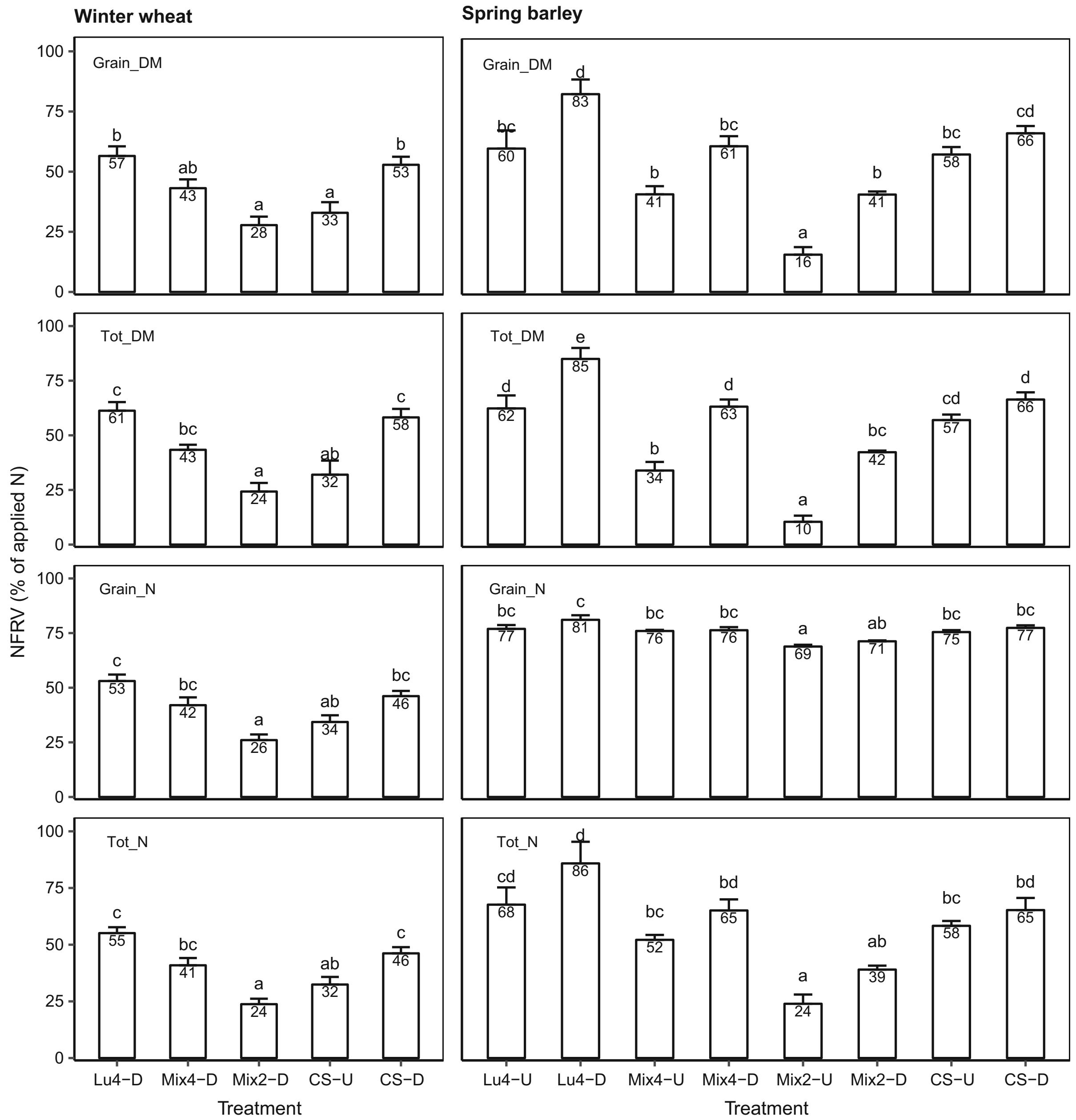


Fig. 4 NFRV of digested (D) and untreated (U) green manures and cattle slurry (% of total applied N) after surface application in winter wheat (left) and direct injection spring barley (right). NFRV was calculated based on different yield response curves: grain dry matter (DM), total DM, grain N, total N. Numbers inside the bars are average values. Error bars indicate standard errors (n = 4), and different letters indicate significant differences ($P < 0.05$) within the same crop and reference yield

Effect of anaerobic digestion

Anaerobic digestion of green manure biomass increased the proportion of NH_4-N/N, which is readily available for plant uptake, as well as the total N concentration, due to a decrease in DM content (Möller and Müller 2012). In spring barley, where both untreated silages and digestates were injected prior to sowing, application of digestate significantly increased DM yield, thus the relative NFRV. This can be attributed to the higher initial N availability (NH_4^+-

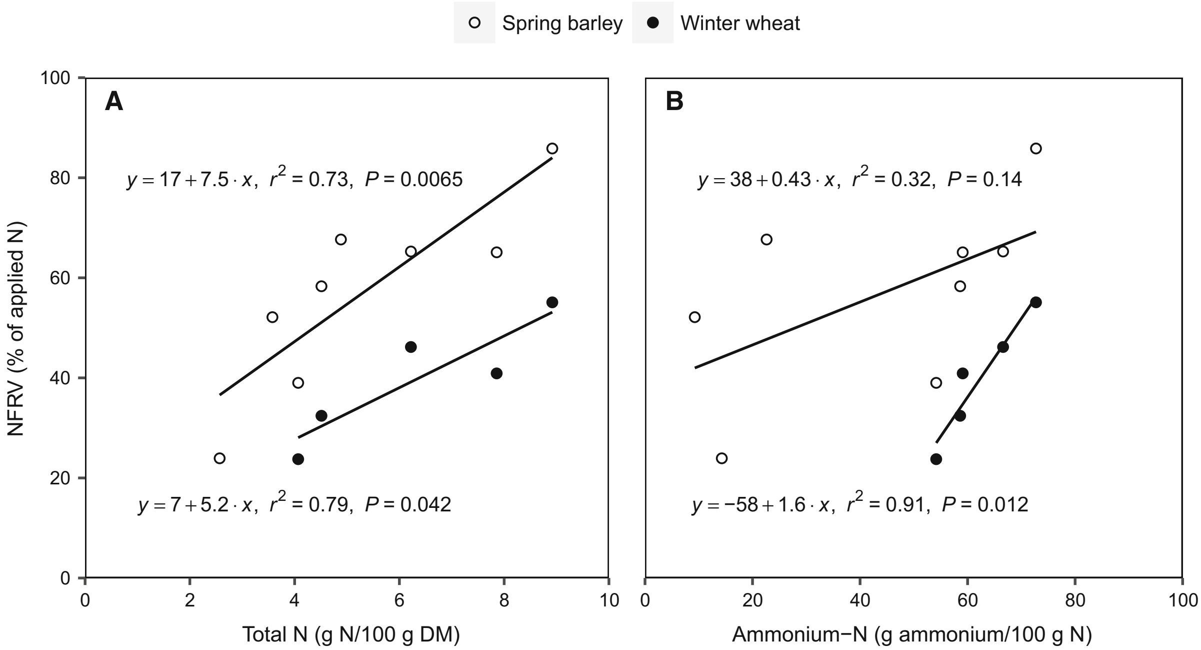


Fig. 5 Correlation between NFRV (% of total applied N), calculated based on total N yield, and **a** total N (g 100 g^{-1} DM) and **b** ammonium-N (g 100 g^{-1} N) of the applied material. Empty dots indicate spring barley, full dots indicate winter wheat. DM = dry matter

N/N) in digestate than in untreated silages. However, anaerobic digestion did not significantly affect grain N yield. This was due to an increase in grain N concentration in the treatments with untreated silages, where the low N content corresponded to a later release of mineral N. This effect counterbalanced the lower DM yield, resulting in similar grain N yields from untreated silages and the corresponding digestates, in agreement with results reported by Froseth et al. (2014). When considering the total N yield, NFRV was on average 15 percentage point higher in digestates than in untreated silages, indicating an overall positive effect of anaerobic digestion on N availability. In general, differences in NFRV were less pronounced among digestates than for untreated silages, suggesting that anaerobic digestion could partly counterbalance the effect of a reduced cutting frequency and of the plant composition.

In the context of organic arable farms, anaerobic digestion of green manure should be considered as an alternative to mulching. Benefits derived from biogas production (Wahid et al. 2018), increase in N recovery (Froseth et al. 2014) and redistribution of N among different crops (Möller and Müller 2012) should be taken into account, in addition to the positive effect on

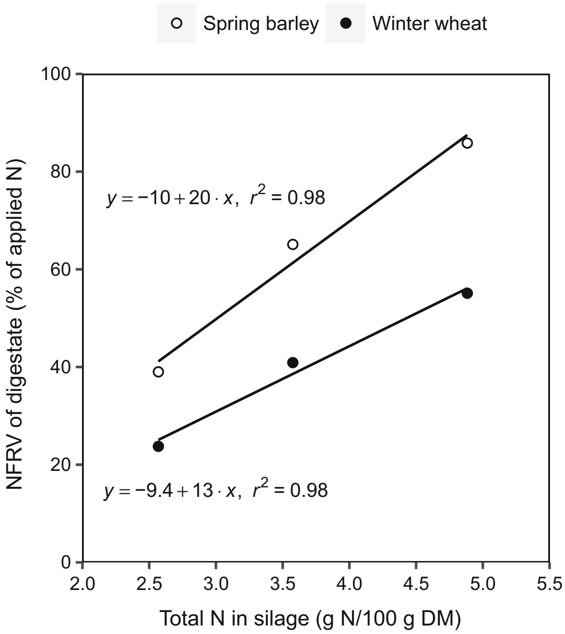


Fig. 6 Correlation between NFRV of the digestates (% of total applied N), calculated based on total N yield, and the total N concentration (g 100 g^{-1} DM) in the corresponding silages. Empty dots indicate spring barley, full dots indicate winter wheat. DM = dry matter

N availability. Moreover, when compared to digested cattle slurry, NFRV of digestates from Lu4 and Mix4 were higher or similar. This suggests that anaerobic digestion of green manure can provide a valuable source of N to organic arable systems, able to substitute imported animal manure and improve N utilization, if compared to mulching. However, the green manure must be relatively rich in legumes and the plant material must be harvested at an early growth stage with low fiber content to obtain fertilizer replacement values above 50%.

Difference between winter wheat and spring barley

NFRV of the applied materials was higher in spring barley than in winter wheat, in agreement with Jensen (2015). This was true not only for the degassed plant materials, but also for untreated and digested cattle slurry. The reasons behind this difference can be several, and include method and timing of application. The application methods used in this study (surface banding in winter wheat and injection in spring barley) are representative of the common practice of organic liquid fertilizer application in Denmark, and were reported also in previous literature (Sørensen and Eriksen 2009). Surface banding is less efficient than injection in regards to ammonia emissions after manure application (Sogaard et al. 2002). The risk of ammonia losses is particularly high when surface banding of manure is done in early spring, like it was done in this study, because of the limited height of the crop (Jensen 2013; Sommer and Hutchings 2001). In addition to the reduced ammonia loss after injection, the materials were applied prior to sowing of spring barley, allowing more time for the organic N to be mineralized and match the crop N demand. The situation was different for winter wheat, which was already growing at the time of fertilization. As N demand is mainly determined by crop growth rate (Limaux et al. 1999), immediate N availability was important for winter wheat, and less for spring barley. This was clearly shown by the correlation between NFRV and NH_4^+-N/N, which was significant and with a high coefficient of determination in winter wheat, while non-significant in spring barley.

NFRV and the importance of the reference yield response

NFRV can be calculated based on the marketable yield (e.g. grain DM yield), on the N yield or on the plant N uptake responses (Jensen 2013; Schröder 2005). Advantages and disadvantages are associated to all possible references, and different arguments can be used in support of one or the other. To calculate NFRV based on the marketable yield seems the most relevant choice from a farmer´s perspective (Jensen 2013; Schröder 2005). However, grain yield response to increasing N fertilization rates is often non-linear, as in the study by Eriksen et al. (2006). A non-linear response means that the calculated NFRV will be influenced by the rate of manure N application, whereas NFRV is independent of the application rate when the response is linear. Below optimal N fertilization rates, N uptake response to N fertilization is usually linear, allowing a more reliable estimation of NFRV. Due to the need for chemical analysis and the difficulty of sampling the entire crop biomass (including below-ground), the NFRV is often calculated just based on the harvested N yield (Jensen 2013). This was done, for example, by Askegaard and Eriksen (2007), who studied the residual effect of different catch crop species on the following spring barley crop.

The results from the present study, however, highlight how different reference yields can provide very different NFRV, which can reflect different processes. This was particularly true for spring barley where, for example, NFRV of Mix2-U varied from 10 to 69% according to the reference used. The variation in NFRV was mainly associated to timing of mineral N release, which was low at the beginning of the growing period (low DM yield) and higher at the end (high grain N concentration). The same cannot be stated for winter wheat, where NFRV was consistent. This difference can be attributed to the different fertilizer application methods and growing rates of the two crops, and underlines how the NFRV of a chosen material cannot be generalized. On the contrary, it is necessary to evaluate carefully the specific conditions and to choose the reference yield response with knowledge of the processes that will be reflected.

Conclusions

Silages of plant materials with different composition and N content may be similar in terms of biomethane production (Wahid et al. 2018), but the N fertilizer value of the digestates can vary considerably. This study showed how NFRV of anaerobically digested green manures could be explained almost exclusively by the total N concentration (based on DM) of the corresponding silages, which varied based on plant species composition and cutting strategy. In particular, inclusion of non-leguminous forbs and a reduced cutting frequency reduced NFRV. On the contrary, a high proportion of legumes and a frequent cutting strategy can ensure a high total N concentration (based on DM) in the plant material leading to a high NFRV of the digestate. In general, anaerobic digestion increased the NFRV of green manure biomass, with a stronger effect for the material with the lowest N concentration (based on DM). The importance of fertilizer application method and timing was highlighted by the higher NFRV in spring barley than in winter wheat. The choice of reference yield (either DM or N) can greatly influence the estimation of the NFRV. This was the case of spring barley, where DM and N yields were affected in different ways by green manure plant species composition, cutting strategy and anaerobic digestion, mainly due to the timing of mineral N release, which determines DM yield and N concentration.

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