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Achieving legislation requirements with different nitrogen fertilization strategies: results from a long term experiment

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Abstract

The Nitrates Directive (91/676/EEC, Anonymous, 1991) was developed in Europe to limit environmental threats from intensive livestock farming and N fertilizer applications to crops. It imposed several rules on farmers and public bodies, one of which was nutrient fertilization plan adoption. Here we use results from the Tetto Frati (Northern Italy) Long-Term Experiment to verify the terms and coefficients in the official Italian guidelines and evaluate the limitations imposed to organic fertilization amounts. For this purpose, we mined long-term experimental data of crop yield, N uptake, N use efficiency, and soil organic matter content from miscellanea cropping systems fertilized with farmyard manure (FYM) and bovine slurry (SLU), typical of a dairy farm in Northern Italy. N fertilization efficiency indicators (Removal to Fertilizer ratio, Apparent Recovery and Nitrogen Fertilizer Replacement Value) indicated that in the long run, FYM behaved similarly to urea, and better than SLU. Even N supply rates as high as 250 kg N ha^{-1} were justified by high rates of crop removal. In fact, among the terms of the mass-balance equation, SOM mineralization was found to be most relevant, followed by meadow rotation residual effects. We conclude that a revised Nitrates Directives application scheme could be more relaxed in its application limit of manure-N, but should be more ambitious in setting efficiency coefficients for manure fertilization.

Keywords

Nitrogen balance; Manure; Maize; Fertilization; Nitrates Directive; N use efficiency; Long-Term Experiment

1. Introduction

During the Eighties many researchers showed that nitrogen (N) amounts applied in Western European agriculture were excessive and risked producing pollution. Furthermore, excesses were unevenly distributed across the continent and linked primarily to areas of intensive livestock farming (Velthof et al., 2014). By all accounts, measures to protect human health, living resources, and aquatic ecosystems were needed. Today, despite reduction in chemical fertilizer usage, the European Union (EU-28) still exhibits excess N supply (the sum of organic and mineral fertilizers, natural deposition, and N-fixation) compared with removal by crop yields (Leip et al., 2011).

Imbalance in the input *vs* output N budget can produce various types of N losses to the environment (Sutton et al., 2011). While initial scientific attention focused mainly on the problem of nitrate leaching, soon other threats linked to excess manure use or imbalance gained attention (Solomon et al., 2007; Galloway et al., 2008), such as ammonia volatilization and N oxide emissions, as well as the inherently-associated massive phosphorous load that can over-enrich the soil to menace fresh and marine waters with eutrophication produced from sediment runoff (Schoumans et al., 2015).

The Nitrates Directive (91/676/EEC, Anonymous, 1991) was approved in Europe 24 years ago to address the excessive distribution and/or discharge of livestock effluents onto agricultural land and the excessive use of mineral fertilizers in several parts of Europe. Northern Italy soon emerged as a high-risk area because of its numerous intensive livestock farms. The Nitrates Directive defined a few but clear monitoring actions and limitations to the application of N: i) member States must extensively monitor fresh waters to identify areas where nitrate content exceeds a threshold of 50 mg NO_3^- l^{-1}; ii) member States must designate Nitrate Vulnerable Zones (NVZs), where actual or potential nitrate pollution of agricultural origin is problematic; iii) farms lying in NVZs must implement Action Programs to adopt sustainable management practices. A core part of the Action Program established constraints for land-application of all nitrogen-containing fertilizers, and in particular, set specific limits for livestock manure application.

A key component of Action Programs is balancing N inputs and outputs. The definition of “balance” rested on a rather simple principle that the difference between expected nitrogen crop requirements and the nitrogen supply from the soil, air, and fertilization approximate one another. However, predicting the equation components (e.g. crop removal, initial N availability when crop growth starts, soil N net mineralization, efficiency of N from livestock manure and chemical fertilizers) is not trivial (Webb et al., 2013; Schröder and Neeteson, 2008).

The Tetto Frati Long Term Experiment (LTE) has been underway about as long as the Nitrates Directive has been in effect in Europe. It was initially created in 1992 to mimic a set of intensive forage systems for a typical Northern Italy dairy farm, where maize is the main fodder crop, and liquid and solid manures are fertilizer resources (Grignani et al., 2007). It was used for several purposes, all of which were connected to the study of N efficiency and the fertilization impacts of highly manured cropping systems (Zavattaro et al., 2012). Studied treatments are semi-static in the sense that zero, low, and high input levels have been applied continuously, with exact N low and high input amounts having been adjusted just twice to produce data relevant to scientific and technical questions on application of the Nitrates Directive in Italy. Prior to 2006, the LTE was used to investigate a frequently applied fertilization strategy that combined manure and chemical fertilizer as inputs. The low and high input levels were determined by fixed amounts of slurry and farmyard manure while actual nitrogen input changed according to manure and slurry characteristics, and all organic treatments were top-dressed with a fixed amount of 100 kg urea-N ha^{-1}. During 2007 to 2011, LTE research centered on the two maximum levels of manure-N inputs mandated within and external to Italian NVZs (170 and 340 kg N ha^{-1}). Top-dressed urea was excluded from manured treatments to test the simple effects of manures and mineral fertilizers. The third phase of LTE research began in 2012 and is still ongoing; it is devoted to verification of the application effects of the 250 kg manure-N ha^{-1} Derogation scheme in Italy (low level is 170 and high level is 250 kg N ha^{-1}).

Results from the first period have been presented in previous papers. Here we utilize Tetto Frati LTE data from 2007 forward to answer two questions.

- Are the coefficients used to estimate the terms of the N balance equation consistent with long-term experimental data?
- Are the limitations imposed by the Nitrates Directive sufficient and/or necessary to guarantee an N-balanced cropping system?

In particular, we wanted to evaluate the N use efficiency of animal manure-N (farmyard manure and bovine slurry) compared to mineral N fertilizers such as urea, and to demonstrate that the application of 250 kg ha^{-1} of manure-N to high demanding crops under adequate environmental conditions increases the value of manure-N and minimizes the need of purchasing additional N fertilizers.

2. Materials and Methods

2.1 LTE description

The long-term experiment of Tetto Frati (44°53′ N; 7°41′ E; 232 m a.s.l.) of the University of Turin, Italy was started in 1992, but the first year of data has been excluded to mitigate the effect of past fertilizations. The climate is temperate sub-continental, the average annual rainfall is 760 mm, and the mean annual temperature is 12°C. The soil, Typic Udifluvent, is deep and calcareous with loam texture in the first horizon and silty texture in deeper horizons (Grignani et al., 2007).

The experiment, designed as a randomized block with three replicates, compares four cropping systems at five nitrogen application levels, plus a rotational maize-lucerne system at a single fertilization level. Cropping systems are: entirely-harvested maize for silage (Ms); maize for grain with residue incorporation (Mg); silage maize+Italian ryegrass double cropping with almost continuous soil cover (Mr); 4-years or 6-years rotation of entirely-harvested maize and long-growing grass ley (Ml); 4-years or 6-years rotation of maize for silage and lucerne, Mu. The five N application levels are arranged in nine distinct fertilization treatments: 0N control, four doses of mineral N as urea (U100 to U400), two doses of bovine slurry (SLU), two doses of farmyard manure (FYM). The Mu system is fertilized as SLULow in the maize phase, while lucerne receives no N fertilizer. Further details on fertilization treatments are reported in Table 1. The target amount of N supplied was based on total N contents of FYM and SLU. Manure was always distributed in spring and incorporated quickly into the soil, a few days before maize sowing. Urea was chosen as the reference mineral fertilizer because it is the most commonly used N fertilizer in maize in Italy, due to its low cost. It was partly distributed few days before maize sowing and partly top-dressed and incorporated through ridging. Phosphorous (P) and potassium (K) were supplied through mineral fertilizers at levels above plant needs to exclude any possibility of nitrogen interaction. Nine different commercial maize hybrids of the 500 or 600 FAO were used throughout the 23 years period. Further details on the crop management are reported in Grignani et al. (2007), Bertora et al. (2009), and Zavattaro et al. (2012). Fertilization amounts were modified from experimental start to conform to a semi-static management, in which the number of levels remained constant. Table 1 reports all fertilization data for completeness, but in this paper we discuss results from the 2007 to 2014 period only, with the following qualifications:

- crop yield, N uptake, and N use efficiency indicators were reported separately for 2007-2011 (five years) and 2012-2014 (three years) according to the two fertilization levels (U300 and U400 were averaged to allow a direct comparison with the 340 kg ha^{-1} dose of manured treatments);
- incorporated crop residue and rotational ley effects were derived by averaging data over the entire 2007-2014 period;

Maize was sown in Ml and Mu systems in 2011 and 2012, while grass or lucerne leys lasted from 2008 to 2010 and from 2013 to 2014. Leys growth failed in 2007, therefore no crop data are reported in that year. Table 2 reports the general characteristics of the organic fertilizers used in the two periods analyzed.

2.2 Sampling and chemical analyses

Values of crop dry matter yield and N content were determined annually at harvest. N analyses were performed on samples pooled from the three replicates, using an elemental analyzer (Flash EA 1112, Thermoquest). In this work, Mg yield was from grain only. Mr was based on the sum of two crops (Italian ryegrass and late-sown maize), whereas Ml came from the average of the two crops.

Organic fertilizers were sampled and analyzed before each distribution to determine several measures: dry matter (oven dried at 105°C), organic carbon, C (NA2100 protein Carlo Erba elemental analyzer), total N (Kjeldahl method after acid mineralization of organic N), ammonium N (Kjeldahl method), phosphorous (P) and potassium (K) contents (nitric or perchloric acid microwave solubilization followed by ICP spectroscopy).

Soil organic C and total N content were determined in all plots at a depth of 0-30 cm (total C and N contents: NA2100 protein Carlo Erba elemental analyzer, mineral C content: Dietrich-Fruhling calcimeter). Sampling was made in the early spring of years 2007, 2010, 2012, and 2015.

2.3 Statistical methods

Simple linear regression analyses were performed using a least-squares procedure, and tested at $p<0.05$. An analysis of variance at $p<0.05$ was performed on yield and N uptake data; means were separated using the post-hoc R-E-G-W-Q test. To separate the effect of organic fertilizers on crop yields and N uptakes for the four cropping systems and two periods, fertilization type and amount (four levels) was tested as a single fixed factor, while year was considered a random factor. Likewise, in order to determine the effect of crop residues on plant N uptake, cropping system (Mg *vs* Ms), as well as fertilization type and amount (all levels) were deemed fixed factors and year was considered random for the test. An ANOVA was used to test rotation on maize N uptake, using the cropping system (Ml *vs* Ms), fertilization type and amount and years post a ley crop as the between-subjects factors for the first two years after ley (1995-96, 2002-03, 2011-12). The entire historical dataset of the LTE was used in this case. A repeated-measures ANOVA was used to test the effect of rotation and of residue incorporation on SON stock, using the cropping system as the between-subjects fixed factor, and time as the within-subjects factor.

2.4 Efficiency coefficients

Nitrogen efficiency coefficients were calculated using experimental data as follows.

- R/F, or Removal to Fertilizer Ratio, was calculated as the amount of N removed as yield (*Nremoval*) divided by the total amount of fertilizer N (*Nfertilizer*);
- AR, or Apparent Recovery, was calculated as:

$$AR = \frac{Nremoval - Nremoval_{0N}}{Nfertilizer}$$

where *Nremoval* and $Nremoval_{0N}$ are the amounts of N removed by a fertilized treatment and the control, respectively;

- AR relative to urea was calculated as the ratio between AR of the manured treatment and AR of urea:

$$AR\_relative\_to\_urea = \frac{Nremoval_{MAN} - Nremoval_{0N}}{Nfertilizer_{MAN}} \times \frac{Nfertilizer_{U}}{Nremoval_{U} - Nremoval_{0N}} =$$

$$= \frac{Nremoval_{MAN} - Nremoval_{0N}}{Nremoval_{U} - Nremoval_{0N}}$$

where and $Nremoval_{MAN}$ and where and $Nremoval_{U}$ are N removals in manured (FYM or SLU) and urea treatments, respectively, while $Nfertilizer_{MAN}$ and $Nfertilizer_{U}$ are the amounts of N supply in the two treatments.

AR relative to urea corresponds to one of the definitions of N Fertilizer Replacement Value (NFRV); specifically, the definition proposed by Schröder et al. (2007).

2.5 N balance according to Italian legislation

Field-assessed N balance according to the Italian Action Programs (Anonymous, 2006) application already considers soil mineralization, atmospheric deposition, previous crop effects, and crop residue effects, while fertilizer supply is taken into consideration with an efficiency coefficient. Quantification of the main terms is suggested to guide the farmer. The equation is:

$(Fc \times Kc) + (Fo \times Ko) + Nf + Nc + Mso + Ad = (Y \times B)$

where:

- Fc and Fo are the amounts of N supplied through mineral and organic fertilizers, respectively;
- Kc is an efficiency coefficient of N mineral fertilizers, which is set “generally equal to 1”;
- Ko is an efficiency coefficient of total N in organic fertilizers, expressed as the R/F indicator. Reference values are provided depending on the type of manure, soil texture, type of distribution (incorporated or not), timing and crop. Suggested values for well-managed bovine manures, both liquid and solid, range from 0.48 to 0.62 depending on the soil texture, but these values are reduced by 75-45% for incorrect timing or missing incorporation;
- Nf is the amount of N from the previous year farmyard manure fertilization, equal to at least 30% of applied N;

- Nc is the amount of N from the previous crop. Suggested values are 80 kg of N ha^{-1} after lucerne or long-lasting (>5 years) grass leys, and 30-40 kg of N after short-duration (≤ 5 years) grass leys. Conversely, when crop residues are incorporated into the soil, the available N is reduced by 30 kg ha^{-1} in the case of cereal straw, and by 40 kg ha^{-1} in the case of maize stalks, due to immobilization;
- Mso accounts for the mineralization of Soil Organic Matter (SOM), calculated as 30 kg ha^{-1} yr^{-1} of N for each percent of SOM for annual and long-lasting crops (maize included), and reduced to a fraction of this amount for short-lasting annual crops;
- Ad is the atmospheric deposition, equal to 20 kg N ha^{-1} per year;
- Y is the expected yield;
- B is the expected N concentration of yield. Suggested values are 17 kg of N t^{-1} of dry matter for maize grain, 10 kg t^{-1} for silage maize, 14 kg t^{-1} for Italian ryegrass, and 18 g t^{-1} for grass ley.

3. Results

3.1 Use efficiency of fertilizer N

The three indicators of N use efficiency showed a dependency on N supply. During the 2007-2011 period (Tab. 3 a), the R/F indicator was consistently high. It ranged between 1.27-1.70 in entirely-harvested systems (Ms, Mr, and Ml) at 170 kg ha^{-1} of N supply, and slightly decreased to a 0.82-1.09 range at 340 kg ha^{-1} of N supply. In other words, R/F decreased by 34% when N supply was doubled. Lower R/F values were observed in the Mg system due to limited removal of grain only. Crops fertilized with SLU exhibited systematically lower R/F values than when FYM was applied. These differences were smaller in Mg and Ms (2 to 7%) and larger in the long-growing cropping systems of Mr and Ml (8 to 31%).

R/F interannual variability, expressed as standard deviation, ranged from 0.06 to 0.51, with small differences among fertilizer types and more marked differences between cropping systems. In fact, the higher the measure of R/F value, the lower the interannual variability ($R^2 = 0.49$, $p=0.000$).

When the soil natural N supply is subtracted from N removal, we obtain the AR indicator. Obviously, experimental AR values (Tab. 3 a, referred to the 2007-2011 period) were lower than those of R/F, and ranged from 0.52 to 0.90 in entirely-harvested systems (Ms and Mr) at 170 kg ha^{-1} of N supply; values decreased to a range of 0.44-0.72 at 340 kg ha^{-1} of N supply. Lower AR values were measured in the Mg system because grain only was removed. As observed in R/F, SLU yielded lower AR values than did FYM, a difference not greatly influenced by N input and cropping system. The overall inter-annual variability of this coefficient ranged between 0.10 and 0.24.

When the apparent recovery of organic fertilizers is expressed relative to that of mineral ones, we obtain one of the definitions of NFRV (Schröder et al., 2007). In the 2007-2011 period and at 170 kg ha^{-1} of N supply, FYM showed a NFRV of 0.83 in monoculture maize (Mg and Ms) and 0.99 in the double-cropping system (Mr). NFRV for SLU was always lower, ranging between 0.72 and 0.80. NFRV was increased at the high dose if compared to the low dose because the AR of urea had decreased to a higher extent (from 0.90 to 0.60 in Ms, from 0.66 to 0.65 in Mr). As a result, NFRV was above 1.00 in all cropping systems, which demonstrated that in the long term and at high doses, the use efficiency of organic fertilizers was higher than that of urea.

Results from the second period 2012-2014 (Tab. 3 b) confirm some effects described for the previous period. In general, the N use efficiency indicators of R/F and AR at the 170 kg ha^{-1} level validated those observed in 2007-2011 (R^2 of R/F = 0.68, p=0.001, R^2 of AR = 0.44, p=0.050). The efficiency of SLU was also found to be lower than that of FYM, even though differences between cropping systems were less clear. NFRV values did not trend alike in the two periods (R^2 = 0.20, p=0.374), but they did affirm the high efficiencies of FYM and SLU relative to urea (0.62-0.98).

The main difference between the two periods is attributed to a lower High fertilization level in 2012-2014 (250 kg N ha^{-1}, corresponding to the maximum organic fertilization amount for farms in the Derogation scheme in Italy). This caused efficiency indicators not to consistently decrease when passing from the low to high level, as previously shown at 340 kg ha^{-1}. They did increase sometimes, such as in Ms and Mr cropping systems fertilized with FYM, indicating the use efficiency remained high up to 250 kg N ha^{-1}. As in the first period, NFRV increased as N supply increased also as a result of a progressively reduced urea efficiency (from 0.78 to 0.78 in Ms, from 0.81 to 0.72 in Mr). The range in the annual variability of the reported indicators was similar in the two periods; specifically, values for the 170 kg dose differed less than one standard deviation between the two time frames.

3.2 Mineralization from the soil

The crop N uptake of non-fertilized plots is frequently used as a proxy for the amount of N mineralized from the soil. The 2007-2014 average N uptake in the Mg, Ms, and Mr systems was 116 kg N ha^{-1} (standard deviation: 29 kg N ha^{-1}), without any substantial difference between Mg, Ms and Mr cropping systems, despite the different soil cover duration. Remarkably higher plant N uptake were recorded in the Ml system (214 kg N ha^{-1} in maize, 163 kg N ha^{-1} in grass), probably biased by an enrichment of soil N due to N-fixation by clover that progressively developed in the 0N sward.

3.3 Role of crop residues

Maize residues (stalks, cobs, and bracts) contained about 100 kg N ha^{-1} in the well-fertilized treatments (between-year s.d. = 23 kg N ha^{-1}). A C:N ratio as high as 60 was recorded for stalks, that represented 85% of residues.

To explore the role of crop residues, we compared their effects on plants and soil in treatments in which crop residues were (Mg) or were not returned to the soil (Ms). Average results are reported in Figure 1 a and b. A higher total plant N uptake in Mg *vs* Ms would show that residues are actively increasing N availability, whereas a higher SON content in Mg than in Ms would attest to N immobilization in stable organic matter.

Surprisingly, the N uptake of the entire plant was not influenced by the extra -N provided by residues ($p=0.810$) over the eight experimental years 2007-2014. Some positive effects seemed to take place in low-fertilized treatments, while a trend toward immobilization was noted in manured treatments at high doses (significant system by level interaction, $p = 0.019$).

On the other hand, the mean SON stock measured on four dates in the 2007-2014 period (Fig. 1 b) confirmed that incorporation of crop residues caused a buildup of SON stock to as much as 584 kg N ha^{-1}

over the years. Differences between the two cropping systems were significant in a repeated measures analysis of variance (p=0.000).

3.4 Rotation with leys

The effect of plowing a grass ley on available N to the subsequent maize crop was explored through a comparison of maize N uptake after the ley phase (Ml) and monoculture maize (Ms) (Fig. 2). A significant positive effect was observed at all fertilization levels, with an average increase of 27 kg N ha^{-1} per year in the first two years after grass plowing. A similar test was performed in rotation after a lucerne ley (Mu *vs* Ms), for which the increase in subsequent maize N uptake was found to be similar.

The incremental change in available N was negatively and linearly related to total fertilization supply (R^2 = 0.86, p=0.001), which indicated that the beneficial effects of rotation were evident at low fertilization inputs and less important at high fertility levels (Fig. 3).

The expected increase in SON stock due to grass and lucerne residues (Fig. 2 b) was not significant to a repeated measures analysis of variance (p=0.256).

3.5 Crop yield and N uptake

Maize fertilized with FYM at the high dose (340 kg ha^{-1}) yielded significantly more dry matter and N removal than at the low dose (170 kg ha^{-1}) (Tab. 4 a). The positive effect of the high N input was more evident in N uptake than in yield. Yield and N removal in SLU treatments were lower than those treated with FYM, and the difference between the two doses was not always significant in SLU. These results show that at an N fertilizer dose of 170 kg ha^{-1}, maize was still in the increasing phase of the fertilizer response curve when N could be used by the crop to a greater extent. The N removal was higher than 170 kg ha^{-1} also in the Mg systems (where removal refers to grain only) and as high as 345 kg ha^{-1} in the best case scenarios.

Comparing the Derogation limit and the 170 kg ha^{-1} limit to manure N (Tab. 4 b) shows that at 250 kg ha^{-1}, maize was apparently still utilizing all fertilizer N. Differences in yield and N uptake between the two doses were always significant when either FYM or SLU was used, and only when SLU was used in the instance of N uptake.

On average, by raising the fertilization level from 170 to 250 kg it was possible to increase the yield by 10-15% in plots that received SLU and 20-25% (as much as 40% in Mr) with FYM. These results were comparable to the increases observed when fertilization levels were raised from 170 to 340 kg ha^{-1} (+20% on average).

Crop N concentration, reported in Table 5, indicates that N concentration in plant tissues positively responded to fertilizer supply.

Crop performances have improved in the last decades because of genetic selection. Over the years in our trial, we have chosen nine different commercial maize hybrids of the 500 or 600 FAO class, following the genetic evolution of marketable hybrids. The genetic improvement of maize has resulted in an average dry matter yield increase of 286 kg ha^{-1} and an improvement of 4.5 kg ha^{-1} of N uptake per year (Fig. 4), as opposed to the relatively stable N concentration of yield over time.

4. Discussion

A balanced fertilization plan, which is the main tool of the Nitrates Directive to reduce water pollution induced by nitrate leaching, requires data that are difficult to obtain and are inherently variable, which may be at odds with an easy to apply and useful tool to guide crop fertilization management. Our results show that a long-term experiment is a valuable source of information to integrate various pieces of knowledge that permit the official equation of N balance to be tested for sufficient robustness such that it guarantees sustainable agricultural practices.

4.1 N use efficiency

The N use efficiency of fertilizers seems a simple concept, but different approaches have led to different evaluations with important effects on N balance, and consequently, on the amount of mineral fertilizers calculated in manure-N limited areas. Different approaches are expressed with different formulae, such as

Schröder et al., 2007 and Lalor et al., 2011. Depending on the formula chosen, results differ. In our case, the efficiency of FYM in Ms applied at a dose of 170 kg manure-N ha^{-1} ranged from 1.45 to 1.09 (if calculated as R/F), from 0.71 to 0.52 (if calculated as AR), and between 0.83 and 0.67 (if calculated as AR relative to urea, or NFRV).

The R/F indicator shows the amount of nutrient utilized by the plant per unit of fertilizer, irrespective of the source of N (fertilizer, SOM mineralization, N-fixation, atmospheric deposition). In the mass-balance formulation, the R/F indicator is particularly useful when the N mineralized from SOM is not quantified separately. A value greater than 1.00 is possible when the plant is utilizing natural reserves from soil mineralization, but then a parallel decrease in SOM content is expected. When soil mineralization is quantified separately, as in the N balance formula used for Italian Action Programs, N use efficiency is better described by the AR coefficient, where the soil supply is estimated from the control and is subtracted from the treatment N removal. When the apparent recovery of organic fertilizers is expressed relative to that of mineral ones, we obtain one NFRV definition of several.

Both R/F and AR result in the Mg system having a lower efficiency than the other systems because of its lower removal, but apart from this, no clear link to soil cover duration, growing season duration, or N removal capacities were noted in the other systems. After long-term repeated additions at 170 kg ha^{-1} of manure-N supply, the average N use efficiency of FYM was similar to that of urea, and the efficiency of SLU was lower than that of FYM, probably because of a slower release of N from FYM (Webb et al., 2013). At higher doses, the efficiencies of both manure types were greater than that of urea, likely due to minor losses.

The NFRV indicator showed a marked response to fertilizer amount—smaller than 1.00 at the low dose and greater than 1.00 at the high doses. This occurred because the efficiency of urea decreased to a higher extent at high N supplies. NFRV values provide a good way to compare fertilizers; however in our case, it failed to add practical value relative to AR. Fertilization strategy efficiencies can be evaluated only in the long run.

All indicators demonstrated important time variability, as the reported between-year standard deviations characterize. The efficiency rose, variability between years fell. Values resulting from the 170 kg dose differed between the two analyzed periods less than one standard deviation.

Guidelines of the Italian Action Programs state that well-managed FYM and SLU produce efficiencies ranging from 0.48 to 0.65, depending on the application of the Derogation and soil texture (higher efficiency in coarse-textured soils); in the case of FYM, the carry-over effect of past year additions could be added. Regions are free to adjust guideline values for local variability; the main regions of Northern Italy (where most of the national NVZs are situated) utilize efficiency coefficients in the range of 0.40 to 0.50 for both liquid and solid manures and fertilizations history is often not considered. Farms in the Derogation scheme utilize 0.50 for FYM and 0.65 for SLU. Our findings confirm that the ARs measured for SLU (0.44-0.66) are in the same range of variation as official values, whereas ARs measured for FYM (0.45-0.89) span a wider and higher range than official values.

4.2 N from soil mineralization and atmospheric deposition

The amount of N mineralized from the soil affects the overall N balance, but it is very difficult to estimate it in non-experimental conditions. Long-term unfertilized treatments estimate it at 116 kg N ha^{-1} yr^{-1}. If we subtract the atmospheric deposition onto the soil (26 kg ha^{-1} yr^{-1} of N at the Tetto Frati LTE, as reported by Bassanino et al., 2011) from the N uptake of unfertilized plots, we obtain 90 kg N ha^{-1}. This corresponds to 50 kg of N per percentage point of SOM content in the 0-30 cm layer of 0N plots (1.81 g $100g^{-1}$ on average). Alternatively, if we relate this amount to SON content, we obtain an annual mineralization rate of 2.0%, which is consistent with the value of 1.7% reported by Bertora et al. (2009), who applied an exponential decay model to the first period of the Tetto Frati LTE.

The Italian Action Programs suggest that if no other estimate is available, annual N mineralization can be calculated as 30 kg of N per point of SOM content, which corresponds to an annual mineralization of 1.3%. This value is justifiable only as a national average across various texture and climate types.

4.3 Crop residues

The high N amount contained in crop residues (100±23 kg N ha^{-1} in well-fertilized treatments) increased stable SOM, but did not modify the N uptake of the entire plant.

The Italian guidelines of Action Programs allow farmers to consider incorporated crop residues as a sink of N, rather than as a source, and quantify immobilization up to 40 kg N ha^{-1} in the case of maize. In fact, some farmers do not return residues to the soil because they fear N immobilization, and this results in a principle barrier to their incorporation (Bechini et al., 2015). Our results show that the incorporation of maize straw into the soil did not increase the fertilization requirement, at least in the fertile environment tested. Therefore, in the long run, the Nc term could be neglected. On the contrary, as crop residues increase SOM content, they are theoretically expected to enhance the Mso term in the long-term (e.g. Lehtinen et al., 2014), but the increase in plant N uptake we observed was limited and not significant.

4.4 Effect of rotation with leys

Plowing a grass or lucerne rotational ley before the subsequent maize crop resulted in an efficient release of 27 kg N ha^{-1} yr^{-1} to the following crop, and as it was relevant for two years, the total net extra supply was 54 kg N ha^{-1} . Due to the low C:N ratio of grass and legume (C/N = 19, on average), residues were easily mineralized and could contribute to maize nutrition to a larger extent than to SOM stock buildup. The opposite effect of crop residues and ley residues on N uptake and stable SOM stock could be related to different N mineralization rates, according to the type of organic matter incorporated in the soil.

Our data align with official Action Programs suggestions, where the N addition of a clover or grass ley lasting less than five years is quantified as 30-40 kg N ha^{-1}; the value for a lucerne ley is 60-80 kg N ha^{-1}. However, for such results, addition is prescribed to occur in the first year after plowing the ley.

4.5 Crop yield and N uptake

In the Northern Italy Po Plain, where the Tetto Frati long-term experiment is situated, maize cropping systems are highly productive. When N fertilization was limited to 170 kg N ha^{-1}, crop yield and N uptake were severely reduced; FYM plots produced only 64% of the yield and 69% of N removal in the best performing treatments at the site. These same values for SLU were 61% and 63%, which indicate that in Nitrate Vulnerable Zones, where the limit of 170 kg ha^{-1} of manure-N is adopted, a relevant integration with mineral fertilizers is needed to sustain yield.

Crop yield and N removal were higher when FYM was applied than when SLU was used, possibly because of a slower release of N and an increase of SON caused by FYM over the long-term (Webb et al., 2013). Comparison of the Derogation (250 kg N ha^{-1}) and standard (170 kg ha^{-1}) manure-N limits for both manure types suggested that at the high dose maize still utilized all manure N. Differences in yield and N uptake between the two doses were always significant when FYM was used, but only in the case of N uptake when SLU was used. Fertilization at 250 kg N ha^{-1} was shown not to be excessive, as it was taken advantage of manure-N, thereby limiting the need for extra mineral fertilization. In this context, a Derogation allowing 250 kg ha^{-1} of manure-N is a good option in high performing systems such as those analyzed. Moreover, relative to the lower dose, the Derogation treatment supplied the soil with an extra 0.2-1.2 t ha^{-1} of C from manures that likely improved C sequestration—an effect that requires future experimental confirmation.

All entirely-harvested maize systems proved to demand high N fertilization levels, regardless of the presence of Italian ryegrass as a secondary crop or of rotational grass/lucerne leys. On the contrary, the harvest of grain alone reduced the fertilization requirement by 32-86 kg N ha^{-1} at the low dose and by 70-140 kg ha^{-1} at the high dose. Indeed, this system does not benefit from the Derogation.

We showed an increase in yield and N uptake, probably due to genetic selection, as opposed to the rather stable N concentration of yield over time (Chen et al., 2015). Over the 23 experimental years at Tetto Frati, the main traits that caused production to increase were resistance to lodging, taller plants, “staying green” longer, and a more effective response to N fertilizer. Consequently, legislative constraints on fertilizer amounts set some decades ago might need to be revised and adapted to evolving crop varieties. Maximum allowable fertilizer application rates also contained in Action Programs that member countries set in NVZs might also need periodic revisions.

5. Conclusions

A correct evaluation of all terms in the N balance equation is necessary to correctly calculate the mineral fertilizer amounts required by a cropping system, as manure is typically supplied at the maximum allowable by the Directive on intensive livestock farms. Apart from N fertilization components, the largest term in the equation was the contribution from SOM mineralization, relevant under all management conditions. The second largest term was the effect of rotation with meadows, whose contribution is limited to specific years and specific rotational cropping systems. The incorporation of maize residues held less importance in modifying soil supply, although the overall N balance was influenced by lower N removal when grain only was harvested. The N use efficiency of FYM, in particular, but also of SLU was proved to be higher than expected when organic fertilizers are well managed (supplied annually, distributed in the spring before a spring-growing crop and immediately incorporated). These conditions over the long run resulted in a manure-N efficiency comparable to that of urea.

An increase of the maximum allowed manure-N supply from in NVZs from 170 kg ha^{-1} to 250 kg ha^{-1}, as in the approved Derogation scheme in Italy, increases the value of manure-N and reduces the need for additional mineral N. Otherwise, the integration of mineral N is required to sustain the yield that maize crop can achieve, especially under the backdrop of continuously increasing maize yield through genetic improvements.

LTEs can provide practical, useful information to establish modern Action Programs. For example, this research showed that a revised Nitrates Directives application scheme could be more relaxed in limiting the application of manure-N, and on the other hand, could be more ambitious in setting higher efficiency coefficients for manure fertilization. LTEs can assist public authorities to reach agri-environmental legislative goals. LTEs can source not only readily-available data, such as those reported in this paper that verified parameter values relevant for legislation, but also basic information for simulation modelling to predict long-term management practice effects.

6. Acknowledgements

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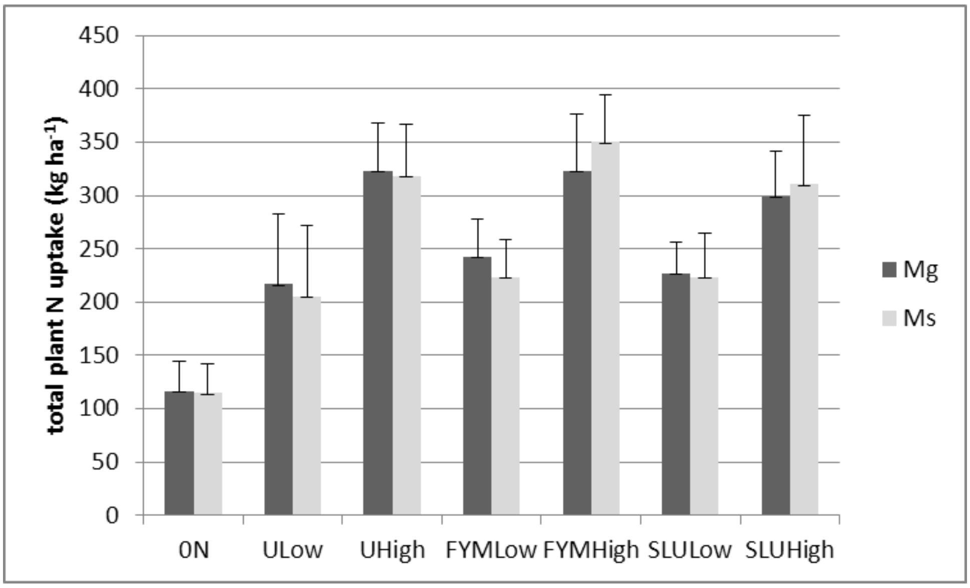
Figure 1. Effects of crop residue incorporation a) on total plant N uptake and b) on SON stock. Residues were incorporated into the soil in the Mg system and removed from the Ms system. Mean (boxes) and annual standard deviation (T-bars) are provided for the 2007-2014 period. ULow is the mean of U100 and U200, UHigh is the mean of U300 and U400.

a)



b)

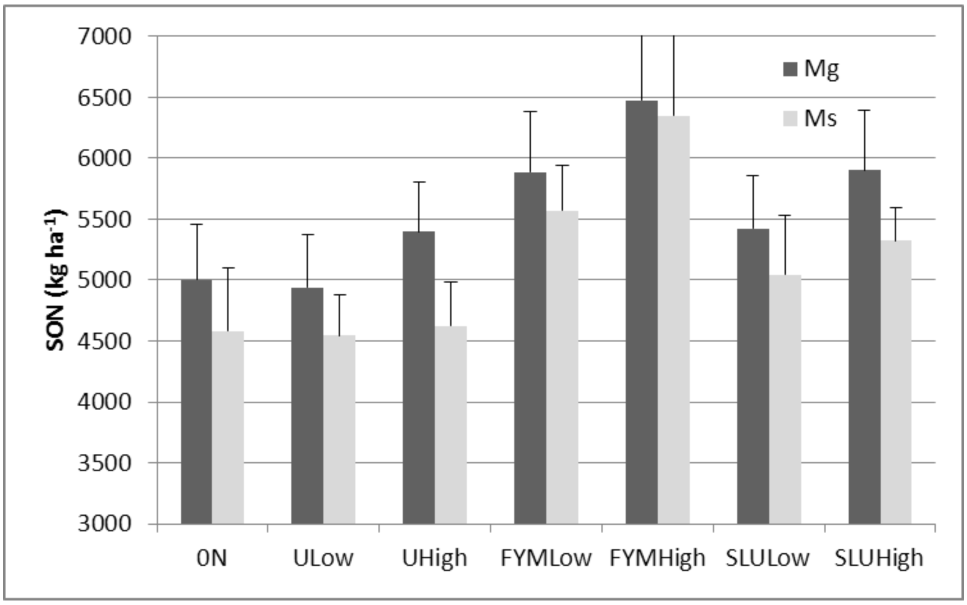
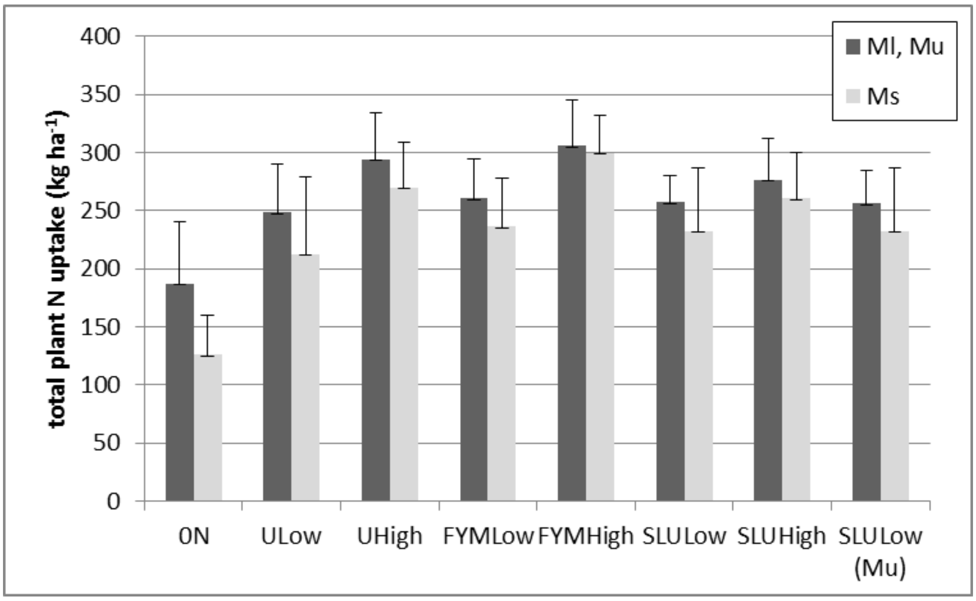


Figure 2. Effects of rotation with grass ley (Ml) or legume ley (Mu) compared with monoculture (Ms) a) on annual maize N uptake in the first two years after ley; b) on SON stock. Means (boxes) and standard deviations (T-bars) of three phases: 1995-1996, 2002-2003, 2011-2012. ULow is the mean of U100 and U200; UHigh is the mean of U300 and U400.

a)



b)

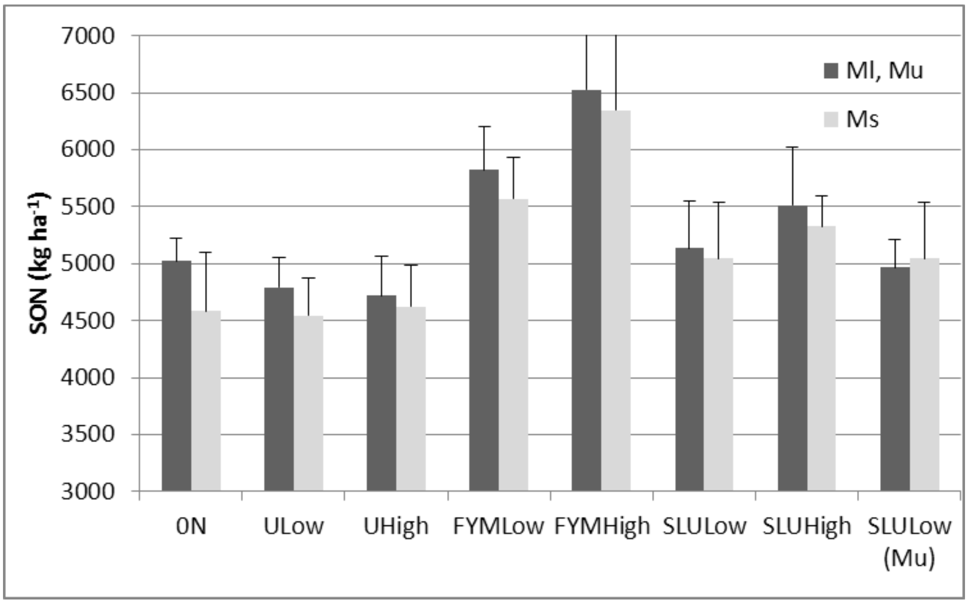


Figure 3. Increment of annual N uptake by rotational maize (Ml, Mu) with respect to monoculture (Ms) as an average of the first two years after ley, as a function of fertilizer N supply.

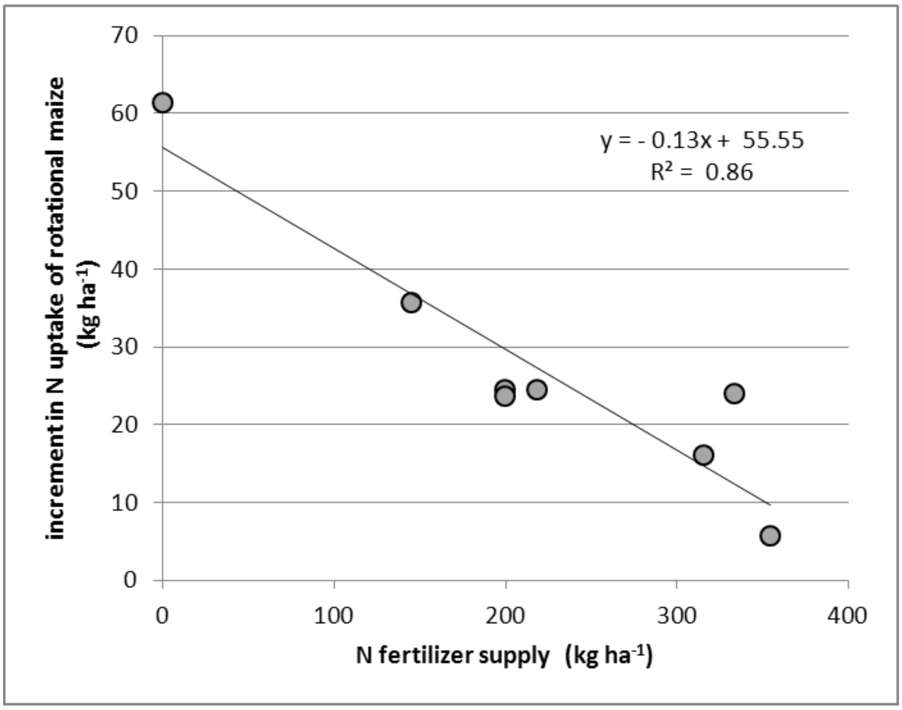
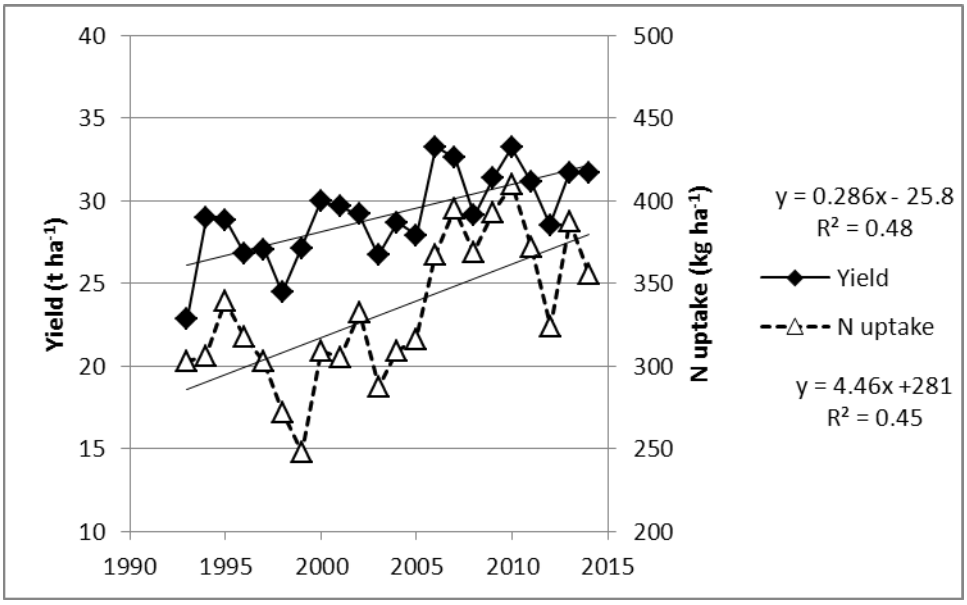


Figure 4. Maximum dry matter yield and N uptake registered in the trial among all treatments across all experimental years.



Table

Table 1. Nitrogen fertilization amounts supplied to treatments in the Tetto Frati LTE. Top dressing was always supplied as urea at ridging. All values are stated in kg N ha^{-1}.

		Period 1992-2006			Period 2007-2011			Period since 2012		
Code	**Fertilizer type**	**Before sowing**	**Top drsg.**	**Total**	**Before sowing**	**Top drsg.**	**Total**	**Before sowing**	**Top drsg.**	**Total**
0N	0N	0	0	**0**	0	0	**0**	0	0	**0**
U100	urea	0	100	**100**	0	100	**100**	0	100	**100**
U200	urea	100	100	**200**	170	0	**170**	170	0	**170**
U300	urea	200	100	**300**	200	100	**300**	250	0	**250**
U400	urea	300	100	**400**	300	100	**400**	250	100	**350**
SLULow	bovine slurry	114	100	**214**	170	0	**170**	170	0	**170**
SLUHigh	bovine slurry	226	100	**326**	340	0	**340**	250	0	**250**
FYMLow	farmyard manure	142	100	**242**	170	0	**170**	170	0	**170**
FYMHigh	farmyard manure	284	100	**384**	340	0	**340**	250	0	**250**

Table 2. Mean and variability (s.d., standard deviation) values of the organic fertilizer chemical characteristics used in the trial during 2007-2014. Data are referred to manure fresh weight.

	Dry matter %		**Total N** g kg^{-1}		**NH_4-N** g kg^{-1}		**P** g kg^{-1}		**K** g kg^{-1}		**C** g kg^{-1}	
Fertilizer	Mean	*s.d.*	Mean	*s.d.*	Mean	*s.d.*	Mean	*s.d.*	Mean	*s.d.*	Mean	*s.d.*
FYM	21.5	*6.1*	5.0	*0.9*	0.4	*0.4*	1.4	*1.1*	6.0	*1.9*	62.6	*15.9*
SLU	3.8	*1.7*	2.1	*0.4*	1.2	*0.3*	0.2	*0.2*	1.3	*0.7*	14.0	*6.9*

Table 3. Values for Nitrogen Removal: Fertilizer ratio (R/F), Apparent Recovery (AR), as such and relative to urea (Fertilizer Replacement value, NFRV). Mean and between-year standard deviation for a) years 2007-2011 and b) years 2012-2014. AR was not calculated in Ml because of a high N uptake of the control, probably due to clover in the sward.

		R/F				AR				AR relative to urea (NFRV)			
		mean	*s.d.*	mean	*s.d.*	mean	*s.d.*	mean	*s.d.*	mean	*s.d.*	mean	*s.d.*
a)		170		340		170		340		170		340	
Mg	FYM	1.01	*0.17*	0.60	*0.11*	0.56	*0.23*	0.51	*0.18*	0.83	0.15	1.30	0.16
	SLU	0.98	*0.12*	0.58	*0.06*	0.53	*0.17*	0.49	*0.10*	0.80	0.09	1.27	0.25
Ms	FYM	1.45	*0.12*	1.09	*0.11*	0.71	*0.22*	0.72	*0.11*	0.83	0.32	1.36	0.59
	SLU	1.39	*0.23*	1.01	*0.18*	0.66	*0.24*	0.64	*0.24*	0.72	0.10	1.07	0.14
Mr	FYM	1.39	*0.24*	1.00	*0.27*	0.65	*0.12*	0.63	*0.23*	0.99	0.09	1.06	0.48
	SLU	1.27	*0.29*	0.82	*0.20*	0.52	*0.19*	0.44	*0.16*	0.77	0.16	0.74	0.28
Ml	FYM	1.70	*0.51*	1.02	*0.14*	-	-	-	-	-	-	-	-
	SLU	1.39	*0.35*	0.79	*0.18*	-	-	-	-	-	-	-	-
b)		170		250		170		250		170		250	
Mg	FYM	1.00	*0.15*	0.81	*0.07*	0.52	*0.07*	0.48	*0.08*	0.98	0.10	1.14	0.05
	SLU	0.89	*0.11*	0.76	*0.09*	0.41	*0.06*	0.44	*0.07*	0.78	0.19	1.04	0.08
Ms	FYM	1.09	*0.06*	1.27	*0.17*	0.52	*0.19*	0.89	*0.22*	0.67	0.11	1.17	0.34
	SLU	1.18	*0.25*	1.03	*0.10*	0.61	*0.21*	0.65	*0.16*	0.84	0.41	0.85	0.21
Mr	FYM	1.04	*0.24*	1.10	*0.29*	0.45	*0.05*	0.70	*0.15*	0.62	0.24	1.03	0.33
	SLU	1.07	*0.23*	0.90	*0.15*	0.48	*0.12*	0.50	*0.03*	0.69	0.35	0.73	0.21
Ml	FYM	1.57	*0.06*	1.45	*0.15*	-	-	-	-	-	-	-	-
	SLU	1.13	*0.32*	1.05	*0.29*	-	-	-	-	-	-	-	-

Table 4. Yield (t ha^{-1} of dry matter) and plant N removal (kg N ha^{-1}) in the marketable yield of Mg (grain only), Mr (sum of maize and ryegrass), and Ml (average of maize and grass ley), as a function of the N fertilizer dose (kg ha^{-1}) supplied through organic fertilizers. Mean and between-years standard deviation (s.d.). Letters indicate significant differences between types and levels of fertilizers in the post-hoc R-E-G-W-Q test for each cropping system separately. a) years 2007-2011 and b) years 2012-2014.

	FYM												SLU											
	Yield			N uptake			Yield			N uptake			Yield			N uptake			Yield			N uptake		
	Mean	*s.d.*		Mean	*s.d.*		Mean	*s.d.*		Mean	*s.d.*		Mean	*s.d.*		Mean	*s.d.*		Mean	*s.d.*		Mean	*s.d.*	
a)	170						340						170						340					
Mg	12.6	*1.3*	b	172	*30*	b	13.7	*1.2*	a	205	*36*	a	13.6	*1.0*	ab	197	*19*	b	13.7	*1.2*	ab	205	*36*	a
Ms	25.7	*2.4*	b	246	*21*	b	31.0	*1.3*	a	369	*35*	a	24.4	*1.7*	b	237	*39*	b	29.7	*3.6*	a	342	*61*	a
Mr	23.8	*2.5*	c	237	*42*	c	29.2	*4.3*	a	341	*93*	a	21.9	*4.1*	c	214	*49*	c	26.5	*4.4*	b	278	*68*	bc
Ml	13.2	*9.3*	ab	232	*87*	b	15.1	*11.0*	a	279	*49*	a	11.5	*9.5*	b	189	*60*	c	13.3	*10.0*	ab	215	*61*	bc
b)	170						250						170						250					
Mg	13.2	*0.8*	bc	170	*26*	bc	14.3	*0.9*	a	202	*16*	a	12.8	*1.3*	c	151	*19*	c	13.9	*1.2*	ab	191	*22*	a
Ms	22.9	*0.7*	b	185	*10*	c	28.7	*3.4*	a	318	*43*	a	22.3	*2.1*	b	200	*42*	c	25.5	*3.5*	ab	258	*26*	b
Mr	21.8	*1.9*	b	190	*24*	c	30.1	*1.0*	a	294	*44*	a	22.0	*1.6*	b	196	*30*	c	24.5	*0.5*	b	236	*17*	b
Ml	18.0	*7.9*	b	255	*76*	bc	21.3	*6.5*	a	345	*101*	a	16.4	*8.7*	b	207	*53*	c	19.3	*6.9*	ab	286	*93*	b

Table 5. N concentration in yield (Mg: grain only), expressed as g $100g^{-1}$, as a function of N fertilizer dose supplied through organic fertilizers. a) years 2007-2011 and b) years 2012-2014. Rotational maize in Ml was not reported because it was referred to a single year only.

	FYM				SLU			
	Mean	*s.d.*	Mean	*s.d.*	Mean	*s.d.*	Mean	*s.d.*
a)	170		340		170		340	
Mg (grain)	1.35	*0.12*	1.48	*0.15*	1.31	*0.07*	1.45	*0.05*
Ms	0.96	*0.09*	1.19	*0.13*	0.97	*0.15*	1.14	*0.07*
Mr - maize	0.99	*0.09*	1.18	*0.19*	0.96	*0.08*	1.06	*0.13*
Mr – It. ryegrass	1.01	*0.23*	1.07	*0.28*	1.03	*0.16*	1.02	*0.27*
Ml – grass (3 years)	2.26	*0.24*	2.42	*0.16*	2.26	*0.24*	2.37	*0.26*
b)	170		250		170		250	
Mg (grain)	1.29	*0.12*	1.41	*0.05*	1.19	*0.04*	1.36	*0.06*
Ms	0.81	*0.07*	1.11	*0.02*	0.89	*0.11*	1.02	*0.06*
Mr – maize	0.91	*0.06*	1.02	*0.14*	0.96	*0.11*	1.03	*0.10*
Mr – It. ryegrass	0.75	*0.05*	0.89	*0.22*	0.71	*0.04*	0.79	*0.14*
Ml – grass (2 years)	1.80	*0.21*	2.19	*0.51*	1.80	*0.21*	1.95	*0.44*