Nutr Cycl Agroecosyst (2011) 89:269–280
DOI 10.1007/s10705-010-9392-2

ORIGINAL ARTICLE

Recovery of mineral fertiliser N and slurry N in continuous silage maize using the ^{15}N and difference methods

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Received: 10 March 2010 / Accepted: 30 July 2010 / Published online: 4 August 2010

Abstract The stable isotope technique and the difference method are common approaches for estimating fertiliser N uptake efficiency. Both methods, however, have limitations and their suitability may depend on N management and environmental conditions. A field experiment was conducted on a humus sandy soil in northern Germany to estimate fertiliser N uptake efficiency of silage maize in the year of application (*Zea mays* L.) by the stable isotope and the difference method as influenced by the type of N fertiliser (mineral vs. cattle slurry), the application mode (separate or combined application), and N rate. Seven N treatments were included (0, 50, 100 and 150 kg mineral N ha^{-1}; 20, 40 m^3 cattle slurry ha^{-1}; 50 kg mineral N ha^{-1} plus 40 m^3 slurry ha^{-1}), where either mineral N or slurry N was labelled, and mineral N was split into two dressings. In addition, 4.1 kg ha^{-1} labelled mineral N was incorporated into otherwise unlabelled treatments (0, 20, 40 m^3 ha^{-1}, and 50 kg mineral N ha^{-1} plus 40 m^3 ha^{-1}) to estimate N uptake from the upper soil layer. Uptake of ^{15}N was followed in leaves, stalk, ear, and the whole crop. Fertiliser N uptake efficiency ($FNUE_{15N}$) of mineral fertiliser N obtained by the isotope technique ranged between 51 and 61%. Recovered fertiliser N was mainly found in the ear, while less labelled N remained in leaves and the stalk. The nitrogen rate tended to increase the amount of recovered N, but the effect was not consistent among plant parts and the whole crop. Plant N uptake from non-fertiliser N was found to increase N input up to 100 kg N ha^{-1}. Nitrogen recoveries of the two mineral N dressings were similar for the different plant parts as well as for the whole crop. Fertiliser N uptake efficiency ($FNUE_{diff}$) of mineral N estimated by the difference method resulted in substantially higher values compared to $FNUE_{15N}$, varying between 56 and 98%. More N was taken up from the upper soil layer with increasing N supply, which is regarded as a major error source of the difference method. Slurry N was taken up less efficient in the year of application than mineral fertiliser N as indicated by recovery rates of 21–22% ($FNUE_{15N}$) and 39–62% ($FNUE_{diff}$), respectively. When mineral N and slurry were applied together, the difference method estimated significantly lower N uptake efficiencies for both mineral and slurry N compared to a single application, while values obtained by the isotope method were not affected.

Keywords Fertiliser N uptake efficiency · ^{15}N · Difference method · Mineral fertiliser · Cattle slurry · Silage maize

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Introduction

In northwestern Europe, silage maize is a key component of ruminant diets in intensive dairy farming due to its high yield and metabolic energy content (O'Mara et al. 1998), and its acreage has increased substantially over the last few decades. Although maize has a high N use efficiency (Schmitt and Edwards 1981), field balances still show considerable N surpluses due to excessive input of organic and mineral fertilisers, which are applied alone or in combination (Neeteson 2000; Schröder et al. 2005; Haas et al. 2005). Adjusting N application rates to crop needs can improve N use efficiency and reduce N leaching. To this end, indicators for assessing the N status of crops or soil can provide valuable information (Schröder et al. 2000; Herrmann and Taube 2005; Samborski et al. 2009). However, nitrogen application tailored to crop demand requires accurate knowledge of fertiliser N recovery, which is especially challenging with respect to slurry since short-term and residual N effects have to be considered (Schröder 2005; Schröder et al. 2005).

Two approaches are commonly used to study the fate of N fertilisers in soil and plant, namely the difference method and the ^{15}N isotope technique. The difference method provides an estimate of fertiliser N uptake efficiency ($FNUE_{diff}$) by determining the extra amount of mineral N fertiliser or slurry N taken up compared to an unfertilised treatment, while assuming that the N transformation processes are not modified by the added N. The isotopic estimate ($FNUE_{15N}$) is based on the recovery of labeled N in shoot organs. Both methods are subject to error sources, which are more pronounced on humous soils as (1) fertiliser application can induce priming effects (Bingemann et al. 1953) resulting in an overestimation of fertiliser N uptake efficiency estimated by the difference method, and (2) ^{15}N fertiliser may be involved in mineralisation-immobilisation turnover (MIT) and biological interchange processes, leading to lower N recovery. Consequently, FNUE estimated by the difference method often exceeds that obtained by isotopic methods. Additional variation of published FNUE rates for mineral N and slurry is due to field site history, N management, and cropping system (Xue et al. 2005; Nissen and Wander 2003; Muñoz et al. 2004; Stevens et al. 2005; Cusick et al. 2006). $FNUE_{15N}$ values found for mineral N fertiliser range from 18 to 49% (Timmons and Cruse 1990; Khanif et al. 1984; Zhou et al. 2000; Stevens et al. 2005; Vesterager et al. 2008), while $FNUE_{diff}$ vary between 22 and 94% (Ma et al. 1999; Schröder 1999; Stevens et al. 2005; Nissen and Wander 2003; Halvorson et al. 2005; Montemurro et al. 2006). Some studies even reported $FNUE_{diff}$ values up to 145% (Maidl et al. 1999). Similar ranges were detected for slurry N with isotopic techniques between 10 and 50% (Muñoz et al. 2003; Chantigny et al. 2004; Muñoz et al. 2004), and $FNUE_{diff}$ varying between 5 and 67% (Schröder 1999; Ma et al. 1999; Muñoz et al. 2003; Schröder et al. 2005), but also down to −16% (Maidl et al. 1999).

The objectives of this research were to

(1) investigate if N rate and application mode influence the short-term FNUE calculated by the difference method and the ^{15}N technique,
(2) quantify the effects of N rate and of application mode, i.e. separate versus combined application of mineral fertiliser and cattle slurry, on short-term FNUE by silage maize, and trace the resulting N partitioning in the various plant parts,
(3) study the contribution of the first and second N dressing of a split mineral N application to total N uptake, and
(4) analyse the impact of N fertiliser type and level on maize N uptake from the upper soil layer

Materials and methods

A field experiment was conducted within the framework of the 'N project Karkendamm' (Taube and Wachendorf 2000) at the research station Karkendamm (53°55′N, 9°55′E; 14 m a.s.l.) in northern Germany during 3 years (1998–2000). The soil was classified as sandy Aquod, with soil characteristics showing a large spatial variation as indicated by the organic carbon content of the plough layer varying between 4.2 and 7.7% and pH values between 5.2 and 5.7. The climate at the experimental site is moderately maritime, characterised by wet, cool summers and mild winters, with moderate seasonal temperature variation. Long-term mean temperature, annual precipitation and climatic water balance (Haude 1955; Wendling 1995) are 8.4°C, 824 and 312 mm,

respectively. Average annual temperatures in the experimental years 1998–2000 were 8.6, 9.6, and 9.3°C, and precipitation amounted to 1,098, 705, and 635 mm. The experimental site had been cropped with forage maize since 1992 and had received 30 to 40 m^3 slurry and 50 kg mineral N ha^{-1} $year^{-1}$ each year.

The present study was embedded in a larger field trial, which was established as a two-factor split-plot design with four randomised blocks as replicates and a plot size of 255 m^2. Treatments were three cattle slurry fertilisation rates (0, 20, 40 m^3 ha^{-1}), factorially combined with four mineral N rates (0, 50, 100, 150 kg N ha^{-1} as calcium ammonium nitrate, CAN). The slurry was applied between spring ploughing and sowing and was immediately incorporated into the soil. Mineral N fertilisation was split into two equal applications at the one-leaf and six-leaf stages. All plots received a starter phosphorus fertilisation at sowing, where the fertiliser (superphosphate) was drilled 5 cm below and 5 cm to the side of the seed at a rate of 30 kg P_2O_5 ha^{-1}. Additionally, 35 kg P_2O_5 ha^{-1} were applied at the 1-leaf stage. Potassium chloride was supplemented at sowing in order to adjust the K supply on all plots to the amount of the largest slurry application. An early maize hybrid (Naxos) was sown between late April and early May in rows that were 0.75 m apart, with a final plant density of 10–11 plants m^{-2}. For more details, see Wachendorf et al. (2006a).

We selected seven of the twelve above-mentioned treatments to include them in the underlying study, namely all mineral-only treatments (0, 50, 100, 150 kg N ha^{-1}), two slurry-only treatments (20, 40 m^3 ha^{-1}), and a combination of slurry and mineral fertiliser (40 m^3 ha^{-1} plus 50 kg mineral N ha^{-1}). Microplots were installed in each field replicate of the seven treatments, where ^{15}N-labelled mineral fertiliser or ^{15}N-labelled slurry was applied to analyse the N recovery of mineral N (experiment 1) and of slurry (experiment 2), and to study the N uptake from the upper soil layer (experiment 3); see Table 1. Locations of microplots within main plots were moved each year in order to exclude any residual effects resulting from ^{15}N applications in the preceding year(s).

Experiment 1: mineral fertiliser N uptake efficiency

Estimation of the short-term mineral N uptake efficiency was based on the mineral-only treatments (0, 50, 100 and 150 kg N ha^{-1}) and the combined mineral and slurry treatment (50 kg mineral N ha^{-1} plus 40 m^3 ha^{-1}); see Table 1. Rates of mineral fertiliser were given in two equal dressings on two separate microplots of 4.5 m^2 in size (three maize rows) at the one-leaf (mid-May) and eight-leaf stage (mid-June), where one microplot received labelled $^{15}NH_4^{15}NO_3$ (1998/1999: 1.43167 at%^{15}N; 2000: 3.84145 at%^{15}N) as a first dressing, and the other microplot as a second dressing. The remaining N amount was applied as unlabelled fertiliser. In the combined mineral and slurry treatment, the mineral part was labelled ($^{15}NH_4^{15}NO_3$), while the slurry (72, 136, and 148 kg N ha^{-1} in 1998, 1999, and 2000, respectively) was unlabelled.

Table 1 Treatments of slurry (S) and mineral (M) N application included in experiments 1 to 3

Slurry N (m^3 ha^{-1})	Mineral N (kg N ha^{-1})	Code	Experiment 1 (mineral N recovery)	Experiment 2 (slurry N recovery)	Experiment 3 (N uptake from upper soil layer)
0	0	Ctrl	X		X
0	50	S0M50	X		
0	100	S0M100	X		
0	150	S0M150	X		
20	0	S20M0			X
40	0	S40M0		X	
40	0	S40M0			X
40	50	S40M50			X
40	50	S40M50	X		
40	50	S40M50		X	

The underlined code denotes the part of the applied fertiliser (mineral or slurry), which was labelled

Experiment 2: slurry N uptake efficiency

Estimation of short-term slurry N uptake efficiency included the slurry-only (0, 40 m^3 ha^{-1}) and the combined mineral and slurry treatments (50 kg mineral N ha^{-1} plus 40 m^3 ha^{-1}); see Table 1. Slurry was labelled (0.72776 at%^{15}N) by feeding ^{15}N-enriched maize silage to steers and by collecting their excrements. Between ploughing and sowing, slurry was applied on microplots of 3.5 m^2 in size (three maize rows) with a watering can at a rate equivalent to 40 m^3 ha^{-1} (1998: 72 kg N ha^{-1}; 1999: 136 kg N ha^{-1}; 2000: 148 kg N ha^{-1}). Slurry was incorporated by hand tools immediately after application to reduce ammonia volatilisation. Mineral fertiliser (CAN) of the combined treatment was unlabelled.

Experiment 3: N uptake from upper soil layer

The experiment included four treatments: control (0 kg N ha^{-1}), 20 m^3 slurry (1998: 36 kg N ha^{-1}, 1999: 68 kg N ha^{-1}, 2000: 74 kg N ha^{-1}), 40 m^3 slurry (1998: 72 kg N ha^{-1}; 1999: 136 kg N ha^{-1}; 2000: 148 kg N ha^{-1}), and 40 m^3 slurry with an additional 50 kg mineral fertiliser N ha^{-1}, where slurry and mineral N were unlabelled. To estimate the N uptake from the upper soil layer, 4.2 m^2-sized microplots (three maize rows) were installed in each treatment, where $K^{15}NO_3$ (10.6 at%15 N; 4.1 kg N ha^{-1}) was applied to the upper soil layer. To this end, the labelled N fertiliser was spread with a hand sprayer on 9 m^3 before ploughing. Subsequently, additional 4 l of water per m^2 were applied with a watering can to facilitate infiltration. One to 3 days later, slurry was applied and incorporated, and afterwards maize was sown.

Crop sampling and processing

At silage maturity (5 Oct 1998, 22 Sept 1999, 29 Sept 2000), five labelled plants were cut at the soil surface from the middle row in each microplot and separated into leaves, stalks and ears. Main plots were harvested by a maize chopper. Samples of main plots and microplots were weighed and dried at 58°C to a constant weight. To obtain a more reliable quantification of dry matter yield for the microplots, the relative proportions of leaves, stalks, and ears of the labelled plants harvested in the microplot were multiplied with the dry matter yield (t DM ha^{-1}) recorded in the respective main plot.

Representative subsamples were ground into 1-mm particles for near-infrared reflectance spectroscopy (NIRS) analysis and homogenised with a ball mill (Retsch MM2000, Haan, Germany) for ^{15}N determination. The total N content of the main plots and plant fractions was estimated by NIRS. Ground samples were scanned on a NIRSystems 5000 scanning monochromator (FOSS GmbH, Rellingen, Germany), and software (ISI-version) for data collection and manipulation was supplied by Infrasoft International® (ISI, Port Matilda, PA, USA). Calibrations were developed separately for vegetative and reproductive plant fractions. Calibration and validation subset samples were analysed for N content using a C/N autoanalyser (Vario Max CN, Elementar Analysensysteme, Hanau, Germany). Standard errors of prediction ranged between 0.68 and 1.97 g N kg^{-1} DM, depending on the plant fraction (Volkers et al. 2003). Due to the experimental design (Table 1), some treatments were included in two (40 m^3 ha^{-1} slurry) or three experiments (40 m^3 ha^{-1} slurry plus 50 kg mineral N ha^{-1}), but with different ^{15}N labelling. In the analysis of the DM and N yield of whole plants and plant parts, averages were calculated for the different labellings. The ^{15}N enrichment of plant parts was determined using a mass spectrometer (ThermoFinnigan Delta C, Bremen, Germany) coupled with an elemental analyser (Carlo Erba 1108, CE Instruments Milano/Italy; Conflo II).

Calculation of N recovery

Using the ^{15}N isotope technique, the amount of N in a given plant part or the whole plant that derived from the labelled mineral fertiliser or slurry (Ndff, kg N ha^{-1}) was estimated by the following equation:

$$\text{Ndff} = \frac{(c-b)}{(a-b)} \cdot p,$$

where a, b, and c denote the atom% ^{15}N concentrations in the fertiliser (mineral or slurry), unlabelled

control, and labelled plant parts or whole plant, respectively, and p is the total N (kg N ha^{-1}) in a given plant part or the whole plant.

Consequently, the N taken up by a plant part or the whole plant originating from non-fertiliser (non-slurry), Ndfs (kg N ha^{-1}), was obtained by

$$\text{Ndfs} = p - \text{Ndff}.$$

Fertiliser N uptake efficiency (FNUE_{15N}) was then calculated by

$$\text{FNUE}_{15N} = \frac{\text{Ndff}}{f} \cdot 100,$$

with f, the amount of labelled N fertiliser (kg N ha^{-1}) applied. The difference method estimates the fertiliser N uptake efficiency ($\text{FNUE}_{\text{diff}}$) by determining the extra amount of mineral N fertiliser or slurry N taken up compared to an unfertilised treatment, and by assuming that N transformation processes are not modified by the added N.

$$\text{FNUE}_{\text{diff}} = \frac{(d - e)}{g} \cdot 100,$$

where d is the nitrogen yield (kg N ha^{-1}) of the fertilised treatment, e is the nitrogen yield (kg N ha^{-1}) of the unfertilised reference treatment, and g denotes the total amount of N fertiliser applied (kg N ha^{-1}). For mineral- or slurry-only treatments, we used the control (0 kg N ha^{-1}) as a reference (e), while S0M50 was used for treatment S40M50.

Statistical analysis

The field trial was conducted over 3 years, where treatments were allocated to fixed plots. It might be argued that this requires the introduction of year as fixed or random effect in the statistical model. Since, however, year had no strong impact on silage maize yield and N recovery, analysis of variance was conducted without any year effects. The effect of N fertiliser amount and fertiliser type was tested for significance using Proc Mixed of SAS®9.0 (SAS Institute Inc 2001). Statistical analysis was conducted separately for each of the three experiments and each plant fraction. Data presented are LSMeans across years produced in the analysis. Significance was declared at $P < 0.05$, and multiple comparisons were conducted using the Tukey-test.

Results

Dry matter and nitrogen yield

Shoot dry matter yield ranged between 7.1 and 14.3 t DM ha^{-1}, with the lowest significant yield found in the control and highest yield obtained in the mineral-only treatment S0M150 (Table 2). Due to the large contribution of the ear to the shoot yield, an identical ranking was found among treatments for the ear DM yield, with values varying between 3.5 and 8.4 t DM ha^{-1}. Also for the stalk DM yield, the treatments showed a similar order, while for the leaf DM yield, less significances were detected. The treatment ranking for N yield corresponded to the observed DM yield. Consistently, the lowest N yield was recorded for the control, while S0M150 achieved the highest N yield for the shoot as well as all plant parts considered. However, differences in N yield among treatments were more pronounced than differences in DM yield, e.g. stalk N yield of the control was only 32% of the maximum N yield achieved in treatment S0M150, while for the DM yield the control still achieved 66% of the S0M150 yield. Notably, the application of 50 kg mineral N ha^{-1} in addition to 40 m^3 of slurry (1998: 72 kg N ha^{-1}; 1999: 136 kg N ha^{-1}; 2000: 148 kg N ha^{-1}) did not increase the DM yield, but resulted in a significantly higher N yield of the shoot, ear, and leaf.

Recovery of mineral fertiliser N

Recovery of labelled N in the aboveground plant parts revealed that the main N sink at silage maturity was the ear. Each of the two mineral N dressings contributed about 7.0 to 30.3 kg N ha^{-1} to the overall N uptake of the ear (Fig. 1a). Stalks and leaves were minor N sinks, with ^{15}N recovery from mineral fertiliser varying between 1.7 and 6.6 kg N ha^{-1} and 3.3 and 10.7 kg N ha^{-1} in each dressing, respectively (Fig. 1b, c).

For leaves, ^{15}N recovery from the first dressing in mineral fertiliser-only treatments was lowest in S0M50 (3.3 kg N ha^{-1}), but similar in S0M100 and S0M150, with 8.0 and 10.7 kg N ha^{-1}. N recovery from the second dressing ranged between 3.8 and 9.6 kg N ha^{-1}. For a given N rate, fertiliser N uptake derived from the first and second dressings did not differ significantly. In stalks, N recovered from the

Table 2 Dry matter yield (t DM ha^{-1}) and nitrogen yield (kg N ha^{-1}) of leaves, stalk, ear, and whole plant as influenced by N fertiliser amount and type

N treatment		Code	Dry matter yield (t DM ha^{-1})				Nitrogen yield (kg N ha^{-1})			
Slurry N (m^3 ha^{-1})	Mineral N (kg N ha^{-1})		Leaf	Stalk	Ear	Shoot	Leaf	Stalk	Ear	Shoot
0	0	Ctrl	1.2 c	2.5 c	3.5 d	7.1 d	7.9 f	7.4 d	31.6 e	46.9 e
0	50	S0M50	2.0 b	3.2 b	6.6 bc	11.8 c	23.7 de	12.6 c	59.6 d	95.8 cd
0	100	S0M100	2.2 ab	3.8 a	7.7 ab	13.6 ab	34.0 bc	18.1 b	80.7 bc	132.8 b
0	150	S0M150	2.2 ab	3.7 a	8.4 a	14.3 a	40.6 a	23.1 a	105.2 a	168.9 a
20	0	S20M0	2.2 ab	3.1 b	6.3 c	11.6 c	21.4 e	11.6 cd	56.7 d	89.7 d
40	0	S40M0	2.2 ab	3.3 b	6.7 bc	12.2 bc	29.3 cd	13.7 bc	68.8 cd	111.8 c
40	50	S40M50	2.3 a	3.5 ab	7.6 ab	13.4 ab	36.6 ab	17.3 b	85.7 b	139.6 b
	S.E.		0.7	0.8	2.3	3.0	1.3	1.0	2.9	3.7

Values generally represent means of 3 years (1998–2000) and four replicates. For treatments S40M0 and S40M50, which were included in different experiments with 2–3 different ^{15}N labellings, averages were calculated over the different labellings

Means followed by different letters within columns are significantly different

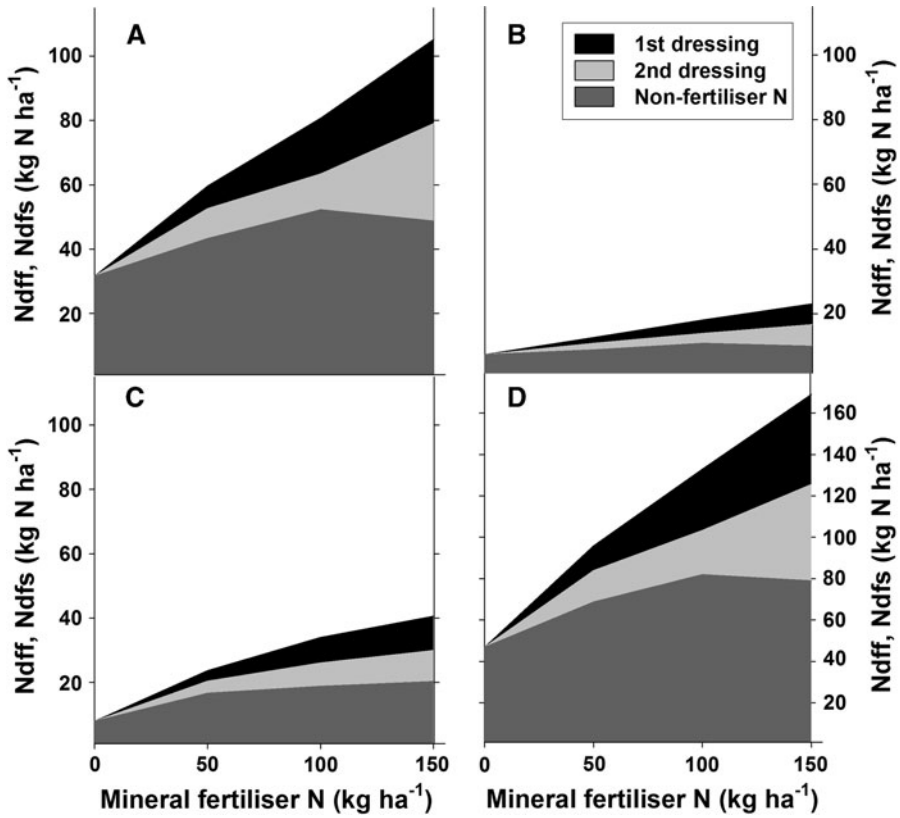

Fig. 1 Effect of mineral N application rate on the ^{15}N recovery (kg N ha^{-1}) of **a** ears, **b** stalks, **c** leaves, and **d** whole plant in the first and second fertiliser dressing (Ndff), and N amount derived from non-fertiliser N (Ndfs). Values represent means of 3 years and 4 replicates

first dressing (1.7–6.5 kg N ha^{-1}) revealed a significant increase with N fertilisation rate (Fig. 1b). N uptake from the second dressing ranged between 2.1 and 6.6 kg N ha^{-1} and was highest in S0M150, but similar in all other mineral fertiliser treatments. As with the leaves, the amounts of N recovered from the first and second dressings were similar within a given treatment. Divergent effects were observed for the ear

(Fig. 1a). Differences in ^{15}N recovery between both dressings were detected only for the S0M100 treatment, where the ear contained more fertiliser N from the first than the second dressing. An increasing N rate resulted in an increased N recovery of the first N dressing for the mineral-only treatments. Nitrogen originating from the second dressing was highest in S0M150, but similar in the other treatments, amounting to 9.3, 9.5 and 11.1 kg N ha^{-1} for S0M50, S40M50 and S0M100, respectively. On a whole plant basis, N recovered from the first dressing amounted to 12.0, 13.7, 29.6 and 43.4 kg N ha^{-1} for S0M50, S40M50, S0M100 and S0M150, respectively (Figs. 1d, 2), and differed between mineral-only treatments, while additional slurry application (S0M50 vs. S40M50) had no impact. Recoveries of mineral fertiliser N in the second dressing were 15.2, 16.3, 21.3 and 46.5 kg N ha^{-1} for S0M50, S40M50, S0M100 and S0M150, respectively, with S0M150 having a significantly higher ^{15}N recovery than the other treatments. For a given fertiliser treatment, N recovery tended towards higher values in the second compared to the first dressing (except for S0M100), but no significant differences became evident. Whole plant N uptake originating from non-fertiliser N (Ndfs) increased with N rate up to treatment S0M100, while further N input showed no further increase or even caused a slight decrease. Consistently, a similar trend was observed for all plant parts.

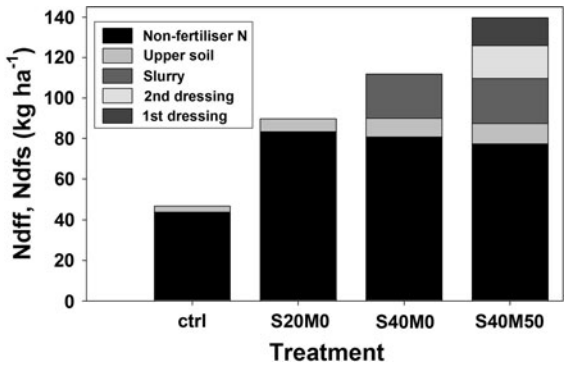

Fig. 2 Whole plant N recovery (kg N ha^{-1}) from first and second dressing of mineral N, slurry, the upper soil layer, and non-fertiliser N as influenced by fertiliser treatment, with ctrl: control, S20M0: slurry only (20 m^3 ha^{-1}), S40M0: slurry only (40 m^3 ha^{-1}), S40M50: combined slurry (40 m^3 ha^{-1}) and mineral N (50 kg N ha^{-1}). In treatment S20M0 only N applied to upper soil layer was labelled. Values represent means of 3 years and 4 replicates

Fertiliser N uptake efficiency ($FNUE_{15N}$) estimated by the isotope technique for the whole plant were 54, 51, 60 and 61% for treatments S0M50, S0M100, S0M150 and S40M50, respectively, and did not differ significantly (Fig. 3a). Corresponding values estimated by the difference method ($FNUE_{diff}$) were considerably higher, except for treatment S40M50, amounting to 98, 86, 81, and 54%. The $FNUE_{diff}$ of mineral-only treatments did not differ substantially, and additional slurry application (S0M50 vs. S40M0) caused a significant decrease of $FNUE_{diff}$.

Recovery of slurry N and of mineral N from the upper soil layer

On the whole plant basis, nitrogen originating from slurry application was similar in the S40M0 and

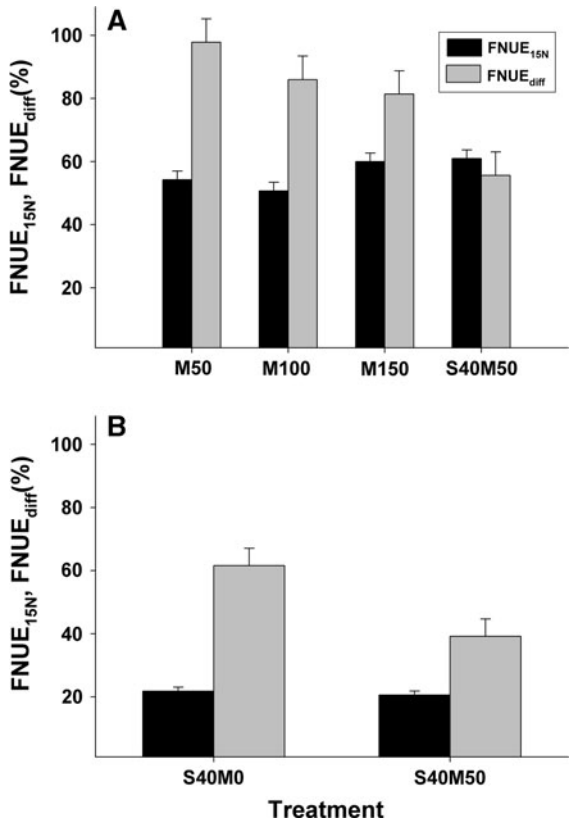

Fig. 3 Impact of N fertiliser treatment on whole plant fertiliser N uptake efficiency estimated by the isotopic technique ($FNUE_{15N}$, %; *black bars*) and by the difference method ($FNUE_{diff}$, %; *grey bars*) for (A) mineral fertiliser N and (B) slurry N. Values represent means of 3 years and 4 replicates. *Error bars* indicate standard errors of the mean values

S40M50 treatments, totalling to 21.8 and 22.2 kg N ha^{-1} (Fig. 2). While 61% of the slurry N was found in ears, leaves and stalk contributed 27 and 13%, respectively (data not presented), indicating that the sink-source relationship for slurry N followed the same pattern as for mineral fertiliser N. Additional slurry application (S40M0 vs. S40M50) caused significantly higher N recovery in stalks, but not in leaves and ears (data not shown).

Uptake of labelled N from the upper soil layer (0–10 cm) was significantly higher in the S40M0 (9.3 kg N ha^{-1}) and S40M50 (10.1 kg N ha^{-1}) treatments than in the control (3.0 kg N ha^{-1}) and the S20M0 treatment (6.4 kg N ha^{-1}); see Fig. 2. In accordance with slurry N recovery, less labelled soil N was found in leaves and stalks than ears (data not presented). Uptake of non-fertiliser N from layers below 10 cm was considerably lower in the control (43.9 kg N ha^{-1}) compared to the S40M0 (80.8 kg N ha^{-1}) and S40M50 (77.3 kg N ha^{-1}) treatments.

Slurry N uptake efficiency ($FNUE_{15N}$) in the whole plant obtained by the isotope technique revealed a similar level for treatments S40M0 (22%) and S40M50 (21%); see Fig. 3b. In contrast, the difference method ($FNUE_{diff}$) resulted in a significantly higher slurry N uptake efficiency for S40M0 (62%) than for S40M50 (39%).

Discussion

Mineral N fertiliser use efficiency

Fertiliser N uptake efficiency of mineral fertiliser varied between 51 and 61% at the whole crop level, which is considerably higher than reported previously for sandy soils (Khanif et al. 1984). In our study, however, the N rate was split to better match the N demand of the crop (López-Bellido et al. 2006). It may therefore be assumed that less fertiliser N was subject to N losses caused by nitrate leaching and nitrous oxide emission during the vegetation period (Torbert et al. 1993; Maidl 1990). The split N application tended to result in higher N recoveries for the second dressing, except for the S0M100 treatment. This finding deviates from several other works, which documented a clear effect of fertiliser N timing on ^{15}N recovery, with substantially higher N use efficiency for N applied sidedressed than at planting (Bigeriego et al. 1979; Russelle et al. 1981; Jokela and Randall 1997; Seo et al. 2006). A high proportion of the nitrogen taken up from the first dressing was found in the ear while recovery in leaves and stalks was low, for instance 60, 25, and 14%, respectively, in the S0M150 treatment. Nitrogen applied in early growth stages and taken up by vegetative plant parts is remobilised during reproductive growth and translocated to the grains. The remobilisation pattern, however, varies among plant parts, with mobilisation of N in stalks starting earlier than in leaves (Weiland and Ta 1992). The later N is taken up during vegetative growth, the more likely it is mobilised to the grains, while N absorbed during grain filling is mainly allocated directly to the grains (Subedi and Ma 2005). Therefore, a higher N recovery of the second dressing compared to the first would have been expected for the ear. In our study, however, differences in N recovery between the first and second dressing were not pronounced, and treatment S0M100 even showed a reverse effect. This may partly be due to the relatively short time interval of 3–4 weeks between the two N dressings.

An increasing N rate neither caused a consistent trend in $FNUE_{15N}$ for single plant parts nor at the whole plant level, which is contrary to previous studies (Bigeriego et al. 1979; Reddy and Reddy 1993; Jokela and Randall 1997; Nissen and Wander 2003). Moreover, we did not find any effect of N rate on ^{15}N distribution among plant parts, while Subedi and Ma (2005) reported the proportion of ^{15}N reaching the grain to be inversely related to N supply. One possible explanation for the deviating results in our study may be that mineral N was not provided in excess, as indicated by the linear increase of total N yields in the mineral-only treatments (Table 2). Support for our findings comes from a meta-analysis of N fate in grain cropping systems conducted by Gardner and Drinkwater (2009). The authors concluded that a reduced N rate had no impact on ^{15}N recovery by the crop, but a positive effect on total ^{15}N recovery of plant and soil, and that the corresponding N rate was an important predictor of unrecovered ^{15}N.

$FNUE_{diff}$ of mineral fertiliser obtained in the present study (81–98%) was in the range reported by Varvel and Peterson (1990), but higher compared to observations by Schröder (1999). This is probably mainly attributed to differences in the yielding performance of the control treatment caused by the

manuring history of the site (Harmsen 2003a, b), but $FNUE_{diff}$ can also be affected by environmental conditions and the maize genotype under evaluation (Coque et al. 2006). Currently available results on the effect of mineral N fertilisation on $FNUE_{diff}$ are ambiguous (Van Dijk and Brouwer 1998; Ma et al. 1999; Nissen and Wander 2003; Halvorson et al. 2005; Stevens et al. 2005; Montemurro et al. 2006). Our own findings strongly support decreasing $FNUE_{diff}$ with increasing N supply. In low N treatments, ^{15}N data revealed a considerable additional non-fertiliser N uptake, which is ascribed to mineral fertiliser when calculating N recovery by $FNUE_{diff}$. In highly fertilised plots, however, the additional N uptake of plants mainly derived from fertiliser N (Fig. 2), leading to a minor discrepancy between both approaches. This may also explain why additional slurry application (S0M50 vs. S40M50) caused a substantial decline of $FNUE_{diff}$ while $FNUE_{15N}$ was nearly constant. Contrary to our hypothesis, $FNUE_{15N}$ data reveal that slurry application obviously did not exert any direct effect on mineral N recovery, which may be due to a sizeable interval between slurry application and mineral N dressings.

The phenomenon of increased soil N uptake in fertilised plots is mainly restricted to N-deficient sites with high soil organic matter content (Harmsen 2003a). In our study, an apparent added nitrogen interaction (ANI) (Jenkinson et al. 1985) may have occurred. Considering the different causes of an apparent ANI (Kuzyakov et al. 2000; Blagodatskaya and Kuzyakov 2008), displacement effects and MIT (mineralisation-immobilisation and turnover) processes seem likely. Furthermore, a stronger and deeper root system in the fertilised treatments may have resulted in additional N uptake.

These effects only apply to N recovery estimation by $FNUE_{15N}$, and $FNUE_{diff}$ thus seems to be a more appropriate tool. When evaluating both approaches, one should, however, keep in mind that different N pools are considered. While $FNUE_{15N}$ detects recently applied labelled N, the difference method includes recently applied N as well as N given in the past, which entered the soil N pool and has been released in exchange with recently applied fertiliser N. The amount of N released from the soil N pool can be considerable, as indicated by significant N leaching losses despite negative field N balances (Wachendorf et al. 2006a, b). The fact that N released from the soil N pool is included in $FNUE_{diff}$ estimation limits its suitability for long-term experiments, where control plots show severe N deficiency and may cause an overestimation of N recovery by $FNUE_{diff}$ (Schindler and Knighton 1999; Stevens et al. 2005) as in, for instance, long-term monoculture cultivation.

Slurry N use efficiency

As expected, for both investigated approaches ($FNUE_{15N}$, $FNUE_{diff}$) fertiliser N uptake efficiency of slurry was lower compared to mineral fertiliser, mainly due to the organically bound N fraction, which makes up about 45% of total nitrogen and is little available in the year of application. Taking the proportion of organically bound N into consideration, the difference in FNUE between slurry and mineral fertiliser will diminish, e.g. to 38–40% $FNUE_{15N}$ for slurry versus 51–61% $FNUE_{15N}$ for mineral N. A further cause might be due to gaseous N losses after slurry spreading. In our study, however, slurry was immediately incorporated after application, so that we may assume ammonia losses to be negligible.

$FNUE_{15N}$ was not affected by additional mineral fertiliser application and varied from 21 to 22% (Fig. 2). Recovery rates reported by Muñoz et al. (2003) for dairy manure in the year of application were somewhat lower (17%), possibly because dairy excreta were mixed with straw, leading to an increased C/N ratio. As is well-known, the C/N ratio is an important determinant of short-term plant availability (Sørensen et al. 2003; Chantigny et al. 2004; Powell et al. 2005). Non-fertiliser N uptake increased by more than 40 kg N ha^{-1} in slurry treatments compared to the control (Fig. 2), yet the uptake of non-labelled N was always lower than unaccounted slurry N. As for mineral N-only treatments, a likely explanation for that finding is an apparent added nitrogen interaction (ANI). Support comes from previous studies (Sørensen and Jensen 1995; Chadwick et al. 2000) documenting that slurry N underlies immobilisation processes and is released in the long term. Our soil labelling data, however, indicate that a real positive ANI might have occurred. With increasing slurry application (control, S20M0, S40M0), the N uptake from the upper soil layer increased linearly by a factor of three ($r^2 = 0.99$). A real ANI would not exclude an apparent ANI since both processes can take place simultaneously.

Slurry $FNUE_{diff}$ of the present study was in the range found in previous studies under similar soil and climatic conditions (Schröder et al. 1993; Schröder et al. 2005), but exceeded the $FNUE_{diff}$ values reported by Schröder (1999). The low $FNUE_{diff}$ in the latter study is somewhat surprising since the field trials were also conducted on sandy soil and since the slurry was banded or incorporated into the soil. Yet, soil N status was higher compared to our study due to regular, intensive slurry application, leading to high-yielding control plots, reflecting the importance of manuring history to $FNUE_{diff}$ estimation (Mallory and Griffin 2007).

In accordance with mineral N recovery estimation, slurry $FNUE_{diff}$ resulted in substantially higher recovery rates than slurry $FNUE_{15N}$, which has also been reported elsewhere (Muñoz et al. 2003). The difference between $FNUE_{diff}$ and $FNUE_{15N}$ was not constant among slurry treatments (Fig. 3b), since different reference treatments were used for estimating $FNUE_{diff}$, i.e. control for S40M0 and S0M50 for S40M50. According to the ^{15}N data, the additional uptake of unlabelled N in S40M0 compared to the reference (control) was higher (43.1 kg N ha^{-1}) than for S40M50 (18.7 kg N ha^{-1}) when compared to its respective reference (S0M50). This led to an underestimation of slurry N recovery for treatment S40M50 and corroborates the assumption that constant plant N uptake in reference and fertilised treatments is one of the major error sources in $FNUE_{diff}$ estimation (Rao et al. 1992). Likewise, the recovered mineral N for treatment S40M50 (27.8 kg N ha^{-1}) that was obtained by the difference method is biased since S40M0 served as a reference. When assuming a constant soil N uptake in all treatments, the sum of soil N and recovered fertiliser N in S40M50 (43.8 kg slurry N, 27.8 kg mineral fertiliser N) was 21.1 kg N ha^{-1} lower than the actual measured N yield (Table 2). In conclusion, the present study gives strong evidence that the difference method does not allow an accurate estimation of N recovery when different N sources are applied together.

Conclusions

The present study substantiated the fact that the approaches used for estimating fertiliser N uptake efficiency, i.e. stable isotopic technique vs. difference method, consider different N pools, differ in magnitude, and have inherent estimation errors. The recovery rates obtained by $FNUE_{15N}$, for instance, exceeded $FNUE_{diff}$ values by a factor of 1.45 in the case of mineral N and by 2.42 in the case of slurry N. It could clearly be demonstrated that the approaches under evaluation come to different conclusions when applying mineral and organic N fertilisers separately compared to a combined application, and correspondingly their suitability depends on the question of interest. While N recovery by $FNUE_{15N}$ was not influenced by additional N sources, be it mineral or organic, $FNUE_{diff}$ resulted in substantially lower values for the combined application. Similarly, an increasing N rate did not influence $FNUE_{15N}$, but led to a considerable decline in $FNUE_{diff}$. We therefore regard $FNUE_{diff}$ as an inappropriate tool for estimating N recovery of a single N source when applied in combination with other N fertiliser types. Recovery rates obtained by $FNUE_{diff}$ should be treated with caution. Future challenges will include the simultaneous labelling of soil N and fertiliser N fractions to facilitate evaluation of their contribution to yield formation and N transformation processes in the soil.

Acknowledgments We are indebted to N. Jovanovic and K. Volkers for data sampling and gratefully acknowledge the excellent technical assistance of B. Biegler. We also thank G. Rave for support in the data analysis.

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