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

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# UNIVERSITÀ DEGLI STUDI DI TORINO

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

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

18

19 **Abstract**

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

34

35 **Keywords**

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

37

38

39 **1. Introduction**

40

41 During the Eighties many researchers showed that nitrogen (N) amounts applied in Western European  
42 agriculture were excessive and risked producing pollution. Furthermore, excesses were unevenly  
43 distributed across the continent and linked primarily to areas of intensive livestock farming (Velthof et al.,  
44 2014). By all accounts, measures to protect human health, living resources, and aquatic ecosystems were  
45 needed. Today, despite reduction in chemical fertilizer usage, the European Union (EU-28) still exhibits  
46 excess N supply (the sum of organic and mineral fertilizers, natural deposition, and N-fixation) compared  
47 with removal by crop yields (Leip et al., 2011).

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

54 The Nitrates Directive (91/676/EEC, Anonymous, 1991) was approved in Europe 24 years ago to address the  
55 excessive distribution and/or discharge of livestock effluents onto agricultural land and the excessive use of  
56 mineral fertilizers in several parts of Europe. Northern Italy soon emerged as a high-risk area because of its  
57 numerous intensive livestock farms. The Nitrates Directive defined a few but clear monitoring actions and  
58 limitations to the application of N: i) member States must extensively monitor fresh waters to identify areas  
59 where nitrate content exceeds a threshold of  $50 \text{ mg NO}_3^- \text{ l}^{-1}$ ; ii) member States must designate Nitrate  
60 Vulnerable Zones (NVZs), where actual or potential nitrate pollution of agricultural origin is problematic; iii)  
61 farms lying in NVZs must implement Action Programs to adopt sustainable management practices. A core  
62 part of the Action Program established constraints for land-application of all nitrogen-containing fertilizers,  
63 and in particular, set specific limits for livestock manure application.

64 A key component of Action Programs is balancing N inputs and outputs. The definition of “balance” rested  
65 on a rather simple principle that the difference between expected nitrogen crop requirements and the  
66 nitrogen supply from the soil, air, and fertilization approximate one another. However, predicting the

67 equation components (e.g. crop removal, initial N availability when crop growth starts, soil N net  
68 mineralization, efficiency of N from livestock manure and chemical fertilizers) is not trivial (Webb et al.,  
69 2013; Schröder and Neeteson, 2008).

70 The Tetto Frati Long Term Experiment (LTE) has been underway about as long as the Nitrates Directive has  
71 been in effect in Europe. It was initially created in 1992 to mimic a set of intensive forage systems for a  
72 typical Northern Italy dairy farm, where maize is the main fodder crop, and liquid and solid manures are  
73 fertilizer resources (Grignani et al., 2007). It was used for several purposes, all of which were connected to  
74 the study of N efficiency and the fertilization impacts of highly manured cropping systems (Zavattaro et al.,  
75 2012). Studied treatments are semi-static in the sense that zero, low, and high input levels have been  
76 applied continuously, with exact N low and high input amounts having been adjusted just twice to produce  
77 data relevant to scientific and technical questions on application of the Nitrates Directive in Italy. Prior to  
78 2006, the LTE was used to investigate a frequently applied fertilization strategy that combined manure and  
79 chemical fertilizer as inputs. The low and high input levels were determined by fixed amounts of slurry and  
80 farmyard manure while actual nitrogen input changed according to manure and slurry characteristics, and  
81 all organic treatments were top-dressed with a fixed amount of 100 kg urea-N ha<sup>-1</sup>. During 2007 to 2011,  
82 LTE research centered on the two maximum levels of manure-N inputs mandated within and external to  
83 Italian NVZs (170 and 340 kg N ha<sup>-1</sup>). Top-dressed urea was excluded from manured treatments to test the  
84 simple effects of manures and mineral fertilizers. The third phase of LTE research began in 2012 and is still  
85 ongoing; it is devoted to verification of the application effects of the 250 kg manure-N ha<sup>-1</sup> Derogation  
86 scheme in Italy (low level is 170 and high level is 250 kg N ha<sup>-1</sup>).

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

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

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

97

## 98 **2. Materials and Methods**

### 99 ***2.1 LTE description***

100 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  
101 started in 1992, but the first year of data has been excluded to mitigate the effect of past fertilizations. The  
102 climate is temperate sub-continental, the average annual rainfall is 760 mm, and the mean annual  
103 temperature is 12°C. The soil, Typic Udifluent, is deep and calcareous with loam texture in the first horizon  
104 and silty texture in deeper horizons (Grignani et al., 2007).

105 The experiment, designed as a randomized block with three replicates, compares four cropping systems at  
106 five nitrogen application levels, plus a rotational maize-lucerne system at a single fertilization level.

107 Cropping systems are: entirely-harvested maize for silage (Ms); maize for grain with residue incorporation  
108 (Mg); silage maize+Italian ryegrass double cropping with almost continuous soil cover (Mr); 4-years or 6-  
109 years rotation of entirely-harvested maize and long-growing grass ley (MI); 4-years or 6-years rotation of  
110 maize for silage and lucerne, Mu. The five N application levels are arranged in nine distinct fertilization  
111 treatments: 0N control, four doses of mineral N as urea (U100 to U400), two doses of bovine slurry (SLU),  
112 two doses of farmyard manure (FYM). The Mu system is fertilized as SLULow in the maize phase, while  
113 lucerne receives no N fertilizer. Further details on fertilization treatments are reported in Table 1. The  
114 target amount of N supplied was based on total N contents of FYM and SLU. Manure was always distributed  
115 in spring and incorporated quickly into the soil, a few days before maize sowing. Urea was chosen as the  
116 reference mineral fertilizer because it is the most commonly used N fertilizer in maize in Italy, due to its low  
117 cost. It was partly distributed few days before maize sowing and partly top-dressed and incorporated  
118 through ridging. Phosphorous (P) and potassium (K) were supplied through mineral fertilizers at levels

119 above plant needs to exclude any possibility of nitrogen interaction. Nine different commercial maize  
120 hybrids of the 500 or 600 FAO were used throughout the 23 years period. Further details on the crop  
121 management are reported in Grignani et al. (2007), Bertora et al. (2009), and Zavattaro et al. (2012).  
122 Fertilization amounts were modified from experimental start to conform to a semi-static management, in  
123 which the number of levels remained constant. Table 1 reports all fertilization data for completeness, but in  
124 this paper we discuss results from the 2007 to 2014 period only, with the following qualifications:

- 125 - crop yield, N uptake, and N use efficiency indicators were reported separately for 2007-2011 (five years)  
126 and 2012-2014 (three years) according to the two fertilization levels (U300 and U400 were averaged to  
127 allow a direct comparison with the 340 kg ha<sup>-1</sup> dose of manured treatments);
- 128 - incorporated crop residue and rotational ley effects were derived by averaging data over the entire  
129 2007-2014 period;

130 Maize was sown in MI and Mu systems in 2011 and 2012, while grass or lucerne leys lasted from 2008 to  
131 2010 and from 2013 to 2014. Leys growth failed in 2007, therefore no crop data are reported in that year.  
132 Table 2 reports the general characteristics of the organic fertilizers used in the two periods analyzed.

133

## 134 ***2.2 Sampling and chemical analyses***

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

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

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

147

### 148 **2.3 Statistical methods**

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

161

### 162 **2.4 Efficiency coefficients**

163 Nitrogen efficiency coefficients were calculated using experimental data as follows.

164 - R/F, or Removal to Fertilizer Ratio, was calculated as the amount of N removed as yield ( $N_{removal}$ )  
165 divided by the total amount of fertilizer N ( $N_{fertilizer}$ );

166 - AR, or Apparent Recovery, was calculated as:

$$167 \quad AR = \frac{N_{removal} - N_{removal_{0N}}}{N_{fertilizer}}$$

168 where  $N_{removal}$  and  $N_{removal_{0N}}$  are the amounts of N removed by a fertilized treatment and the control,  
169 respectively;

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

$$\begin{aligned} 171 \quad AR\_relative\_to\_urea &= \frac{Nremoval_{MAN} - Nremoval_{0N}}{Nfertilizer_{MAN}} \times \frac{Nfertilizer_U}{Nremoval_U - Nremoval_{0N}} = \\ 172 \quad &= \frac{Nremoval_{MAN} - Nremoval_{0N}}{Nremoval_U - Nremoval_{0N}} \end{aligned}$$

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

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

178

## 179 **2.5 N balance according to Italian legislation**

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

$$184 \quad (F_c \times K_c) + (F_o \times K_o) + N_f + N_c + M_{so} + A_d = (Y \times B)$$

185 where:

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

- 195 •  $N_c$  is the amount of N from the previous crop. Suggested values are 80 kg of N ha<sup>-1</sup> after lucerne or long-  
196 lasting (>5 years) grass leys, and 30-40 kg of N after short-duration (≤ 5 years) grass leys. Conversely,  
197 when crop residues are incorporated into the soil, the available N is reduced by 30 kg ha<sup>-1</sup> in the case of  
198 cereal straw, and by 40 kg ha<sup>-1</sup> in the case of maize stalks, due to immobilization;
- 199 •  $M_{so}$  accounts for the mineralization of Soil Organic Matter (SOM), calculated as 30 kg ha<sup>-1</sup> yr<sup>-1</sup> of N for  
200 each percent of SOM for annual and long-lasting crops (maize included), and reduced to a fraction of  
201 this amount for short-lasting annual crops;
- 202 •  $A_d$  is the atmospheric deposition, equal to 20 kg N ha<sup>-1</sup> per year;
- 203 •  $Y$  is the expected yield;
- 204 •  $B$  is the expected N concentration of yield. Suggested values are 17 kg of N t<sup>-1</sup> of dry matter for maize  
205 grain, 10 kg t<sup>-1</sup> for silage maize, 14 kg t<sup>-1</sup> for Italian ryegrass, and 18 g t<sup>-1</sup> for grass ley.
- 206

### 207 **3. Results**

#### 208 **3.1 Use efficiency of fertilizer N**

209 The three indicators of N use efficiency showed a dependency on N supply. During the 2007-2011 period  
210 (Tab. 3 a), the R/F indicator was consistently high. It ranged between 1.27-1.70 in entirely-harvested  
211 systems (Ms, Mr, and MI) at 170 kg ha<sup>-1</sup> of N supply, and slightly decreased to a 0.82-1.09 range at 340 kg  
212 ha<sup>-1</sup> of N supply. In other words, R/F decreased by 34% when N supply was doubled. Lower R/F values were  
213 observed in the Mg system due to limited removal of grain only. Crops fertilized with SLU exhibited  
214 systematically lower R/F values than when FYM was applied. These differences were smaller in Mg and Ms  
215 (2 to 7%) and larger in the long-growing cropping systems of Mr and MI (8 to 31%).

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

219 When the soil natural N supply is subtracted from N removal, we obtain the AR indicator. Obviously,  
220 experimental AR values (Tab. 3 a, referred to the 2007-2011 period) were lower than those of R/F, and

221 ranged from 0.52 to 0.90 in entirely-harvested systems (Ms and Mr) at 170 kg ha<sup>-1</sup> of N supply; values  
222 decreased to a range of 0.44-0.72 at 340 kg ha<sup>-1</sup> of N supply. Lower AR values were measured in the Mg  
223 system because grain only was removed. As observed in R/F, SLU yielded lower AR values than did FYM, a  
224 difference not greatly influenced by N input and cropping system. The overall inter-annual variability of this  
225 coefficient ranged between 0.10 and 0.24.

226 When the apparent recovery of organic fertilizers is expressed relative to that of mineral ones, we obtain  
227 one of the definitions of NFRV (Schröder et al., 2007). In the 2007-2011 period and at 170 kg ha<sup>-1</sup> of N  
228 supply, FYM showed a NFRV of 0.83 in monoculture maize (Mg and Ms) and 0.99 in the double-cropping  
229 system (Mr). NFRV for SLU was always lower, ranging between 0.72 and 0.80. NFRV was increased at the  
230 high dose if compared to the low dose because the AR of urea had decreased to a higher extent (from 0.90  
231 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  
232 demonstrated that in the long term and at high doses, the use efficiency of organic fertilizers was higher  
233 than that of urea.

234 Results from the second period 2012-2014 (Tab. 3 b) confirm some effects described for the previous  
235 period. In general, the N use efficiency indicators of R/F and AR at the 170 kg ha<sup>-1</sup> level validated those  
236 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  
237 found to be lower than that of FYM, even though differences between cropping systems were less clear.  
238 NFRV values did not trend alike in the two periods ( $R^2 = 0.20$ ,  $p=0.374$ ), but they did affirm the high  
239 efficiencies of FYM and SLU relative to urea (0.62-0.98).

240 The main difference between the two periods is attributed to a lower High fertilization level in 2012-2014  
241 (250 kg N ha<sup>-1</sup>, corresponding to the maximum organic fertilization amount for farms in the Derogation  
242 scheme in Italy). This caused efficiency indicators not to consistently decrease when passing from the low  
243 to high level, as previously shown at 340 kg ha<sup>-1</sup>. They did increase sometimes, such as in Ms and Mr  
244 cropping systems fertilized with FYM, indicating the use efficiency remained high up to 250 kg N ha<sup>-1</sup>. As in  
245 the first period, NFRV increased as N supply increased also as a result of a progressively reduced urea  
246 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

247 reported indicators was similar in the two periods; specifically, values for the 170 kg dose differed less than  
248 one standard deviation between the two time frames.

249

### 250 **3.2 Mineralization from the soil**

251 The crop N uptake of non-fertilized plots is frequently used as a proxy for the amount of N mineralized from  
252 the soil. The 2007-2014 average N uptake in the Mg, Ms, and Mr systems was 116 kg N ha<sup>-1</sup> (standard  
253 deviation: 29 kg N ha<sup>-1</sup>), without any substantial difference between Mg, Ms and Mr cropping systems,  
254 despite the different soil cover duration. Remarkably higher plant N uptake were recorded in the MI system  
255 (214 kg N ha<sup>-1</sup> in maize, 163 kg N ha<sup>-1</sup> in grass), probably biased by an enrichment of soil N due to N-fixation  
256 by clover that progressively developed in the ON sward.

257

### 258 **3.3 Role of crop residues**

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

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

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

271 On the other hand, the mean SON stock measured on four dates in the 2007-2014 period (Fig. 1 b)

272 confirmed that incorporation of crop residues caused a buildup of SON stock to as much as 584 kg N ha<sup>-1</sup>

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

275

### 276 **3.4 Rotation with leys**

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

282 The incremental change in available N was negatively and linearly related to total fertilization supply ( $R^2 =$   
283  $0.86$ ,  $p=0.001$ ), which indicated that the beneficial effects of rotation were evident at low fertilization  
284 inputs and less important at high fertility levels (Fig. 3).

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

287

### 288 **3.5 Crop yield and N uptake**

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

296 Comparing the Derogation limit and the  $170 \text{ kg ha}^{-1}$  limit to manure N (Tab. 4 b) shows that at  $250 \text{ kg ha}^{-1}$ ,  
297 maize was apparently still utilizing all fertilizer N. Differences in yield and N uptake between the two doses

298 were always significant when either FYM or SLU was used, and only when SLU was used in the instance of N  
299 uptake.

300 On average, by raising the fertilization level from 170 to 250 kg it was possible to increase the yield by 10-  
301 15% in plots that received SLU and 20-25% (as much as 40% in Mr) with FYM. These results were  
302 comparable to the increases observed when fertilization levels were raised from 170 to 340 kg ha<sup>-1</sup> (+20%  
303 on average).

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

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

311

## 312 **4. Discussion**

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

319

### 320 **4.1 N use efficiency**

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

324 Schröder et al., 2007 and Lalor et al., 2011. Depending on the formula chosen, results differ. In our case,  
325 the efficiency of FYM in Ms applied at a dose of 170 kg manure-N ha<sup>-1</sup> ranged from 1.45 to 1.09 (if  
326 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  
327 relative to urea, or NFRV).

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

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

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

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

352 Guidelines of the Italian Action Programs state that well-managed FYM and SLU produce efficiencies  
353 ranging from 0.48 to 0.65, depending on the application of the Derogation and soil texture (higher  
354 efficiency in coarse-textured soils); in the case of FYM, the carry-over effect of past year additions could be  
355 added. Regions are free to adjust guideline values for local variability; the main regions of Northern Italy  
356 (where most of the national NVZs are situated) utilize efficiency coefficients in the range of 0.40 to 0.50 for  
357 both liquid and solid manures and fertilizations history is often not considered. Farms in the Derogation  
358 scheme utilize 0.50 for FYM and 0.65 for SLU. Our findings confirm that the ARs measured for SLU (0.44-  
359 0.66) are in the same range of variation as official values, whereas ARs measured for FYM (0.45-0.89) span a  
360 wider and higher range than official values.

361

#### 362 ***4.2 N from soil mineralization and atmospheric deposition***

363 The amount of N mineralized from the soil affects the overall N balance, but it is very difficult to estimate it  
364 in non-experimental conditions. Long-term unfertilized treatments estimate it at  $116 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ . If we  
365 subtract the atmospheric deposition onto the soil ( $26 \text{ kg ha}^{-1} \text{ yr}^{-1}$  of N at the Tetto Frati LTE, as reported by  
366 Bassanino et al., 2011) from the N uptake of unfertilized plots, we obtain  $90 \text{ kg N ha}^{-1}$ . This corresponds to  
367  $50 \text{ kg of N per percentage point of SOM content in the 0-30 cm layer of 0N plots (1.81 g } 100\text{g}^{-1} \text{ on average)}$ .  
368 Alternatively, if we relate this amount to SON content, we obtain an annual mineralization rate of 2.0%,  
369 which is consistent with the value of 1.7% reported by Bertora et al. (2009), who applied an exponential  
370 decay model to the first period of the Tetto Frati LTE.

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

374

375 **4.3 Crop residues**

376 The high N amount contained in crop residues ( $100\pm 23$  kg N ha<sup>-1</sup> in well-fertilized treatments) increased  
377 stable SOM, but did not modify the N uptake of the entire plant.

378 The Italian guidelines of Action Programs allow farmers to consider incorporated crop residues as a sink of  
379 N, rather than as a source, and quantify immobilization up to 40 kg N ha<sup>-1</sup> in the case of maize. In fact, some  
380 farmers do not return residues to the soil because they fear N immobilization, and this results in a principle  
381 barrier to their incorporation (Bechini et al., 2015). Our results show that the incorporation of maize straw  
382 into the soil did not increase the fertilization requirement, at least in the fertile environment tested.  
383 Therefore, in the long run, the N<sub>c</sub> term could be neglected. On the contrary, as crop residues increase SOM  
384 content, they are theoretically expected to enhance the M<sub>so</sub> term in the long-term (e.g. Lehtinen et al.,  
385 2014), but the increase in plant N uptake we observed was limited and not significant.

386

387 **4.4 Effect of rotation with leys**

388 Plowing a grass or lucerne rotational ley before the subsequent maize crop resulted in an efficient release  
389 of 27 kg N ha<sup>-1</sup> yr<sup>-1</sup> to the following crop, and as it was relevant for two years, the total net extra supply was  
390 54 kg N ha<sup>-1</sup>. Due to the low C:N ratio of grass and legume (C/N = 19, on average), residues were easily  
391 mineralized and could contribute to maize nutrition to a larger extent than to SOM stock buildup. The  
392 opposite effect of crop residues and ley residues on N uptake and stable SOM stock could be related to  
393 different N mineralization rates, according to the type of organic matter incorporated in the soil.

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

397

398 **4.5 Crop yield and N uptake**

399 In the Northern Italy Po Plain, where the Tetto Frati long-term experiment is situated, maize cropping  
400 systems are highly productive. When N fertilization was limited to 170 kg N ha<sup>-1</sup>, crop yield and N uptake

401 were severely reduced; FYM plots produced only 64% of the yield and 69% of N removal in the best  
402 performing treatments at the site. These same values for SLU were 61% and 63%, which indicate that in  
403 Nitrate Vulnerable Zones, where the limit of 170 kg ha<sup>-1</sup> of manure-N is adopted, a relevant integration with  
404 mineral fertilizers is needed to sustain yield.

405 Crop yield and N removal were higher when FYM was applied than when SLU was used, possibly because of  
406 a slower release of N and an increase of SON caused by FYM over the long-term (Webb et al., 2013).

407 Comparison of the Derogation (250 kg N ha<sup>-1</sup>) and standard (170 kg ha<sup>-1</sup>) manure-N limits for both manure  
408 types suggested that at the high dose maize still utilized all manure N. Differences in yield and N uptake  
409 between the two doses were always significant when FYM was used, but only in the case of N uptake when  
410 SLU was used. Fertilization at 250 kg N ha<sup>-1</sup> was shown not to be excessive, as it was taken advantage of  
411 manure-N, thereby limiting the need for extra mineral fertilization. In this context, a Derogation allowing  
412 250 kg ha<sup>-1</sup> of manure-N is a good option in high performing systems such as those analyzed. Moreover,  
413 relative to the lower dose, the Derogation treatment supplied the soil with an extra 0.2-1.2 t ha<sup>-1</sup> of C from  
414 manures that likely improved C sequestration—an effect that requires future experimental confirmation.

415 All entirely-harvested maize systems proved to demand high N fertilization levels, regardless of the  
416 presence of Italian ryegrass as a secondary crop or of rotational grass/lucerne leys. On the contrary, the  
417 harvest of grain alone reduced the fertilization requirement by 32-86 kg N ha<sup>-1</sup> at the low dose and by 70-  
418 140 kg ha<sup>-1</sup> at the high dose. Indeed, this system does not benefit from the Derogation.

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

426

## 427 **5. Conclusions**

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

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

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

451

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457

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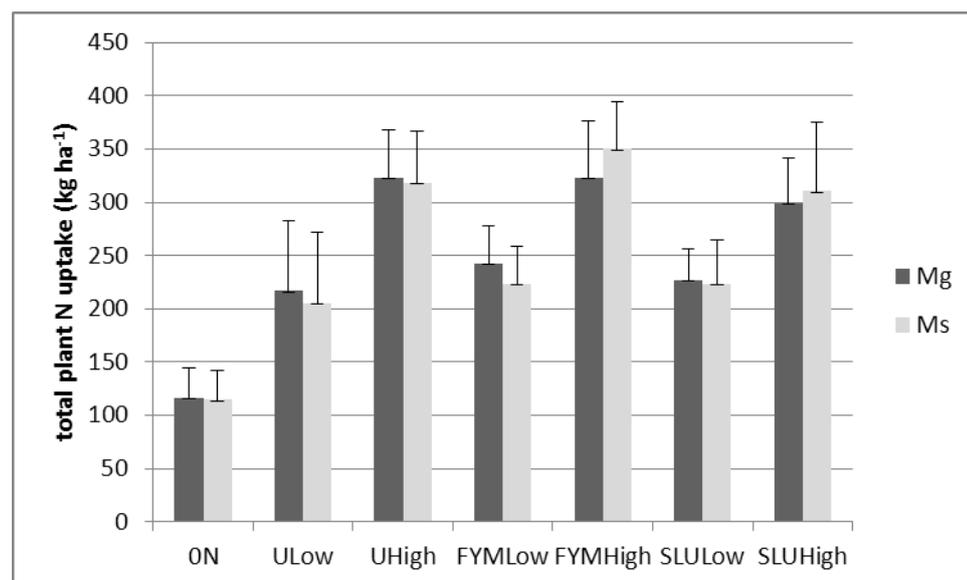
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Figure 1. Effects of crop residue incorporation a) on total plant N uptake and b) on SON stock. Residues were incorporated into the soil in the Mg system and removed from the Ms system. Mean (boxes) and annual standard deviation (T-bars) are provided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

a)



b)

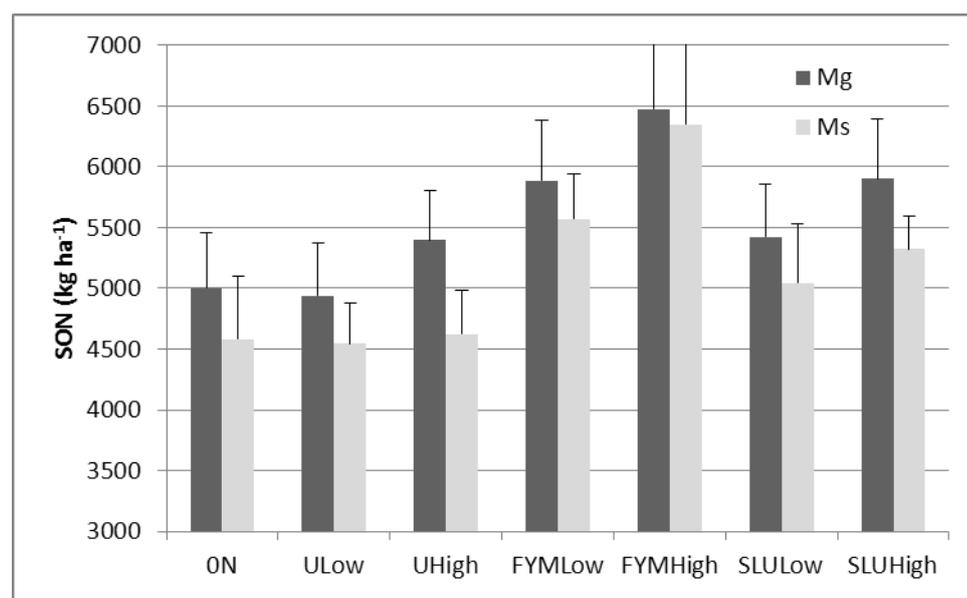
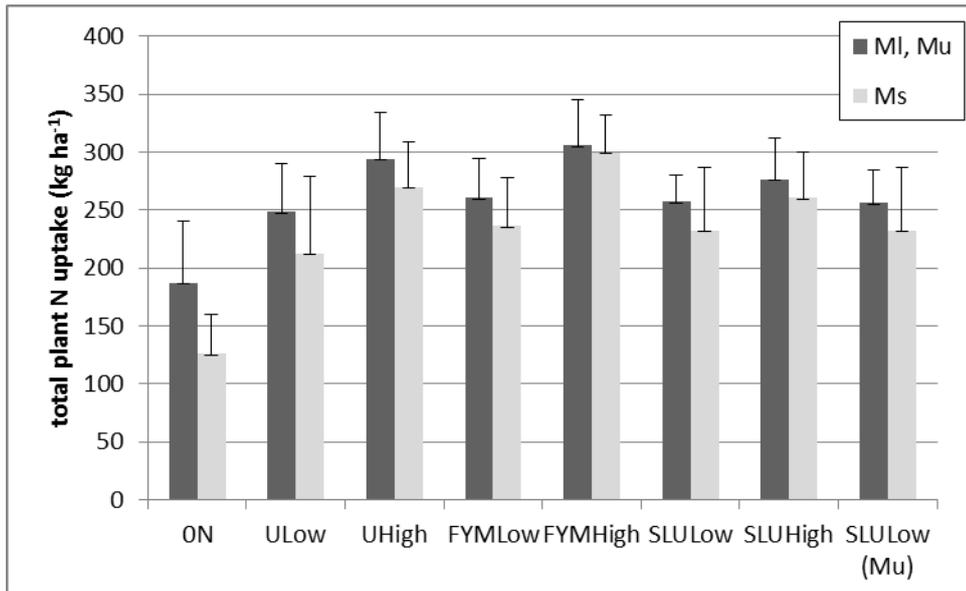


Figure 2. Effects of rotation with grass ley (MI) or legume ley (Mu) compared with monoculture (Ms) a) on annual maize N uptake in the first two years after ley; b) on SON stock. Means (boxes) and standard deviations (T-bars) of three phases: 1995-1996, 2002-2003, 2011-2012. ULow is the mean of U100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

a)



b)

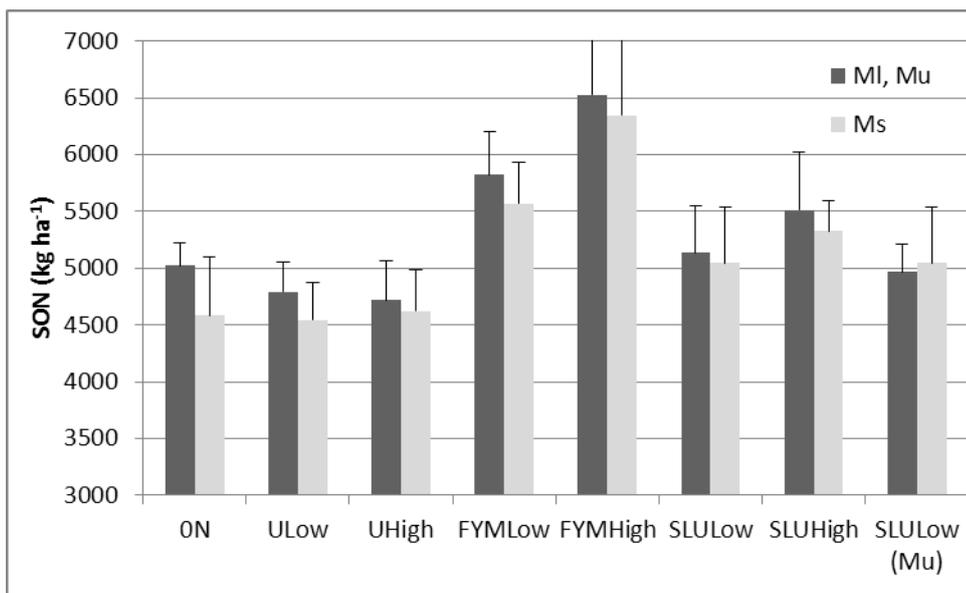


Figure 3. Increment of annual N uptake by rotational maize (MI, Mu) with respect to monoculture (Ms) as an average of the first two years after ley, as a function of fertilizer N supply.

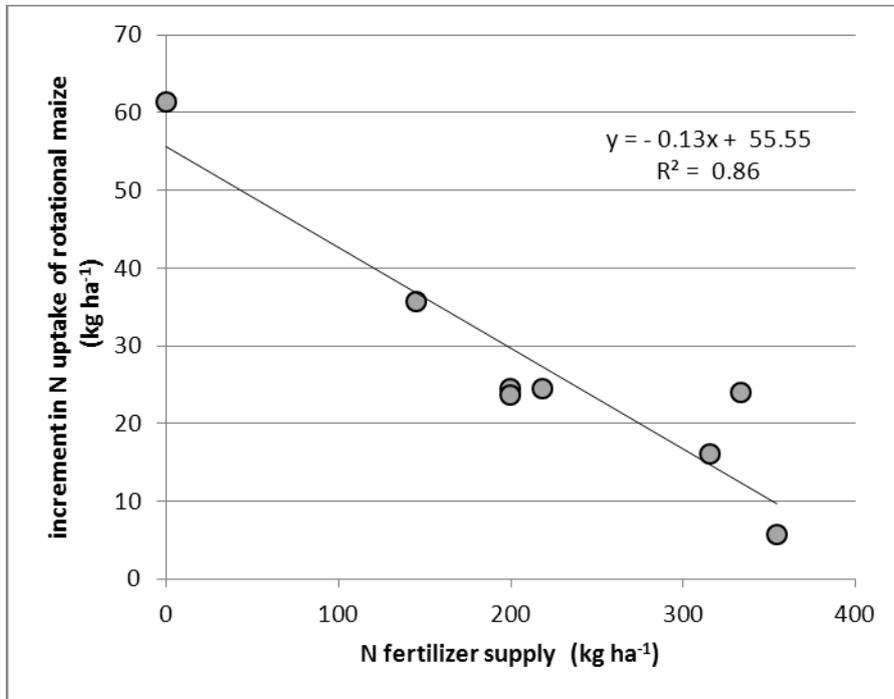


Figure 4. Maximum dry matter yield and N uptake registered in the trial among all treatments across all experimental years.

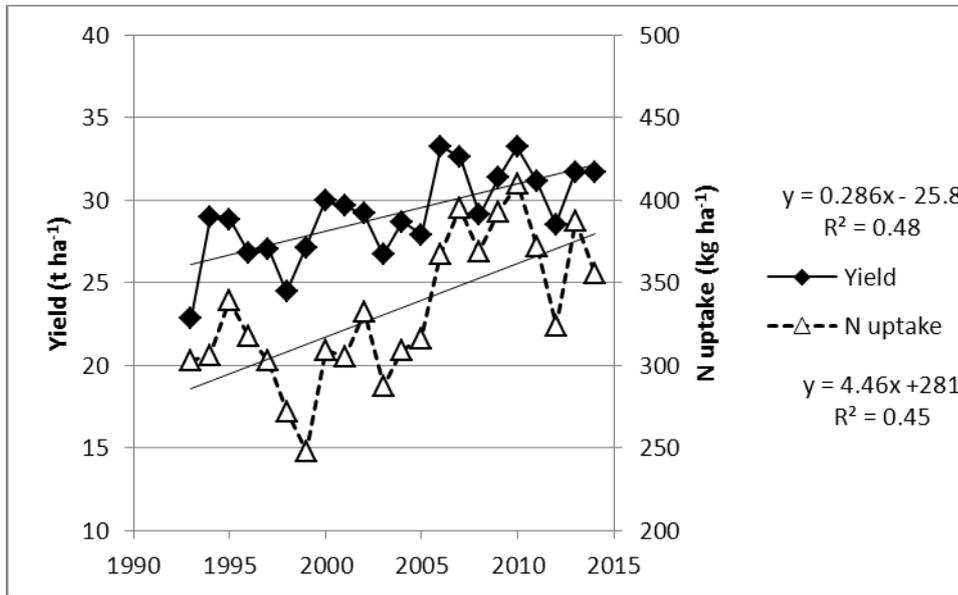


Table 1. Nitrogen fertilization amounts supplied to treatments in the Tetto Frati LTE. Top dressing was always supplied as urea at ridging. All values are stated in kg N ha<sup>-1</sup>.

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

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.

Fertilizer	Dry matter %		Total N g kg <sup>-1</sup>		NH <sub>4</sub> -N g kg <sup>-1</sup>		P g kg <sup>-1</sup>		K g kg <sup>-1</sup>		C g kg <sup>-1</sup>	
	Mean	<i>s.d.</i>	Mean	<i>s.d.</i>	Mean	<i>s.d.</i>	Mean	<i>s.d.</i>	Mean	<i>s.d.</i>	Mean	<i>s.d.</i>
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 4. Yield ( $\text{t ha}^{-1}$  of dry matter) and plant N removal ( $\text{kg N ha}^{-1}$ ) in the marketable yield of Mg (grain only), Mr (sum of maize and ryegrass), and MI (average of maize and grass ley), as a function of the N fertilizer dose ( $\text{kg ha}^{-1}$ ) 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								
	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
MI	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
MI	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  $\text{g } 100\text{g}^{-1}$ , as a function of N fertilizer dose supplied through organic fertilizers. a) years 2007-2011 and b) years 2012-2014. Rotational maize in MI 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 - lt. ryegrass	1.01	0.23	1.07	0.28	1.03	0.16	1.02	0.27
MI - 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 - lt. ryegrass	0.75	0.05	0.89	0.22	0.71	0.04	0.79	0.14
MI - grass (2 years)	1.80	0.21	2.19	0.51	1.80	0.21	1.95	0.44