Organic and mineral fertilization management improvements to a double-annual cropping system under humid Mediterranean conditions

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Abbreviations: CM, cattle manure; DM, dry matter; MN, mineral nitrogen fertilizer; OM: organic matter.

Abstract

The efficient use by crops of nitrogen from manures is an agronomic and environmental issue, mainly in double-annual forage cropping systems linked to livestock production. A six-year trial was conducted for a biennial rotation of four forage crops: oat-sorghum (first year) and ryegrass-maize (second year) in a humid Mediterranean area. Ten fertilization treatments were introduced: a control (without N); two minerals equivalent to 250 kg N ha^{-1} yr^{-1} applied at sowing or as sidedressing; dairy cattle manure at a rate of 170, 250 and 500 kg N ha^{-1} yr^{-1} and four treatments where the two lowest manure rates were supplemented with 80 or 160 kg mineral N ha^{-1} yr^{-1}. They were distributed according to a randomized block design with three blocks. The highest N mineral soil content was found in the summer of the third rotation, in plots where no manure was applied. The yearly incorporation of manure reduced, in successive cropping seasons, the amount of additional mineral N needed as sidedressing to achieve the highest yields. Besides, in the last two years, there was no need for mineral N application for the manure rate of 250 kg N ha^{-1} yr^{-1}. This amount always covered the oat-sorghum N uptake. In the ryegrass-maize sequence uptakes were as high as 336 kg N ha^{-1} yr^{-1}. In the medium term, the intermediate manure rate (250 kg N ha^{-1} yr^{-1}) optimizes nutrient recycling within the farming system, and it should be considered in the analysis of thresholds for N of organic origin to be applied to systems with high N demand.

Keywords: Maize, Manure, Oat, Rotations, Ryegrass, Sorghum.

1. Introduction

Double-annual forage crop production where crops are grown in sequence is a very intensive agricultural management system. It is usually associated with dynamic livestock farming where animals are fed with forages and their feces, usually mixed with straw, are applied to crops as fertilizers. Thus, nutrients are recycled as much as possible within the system. This practice also has economic advantages since it reduces the application of mineral fertilizers.

Despite these advantages, crop intensification requires a better understanding of fertilization management of such animal waste, in order to maintain high productivity while reducing environmental impacts. This is a complex issue, as organic materials show different constraints: high variability in nutrient content depending on many management factors (Klausner et al., 1994; Yagüe et al., 2012), residual effects in the years following application due to organic components (Ketterings et al., 2013; Sieling et al., 2014), as well as some difficulties when applied at different crop development stages as sidedressing, or due to inability to incorporate manure (Thilakarathna et al., 2015). Nutrient use efficiencies from manures are lower, mainly the nitrogen efficiency, when compared with those obtained under mineral fertilization (Schröder, 2005).

Improvements to organic fertilization require several years of experimentation. Results must not be influenced by initial soil fertility (Guillaumes, 2006), and the residual effects of fertilization undertaken in previous years should be included, meaning a stable situation (equilibrium) at the end of the field experiment (Schröder, 2005).

When a double-annual cropping system is established, there is an almost permanent N extraction by the plants that restricts N leaching loss. Under humid temperate conditions, it is possible to take advantage of the manure's residual N effect if soil is covered in autumn (Sørensen, 2004). These climate characteristics also mean an important increment in soil mineral N content as temperatures rise, and this N can be leached if a summer crop is not established. Zavattaro et al. (2012) found that the double-annual cropping system of ryegrass followed by maize increased the efficiency of organic fertilizers and reduced N leaching when compared with a maize monoculture system. Crop rotations compared with a monoculture allow better N use efficiency (Kurtz, 1984) and yield increments, as described for maize by Borrelli et al. (2014). In addition, double-cropping results in more efficient use of land, labor and equipment resources than monocropping (Crabtree, 1990). Production of two crops per year draws attention to the importance of the use of residual soil nutrients from the preceding crop, which includes organic matter mineralization from the manures or slurries applied to it (Yagüe and Quílez, 2013).

The implementation of Directive 91/676/CEE (European Union, 1991) on water quality protection across Europe designates part of the Garrotxa region (NE of Spain) as a nitrate vulnerable zone (Generalitat de Catalunya, 2009a), and N fertilization practices are regulated. One of the agronomic measures establishes the amount of 170 kg N ha^{-1} yr^{-1} as the maximum to be applied from organic fertilizers. This limit seems quite reasonable if only one crop is cultivated annually, but when two crops a year are introduced in a fodder rotation, there is a considerable increase in N demand, and this limit may prevent appropriate nutrient supply. Indeed, the European Union (EU) has allowed derogations of the Directive concerning this specific aspect (Van der Straeten et al., 2012), and in some regions or countries it allows the application of higher N amounts of organic origin, up to a maximum of 250 kg N ha^{-1} year^{-1} (European Union 2005, 2006, 2007a, 2007b, 2008 and 2011). The demand from an EU member state must be reasonable in the sense that the objectives of the Directive are still met.

Situations for derogation include crop rotations with long growing seasons, crops with high N uptake, or soils with high denitrification capacity.

In the Garrotxa region, there is high and diversified stockbreeder activity, bovine and porcine stock being the most abundant. This stock equals 75% of the livestock census (Perramon et al., 2011). Therefore, organic materials (manure and slurry) are readily available and constitute an agronomic opportunity, mainly in double-annual forage cropping systems which are directly related to the stockbreeder. Some information about N management in similar intensive agricultural systems (double-annual cropping) is available. Nevertheless, some of the existing studies were set up in different environments, such as the one of Chataway et al. (2011) for oat-sorghum in a semiarid region of Australia, or they include different varieties with different crop cycles, such as sorghum in Goff et al. (2010), or the crops have different management requirements, as described in Tomasoni et al. (2011) for ryegrass and silage maize.

Our hypothesis is that, in nitrate vulnerable zones associated with high animal density, if a double-annual forage cropping system is established, it ought to be possible to surpass the threshold of the amount of N (from an organic origin) to be applied, while maintaining high productivity, minimizing the environmental impact to underground waters, and maximizing the nutrient recycling from animal manures within the agricultural system. The aim of this study is to evaluate different fertilization strategies with manures, mineral fertilizers or a combination of both, in a medium term (six years) double-annual forage cropping system. The evaluation is undertaken in terms of crop yields and their N uptake, as well as the evolution of the mineral N soil content.

2. Materials and methods

The experiment was conducted over six years (from October 2007 to September 2013) on the same agricultural area in La Garrotxa Volcanic Zone Natural Park (Catalonia, NE Spain; 42° 08' 32" N, 2° 30' 10" E, altitude 534 masl).

2.1. Soil and climate description

The climate is humid Mediterranean; average annual rainfall is about 1000 mm (Fig. 1) without significant dry periods in summer. Weather data were collected from a meteorological station four kilometers away from the experimental field. Crops are usually grown under rainfed conditions, and only in long periods of dry weather can irrigation be used to maintain yields. In this experiment, plots were not irrigated.

For the period 2000-14, the annual average temperature was 12.4°C, with a maximum daily average of 21.0°C in July, and a minimum daily average of 4.1°C in December (Fig. 1). Average annual precipitation was 914.3 mm, with a maximum of 1431 mm in 2011 and a minimum of 606 mm in 2012. Although the precipitation distribution is quite regular over the year, most rain falls in spring and autumn (Fig. 1).

The soil is a well-drained sandy loam, a Fluventic Eutrudept (Soil Survey Staff, 2014), without superficial stoniness. At the start of the experiment, organic matter (OM) content and available phosphorus were medium and available potassium low (Table 1).

2.2. Crop management

During the first year, oat (*Avena sativa* L.) - sorghum (*Sorghum bicolor* L.) was the crop sequence, and in the second year, ryegrass (*Lolium multiflorum* L.) - maize (*Zea mays* L.). This biennial rotation was common in the area. Winter crops are maintained in the field from October to May. Summer crops are maintained from May to October.

Plants from winter and summer crops are cut and used as fodder (ensilage) for bovines (meat production). The experiment was maintained over three full rotations (six years).

Oat varieties were Prevision, the first two cropping seasons, and Blond (a local variety) the third. Sorghum varieties were Digestive (1st and 3rd cropping seasons) and Sweet (local sorghum) the second. The ryegrass variety was Trinova and maize varieties were: PR34P88, PR34B39 and P1114 (all of them belong to a 500 FAO cycle)**.** We used certified seeds. Sowing was done mechanically. Oat seeds were treated with an authorized fungicide. Maize seeds were treated with an authorized fungicide and insecticide while sorghum and ryegrass seeds were not treated with any pesticide. Seed densities at sowing were: 180-190 kg ha^{-1} for oats, 40-45 kg ha^{-1} for sorghum, 35-40 kg ha^{-1} for ryegrass, and 67000 seeds ha^{-1} for maize (distance between rows is 0.75m and between plants 0.2 m). During the different crop cycles plant health controls were set up but it was not necessary to apply any pesticide. Crops were harvested at the development stage, where the equilibrium yield vs. forage quality was considered to be the best: oat at milky grain stage, sorghum when 50% of inflorescences were visible, ryegrass at maximum biomass before coming into ears and maize at the doughy grain stage. The experiment was conducted with a similar management system to that used in commercial practice.

2.3. Experimental design

The experiment was designed as a randomized complete block, with ten annual fertilizer treatments (Table 2) and three replicates. Each plot was 5 m wide by 10 m long. Fertilization was scheduled on a yearly basis (two crops per year) according to sowing and harvesting dates (Table 3) of different crops. The fertilizer applications were made onto the same experimental plots during every year.

Fertilization treatments included a control (no N applied, T1) plus nine N treatments. Two mineral nitrogen (MN) treatments: 250 kg mineral N ha^{-1} at sowing (250MN, T2.1) or after crop emergence (250MN, T2.2). As sidedressing (after crop emergence), it was annually split into 100 kg N ha^{-1} for the winter crop and 150 kg N ha^{-1} for the summer one. Seven treatments included cattle manure (CM), with a range of N rates of 170, 250 and 500 kg N ha^{-1} (T3, T4 and T5, respectively). The lowest manure rate was applied at sowing of the second crop (summer crop), but the two higher rates were annually split into the two sowings (first and second crop). Also, the two lowest CM rates (170 and 250 kg N ha^{-1}) were complemented (as four new fertilizer treatments), with 80 or 160 kg N ha^{-1} $year^{-1}$ from mineral fertilizer (T3.1, T3.2 and T4.1, T4.2, respectively). The mineral complement was split between the winter and summer crops as a sidedressing (Table 2). Sidedressings were established as close as possible to commercial farming practices which apply fertilizers using different machines. This equipment does not allow the application of N fertilizers late in the cropping season, mainly in summer crops.

2.4.Manure and fertilizer applications

Prior to the establishment of the experiment, the field was fertilized, for many years, only with mineral fertilizers. In this experiment manures were obtained from bovine livestock close to the experimental plots. In this agricultural system bovine livestock are fed with the double-annual forage crop production. Manure and mineral fertilizers were applied by hand and buried (~0.15 m) mechanically by a chisel plough up to 24 h after application, although legislation (point 13 from Catalan order 136/1009; Generalitat de Catalunya, 2009b) allows a maximum delay of 4 days (for manure applications from October to April) or 2 days (for manure applications from May to September) before manure burying was done. Minerals applied as sidedressing were not buried. The amount of bovine manure to be applied was established according to the characteristics of the material (Table 3), which were previously determined through analysis. Mineral N fertilizers were applied as ammonium nitrosulfate at sowing time and as urea as sidedressing, following the common practices of the commercial agricultural fields of the area.

In some treatments we also applied P and K as a binary fertilizer (0-14-14), plus potassium sulfate (50% K_2O). They were applied at different rates according to the treatment in order to achieve, in all plots, a total supply of 130 kg P_2O_5 ha^{-1} $year^{-1}$ and 260 kg K_2O ha^{-1} $year^{-1}$. These quantities covered the crop nutrient uptake. This was carried out to ensure that any yield response observed could not be attributed to any nutrient other than N.

2.5. Dry-matter yield, nitrogen concentration of crops, nitrogen uptake by crops

Oats, sorghum and ryegrass were harvested by a mower from an area of 12 m^2 (1.2m*10m). In maize plots, plants were harvested from an area of 15 m^2 (1.5m*10m). Total fresh crop yield for each harvested area was obtained directly in the field. From them, one crop subsample per plot was collected and stored at 4°C for a maximum of 48 hours before analyzing dry matter (DM) and N content. The DM was determined by drying at 60ºC and its N content was determined by Kjeldahl digestion in the first year and by an NIR spectroscopy procedure (after calibration) from the second year onwards. The nitrogen was analyzed in the total plant biomass for oats, sorghum and ryegrass because the entire plant is ground up for silage. In maize plants, the green part of the plant and the ears of maize were ground separately; afterwards, they were mixed following the weight proportion of each part before the N analysis was done. The N uptake by the crop was calculated.

2.5. Soil NO_3^--N content

Soil NO_3^--N content was measured at sowing and just before sidedressing fertilization of every crop, which means four times a year. Soil samples were collected in all plots from 0 to 0.9m. For each plot, two points were sampled and a composite sample was obtained. These soil samples were stored at 4^{o}C until nitrate content was analyzed by ionic chromatography after nitrate extraction with demineralized water.

2.6. Data analysis

For each rotation and crop, the yield response to N rates from manures was fitted to a linear model (y= a+bx) or a linear-threshold model (y=a+bx if $x \leq x_t$ value associated to the plateau value; y=constant if $x > x_t$ value associated to the plateau value). The simple linear regressions were compared between rotations, for each crop, in terms of the intercept (yield in each rotation when no manure was applied) and slope values (response to the applied manure rates). Similar models were fitted for a fixed manure rate combined with mineral N sidedressings in each rotation and crop, In this case, the intercept was the response to the applied manure rate and the slope the response to the different mineral N rates applied as sidedressing.

The analysis of variance (two-way ANOVA) was established for yields, N content and N uptake, according to a GLM procedure for each crop. If the interaction of year and treatment was significant, then analysis were done separately for each crop and year (one-way ANOVA) in order to detect differences associated with fertilization treatment. If the analysis of variance was significant, separation of means was carried out following the Duncan Multiple Range Test ($\alpha = 0.05$).

The statistical analysis was performed using the statistical package SAS v9.4 (SAS Institute Inc. 2002-2012) for ANOVA analysis and JMP 9 statistical software (SAS Institute, 2010) for regressions.

3. Results

3.1. Yields

In this rainfed system, the yield response to fertilization treatments interacted with the year factor, as climatic factors, mainly rainfall and its distribution, varied between years (Fig.1). The effect was more evident in the ryegrass-maize sequence than in the oat-sorghum one, because of the differences in summer biomass productivity, which were higher in maize (Table 4, Figs. 2, 3, 4). The yields of the fifth year stressed biomass variability as a consequence of summer drought. Maximum dry matter yields averaged over 3 years were: 10166, 8477, 8274 and 16235 kg ha^{-1} for oats, ryegrass, sorghum and maize, respectively (Table 4). For manures, at the lowest rate (T3) a mineral complement was always needed (Table 4, Fig. 2). However, this complement can be reduced after the fourth year (T3.1). The mineral sidedressing could be saved at the highest rate (T5) from the second year and at the intermediate one (T4) from the fourth year. In this last case, the mineral complement could be reduced by half (T4.1) from the second year onwards (Fig. 3, Table 4).

In manured plots (with or without mineral sidedressings), linear equations (yield vs N rate) were established (Table 5). When compared between rotations (Table 6), it was possible to observe the constraint of water availability for maize (Fig. 4D), which reduced the intercept value (yield when no mineral N was applied) in the second rotation compared with the first one, without affecting the slope (small yield increment vs N increment). A similar effect was also observed for ryegrass when comparing with the third rainy rotation (Table 6, Fig. 4C), or for ryegrass and maize (Table 6, Figs. 2C and 2D) when the T3 manure rate was complemented with minerals (second vs. first rotation). Oats could be also sensitive to such constraints in intercept values (Table 6, Figs, 2A and 4A), although the evolution of the slope (rotation 1 vs rotation 2), higher in the second year (Table 6, Fig. 2A), indicated that the initial constraint was overcome during the crop cycle. If yields of winter and summer crops were compared when manures were complemented with minerals (Table 6, Figs. 2 and 3) it was clear that the slope of the relationship (yields vs N as sidedressing) tended to be higher in winter crops. This behavior substantiated the greater value of N sidedressing in winter crops than in summer ones.

The N residual effect of manures was observed when initial linear functions derived to linear-threshold functions, mainly in the third rotation where maize (the highest demanding N crop) was included (Table 5, Figs. 2D, 3D, 4D). It was also observed for ryegrass, which reduced or suppressed the need for additional N sidedressings, depending on the manure rate applied (Table 5, Figs. 2C, 3C). In oats, this effect was clearly observable at the intermediate manure rate (Table 5, Fig. 3A). Sorghum was an exception as no N sidedressing was applied the first year, and because of the drought recorded in the third rotation. When manures were the only N supplier, linear functions were maintained, with the exception of maize (Fig.4).

3.2. Nitrogen uptake

Nitrogen content and uptake were affected by fertilization rates (Tables 7, 8), although in maize they interacted with the cropping season. The great variations in maize yield were associated with N uptake (Table 8), which oscillated (as annual average) from 101 and 115 kg N ha^{-1} (first and second season) to 235 kg N ha^{-1} in the third. In sorghum, N content was very variable, from 7.7-8.8 kg N Mg^{-1} dry matter (averages of the first and second year) to 23.9 in the third season. The average maximum values of N uptake for each crop were recorded in the T2.2 treatment, reaching 106, 98, 122 and 181 kg N ha^{-1} for oats, sorghum, ryegrass and maize, respectively (Table 8). This means a maximum of 215 kg N ha^{-1} for the oat-sorghum annual sequence and 443 kg N ha^{-1} for ryegrass-maize (Table 8). Summer crops removed more N than winter crops. In the oat-sorghum sequence, N uptake by sorghum equalled 53% of the total N removed, and in the ryegrass-maize sequence, N uptake by maize equalled 67%.

3.3. Evolution of soil nitrate content

The average nitrate soil content (0-0.9m depth) was lowest at the start of the experimentation (39.4 kg N-NO_3^- ha^{-1}), and the highest levels were found in years 3, 5 and 6, with 82.7, 88.3 and 89.2 kg N-NO_3^- ha^{-1}, respectively (Fig. 5). The control maintained the lowest average content of 55.9 kg N-NO_3- ha^{-1}, and treatment T2.1 the highest (94.7 N-NO_3^- ha^{-1}), although differences were not statistically analyzed. Manures controlled the N build up in soil (Fig. 5B) in spite of the occurrence of N residual effects over the cropping seasons. This observation was most obvious when manures were compared with mineral treatments alone (Fig. 5A) and at manure rates as high as 500 kg N ha^{-1}. Nevertheless, when manures were complemented with minerals (Figs. 5C, 5D), there was a tendency to increase N soil contents.

4. Discussion

4.1. Yields

Oats as a double-crop with sorghum were highly productive: maximum yields (16.7 Mg ha^{-1}, Table 4) were close to maximum values (16.2 Mg ha^{-1}) found in similar systems (Chataway, 2011) in Australia. Also, maximum rotation averages for the ryegrass-maize sequence (24.04 Mg ha^{-1}, Table 4) were close to maxima (24.6-27.6 Mg ha^{-1}) obtained by Grignani et al. (2007), under similar environmental conditions, after 11 years of experimentation, or by Tomasoni (2011), when combining manures and minerals (25.75 Mg ha^{-1}).

The yield variability can be explained by the irregular annual rainfall distribution (Fig. 1) which is not unusual in these rainfed systems, as reported by other authors (Trindade et al., 2008). The summer drought in 2012 affected sorghum yields. Besides, in maize, it is known that drought during the reproductive stage affects kernel number and weight (Di Paolo and Rinaldi, 2008). Also, in July and August 2010 and 2012, average temperature was higher than 22.6ºC, the threshold from which maize development can be negatively affected (Thompson, 1986).

In winter crops, yields were influenced by N fertilization management. Yields increased when N mineral was applied as sidedressing versus applications at sowing time, due to the importance of N availability at the period of highest N demand (Schröder et al., 2004; Schröder, 2005). This effect was not observed in summer crops, probably because of the additional N associated with the mineralization of organic matter (Magdoff, 1978).

Nitrogen residual effects of manures were observed because of higher yields for a similar manure rate in successive rotations, mainly when water availability was not a constraint (Fig. 4). Thus, they took advantage of the previous organic N applied, which became available to plants with time (Klausner et al., 1994). We should point out that no organic fertilizer had been applied before the start of the experiment, and the mineral N (ammonium) from the CM applied just accounted for 25% of its total N content (Table 3). Whatever the manure rate applied, the residual effect was more evident in summer crops than in winter ones (Table 6, Figs. 2, 3). This evidence could be explained by the effect of temperatures on the OM mineralization, which increases as temperatures increase (Schvartz et al., 2005), resulting in higher N availability to plants. From the management point of view, this means that the complementary mineral N, to be applied to manured fields, can be reduced with time until the point that it can be suppressed in the CM rate equivalent to 250 kg N ha^{-1} from the second biennial rotation onwards.

Nevertheless, according to current legislation (European Union, 1991), if a maximum of 170 kg N ha^{-1} yr^{-1} from manures is applied, a complement of mineral N will be necessary to achieve the highest yields (from 160 kg N ha^{-1} to a minimum of 80 kg N ha^{-1}, after the second rotation).

4.2. Nitrogen uptake

Differences in the crop development stage at harvest, under different management systems, make it difficult to compare results in N content with references (Table 7), such as for the oat-sorghum sequence (Chataway et al., 2011). The high N concentration in sorghum (26.1 g N kg^{-1}), in the third rotation (Table 7), was mainly due to the extreme 2012 summer drought which reduced yields (Table 4), although it coincided with maximum values (~26.86 g N kg^{-1}) recorded from Restelatto et al. (2013). In previous years, our maximum N concentration (12.9 g N kg^{-1}), was close to the one reported by Buxton et al. (1999) who achieved 11.5 g N kg^{-1} at the highest N rate (280 kg N ha^{-1}).

Average N concentrations in ryegrass (from 9.2 to 14 g N kg^{-1}) were close to the ones (8-10 g N kg^{-1}) from Grignani et al. (2007), and they were in the range obtained by Trindade et al., (2008) with values from 9.2 to 20.5 g N kg^{-1}. Furthermore, average N concentrations in maize (from 7.7 to 11.8 g N kg^{-1}) coincided with Richards et al. (1999), Grignani et al. (2007) and Trindade et al. (2008) with 11.7-13.1, 8-11 and 9-12.5 g N kg^{-1}, respectively.

Broadly, the N concentration in plants from controls (no N added) was in the group of the lowest concentrations (Table 7). By contrast, the N concentration in plants from the T2.2 treatment was always among the highest ones.

As the annual oat-sorghum sequence is less N demanding than the ryegrass-maize one, N uptake is much higher when maize is present (Table 8). However, manure at an equivalent rate of 500 kg N ha^{-1} yr^{-1} (T5) is not sustainable, because the N applied doubled uptakes (Table 8). In ryegrass and maize, the N uptake (Table 8) was similar to values obtained by Grignani et al. (2007) and Trindade et al. (2008), also in terms of N uptake distribution, being around 33% of the annual N uptake for ryegrass.

4.3. Evolution of soil nitrate content

At the initial growing stages, the nitrate values recorded when mineral N was applied at sowing (T2.1) indicate its potential low efficiency when crop N uptake is low. The evolution of nitrates in mineral treatments (T2.1 and T2.2) and in the manure treatment (T4) with the same total amount of applied N (250 kg N ha^{-1}), indicates that the evolution in T4 is similar when minerals are applied when crop uptake is most vigorous, thus, at side-dressing (T2.2). In general, the highest recorded values in summer (Fig. 5) coincided with a high OM mineralization period (Richards et al. 1999). Furthermore, maximum values were recorded after a dry month of July, in August 2012 with a monthly rainfall close to 100 mm (Fig. 1). By contrast, the lowest soil N mineral content (46.6 kg N ha^{-1}) was recorded at the summer crop sowing time (late May – June, Table 3); thus, after the winter crop cycle. This behavior agrees with Cavalli et al. (2014), in North Italy, in a double-annual crop of barley and maize fertilized with dairy slurries. The tendency to increase soil nitrate content over the six years of experimentation reinforces the evidence of residual effects from manures (Fig.5). At the highest manure rate (T5, 500 kg N ha^{-1} yr^{-1}), the nitrate soil evolution with time (two first rotations) was quite similar to that found with the lower N manure rate and the control. Nevertheless, at the start of the third rotation, it was possible to observe a tendency of differentiation in the amount of nitrate soil content from the control (low values) vs manured treatments. This fact could be a symptom of the effects of soil organic matter mining in the control plots. This aspect deserves further research based on N and water balances, nature of OM and changes in the soil's content

5. Conclusions

In a biennial rotation of a double-annual forage cropping system, manures applied every year at rates equivalent to 170-250 kg N ha^{-1} reduce the amount of mineral N needed as sidedressing in successive growing seasons, while maintaining the highest yields. After the second rotation, only the rate of 250 kg N ha^{-1} makes it possible to save the supplementary mineral N, while maximizing the nutrient recycling within farms with animal husbandry activity. Furthermore, this rate controls soil mineral content, in the first 90 cm depth, generally below 100 kg N ha^{-1} .When compared with the same rate of mineral fertilizer, figures in minerals can easily double over the summer cropping period, apart from an important fluctuation in the nitrate soil content. Our results make it clear that, in these high demanding N systems, it is possible to increase the amount of N applied in organic form up to 250 kg N ha^{-1}. This last point offers a new insight to be discussed in the framework of the legislation concerning fertilization in nitrate vulnerable zones.

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Legends to Figures

Figure 1. Mean air temperature and total monthly precipitation, for the experimental period (2007-2013; bars), on a monthly basis. Historical patterns (2000-2014) are reported as solid lines. Data were recorded by an automatic meteorological station in the vicinity of the experimental plot.

Figure 2. Yields of different winter (A: oat; C: ryegrass) and summer (B: sorghum; D: maize) crops related to the N applied as sidedressing when 170 kg N ha^{-1} yr^{-1} were applied as manure at sowing of the summer crop (in 2008-09, no manure was applied before the oat crop). The relationships, as equations, are described in Table 5.

Figure 3. Yields of different winter (A: oat; C: ryegrass) and summer (B: sorghum; D: maize) crops related to the N applied as sidedressing when 250 kg N ha^{-1} year^{-1} were applied as manure. Manure rate was fractioned in both annual sowings: 100 kg N ha^{-1} to the winter crop and 150 kg N ha^{-1} yr^{-1} to the summer crop. The relationships, as equations, are described in Table 5.

Figure 4. Yields of different winter (A: oat; C: ryegrass) and summer (B: sorghum; D: maize) crops related to the N applied as manure (170, 250 and 500 kg N ha^{-1} yr^{-1}). A manure rate equivalent to 170 kg N ha^{-1} year^{-1} was applied at sowing of the summer crop (which means that, in 2008-09, no manure was applied before the oat crop). The manure rates of 250 and 500 kg N ha^{-1} year^{-1} were fractioned in both annual sowings: 100 or 250 kg N ha^{-1} for the winter crop and 150 or 250 kg N ha^{-1} year^{-1} for the summer crop, respectively. The relationships, as equations, are described in Table 5.

Figure 5. Soil NO_3^-N content (0-0.9m) in different treatments over the period of six years (three rotations) and for the different crops (O: oat; S: sorghum; R: ryegrass; M: maize). Code letters of treatment groups are given in Table 2.

Table 1. Soil chemical characteristics, at the beginning of the experiment in 2007.

Parameter	Layer			
	cm			
	0-30	30-60	60-90	90-120
pH (potentiometry, water 1:2.5)	8.1	8.1	8.2	8.2
Electrical conductivity (1:5, dS m^{-1}, 25 ^{o}C)	0.14	0.13	0.13	0.13
Calcium carbonate (Calcimeter Bernard method, g kg^{-1})	30	30	30	20
Organic matter (Walkley -Black, g kg^{-1})	15	8	6	3
Total N (Kjeldahl, g kg^{-1})	1.18	0.86	0.62	0.49
Available P (Olsen, mg kg^{-1})	19	8	5	5
Available K (NH_4AcO 1N, mg kg^{-1})	76	60	53	49
Particle size distribution (g kg^{-1})				
Sand (0.05-2 mm)	568	567	580	623
Silt (0.002-0.05 mm)	274	286	285	235
Clay (<0.002 mm)	158	147	135	142

Table 2. Description of the fertilization treatments. Cattle manures (CM)[a] and mineral fertilizers (MN)[b] were annually applied at sowing or/and as sidedressing in winter and summer crops according to the double-annual cropping system (oat-sorghum or ryegrass-maize).

Treatment		Nitrogen fertilization of the winter crop (oat or ryegrass)			Nitrogen fertilization of the summer crop (sorghum or maize)			Annual nitrogen fertilization		
		kg N ha^{-1}								
Acronym	Description	Manure at sowing	Mineral at sowing	Mineral as sidedressing	Manure at sowing	Mineral at sowing	Mineral as sidedressing	Manure	Mineral	Total
T1	Control							0	0	0
T2.1	Mineral at sowing (250MN)		100			150		0	250	250
T2.2	Mineral as sidedressing (250MN)			100			150	0	250	250
T3	Manure (170CM)				170			170	0	170
T3.1	170CM + 80MN			30	170		50	170	80	250
T3.2	170CM + 160MN			60	170		100	170	160	330
T4	Manure (250CM)	100			150			250	0	250
T4.1	250CM + 80MN	100		30	150		50	250	80	330
T4.2	250CM + 160MN	100		60	150		100	250	160	410
T5	Manure (500CM)	250			250			500	0	500

[a] Numbers before the CM acronym indicate the nitrogen applied (kg N ha^{-1}) from cattle manure.
[b] Numbers before the MN acronym indicate the nitrogen applied (kg N ha^{-1}) from mineral fertilizers. Ammonium nitrosulfate (26% N) was applied at sowing and urea (46% N) as sidedressing.

Table 3. Date of sowing and harvest of the different crops along a period of six years. Application dates for manures and their N composition[a] are also included.

Established crop	Agronomic year	Application date of the manure	Date of sowing	Date of harvest	Composition of the applied manure		
					Total N	Organic N	Mineral N-NH_4^+
		dd/mm/yr			g kg^{-1}		
Oat	2007-08	15-11-07	17-11-07	10-06-08	3.76	2.36	1.40
Sorghum		20-06-08	26-06-08	19-09-08	3.10	1.70	1.40
Ryegrass	2008-09	30-09-08	04-10-08	13-05-09	5.14	4.70	0.44
Maize		25-05-09	27-05-09	01-10-09	2.95	1.91	1.04
Oat	2009-10	20-10-09	10-11-09	27-05-10	5.84	5.22	0.62
Sorghum		22-06-10	22-06-10	07-09-10	4.23	3.34	0.89
Ryegrass	2010-11	15-09-10	30-09-10	05-05-11	5.42	4.86	0.56
Maize		11-05-11	21-05-11	14-09-11	3.35	2.25	1.10
Oat	2011-12	30-11-11	07-12-11	06-06-12	3.39	2.01	1.39
Sorghum		25-06-12	26-06-12	21-09-12	6.15	3.45	2.70
Ryegrass	2012-13	04-10-12	09-10-12	06-05-13	5.46	4.35	1.12
Maize		14-05-13	07-06-13	24-09-13	4.51	3.28	1.23

[a] Nutrient composition of manures is expressed in a fresh weight basis.

In some treatments we also applied P and K fertilizers (0-14-14) plus potassium sulfate (50% K_2O). They were applied at different rates according to the treatment in order to achieve, in all plots, 130 kg P_2O_5 ha^{-1} $year^{-1}$ and 260 kg K_2O ha^{-1} $year^{-1}$. These quantities covered the crop nutrient uptake.

Table 4. Annual yields (Mg ha^{-1} on a dry matter basis) and for each year (Yr) of experimentation (from 1 to 6) and for two or three rotations, according to the fertilization treatment[a].

Treatment (Tr)	Oat				Sorghum					Ryegrass				Maize			
	Yr 1	Yr 3	Yr 5	3 rotations	Yr 1	Yr 3	Yr 5	2 last rotations	3 rotations	Yr 2	Yr 4	Yr 6	3 rotations	Yr 2	Yr 4	Yr 6	3 rotations
T1	6.17bc	2.52d	5.10c	4.59	6.06c	5.95c	3.00	4.47	5.00	1.95d	1.82c	6.03c	3.27	6.93b	7.90c	15.12c	9.99
T2.1	7.89bc	8.85ab	8.71ab	8.49	11.27a	9.85a	3.70	6.78	8.27	6.45ab	5.59ab	10.50a	7.51	12.50a	13.55ab	21.85ab	15.97
T2.2	10.72a	10.08a	9.70a	10.17		9.37a	3.13	6.25		8.28a	6.21a	10.95a	8.48	12.03a	12.98ab	21.21ab	15.41
T3	6.35bc	3.38d	6.08bc	5.27	8.16bc	7.66b	3.85	5.75	6.56	2.11d	3.27bc	7.72bc	4.37	7.4b	9.83abc	19.36b	12.22
T3.1	7.81bc	6.89bc	8.40ab	7.70		8.50ab	3.87	6.18		4.35bcd	5.54ab	10.44ab	6.93	10.69ab	11.94ab	23.50a	15.38
T3.2	8.46abc	8.46ab	9.14a	8.68		9.00ab	3.42	6.21		6.46ab	6.90a	10.18ab	7.85	10.84ab	12.97ab	21.57ab	15.12
T4	5.93c	5.00cd	7.22abc	6.05	7.74bc	8.23ab	3.91	6.07	6.63	3.60cd	4.13abc	8.97ab	5.57	7.84b	9.72bc	22.94ab	13.50
T4.1	7.26bc	7.85ab	9.38a	8.16		9.35a	3.74	6.55		5.90abc	5.98ab	10.61a	7.49	12.32a	12.96ab	22.37ab	15.88
T4.2	8.69ab	8.23ab	9.54a	8.82		9.80a	3.61	6.71		7.46a	6.51a	9.45ab	7.81	1.193a	13.73a	23.04ab	16.23
T5	6.92bc	7.90ab	9.57a	8.13	9.78ab	9.19ab	4.45	6.82	7.81	6.04abc	6.34a	10.95a	7.37	12.21a	12.23ab	24.17a	16.20
Significance	0.0002	<0.0001	<0.0001		0.0085	<0.0001	0.1982			<0.0001	<0.0001	0.0021		<0.0001	<0.0001	<0.0001	
Significance of the Yr* Tr interaction				0.0001				<0.0001	0.0118				0.0146				0.0019

[a] Acronyms of treatments are described in Table 2.

Means within a column followed by a different letter are significantly different (α=0.05) according to Duncan's multiple range test.

Table 5. Linear and linear-threshold equations describe the yield (y, kg ha^{-1}) relationship to N applied (x, kg ha^{-1}) as manure (CM). The yield relationship to the mineral N fertilizer (MN, x), applied as a complement (sidedressing) in the two lowest manure rates, is also included.

Fertilization[a]	Crop	First rotation			Second rotation			Third rotation		
		Equation		R^2	Equation		R^2	Equation		R^2
170CM + different mineral rates (MN)	Oat	$y = 35.23x + 6541$	*	0.57	$y = 84.56x + 3635$	***	0.87	$y = 50.92x + 6243$	**	0.80
	Sorghum[b]				$y = 13.38x + 7718$	*	0.54		ns	
	Ryegrass	$y = 72.62x + 2137$	***	0.98	$y = 60.55x + 3384$	***	0.95	$y = 110.20x + 7724 \quad x \leq 23.5$ $y = 10313 \quad x > 23.5$	*	0.53
	Maize	$y = 33.60x + 7986$	*	0.58	$y = 31.44x + 10006$	**	0.78	$y = 71.45x + 19357 \quad x \leq 44.5$ $y = 22537 \quad x > 44.5$	*	0.60
250CM + different mineral rates (MN)	Oat	$y = 46.06x + 5913$	***	0.82	$y = 95.05x + 5002 \quad x \leq 34.0$ $y = 8234 \quad x > 34.0$	*	0.72	$y = 71.95x + 7220 \quad x \leq 32.2$ $y = 9537 \quad x > 32.2$	*	0.69
	Sorghum[b]				$y = 15.66x + 8347$	*	0.44		ns	
	Ryegrass	$y = 64.28x + 3724$	**	0.8	$y = 39.67x + 4351$	***	0.84		ns	
	Maize	$y = 106.10x + 7841 \quad x \leq 40.4$ $y = 12127 \quad x > 40.4$	**	0.74	$y = 64.89x + 9717 \quad x \leq 61.8$ $y = 13727 \quad x > 61.8$	**	0.85		ns	
Different manure rates (CM)	Oat		ns		$y = 10.55x + 2199$	***	0.94	$y = 9.51x + 4845$	***	0.92
	Sorghum	$y = 8.49x + 6158$	**	0.66	$y = 6.89x + 6246$	***	0.85		ns	
	Ryegrass	$y = 8.98x + 1396$	***	0.83	$y = 9.90x + 1714$	***	0.96	$y = 10.05x + 6123$	***	0.83
	Maize	$y = 11.29x + 6070$	**	0.70	$y = 9.10x + 7912$	***	0.78	$y = 30.27x + 14901 \quad x \leq 306.3$ $y = 24173 \quad x > 306.3$	**	0.77

ns: not significant, * $p<0.05$, ** $p<0.01$, *** $p<0.001$. R2 = coefficient of determination.

[a] Numbers before the acronyms indicate the nitrogen applied previously (kg N ha^{-1}) as cattle manure (CM).

[b] Mineral N (MN) was not applied to the sorghum in the first year.

Table 6. Comparison[a] between years of the lineal functions described in Table 5. Comparisons were set up using fictitious variables and are based on the equation[b]: $\text{yield} = \alpha + \gamma + \beta{*}\text{N} + \lambda{*}\text{N}$.

Fertilization[c]	Crop	first vs second rotation				first vs third rotation				second vs third rotation			
		α	γ	β	λ	α	γ	β	λ	α	γ	β	λ
170CM + different mineral rates (MN)	Oat	***	**	***	*	***	ns	***	ns	***	**	***	0.07
	Sorghum	-	-	-	-	-	-	-	-	-	-	-	-
	Ryegrass	***	***	***	0.09	-	-	-	-	-	-	-	-
	Maize	***	**	**	ns	-	-	-	-	-	-	-	-
250CM + different mineral rates (MN)	Oat	-	-	-	-	-	-	-	-	-	-	-	-
	Sorghum	-	-	-	-	-	-	-	-	-	-	-	-
	Ryegrass	***	ns	***	ns	-	-	-	-	-	-	-	-
	Maize	-	-	-	-	-	-	-	-	-	-	-	-
Different manure rates (CM)	Oat	-	-	-	-	-	-	-	-	***	***	***	ns
	Sorghum	***	ns	***	ns	-	-	-	-	-	-	-	-
	Ryegrass	***	0.08	***	ns	***	***	***	ns	***	***	***	0.09
	Maize	***	*	***	ns	-	-	-	-	-	-	-	-

ns: not significant, * $p<0.05$, ** $p<0.01$, *** $p<0.001$. The p values between 0.05 and 0.1 are also shown.

[a] If γ is not significant, the intercept term of the equation does not differ between years. If λ is not significant, the slope term of the equation does not differ between years.

[b] N is the amount of nitrogen applied as manure (CM) or just as a mineral complement (sidedressing) in the two lowest manure rates.

[c] Numbers before the acronyms indicate the nitrogen applied (kg N ha^{-1}) previously from cattle manure.

Table 7. Nitrogen concentration in crops (g N kg^{-1} dry matter) for each year (Yr) included in the experimentation (from 1 to 6), and for two or three rotations, according to the fertilization treatment[a].

Treatment (Tr)	Oat				Sorghum					Ryegrass				Maize			
	Yr 1	Yr 3	Yr 5	3 rotations[b]	Yr 1	Yr 3	Yr 5	2 last rotations[b]	3 rotations[b]	Yr 2	Yr 4	Yr 6	3 rotations[b]	Yr 2	Yr 4	Yr 6	3 rotations
T1	8.1	8.8	9.3	8.8c	6.7	6.4	19.7	13.0e	10.9d	8.8	9.1	9.6	9.2d	7.1d	7.7b	8.4b	7.7
T2.1	8.6	8.4	11.6	9.5abc	8.9	8.1	25.9	17.0ab	14.3a	6.9	7.5	13.6	9.3d	9.5bcd	8.5ab	11.9a	10.0
T2.2	9.1	9.3	13.0	10.4a		12.9	24.3	18.6a		13.1	12.4	16,6	14.0a	12.4a	11.1a	11.8a	11.8
T3	9.1	8.6	10.4	9.4bc	6.8	6.0	21.5	13.7de	11.4cd	10.2	8.6	10.2	9.7d	7.8cd	8.4ab	9.2ab	8.5
T3.1	8.2	8.3	10.2	8.9c		9.7	24.5	17.1ab		9.6	9,2	13.2	10.8cd	9.7abcd	10.2ab	10.3ab	10.1
T3.2	9.9	8.4	12.1	10.1ab		11.2	25.5	18.3a		12.7	11.1	15.8	13.2ab	10.3abc	10.8a	11.5a	10.9
T4	8.9	8.7	10.2	9.3bc	6.8	7.2	22.9	15.0cd	12.3bc	8.1	8.4	11.1	9.2d	8.5cd	8.5ab	10.4ab	9.1
T4.1	9.0	8.1	12.0	9.7abc		9.2	24.8	17.0ab		8.5	9.0	13.5	10.3cd	9.5bcd	10.6a	11.0ab	10.4
T4.2	9.6	9.1	12.6	10.5a		9.9	26.1	18.0a		10.8	9.5	15.3	11.9bc	11.3ab	10.2ab	11.7a	11.0
T5	8.2	9.0	10.4	9.2bc	8.3	7.5	23.8	15.7bc	13.2ab	7.4	8.1	14.7	9.5d	8.7bcd	9.9ab	11.7a	10.1
Significance				0.0029				<0.0001	<0.0001				<0.0001	<0.0001	<0.0001	0.0052	
Significance of the Yr* Tr interaction				0.1822				0.0678	0.1301				0.0548				0.0374

[a] Acronyms of treatments are described in Table 2.

[b] If the interaction treatment*year was not significant, separation of means was just done in the three year average and also in years 3 and 5 mean in the sorghum crop.

Means within a column followed by a different letter are significantly different (α=0.05) according to Duncan's multiple range test.

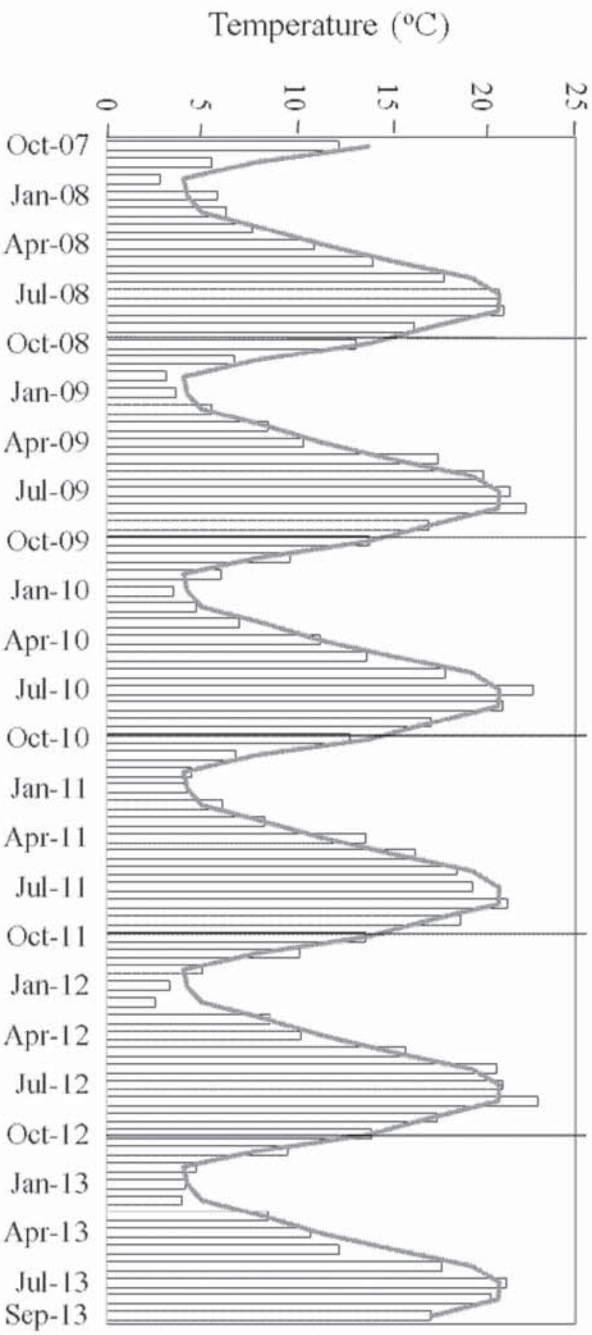
Table 8. N uptake by crops (kg N ha^{-1} dry matter) for each year (Yr) of experimentation (from 1 to 6), and for two or three rotations, according to the fertilization treatment[a].

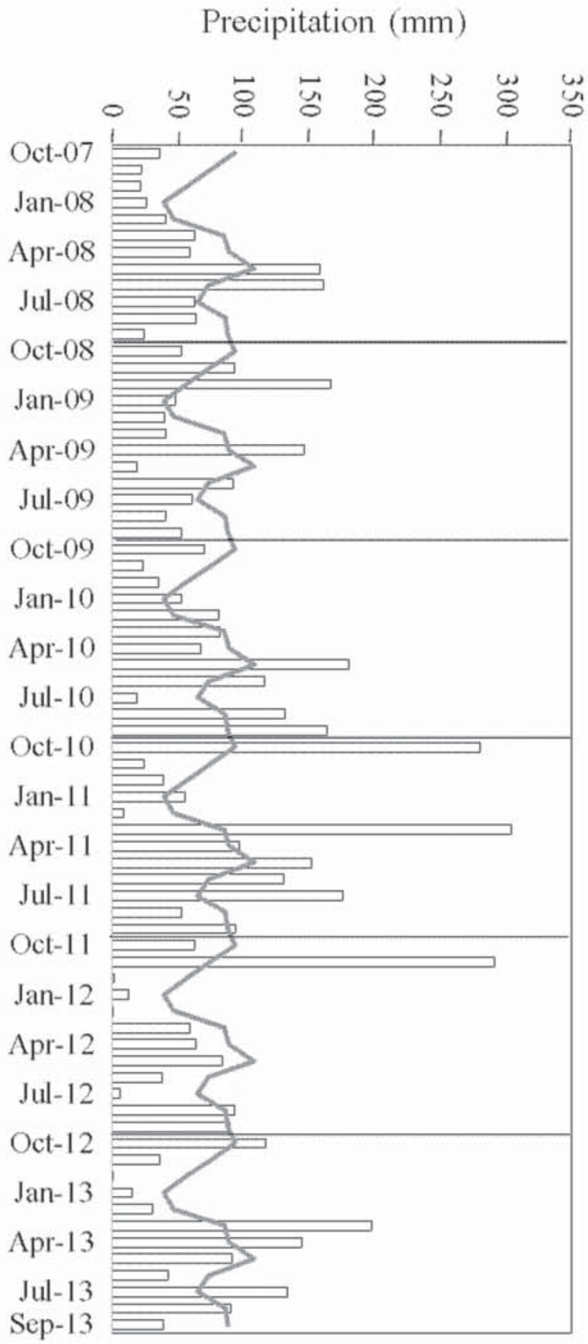
Treatment (Tr)	Oat				Sorghum					Ryegrass				Maize			
	Yr 1	Yr 3	Yr 5	3 rotations[b]	Yr 1	Yr 3	Yr 5	2 last rotations	3 rotations	Yr 2	Yr 4	Yr 6	3 rotations[b]	Yr 2	Yr 4	Yr 6	3 rotations
T1	50	22	48	40g	40	38d	59	49	46c	17	17	58	31f	49c	61b	131c	80
T2.1	68	75	101	81cd	100	80abcd	96	88	92a	44	42	144	77d	118ab	115ab	259a	164
T2.2	98	93	126	106a		120a	76	98		109	77	181	122a	148a	144a	251a	181
T3	58	29	64	50gf	56	45cd	83	64	62b	22	28	79	43ef	59bc	83ab	179bc	107
T3.1	65	57	85	69e		83abcd	95	89		42	50	137	79cd	104abc	121ab	243a	156
T3.2	84	72	110	89bc		101ab	88	94		82	77	163	107ab	112ab	140a	249a	167
T4	53	44	73	57f	52	60bcd	90	75	67b	29	35	98	54e	66bc	83ab	238ab	129
T4.1	65	64	112	80cde		87abc	93	90		50	54	143	82cd	116ab	138a	245a	166
T4.2	84	75	121	93b		97ab	94	95		81	62	145	96bc	133a	139a	269a	181
T5	57	71	99	76de	81	70bcd	106	88	85a	45	51	160	76d	107abc	121ab	283a	170
Significance				<0.0001		<0.0001	0.0819						<0.0001	<0.0001	<0.0001	<0.0001	
Significance of the Yr* Tr interaction				0.0620				0.0012	0.3486				0.0540				0.0071

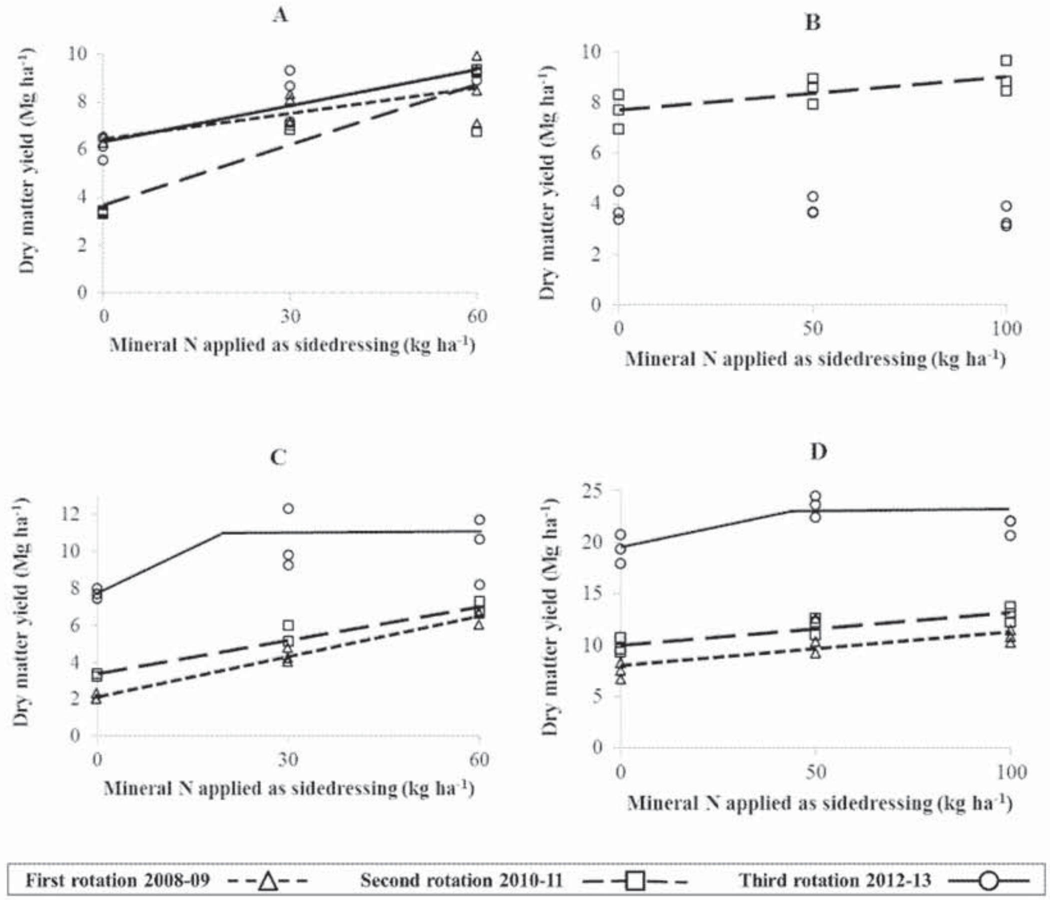
[a] Acronyms of treatments are described in Table 2.

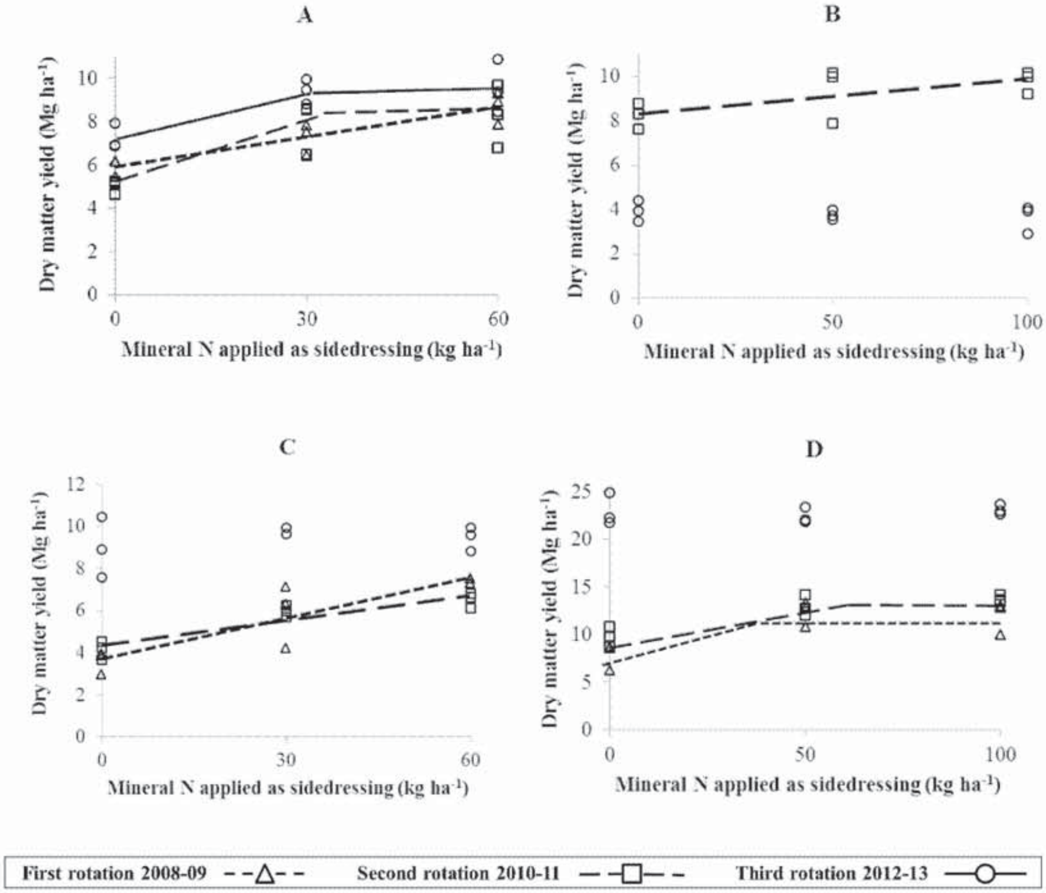
[b] If the interaction treatment*year was not significant, separation of means was just done in the three year average.

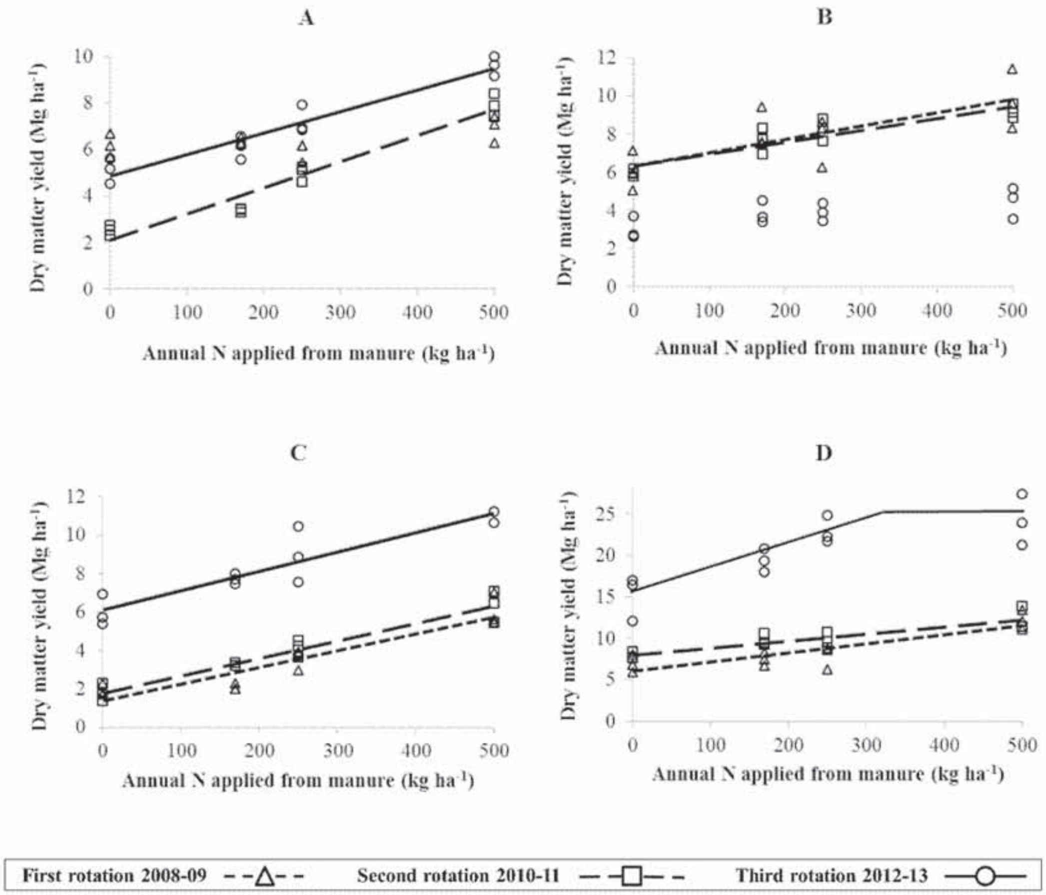
Means within a column followed by a different letter are significantly different (α=0.05) according to Duncan's multiple range test.

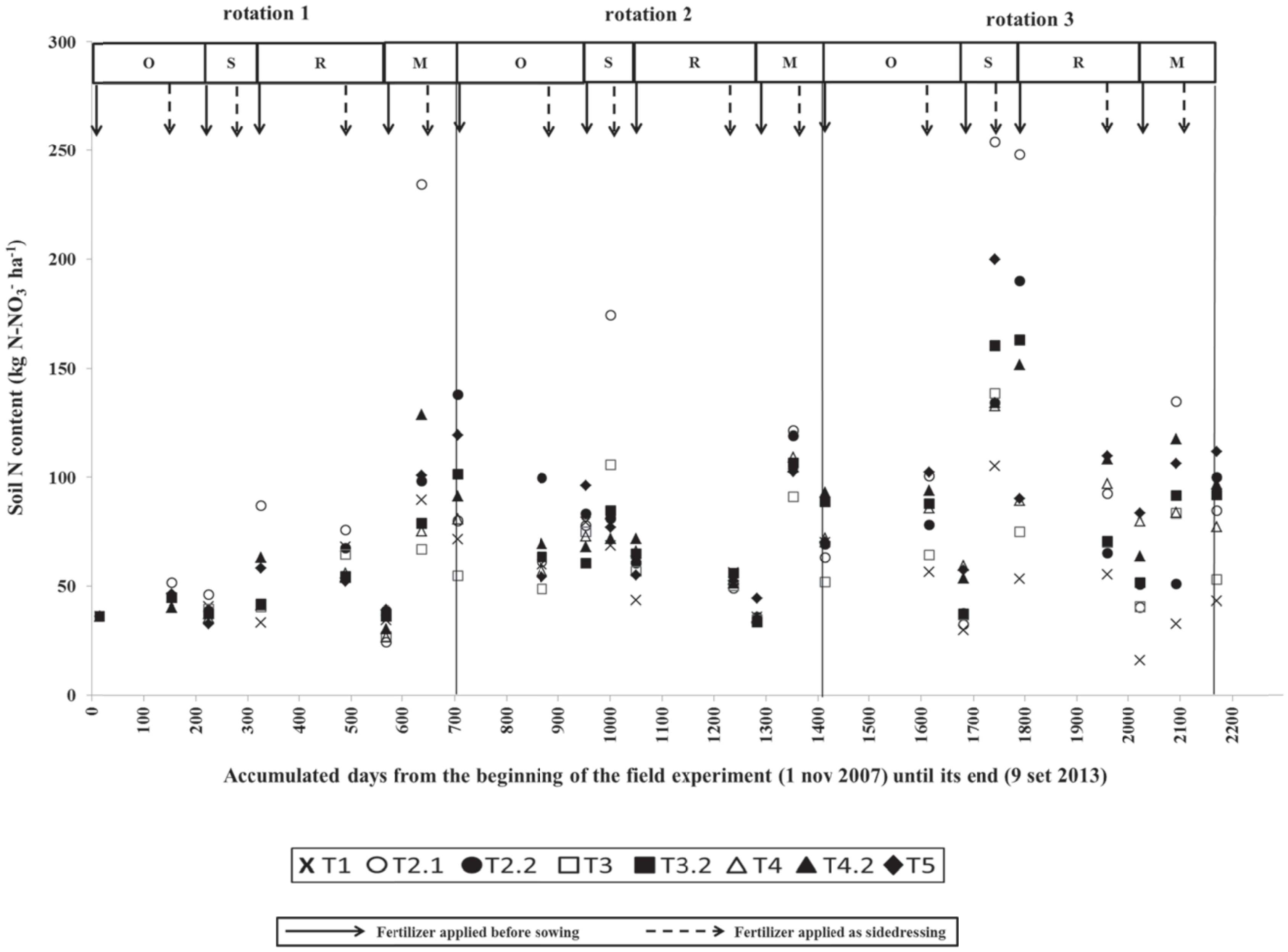

Temperature (ºC)
0
5
10
15
20
25
Oct-07
Jan-08
Apr-08
Jul-08
Oct-08
Jan-09
Apr-09
Jul-09
Oct-09
Jan-10
Apr-10
Jul-10
Oct-10
Jan-11
Apr-11
Jul-11
Oct-11
Jan-12
Apr-12
Jul-12
Oct-12
Jan-13
Apr-13
Jul-13
Sep-13


Precipitation (mm)
0
50
100
150
200
250
300
350
Oct-07
Jan-08
Apr-08
Jul-08
Oct-08
Jan-09
Apr-09
Jul-09
Oct-09
Jan-10
Apr-10
Jul-10
Oct-10
Jan-11
Apr-11
Jul-11
Oct-11
Jan-12
Apr-12
Jul-12
Oct-12
Jan-13
Apr-13
Jul-13
Sep-13


A
B
C
D
Dry matter yield (Mg ha^{-1})
Mineral N applied as sidedressing (kg ha^{-1})
0
2
4
6
8
10
12
5
15
20
25
30
50
60
100
First rotation 2008-09
Second rotation 2010-11
Third rotation 2012-13


A
B
C
D
Dry matter yield (Mg ha^{-1})
Mineral N applied as sidedressing (kg ha^{-1})
0
2
4
6
8
10
12
0
5
10
15
20
25
30
50
60
100
First rotation 2008-09
Second rotation 2010-11
Third rotation 2012-13


A
B
C
D
Dry matter yield (Mg ha^{-1})
Annual N applied from manure (kg ha^{-1})
0
2
4
6
8
10
12
5
15
20
25
0
100
200
300
400
500
First rotation 2008-09
Second rotation 2010-11
Third rotation 2012-13


rotation 1
rotation 2
rotation 3
O
S
R
M
300
250
200
150
100
50
0
Soil N content (kg N-NO$_3^-$ ha^{-1})
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
1800
1900
2000
2100
2200
Accumulated days from the beginning of the field experiment (1 nov 2007) until its end (9 set 2013)
✕ T1
○ T2.1
● T2.2
□ T3
■ T3.2
△ T4
▲ T4.2
◆ T5
Fertilizer applied before sowing
Fertilizer applied as sidedressing