

Replacing organic with mineral N fertilization does not reduce nitrate leaching in double crop forage systems under Mediterranean conditions

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Abstract: This research evaluated the impact of four nitrogen (N) fertilizer management systems on nitrate losses in an irrigated forage system under Mediterranean conditions within a Nitrate Vulnerable Zone (NVZ). The experiment was conducted from June 2009 to May 2012 in an intensive dairy cattle farm that produces silage maize and Italian ryegrass in a double cropping system. A monthly monitoring of the nitrate concentrations in the soil solution was carried out using 10 cm diameter disc lysimeters. The N fertilization systems had a target N application of 316 and 130 kg ha⁻¹ for maize and Italian ryegrass, respectively. Four systems were compared: cattle manure (MA); cattle slurry (SL); cattle slurry + mineral N fertilizer (SM); mineral N fertilization (MI). A clear seasonal dynamics of nitrate concentration was observed in the three years and was similar among treatments, with maximum occurring in autumn-winter. On average, at soil depths between 50 and 90 cm, nitrate concentrations in the soil solution were intermediate for the MI treatment (146 ± 10.4 mg L⁻¹), in between those of SL or SM (202 ± 11.3 and 164 ± 9.4 mg L⁻¹ respectively) and MA (78 ± 4.9 mg L⁻¹). Despite the high average concentrations, only in some sampling dates the nitrate concentration was significantly higher than 50 mg L⁻¹. The estimated annual N leaching losses below 90 cm soil depth ranged from 42 (MA) to 110 (SL) kg N ha⁻¹.

These findings highlighted that, under Mediterranean conditions and with the N input rates, nitrate leaching in autumn-winter cannot be easily controlled through N fertilizer management because it is mainly associated to the natural water surplus and the low N uptake from the winter crop. The cattle manure has proved to be the most conservative in terms of N leaching, while replacing organic with mineral sources of N did not reduce nitrate leaching.

Highlights

- The impact of mineral and organic fertilizers on nitrate leaching was assessed
- Disc lysimeters were used for the nitrate content monitoring in the soil solution
- Cattle manure proved to be the most conservative in terms of nitrate content
- The replacement of organic with mineral fertilizer did not reduce nitrate leaching
- Autumn-winter soil water nitrates were almost independent from fertilizer systems

1 Replacing organic with mineral N fertilization does not reduce nitrate leaching in double crop
2 forage systems under Mediterranean conditions

3

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20 **Abstract**

21 This research evaluated the impact of four nitrogen (N) fertilizer management systems on nitrate
22 losses in an irrigated forage system under Mediterranean conditions within a Nitrate Vulnerable
23 Zone (NVZ). The experiment was conducted from June 2009 to May 2012 in an intensive dairy
24 cattle farm that produces silage maize and Italian ryegrass in a double cropping system. A
25 monthly monitoring of the nitrate concentrations in the soil solution was carried out using 10 cm
26 diameter disc lysimeters. The N fertilization systems had a target N application of 316 and 130
27 kg ha⁻¹ for maize and Italian ryegrass, respectively. Four systems were compared: cattle manure
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30 among treatments, with maximum occurring in autumn-winter. On average, at soil depths
31 between 50 and 90 cm, nitrate concentrations in the soil solution were intermediate for the MI
32 treatment ($146 \pm 10.4 \text{ mg L}^{-1}$), in between those of SL or SM (202 ± 11.3 and $164 \pm 9.4 \text{ mg L}^{-1}$
33 respectively) and MA ($78 \pm 4.9 \text{ mg L}^{-1}$). Despite the high average concentrations, only in some
34 sampling dates the nitrate concentration was significantly higher than 50 mg L^{-1} . The estimated
35 annual N leaching losses below 90 cm soil depth ranged from 42 (MA) to 110 (SL) kg N ha⁻¹.
36 These findings highlighted that, under Mediterranean conditions and with the N input rates,
37 nitrate leaching in autumn-winter cannot be easily controlled through N fertilizer management
38 because it is mainly associated to the natural water surplus and the low N uptake from the winter
39 crop. The cattle manure has proved to be the most conservative in terms of N leaching, while
40 replacing organic with mineral sources of N did not reduce nitrate leaching.

41

42 **Key words:** disc lysimeter, silage maize, Italian ryegrass, cattle slurry, manure, Nitrate
43 Vulnerable Zone.

44

45 **Introduction**

46 Intensive, grain-fed livestock production systems are considered a major source of nitrate (NO_3^-)
47 leaching and pollution. The mitigation of NO_3^- leaching from agricultural soils has become of
48 global outstanding relevance (Hooda et al., 2000). The European Nitrate Directive (ND,
49 1991/676/EEC) intends to reduce diffuse NO_3^- pollution of water bodies by designating Nitrate
50 Vulnerable Zones (NVZs) that need to be managed in a way that prevent NO_3^- buildup. The ND
51 established a 50 mg L^{-1} threshold for NO_3^- concentration in ground and surface water (European
52 Union, 1998). In NVZs, the use of organic fertilizers is restricted to 170 kg N ha^{-1} with a
53 restricted time window for its application (e.g. between November and February in the
54 Mediterranean area). The only restriction for the mineral fertilizer supply is that of not exceeding
55 crop requirements. This is because mineral fertilization is assumed to have higher efficiency than
56 organic fertilizers (Hernández et al., 2013). Moreover, the ND does not make any distinction in
57 terms of amount or timing between different types of organic fertilizers, assuming that the
58 amount of leached N is not influenced by the organic N source and management.

59 The implementation of ND prescriptions has also often resulted in increased costs for crop
60 fertilization and particularly for disposing the excess N from the farm effluents out of the NVZ
61 (de Roest et al., 2007).

62 The impact of different N fertilization sources on NO_3^- leaching is not clearly established since
63 NO_3^- dynamics are controlled mainly by a complex set of interrelationships between rainfall
64 patterns (Fang et al., 2013), irrigation (Jamali et al., 2015) and fertilization management
65 practices, as well as by soil type and cropping systems (Jabloun et al., 2015; Barros et al., 2012).

66 In many NVZs, the increased demand for high-quality milk has led to an evolution of the dairy
67 cattle housing from the bedded-pack to the cubicle housing system. This resulted in a higher
68 production of slurry than farmyard manure (Phillips, 2010), even though the bedded pack system
69 was recognized to be compatible with high quality milk and better animal welfare (Atzori et al.,
70 2009 a).

71 The development of site-specific sustainable fertilization management options for mitigating
72 NO_3^- pollution is therefore a key priority for policy decision makers and land managers
73 (Zavattaro et al., 2012).

74 The NO_3^- pollution is an issue in Mediterranean semi-arid countries (Daudén et al., 2004). In the
75 Mediterranean regions of the northern hemisphere, 50-90% of total NO_3^- losses by leaching were
76 found to occur particularly between October and February (De Paz et al., 2009; Trindade et al.,
77 1997; Goss et al., 1988). The application of N fertilizers in autumn reinforces the natural
78 accumulation of NO_3^- in the soil at the start of the rainy period (Trindade et al., 2009), thus
79 promoting significant N leaching (Trindade et al., 1997). The dynamic of these processes relates
80 to both the N source and application scheme.

81 Experiments in Mediterranean conditions showed that the NO_3^- leaching potential from the
82 application of slurries could be lower than those from N mineral fertilizer application (Trindade
83 et al., 2009; Daudén and Quílez, 2004). However, it was also recognized that NO_3^- leaching can
84 be a serious problem when organic effluents are used (Giola et al., 2012; Daudén et al., 2004;
85 Chambers et al., 2000; Trindade et al., 1997). Hence it is still an open question whether the
86 replacement of organic with mineral N fertilizers can effectively mitigate NO_3^- leaching in NVZ
87 and if the type of organic effluent can make a difference in improving the environmental
88 sustainability of intensive dairy farming under Mediterranean conditions. This issue is
89 particularly relevant for intensive livestock farming areas under Mediterranean conditions, like
90 the district of Arborea (Sardinia, Italy). This farming district is characterized by very high
91 stocking rate and high productivity of its dairy cattle system, one of the highest in Europe
92 (Manca, 2009), with a stocking rate of some 5 livestock unit ha^{-1} . In these conditions, the annual
93 animal effluent production is somehow double than the maximum applicable to farmland
94 according to the ND (Nguyen et al, 2014). This study showed that to comply with the ND,
95 Arborea farmers must buy additional mineral N fertilizer to meet the total N crop requirements
96 and must also rent additional land or pay to dispose the cattle effluents outside the NVZ, thus

97 increasing the production costs. This situation is clearly unsustainable and has given rise to
98 controversy about the effectiveness of the ND measures to mitigate NO_3^- pollution while
99 maintaining the profitability of the dairy farming.

100 The objective of this study was to compare a range of N fertilization options for dairy farming
101 systems in NVZ under Mediterranean conditions, to support the development of sustainable
102 fertilization strategies by exploiting the recycling of the farm N resources.

103 In this paper, we tested two hypotheses: i) in the Mediterranean intensive forage systems, the
104 seasonal dynamics of the NO_3^- concentrations in the soil solution is very wide and can be
105 effectively controlled by the fertilization management systems; ii) the mineral fertilizers are less
106 impacting on NO_3^- losses than organic fertilizers in NVZ.

107 We measured the dynamics of the NO_3^- concentration in the soil solution and assessed the
108 potential N leaching as influenced by four N fertilization management systems for an irrigated
109 double forage cropping system in sandy soil under Mediterranean conditions.

110

111 1. **Material and methods**

112 1.1. **Site and crop management**

113 The field experiment was conducted from June 2009 until May 2012 in a private farm located in
114 the NVZ of Arborea, Italy (39°47' N, 8°33' E, 3 m asl). In the district of Arborea, some 28,000
115 Bovine Livestock Units are reared by 160 farms on a 5,000 ha irrigated plain. This plain was
116 drained in the 1930's from wetlands and salt marshes. The groundwater table dynamics is
117 somehow regulated by the local Water User Association dewatering pump system, which
118 controls the drainage of the whole area. The groundwater table depth was monitored during the
119 three year of experiment through 12 piezometers installed in the experimental field.

120 In this area, about two thirds of the livestock effluent volume is represented by slurry and one
121 third by manure. Considering the N content of the two effluents, the N load from manure and
122 slurry in this NVZ is approximately 38 % and 62 %, respectively.

123 The climate is Mediterranean and the mean annual temperature and precipitation are 16.7 °C and
124 568 mm, respectively (1959-2012). Some 73 % of the annual rainfall occurs between October
125 and March and the average annual aridity index (rainfall/reference evapotranspiration) is 0.49
126 (semi-arid area). The soil was classified as Psammentic Palexeralfs (USDA, 2006) and the soil
127 slope was less than 0.5 %. The soil properties are reported in Table 1. The field capacity of soil
128 profile was determined according to Romano and Santini (2002). The water balance dynamic
129 from May 2009 to June is shown in Figure 1. The common forage cropping system is based on
130 the double-crop rotation of silage maize (*Zea mays* L.) grown from June to September and Italian
131 ryegrass (*Lolium multiflorum* Lam.), grown from October to May. In this cropping system,
132 organic fertilizers (slurry and manure) are applied regularly prior to sowing of each crop and
133 combined with mineral fertilization. During the Italian ryegrass cycle, mineral fertilizers are
134 often applied at the end of the winter to compensate for insufficient soil mineral N availability,
135 while maize is fertilized in summer either at seeding or at the 4-6 leaves stage.

136 We compared four fertilization systems at the same target N rate (316 and 130 kg ha⁻¹ for maize
137 and ryegrass respectively), set on the basis of the N fertilization prescriptions for NVZs, and of
138 the business as usual local fertilization practices, which should correspond to the expected N
139 uptakes of silage maize and Italian ryegrass. The experimental design was a randomized
140 complete block design with four replicates and a plot size of 12 m × 60 m. The treatments were:

141 i) manure (MA): cattle farmyard manure applied before the sowing of each crop with a
142 conventional spreader and followed by rotary tillage. About 70 % of the total amount was spread
143 to maize at the end of May and 30 % to ryegrass in October;

144 ii) slurry (SL): cattle slurry applied before sowing with a conventional spreader and immediately
145 incorporated into the soil through rotary tillage using the same proportions as the MA treatment
146 for maize and ryegrass;

147 iii) slurry+mineral (SM): following the NVZs prescriptions, slurry was applied at a
148 corresponding target rate of 100 and 70 kg ha⁻¹ of N for maize and ryegrass respectively and

149 mineral fertilizer (ENTEC 26[®]) at a rate of 216 and 60 kg ha⁻¹ of N applied before sowing for
150 maize and at the end of ryegrass tillering respectively;

151 iv) mineral (MI): mineral fertilizer (ENTEC 26[®]) applied before sowing for maize and at the end
152 of ryegrass tillering (mid February - beginning of March).

153 The amount of total N rate actually supplied with organic fertilizers (Table 2) was calculated *ex*
154 *post* on the basis of the volume of fertilizer distributed and its actual nutrient concentration. The
155 fertilizer was sampled from the field spreader at each distribution event, for a total of six
156 sampling dates. Four and eight samples per sampling date were collected respectively for manure
157 and slurry and stored frozen until the determination of total N content. The cattle slurry was
158 stored in concrete storage pits at the farm and was homogenized prior to surface application
159 using a vacuum-tank spreader. The cattle manure was stored for five months before spreading
160 and it was sampled just before application.

161 A mixture of four varieties and hybrids of Italian ryegrass (cultivars Meritra, Ivan, Littorio and
162 Mowester) was sown around mid-October each year. Seedbed was prepared by harrowing and
163 milling. Auxiliary sprinkler irrigation was provided to ryegrass when necessary to minimize crop
164 water stress (a total of about 80 mm per cycle). Ryegrass hay was harvested in mid-May at early
165 earing stage. The Calcio hybrid (FAO 700) of maize was sown during the first ten days of June
166 and it was harvested between the 15th and the 20th of September at the dough stage (target of 33
167 % DM for the whole plant). The maize seedbed was prepared using a rotavator, ripper and
168 harrow. The sprinkler irrigation for maize started at seeding in June until one week before
169 harvest. The total water volumes were on average 4600 m³ ha⁻¹. Weeds were controlled with the
170 pre-emergence herbicide Lumax[®].

171 **1.2. Soil solution sampling and analysis**

172 Two tension disc lysimeters (10 cm diameter, PRENART[®]) were installed in each plot below the
173 Ap soil horizons, at soil depths between 50 and 90 cm (average depth 68 cm). A total of 32 disc
174 lysimeters were installed. Special care was taken in repacking the soil from different layers in the

175 original position. The disc lysimeters were connected to aboveground with plastic pipes at the
176 border of each plot. Near the soil surface a 20 cm rubber collar was put around the plastic pipe to
177 prevent water from seeping from the surface down through the pipe. The soil solution was
178 sampled through the disc lysimeters at monthly intervals by applying a suction of -70 kPa for
179 about 45 minutes using an electric pump. Before collecting the sample to be analyzed, pipes
180 were cleaned out of the water left from the previous sampling in the connecting pipe and disc
181 lysimeter. Collected samples were stored under cooled conditions (4 °C) and filtered in the lab
182 through a 0.2 μm membrane filter. The NO_3^- concentration was determined by ion
183 chromatography (anion column Alltech model allsep anion 7 μm , 100 mm).

184 **1.3. Water balance**

185 The water balance of the maize-Italian ryegrass cropping system was estimated using the EPIC
186 model (Williams, 1995). The model was set using local soil and weather data, information about
187 the actual management, including irrigation volumes and amount of fertilizers distributed, and
188 crop related data such as plants density and crop growing period. EPIC simulated actual
189 evapotranspiration from the Penman Monteith equation considering the actual crop management.
190 Daily weather data were collected from a weather station located about 5 km away from the
191 experimental field ($39^{\circ}45'$ N; $8^{\circ}34'$ E. 13 m asl, OR, Italy). Soil characteristics were obtained
192 analyzing soil samples collected in the experimental field and water holding capacity was
193 measured in the field through systematic gravimetric and tensiometric measurements between
194 saturation and field capacity. The actual physical and chemical soil characteristic (including the
195 hydraulic properties) were used as inputs in the EPIC model. Because the groundwater table
196 depth is controlled by the local Water Use Association (WUA), part of the subroutine that
197 simulates the groundwater table dynamics in EPIC was modified considering a deeper
198 groundwater table in spring and summer and a shallower groundwater table in autumn and
199 winter. The modification in EPIC allowed to overwrite the initial maximum and minimum depth
200 for the groundwater table fluctuation and to use different maximum and minimum depths in

201 different periods of the year. The model was calibrated for the crops yield and ground water table
202 dynamics. For each crop a total of 12 yield observations (3 years \times 4 replicates) were available.
203 Six observations were used for the calibration and the remaining six observations were used for
204 the validation. For the groundwater table depth, a total of 24 observations were available from
205 November 2009 to May 2012. The first 12 observations were used for the calibration and the last
206 12 observations were used for the validation. The calibration and validation were evaluated using
207 relative root mean square error (RRMSE; Bellocchi et al., 2002), modeling efficiency (EF;
208 Loague and Green, 1991), slope and intercept of the regression line and coefficient of
209 determination (R^2). After the calibration and validation, the EPIC model was used to estimate the
210 percolation below the average lysimeters depth. The calculation of the total percolation,
211 including groundwater table fluctuations, was performed for those days in which a water surplus
212 (Precipitation + Irrigation $>$ ET) occurred.

213 **1.4. Nitrate leaching and N balance**

214 Assuming that the soil water percolation between two soil water sampling dates had the average
215 NO_3^- concentration of the sampled soil water, the amount of NO_3^- leached (kg N ha^{-1}) was
216 calculated using the average NO_3^- concentration between two subsequent sampling dates times
217 and the volume of percolation between the two dates as estimated with EPIC.

218 The cumulative N leaching was estimated on the basis of average NO_3^- concentration and the
219 soil water percolation (as simulated by the EPIC model) for each sampling date.

220 Crop yield, aboveground biomass at harvest and N removal were measured every year but in this
221 paper we report only the data on crop N removal. The entire plots were harvested using farm
222 machineries and the removed products (fresh aboveground maize or ryegrass hay) were weighed
223 immediately using electronic weighing cells positioned in a flat place under the tractor cart. The
224 biomass dry matter content at harvest was assessed by sampling 1 kg of ryegrass hay or fresh
225 chopped maize that was immediately cooled in plastic bags and hence dried in a forced-air oven
226 at 65 °C for 72 h. The crop N removal was estimated by multiplying the crop yield and N

227 concentration determined on harvested biomass samples by the Kjeldahl method (Mipaaf, 2006).
228 N surplus was defined as the difference between N fertilizer input at plot scale and crop N
229 removal (Grignani and Zavattaro, 2000). While, combining the data on crop N removal and
230 leached N we also estimated by difference the residual soil N pools available for N
231 immobilization, volatilization or denitrification.

232 **1.5. Statistical analysis**

233 The NO_3^- concentration and yearly cumulative N (removal, leaching and residual) were analyzed
234 with the PROC MIXED procedure in SAS (SAS institute, 1999), suitable for analyzing mixed
235 effects and repeated measures with non-constant variance and any covariance structure models.
236 The independence assumption on the error terms required for the ANOVA of a factorial model
237 (Montgomery, 1997) was likely not met. Therefore, the appropriate assumption on the error
238 terms for this experiment was the non normal distribution with heterogeneous (non-constant)
239 variance by sampling date and a given covariance structure. The appropriate covariance structure
240 for this particular experiment was determined using -2 Log Likelihood (-2RLL), Akaike's
241 Information Criterion (AIC), Corrected Akaike's Information Criterion (AICC) and Schwarz's
242 Bayesian Criterion (BIC), which are essentially log likelihood values penalized for the number
243 of parameters estimated (Littell et al., 1996). Also, according to the fit statistic -2RLL , AIC
244 AICC and BIC, the best covariance structure was determined to be the Compound Symmetry
245 covariance type. The statistical test results given in the following section are based on the
246 transformation of original NO_3^- concentration data as reported Shen et al. (2006).
247 A one-tailed t test was performed on data from each sampling date to determine whether
248 treatments significantly exceed the EU threshold of 50 mg L^{-1} of NO_3^- concentration using the
249 pooled error variances when appropriate.

250 All data analyses were performed using Microsoft Excel® and SAS Statistical Package (SAS
251 institute, 1999).

252

253 2. Results

254 2.1. Nitrate concentration in the soil solution

255 The average concentration of NO_3^- in the investigated soil depths showed a high variability and
256 it fluctuated from 0.8 ± 0.3 (mean \pm SE, June 2010) to 586 ± 122 (November 2011) mg L^{-1} in
257 MA, from 6 ± 3 (May 2010) to 593 ± 104 (January 2012) mg L^{-1} in SL, from 13 ± 3 (June 2009)
258 to 460 ± 54 (December 2011) mg L^{-1} in SM and from 2.6 ± 0.4 (June 2009) to 390 ± 25
259 (December 2010) mg L^{-1} in MI (Figs. 2-4).

260 The dynamics of NO_3^- concentration (mg L^{-1}) in the soil solution showed a strong seasonal
261 pattern for all treatments, characterized by two maximum values, one in the winter, during the
262 ryegrass crop phase, and one in the summer, during the maize crop phase (Figs. 2-4). Minimum
263 values were typically observed at ryegrass and maize harvest, in late spring and September. In all
264 treatments, the NO_3^- concentrations decreased to reach a minimum in April and May, with the
265 exception of spring 2012 (Fig. 4), when the observed NO_3^- concentrations remained high until
266 the end of April in all treatments. Plots receiving the MI treatment showed high NO_3^-
267 concentration in all autumns, even though N fertilizer was not applied until February.

268 In the three years, the mean NO_3^- concentration significantly exceeded the 50 mg L^{-1} on 5, 14,
269 15 and 8 dates out of the total 33 sampling dates in MA, SL, SM and MI respectively (Figs. 2-4).

270 Of these dates, 5/5, 8/14, 7/15 and 7/8 respectively in MA, SL, SM and MI occurred in the
271 November-February period and the remaining occurred in the spring 2012.

272 Of the 33 sampling dates, only 6 showed a significant difference between treatments. In
273 particular, 4 dates in the third year (Jan-Mar-Apr-May) had higher NO_3^- concentration in SL
274 with respect to MI and MA, while SM was not significantly different from all the other
275 treatments. In December 2010 the NO_3^- concentration was lower in MA treatment than in SL
276 and MI treatments, despite the mineral fertilizer was not applied yet.

277 The lowest average NO_3^- concentration was observed in MA. In 2009 and 2010 MA showed an
278 average annual NO_3^- concentration of about $46 \pm 16 \text{ mg L}^{-1}$, but in 2012 the NO_3^- dynamics of
279 MA was similar to that observed in the other treatments (Fig. 4).

280 **2.2. EPIC model calibration and validation**

281 Model performance for crop yields and groundwater table dynamics were satisfactory for all the
282 data considered and for both the calibration and validation phases (Table 3). For silage maize the
283 RRMSE values indicate small deviations of the simulated values from the observed ones and the
284 $\text{EF} > 0$ indicates that the model is a good predictor of the observed values. For Italian ryegrass
285 the deviations of the model results from the observed values were acceptable in terms of RRMSE
286 and good for EF values. The model was also able to reasonably simulate the variation during
287 time of the groundwater table depth. The deviation of the simulated data from the observed data
288 was about 26 % and 20 % during the calibration and validation process respectively and the EF
289 value was of 0.37 for the calibration and 0.54 for the validation. Moreover, the slope and
290 intercept of the regression line were close to the optimal values.

291 **2.3. Soil water percolation**

292 The estimated average annual percolation simulated by EPIC was 252 mm, 292 mm and 268 mm
293 during June-May in 2009-2010, 2010-2011 and 2011-2012, respectively. In all years, percolation
294 events occurred almost exclusively from October to February with an influence of cumulative
295 monthly precipitation (y) on estimated cumulative monthly percolation (x), as evidenced by the
296 significant linear regression between these two variables ($y = 0,327x + 4,891$; $R^2=0.66^{**}$). The
297 average soil water percolation in October-January was about 130 mm and it represented 1/2 of
298 the total annual percolation (60 % between Oct-Feb). The rainfall in autumn 2010 (392 mm in
299 Nov-Dec) was considerably higher than the long term average recorded for the study area (158
300 mm) and this was mirrored by the calculated drainage volumes (Fig. 3). From April to
301 September no relevant drainage was estimated, the percolation was on average about 60 mm as
302 the total water supply (730 mm = 194 mm rain and 536 mm irrigation) rarely exceeded crop

303 transpiration demands (670 mm). The groundwater table depth ranged between 0.25m (in
304 January-February) and 1.80 m in July-August. Groundwater table depth reached the highest
305 values from June to September, while the water table was the shallowest during the autumn from
306 November to February in coincidence with rainfall events.

307 **2.4. Nitrate leaching and N balance**

308 The average N concentration of slurry was 0.26 % but ranged between 0.09 and 0.47, while that
309 of manure was 0.71 % (0.45 – 0.83). The average ammonia N concentration was 0.12 % in slurry
310 and 0.17 % in farmyard manure. The nitric nitrogen was 0.002 % in slurry and 0.01 % in
311 manure.

312 The N input of organic fertilizers varied widely (Tab. 2) according to the variable N
313 concentration of the animal effluent.

314 The C:N ratio and the organic N concentration in the animal effluents were respectively $8.01 \pm$
315 0.85 (mean \pm SE) - 0.14 ± 0.01 % for slurry and 13.02 ± 1.17 - 0.51 ± 0.04 % for manure.

316 The estimated N leaching and residual N were significantly influenced by the fertilization
317 treatment ($P < 0.05$) and no significant year \times treatment interaction was observed (data not
318 shown). The estimated residual N was variable among years, particularly for SL. The exception
319 was the MI treatment, in which residual N variability was much lower than all other treatments,
320 likely due to a lower and more constant rate of N inputs relative to that observed for organic
321 fertilizers ($r = 0.47$, ns). For this reason, N inputs on SL and SM explained as much as 97 % of
322 the variability of the residual N ($P < 0.01$), while there was no linear correlation between N input
323 and N leaching considering all treatments ($r = 0.42$) or between N surplus and N leaching ($r =$
324 0.30 , ns). The estimated annual N leaching losses below 90 cm soil depth were reported in Table
325 4. The relative proportion of the N surplus lost with leaching was high (62 %) just for MI while
326 it represented less than about 17 % of the surplus when organic fertilizers were used.

327 On average, N removal roughly represented between 1/3 (SL) and 1/2 (SM) of the N input in all
328 treatments with organic fertilizers, while it reached 2/3 of the total N input in MI (Tab. 4). N
329 leaching in MA was 32 % lower than the average of the other fertilization systems.

330 The dynamics of the cumulated N leaching showed the same pattern in the three years of
331 experiment (Fig.5). On average, 78 % of the total N leaching was in autumn (44 %) and winter
332 (34 %). Almost no soil water percolation and hence no leaching was recorded during the maize
333 crop phase, despite the relatively high NO_3^- concentration in soil solution, but we estimated a
334 high percolation in April.

335 The total cumulated annual water drainage simulated by EPIC was reported in previous
336 paragraph. If the NO_3^- losses were expressed as a function of the cumulative drainage (data not
337 shown), the loss of NO_3^- corresponding to a drainage concentration of 50 mg L^{-1} of NO_3^- was
338 28.4, 33.1 and 25.9 kg ha^{-1} in year 1, 2 and 3 respectively. The estimated NO_3^- losses reached
339 these thresholds between 125 mm and 170 mm of drainage in MI and MA respectively in the
340 first year; between 60 mm and 125 mm in SL and MA in the second year and about 35 mm for
341 all treatments in the third year.

342 **3. Discussion**

343 The NO_3^- concentration dynamics in the soil solution was very variable during the year and was
344 influenced by weather and crop N uptake. This was consistent with our first hypothesis. About
345 68 % of the average total annual NO_3^- leaching occurred between October and February, which
346 corresponds to the period in which 57 % of the annual percolation occurred, as a consequence of
347 the unbalance between rainfall and evapotranspiration, as usual under Mediterranean conditions
348 (Carneiro et al., 2012; Arregui and Quemada, 2006). In this period, N uptake of Italian ryegrass
349 is very low as the crop is in the establishment-tillering phase (Carneiro et al., 2012; De Paz et al.,
350 2009).

351 A wide variation in N concentrations in soil water collected using the same methodology under
352 similar agro-environmental conditions of this study was also observed by Carneiro et al.(2012)

353 and Trindade et al. (1997). The wide variation in the soil water NO_3^- concentration at a given
354 sampling time was attributed to the heterogeneous distribution of fertilizers into the soil, to the
355 soil composition variability and the corresponding expected uneven infiltration pattern (Cuttle et
356 al., 1992). However, the wide variability of soil water NO_3^- concentration may also be related to
357 microbiological processes (Liang et al., 2010). Moreover, the high N content variability of
358 manure and slurry determined *a posteriori* in our study, led to application of a higher N supply
359 than the amount planned, and, hence, higher than crop requirements. This unintentional over-
360 application led to a high N surplus, as similarly reported also by Trindade et al. (2009),
361 generating a source of difficulty for the accurate comparison of NO_3^- concentration data in the
362 soil solution among the four N fertilization systems. The N input from SL and SM treatments
363 was significantly higher than that from MI treatment, while the nitrate leaching rates for SL, SM
364 and MI were similar. This was interpreted as possibly due to the ammonium N immobilization
365 through microbial decomposition of organic matter in the slurry after application, that plays a
366 significant role in the organic N retention in soil (Sørensen, 2004). This immobilised N is
367 stabilised and slowly released a few months after application (Sørensen, 2004). We can then
368 assume that much more N was immobilized after slurry or manure incorporation than after
369 mineral fertilizer application. Moreover, the significantly lower N leaching achieved from MA
370 treatment could be associated to the higher C:N ratio of farmyard manure than slurry. However,
371 neither the N input nor the N surplus showed a significant linear correlation with N leaching
372 considering all treatments, similarly to what reported by Morari et al. (2012) and Zavattaro et al.
373 (2012). In contrast to our results, Silgram et al. (2001) reported that the leaching losses are
374 linearly related to N inputs, over-simplifying a complex N loss function which depends on the
375 interactions between over-winter rainfall, soil type, cropping, and the rate/timing of
376 fertilizer/manure applications. Since in our study the NO_3^- leaching was estimated considering
377 only losses associated to percolation in the days when water surplus occurred (i.e.
378 Rain+Irrigation-ET), it is likely that the total N losses were underestimated. However, the rate of

379 estimated N leaching was not affected neither by the N input or N surplus and the percolation
380 was not very high, on average 271 mm per year. The shallow groundwater in autumn-winter
381 could have contributed with washing away to the N leaching rate, in particular under the winter
382 crop as shown by the drop of NO_3^- concentration at the end of the winter, while the contribution
383 of the deep groundwater table to NO_3^- leaching and maize water requirements was assumed to
384 be negligible.

385 The average monthly NO_3^- concentration was lower in April and May, except for the third year,
386 when we observed low winter rainfall (and percolation) and higher NO_3^- concentrations from
387 December to February and in July, as found for other irrigated Mediterranean cropping systems
388 (Ibrikci et al., 2015). In particular, the high NO_3^- concentrations were recorded after fertilization
389 and precipitation/irrigation events, in agreement with what found by Perego et al. (2012) in
390 summer on maize.

391 In the present study, the observed high soil water NO_3^- concentration in the autumn was
392 independent of the fertilization system, hence the NO_3^- concentrations in the soil solution were
393 not effectively controlled by the fertilization management. The potential impact of the presence
394 of the nitrification inhibitor under MI was assumed to be negligible below a soil depth of 40 cm
395 as reported Yu et al. (2007). In this period, the MI treatment had not been fertilized yet, but
396 showed high NO_3^- concentration. This could be interpreted hypothesizing that the maximum
397 NO_3^- concentrations observed in autumn were the outcome of the accumulation of NO_3^- in the
398 soil after the maize harvest, in a period in which soil temperature and water content were not
399 limiting for microbial activity (Liang et al., 2010), crop uptake was zero and the maize crop
400 residues were incorporated into the soil before ryegrass seeding. Although the residual amount of
401 soil NO_3^- after maize harvest was not measured in this experiment, we estimated it from the
402 NO_3^- concentration between two subsequent sampling dates (September and November) across
403 maize harvest dates and the soil water percolation volume estimated by EPIC. The outcomes of
404 this estimate showed that on average the estimated NO_3^- at maize harvest ranged from 10 kg N

405 ha^{-1} to 30 kg N ha^{-1} and this is in accordance to the results obtained from the application of the
406 linear relationship proposed by Andrasky et al. (2000) for 1.3-1.5 m soil depth. Several studies
407 reported that silage maize often shows high residual mineral soil N amounts ranging from 48 kg
408 N ha^{-1} under low N input up to 278 kg N ha^{-1} under high fertilized treatments (Morari et al.,
409 2012; Kayser et al., 2011) leading to high over-winter NO_3^- leaching (Trindade et al., 2009; Le
410 Gall et al., 1997; Simon and Le Corre, 1988).

411 The estimated N leaching loss is within the range reported by Carneiro et al. (2012), while
412 sometimes we found that the mean annual leaching was lower than that reported by Perego et al.
413 (2012) in Northern Italy, where the annual drainage (about 350 mm year^{-1}) was higher than that
414 observed in our experiment (about 271 mm year^{-1}). The magnitude of the losses was mainly
415 determined by the NO_3^- concentration in the soil solution in November and December.

416 The N mineralized between October and March may also have represented an important NO_3^-
417 source. Vertes and Decau (1992) estimated that 90 % of N leaching losses were originated from
418 N mineralization. Therefore, these authors recommended for preventing high soil NO_3^-
419 concentration in the autumn to apply conservative fertilization and cropping practices for the
420 summer crop to avoid high residual- NO_3^- values, in order to favour the early establishment of
421 the autumn–winter crops or to minimize the amount of N supplied at the winter crop, particularly
422 when sown late (Carneiro et al., 2012). The same authors suggested that the mineral N should be
423 applied only through top-dressing applications. This may explain why in similar agro-
424 environmental conditions, it was observed that N applied at tillering was recovered more
425 efficiently than that applied at emergence (López-Bellido et al., 2005; Kirda et al., 2001). In
426 agreement with this study, Trindade et al. (2009) reported that the treatments where mineral-N
427 fertilizer was applied had higher soil NO_3^- -leaching potential after the maize crop. Moreover,
428 Smith and Chambers (1993) suggested that wherever cool and wet winters prevail, N leaching
429 can be reduced using organic fertilizers with a low mineral to organic N ratio, as farmyard
430 manure. On the contrary, Shepherd and Newell-Price (2013) reported that annual applications of

431 farmyard manure increased NO_3^- leaching by 39 % more than the inorganic fertilizer, under a 7-
432 yr rotation (wheat-potatoes, wheat-winter barley, sugar beet- wheat). This could be related to the
433 site-specific dynamics of the soil organic matter: in this study, the soil organic matter
434 accumulated over the years from long-term organic fertilization may have acted as a buffer of
435 organic N available for mineralization (Indraratne et al., 2009; Kaur et al., 2008; Gutser et al.,
436 2005; Schröder et al., 2005; Hao et al., 2003).

437 The findings of this study suggest that under Mediterranean conditions N fertilization in autumn
438 could be omitted to mitigate the leaching of NO_3^- in the autumn-winter period without affecting
439 crop yields. López-Bellido et al. (2012) showed that the postponing of the N fertilizer application
440 to tillering-stem elongation phases for winter crops is usually more efficient in terms of plant N
441 uptake. Besides, this is confirmed by the findings of Trindade et al. (2009) who reported no
442 significant effects on Italian ryegrass annual dry matter yields of the N application in October
443 compared to no N fertilization using similar total N inputs to our study. Moreover, despite the
444 ND prescriptions are forbidding the supply of organic or mineral fertilization from November to
445 February , this practice did not mitigate the NO_3^- leaching.

446 Studies conducted in the Netherlands under maize and grassland crops reported that about 25 %
447 of the total N input applied as cattle slurry was lost by leaching and it was independent of its rate
448 application (Schröder and Dilz, 1987). So that the N losses depends on the source as well. An
449 investigation by Worrall et al. (2009) showed the lack of objective success in the majority of the
450 32 NVZs studied in the United Kingdom. This has called into doubt the use of input management
451 as a means of limiting NO_3^- pollution, especially when the areas designated are small. The lack
452 of objective success for NVZ designation suggests that NO_3^- pollution control strategies based
453 only on input management need to be rethought.

454 The residual N input that is not uptaken by the crop or leached, can be subjected to various
455 processes such as volatilization (Atzori et al, 2009), immobilization (Divito et al., 2011; Kaur et
456 al., 2008) or denitrification (Morari et al., 2012). However the assessment of the relative

457 contribution of these processes to the fate of the residual N was not the object of this study and is
458 worthy of further studies.

459 The lowest average NO_3^- concentration was observed in MA and this was associated to the
460 higher C:N ratio and proportion of organic N in farmyard manure than in slurry. Similar findings
461 were reported by several scholars under a range of agro-environmental conditions (Sanger et al.,
462 2010; Bertora et al., 2008; Moral et al., 2005). Ever more frequently the amount of manure
463 produced in this farming livestock system decreases when cattle management shifts from a bed-
464 pack system to cubicle housing as occurred in this study area. In fact the production of manure
465 instead of slurry is related to structural changes of the livestock housing system, which in turn is
466 indirectly related to farming targets of milk quality and animal welfare.

467 However, the bed-pack management and particularly the straw availability are key components
468 for achieving good milk quality (Wenz et al., 2007) and animal welfare standards (ADAS, 1994;
469 Singh et al., 1994) that can be compatible with the production of manure, which proved to be
470 much more sustainable in terms of environmental impacts of intensive dairy farming under
471 Mediterranean conditions.

472

473 **4. Conclusions**

474 The study supported the hypothesis that the NO_3^- dynamics in the soil water in a maize-ryegrass
475 double cropping forage system under Mediterranean conditions are variable, independently of
476 the fertilization system. This implies that the NO_3^- losses can be only partially controlled by the
477 fertilization system, while the complex interactions between the winter rainfall, the crop
478 sequence and the soil organic N buffer are far more important.

479 Among the fertilization systems under comparison, the cattle manure proved to be the most
480 conservative, while the replacement of organic with mineral N fertilization systems was not
481 effective in mitigating NO_3^- leaching, despite the lower yearly residual N estimated.

482 The maximum soil water NO_3^- concentrations observed in the autumn-winter were almost
483 independent from the type of fertilizer and actual N input rate. This was explained by the
484 impossibility of controlling effectively the leaching associated to the natural water surplus in
485 winter and the observed soil water NO_3^- concentration dynamics in autumn-winter. It seems that
486 the autumn-winter NO_3^- leaching in the maize-ryegrass double cropping is unavoidable in highly
487 fertilized systems. The fertilization with mineral N instead of organic N does not reduce N
488 leaching in winter. The average annual NO_3^- losses and soil water NO_3^- concentrations were
489 significantly lower using 100% farmyard manure as N fertilizer. This may have relevant
490 implications on the livestock effluent management system at the farm scale (e.g. from cubicle
491 housing to straw bedded-pack), that could be addressed to obtain fertilizers with higher C:N ratio
492 in order to minimize the release of NO_3^- during the leaching period. However the organic
493 fertilizers must be carefully managed to prevent over- or under-application and to account for the
494 cumulative environmental effects of application as well as storage.

495

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680

681 Figure 1. Water balance dynamic from May 2009 to June 2012 under Maize-Italian Ryegrass
682 system.

683

684 Figure 2. Dynamics of the NO_3^- concentration (mg L^{-1} , transformed by Shen et al., 2006) in the
685 soil solution and soil water percolation as simulated by EPIC model during the silage maize –
686 Italian ryegrass rotation (2009–2010) for the different treatments (MA = Cattle Manure; SL =
687 Cattle Slurry; SM = Cattle Slurry+ Mineral; MI = Mineral). Vertical black arrows indicate the
688 dates of fertilizers distribution. The horizontal black line indicates the legal threshold of 50 mg
689 L^{-1} for drinkable water and the rectangles indicate the duration of cropping cycle. The asterisks
690 indicate the sampling dates when the NO_3^- concentration significantly exceeded 50 mg L^{-1} .

691

692 Figure 3. Dynamics of the NO_3^- concentration (mg L^{-1} , transformed by Shen et al., 2006) in the
693 soil solution and soil water percolation as simulated by EPIC model during the silage maize –
694 Italian ryegrass rotation (2010–2011) for the different treatments (MA = Cattle Manure; SL =
695 Cattle Slurry; SM = Cattle Slurry+ Mineral; MI = Mineral). Vertical black arrows indicate the
696 dates of fertilizers distribution. The horizontal black line indicates the legal threshold of 50 mg
697 L^{-1} for drinkable water and the rectangles indicate the duration of cropping cycle. The asterisks
698 indicate the sampling dates when the NO_3^- concentration significantly exceeded 50 mg L^{-1} .

699

700 Figure 4. Dynamics of the NO_3^- concentration (mg L^{-1} , transformed by Shen et al., 2006) in the
701 soil solution and soil water percolation as simulated by EPIC model during the silage maize –
702 Italian ryegrass rotation (2011–2012) for the different treatments (MA = Cattle Manure; SL =
703 Cattle Slurry; SM = Cattle Slurry+ Mineral; MI = Mineral). Vertical black arrows indicate the
704 dates of fertilizers distribution. The horizontal black dotted line indicates the legal threshold of
705 50 mg L^{-1} for drinkable water and the rectangles indicate the duration of cropping cycle. The

706 asterisks indicate the sampling dates when the NO_3^- concentration significantly exceeded 50 mg
707 L^{-1} .
708 Figure 5. Distribution of cumulated N leaching (kg N ha^{-1}) from June 2009 to May 2012 for the
709 different treatments.

710 Table 1. Physical and chemical characteristics of the soil at the beginning of the experiment
 711 (2009).

Trait	Soil depth (cm)			
	0-45	46-70	71-100	>100
Clay, g kg ⁻¹	25 ±0.16	20 ±0.15	28 ±0.48	66 ±1.04
Sand, g kg ⁻¹	953 ±0.20	940 ±1.44	954 ±0.47	912 ±1.01
Silt, g kg ⁻¹	21 ±0.14	19 ±0.13	22 ±0.22	22 ±0.31
Bulk density, g cm ⁻³	1.55 ±0.02	1.35 ±0.11	1.22 ±0.17	1.25 ±0.18
Water holding capacity, % Vol 0 kPa	41.6	49.2	52.5	52.3
Field capacity, % Vol 33kPa	6.6	7.8	8.8	9.7
Field capacity, % Vol 23kPa	17.7	20.5	22.3	23.2
Wilting point, % Vol 1500 kPa	3.8	3.2	3.8	3.8
Soil Organic Matter, g kg ⁻¹	23.4 ±0.10	8.0 ±0.19	4.7 ±0.14	4.1 ±0.10
Tot N, g kg ⁻¹	1.3 ±0.04	0.4 ±0.08	0.3 ±0.05	0.4 ±0.07

712 Table 2. N inputs (kg N ha⁻¹) from organic and mineral fertilizers (three-years average \pm std
 713 error) for Italian ryegrass and maize (MA = Cattle Manure; SL = Cattle Slurry; SM = Cattle
 714 Slurry + Mineral; MI = Mineral).

Treatments	Italian ryegrass	Maize
	2009-12	2009-2011
MA	151 \pm 24	379 \pm 38
SL	317 \pm 115	469 \pm 213
SM	231 \pm 53	374 \pm 60
MI	130 \pm 0	316 \pm 0

715
 716 Table 3. Indices used for the evaluation of the model performances (RRMSE= relative root mean
 717 square error; EF=modeling efficiency) for calibration and validation in Italian ryegrass, maize
 718 and ground water table depth.

		RRMSE	EF	Slope	Intercept	R ²
Best		0.0	1.0	1.0	0.0	1.0
Min		0.0	$-\infty$	$-\infty$	$-\infty$	$-\infty$
Max		$+\infty$	1.0	$+\infty$	$+\infty$	$+\infty$
Silage maize yield	Calibration	5.90	0.50	0.70	6.95	0.57
	Validation	8.02	0.24	0.46	12.00	0.31
It. ryegrass yield	Calibration	17.55	0.55	0.65	2.68	0.64
	Validation	19.40	0.25	0.75	2.21	0.57
Groundwater table depth	Calibration	25.75	0.37	1.13	-0.01	0.72
	Validation	19.50	0.54	0.89	0.17	0.67

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723 Table 4. Average three years N balance estimated from N input, removal and leaching as influenced by the treatments (MA = Cattle Manure; SL =
724 Cattle Slurry; SM = Cattle Slurry+ Mineral; MI = Mineral). The residual N is calculated by difference. Means followed by the same letter were not
725 significantly different (P<0.05). Means followed by standard errors refer to variables with heteroschedastic error variances.

Treatments	N input		N removal		N leaching		Residual N	
	(kg N ha ⁻¹ ± err std)	(%)	(kg N ha ⁻¹)	(%)	(kg N ha ⁻¹)	(%)	(kg N ha ⁻¹ ± err std)	(%)
MA	530 ± 64	100	216 b	41	42 b	8	272 ± 41 c	51
SL	813 ± 249	100	255 ab	31	110 a	14	447 ± 246 a	55
SM	602 ± 88	100	281 a	47	94 a	16	226 ± 95 b	38
MI	446 ± 0	100	301 a	68	89 a	20	56 ± 44 c	12
Mean	599	100	264	47	84	15	250	39
CV (%)	20	-	14	-	29	-	83	-

726

Figure 1
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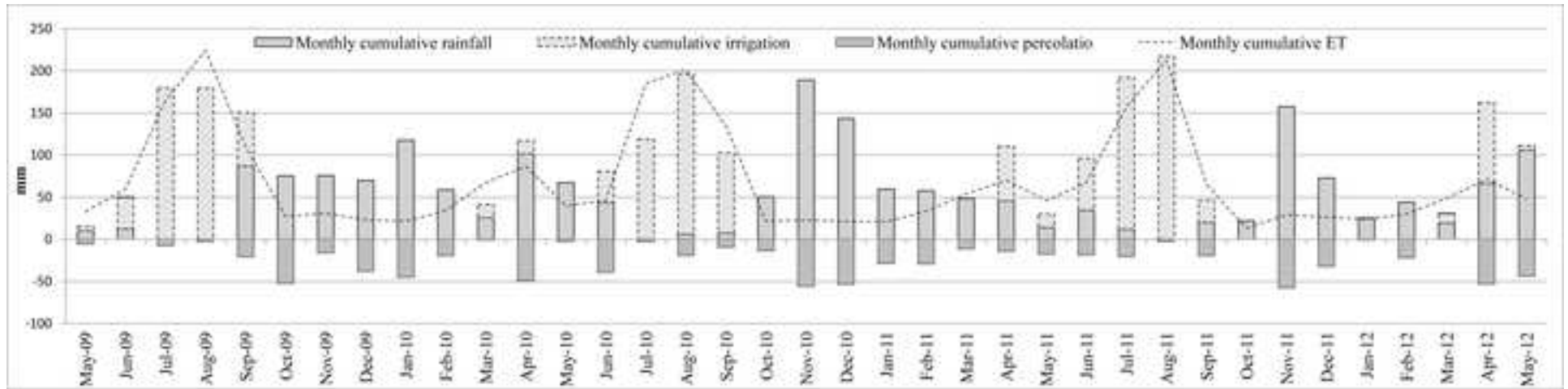


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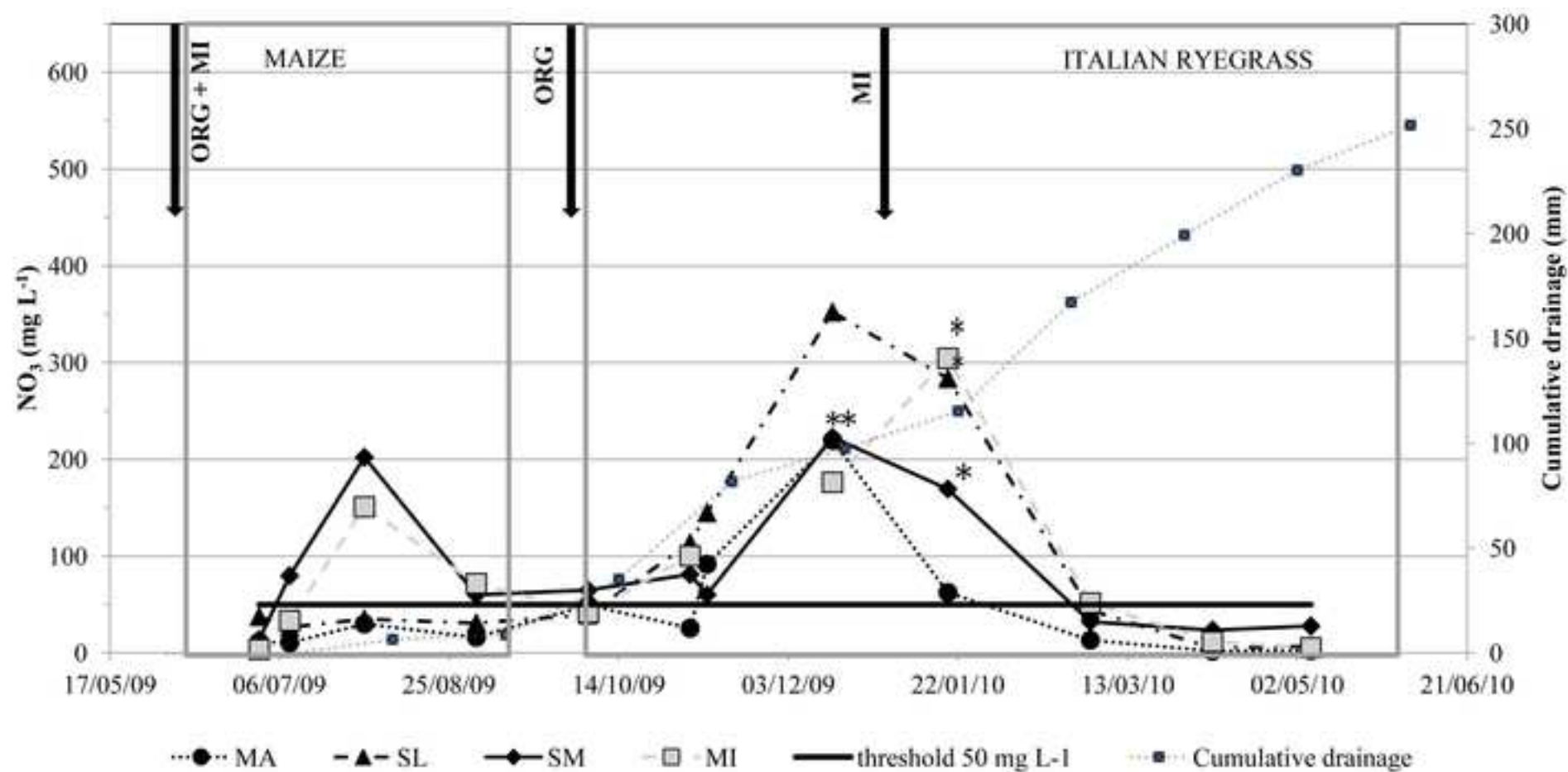


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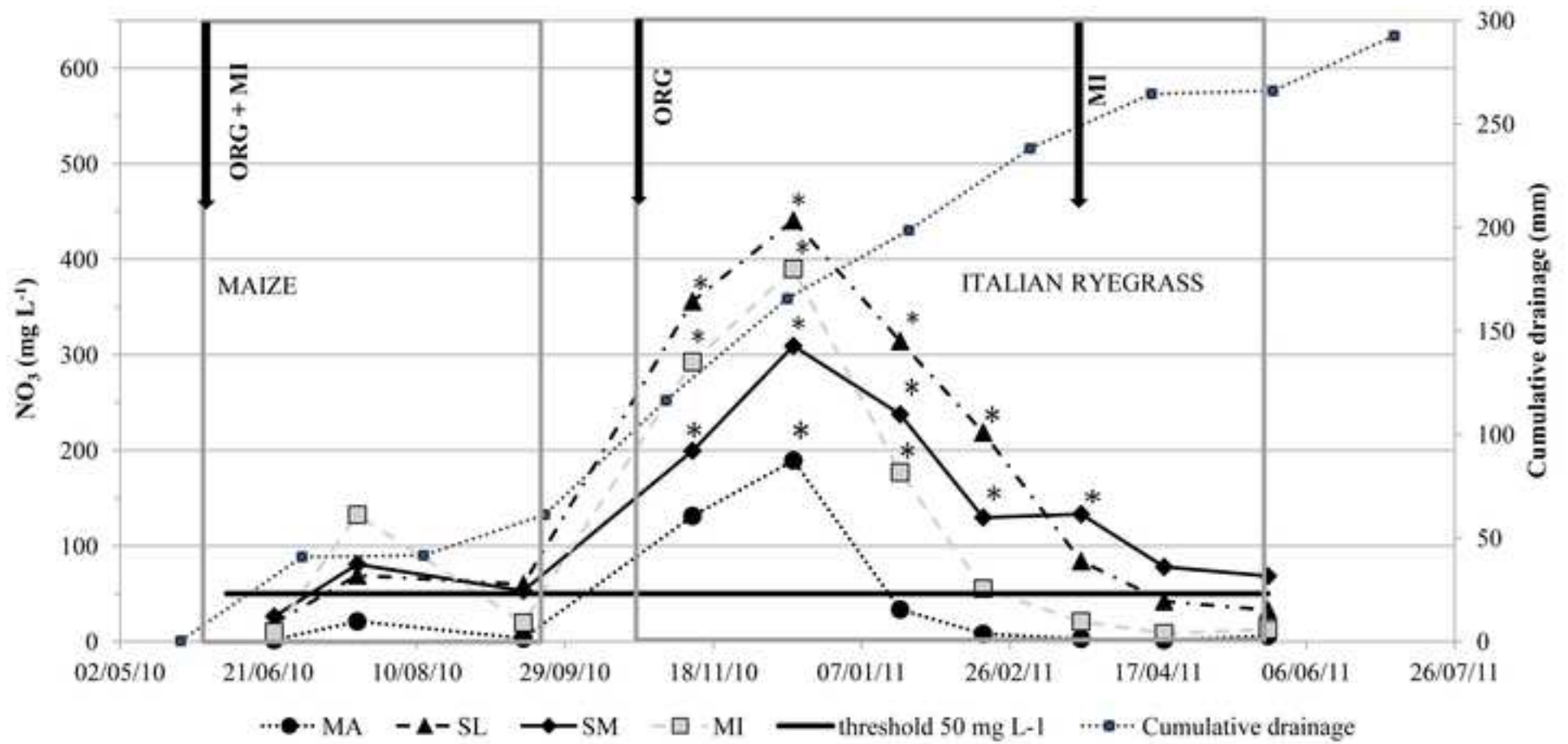


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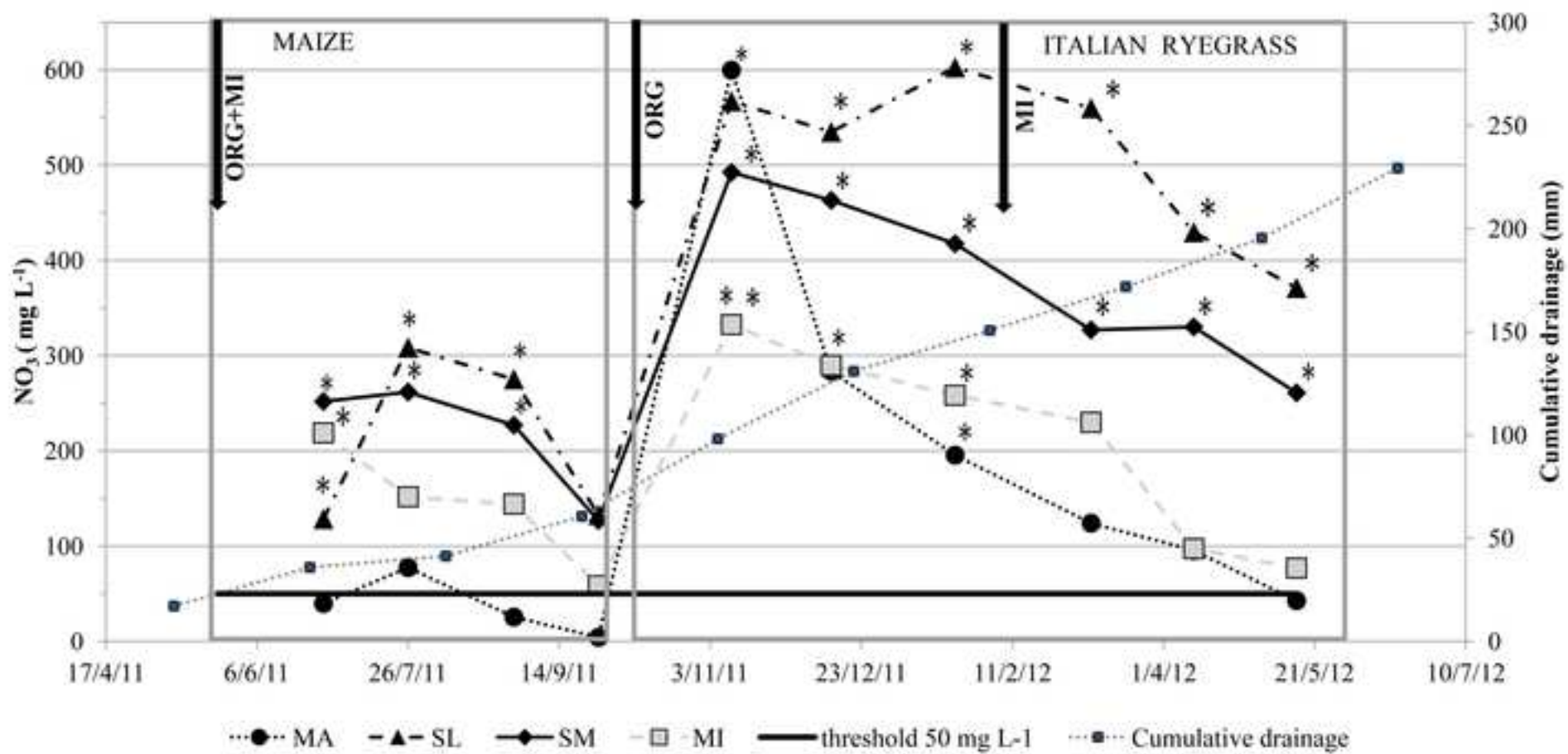


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