Long-term effects of pig slurry on barley yield and N use efficiency under semiarid Mediterranean conditions

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12 **Abstract**

13 Intensive pig farming is a relevant economic activity in Mediterranean areas, which
14 generates large amounts of pig slurry (PS) as a by-product. Pig slurry represents a
15 valuable resource for low-fertility Mediterranean soils, capable of supplying organic
16 matter and plant nutrients, particularly N. The cumulative and residual effects of PS on
17 winter barley (*Hordeum vulgare* L.) yield and N use efficiency were investigated in a
18 seven-year field experiment under semiarid Mediterranean conditions. Treatments
19 included five rates of PS ranging from 30 to 150 m³ ha⁻¹ applied either every year or
20 only once at the beginning of the experiment, an annual mineral N fertilization
21 (traditional in the study area), and a control with no fertilization. Plant-available N
22 content in soils after harvest increased with the annual application of PS with respect to
23 the mineral fertilization and the control treatment, especially in dry years. Yearly
24 variations in water availability also shaped the response of barley to PS fertilization. In
25 general, the annual application of PS affected positively grain yield and biomass
26 production. Residual effects were evident for the second and third barley crop.
27 Cumulative N uptake by the barley crop increased with increasing the rate of PS (up to
28 120 t ha⁻¹), while N use efficiency decreased, which suggested an increasing risk of
29 leaching and contamination of ground water. As a whole, our data indicate that the
30 annual application of 30 m³ ha⁻¹ of PS (equivalent to an average of 67 kg N ha⁻¹) may
31 result in barley yields similar to those obtained with the traditional mineral fertilization,
32 while safely adhering to European regulations for the use of N in agriculture. The
33 application of PS at rates higher than 60 m³ ha⁻¹ may result in better yields, but also in
34 significantly lower N use efficiency and a higher potential environmental impact.

35

36 *Keywords:* *Hordeum vulgare* L.; Mineral fertilization; Liquid manure; N mineralization;
37 N immobilization; N uptake.
38

39 **1. Introduction**

40

41 Intensive pig farming has developed significantly in Spain in the last decades.

42 According to current data (MARM, 2010), Spain is the second largest producer of pigs

43 in the European Union (after Germany) with more than 25 million heads. This activity

44 is estimated to generate more than 65 million m³ of pig slurry (PS) per year. Since

45 intensive pig farming is concentrated in certain areas, an effort to adequately manage PS

46 must be done to avoid environmental and health hazards.

47 The application of PS to agricultural soils has the benefit of providing organic matter

48 (OM) and plant nutrients, especially N (Sánchez and González, 2005), and hence may

49 help reduce the use of mineral N fertilizers. Most N in PS is contained in inorganic

50 forms, mainly as NH₄⁺ (Sánchez and González, 2005). The organic N applied with PS is

51 incorporated into the soil OM pool and can be converted to NH₄⁺ through

52 mineralization or absorbed by soil microorganisms for their growth. Inorganic N (NH₄⁺

53 and NO₃⁻) can be taken up by the plants or immobilized by the microbial biomass.

54 When microbial growth is limited by organic C, net N mineralization occurs, whereas

55 immobilization occurs to a greater extent when microbial growth is limited by N

56 (Bardgett, 2005). Inorganic N in PS-amended soils not taken up by the plants and not

57 immobilized by the microbial biomass may be lost from the soil through denitrification,

58 volatilization, and leaching processes. Thus, excessive or inappropriate application of

59 PS to agricultural soils may seriously endanger air and ground water quality (Díez et al.,

60 2004; Vallejo et al., 2004; Misselbrook et al., 2005).

61 Plant-available N in soils fertilized with mineral and organic fertilizers can differ

62 markedly (Gutser et al., 2005). While plants can take up most of the N added with

63 mineral fertilizers during the year of application (Gutser et al., 2005), a large part of the

64 mineral N applied with PS can be immobilized by microorganisms shortly after its
65 incorporation into the soil during the degradation of readily available organic
66 compounds also added with the PS (Sørensen and Amato, 2002); further, part of the PS-
67 derived NH_4^+ can be fixed on clay surfaces or between clay layers. As a result, N
68 utilization by crops in the year of application may be relatively small. However, part of
69 the immobilized N can be mineralized in subsequent years, releasing N that becomes
70 available for plant uptake and thus inducing residual effects generally not accounted for
71 in short-term experiments (Sørensen and Amato, 2002). When organic fertilizers are
72 used repeatedly, residual effects accumulate, N availability increases, and crop
73 responses may be significantly affected (Schröder et al., 2005).

74 A number of studies in the literature have evaluated the effects of PS on crops (Petersen,
75 1996; Nicholson et al., 1999; Mooleki et al., 2002, 2004; Edmeades, 2003; Schröder et
76 al., 2005, 2007). However, the available information on crop responses to PS
77 fertilization under Mediterranean conditions is scarce (Daudén and Quílez, 2004; Yagüe
78 and Quílez, 2010). Mediterranean areas are characterized by a warm and dry climate,
79 with erratic and scarce precipitations, which renders water availability the main limiting
80 factor for crop production. Accordingly, soils feature low OM contents due to high
81 mineralization rates enhanced by high temperatures. Some agricultural practices, such
82 as intensive tillage and harvesting of crop residues, often lead to an intensification of the
83 loss of soil OM, resulting in soils with low fertility levels and high vulnerability to
84 erosion and degradation. In consequence, managing soil OM and plant nutrients
85 properly through the use of organic amendments, such as PS, is especially important in
86 Mediterranean agricultural areas (Zdruli et al., 2004).

87 In this work, a seven-year field experiment was used with the aim of evaluating the
88 response of winter barley (*Hordeum vulgare* L.) to PS fertilization under Mediterranean

rain-fed conditions. Cumulative (annual applications) and residual (only one initial addition) effects of five increasing rates of PS (from 30 to 150 m³ ha⁻¹) on plant-available N, crop yield, N uptake, and N use efficiency were assessed to improve our fundamental knowledge of the potential of this material as a N source; this is essential for proper recommendations regarding application rates while reducing the risks of soil, water, and air contamination. In Spain, barley grain yield in rain-fed conditions is estimated to vary between an average of 2000 kg ha⁻¹ in arid regions and an average of 5300 t ha⁻¹ in humid regions; hence the average optimum N rate can vary greatly, between 50 to 140 kg N ha⁻¹ based on the assumption that barley requires an average of 25 kg of N to produce 1000 kg of grain (López, 2010). Here a mineral fertilization of about 100 kg N ha⁻¹, which is traditional in the study area, and a control treatment with no fertilization were used as references for comparison.

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103 **2. Materials and methods**

104

105 *2.1. Site, experimental design, soil, and pig slurry*

106

107 A field plot experiment was conducted on rain-fed winter barley (var. Reinette) over
108 seven seasons from 1997-1998 to 2003-2004 in the experimental farm “La Higuera”
109 of the Spanish National Research Council (CSIC), located in Santa Olalla, Toledo
110 province, Spain. The site is characterized by a continental semiarid climate with an
111 annual rainfall of 487 mm and an average annual temperature of 14 °C. Monthly mean
112 temperatures and monthly precipitation during the experiment are shown in Fig. 1.
113 At the beginning of the first season (mid-October, 1997), plots (10 m × 8 m) were

114 amended with PS at rates of 30 (PS30), 60 (PS60), 90 (PS90), 120 (PS120), and 150
 115 (PS150) m³ ha⁻¹ before barley sowing (early November). Six plots were set up for each
 116 rate of PS. From the second season on, three plots of each PS rate were left unamended
 117 in order to study the residual effects of PS fertilization over the rest of the study period,
 118 whereas the other three plots were amended with PS at the same rate in October of
 119 every year until 2003 in order to study the cumulative effects. To compare with the PS
 120 treatments, a mineral fertilization (MF) traditionally used in the area was annually
 121 applied in triplicate as 400 kg ha⁻¹ of N-P-K 15-15-15 prior to sowing and 150 kg ha⁻¹ of
 122 NH₄NO₃ (equivalent to 53 kg ha⁻¹ y⁻¹ of N) in mid-March at the beginning of tillering.
 123 A control treatment with three replicates with no fertilization was also included in the
 124 experimental design (C). A scheme of the field plot experimental is shown in Fig. 2. For
 125 the sake of clarity, crop seasons are denoted herein by the year of the crop harvest (from
 126 1998 to 2004).
 127 The soil of the experiment was classified as Typic Haploxeralf (Soil Survey Staff,
 128 2003). The main characteristics of the top layer (0-15 cm) measured at the beginning of
 129 the experiment were the following: sandy loam texture (sand, 590 g kg⁻¹; silt, 220 g kg⁻¹;
 130 clay, 190 g kg⁻¹); pH (H₂O), 5.8; electrical conductivity (EC), 0.06 dS m⁻¹; Kjeldahl N
 131 content, 1.2 g kg⁻¹; NH₄⁺-N, 86.2 mg kg⁻¹; NO₃⁻-N, 19.9 mg kg⁻¹; total organic C (TOC),
 132 13.2 g kg⁻¹; available P, 60 mg kg⁻¹; and available K, 491 mg kg⁻¹. These values are
 133 indicative of slight acidic pH, low EC and TOC content, medium available N and P
 134 contents, and high K content (Cadahía and Lucena, 1998; López and Grau, 2004).
 135 The pig slurry was collected every year from a pig-breeding farm which employed a
 136 closed-cycle production system. The slurry in the storage pit was stirred the day of
 137 application and pumped to a tanker equipped with a splash plate. The PS was applied by
 138 surface spreading and immediately incorporated by disking into the top 15 cm of soil.

139 Samples of PS were taken from the tank. The main characteristics of the PS applied
140 each year are listed in Table 1.

141

142 *2.2. Sampling and analysis of plant and soil*

143

144 Every year in late June (from 1998 to 2004), soon after grain maturity was reached, the
145 above-ground barley biomass (AGB) of six 0.5-m-long rows, equivalent to an area of
146 0.45 m², was collected randomly from each plot. The plant samples were weighed,
147 threshed, and then the grain weighed. Sub-samples of grain and straw were ground to
148 pass a 0.5-mm sieve and analyzed for total N by inductively coupled plasma-atomic
149 emission spectroscopy (PerkinElmer ICP/5500, Waltham, MA) after nitric-perchloric
150 acid digestion.

151 Soon after harvest, soil samples were prepared by mixing ten surface (0-15 cm) soil
152 subsamples randomly collected from each plot. Plant-available N (NH₄⁺-N plus NO₃⁻-
153 N) content was determined by extraction with 2 M KCl (soil to extractant ratio of 1:10)
154 and colorimetry (Keeney and Nelson, 1982) with an automated analyzer (Technicon
155 AutoAnalyzer AAI, Buffalo Grove, IL).

156

157 *2.3. Parameters of N use efficiency and statistics*

158

159 Nitrogen uptake by the barley crop was estimated as the sum of grain plus straw N
160 uptake, which is considered to be a good indicator of the total plant N uptake (Mooleki
161 et al., 2002). Grain and straw uptake was calculated by multiplying N concentrations by
162 yields.

163 Cumulative N use efficiency (NUE) for each treatment (t) and year (i) was calculated

164 as

$$165 \quad N U E_{t,i}(\%) = \left(\sum_{i=1998}^{2004} C N U_{t,i} - \sum_{i=1998}^{2004} C N U_{C,i} \right) / \sum_{i=1998}^{2004} C N A_{t,i} \quad (1)$$

166 where CNU is the cumulative N uptake by the crop on the treated plots, CNU_C is the
167 cumulative N uptake by the crop on the control plots, and CNA is the cumulative
168 amount of N applied with the PS or MF treatment (Mooleki et al., 2002).

169 Agronomic efficiency (AE) was calculated as

$$170 \quad A E_{t,i}(k g \text{ grain } k g^{-1} N) = \left(\sum_{i=1998}^{2004} G Y_{t,i} - \sum_{i=1998}^{2004} G Y_{C,i} \right) / \sum_{i=1998}^{2004} C N A_{t,i} \quad (2)$$

171 where GY and CY_C are the cumulative grain yields on the treated and control plots,
172 respectively (Mengel and Kirkby, 2001).

173 Finally, to compare the N availability from the PS amendment with that from the
174 mineral fertilization, we calculated the apparent mineral-fertilizer-N equivalents (MFE)
175 according to Gutser et al. (2005) as

$$176 \quad M F E_{t,i}(\%) = \left(\sum_{i=1998}^{2004} C N U_{t,i} - \sum_{i=1998}^{2004} C N U_{C,i} \right) / \left(\sum_{i=1998}^{2004} C N U_{M F,i} - \sum_{i=1998}^{2004} C N U_{C,i} \right) (3)$$

177 Statistical calculations were performed with the SPSS 18.0 software for Windows
178 (Statistical Product and Service Solutions Inc., Chicago, IL). Data from dependent
179 measures (available N, grain yield, AGB, grain N content, N uptake, NUE, AE, and
180 MFE) were subjected to one-way ANOVA within each year. Cumulative and residual
181 PS treatments were analyzed independently and compared to C and MF treatments. The
182 Tukey's Honestly Significant Difference (HSD) test at the 0.05 probability level was
183 used for mean separation.

184

185

186 3. Results

187

188 3.1. *Plant-available N*

189

190 In general, plant-available N contents in the top layer (0-15 cm) of the C and MF soils
191 after harvest were similar to each other and tended to decrease over the years (Fig. 3). In
192 1998 and 2001 (693 and 676 mm of precipitation, respectively), the available N content
193 of the PS-amended soils (except PS150) did not differ significantly from those of the C
194 and MF soils. By contrast, in 2002, 2003, 2004, and especially in 1999 and 2000 (552,
195 489, 563, 328, and 410 mm of precipitation), differences in available N content between
196 the PS soils and the MF and C soils were more evident. Compared to C and MF, no
197 significant residual effects of PS on available N were detected.

198

199 3.2. *Grain yield and above-ground biomass*

200

201 Barley grain yield and AGB as affected by the different treatments are shown in Fig. 4.
202 The extent of the crop response to PS application fluctuated over the years. In the first
203 season (1998), with high precipitation in November and May, grain yields for the PS90
204 and PS120 plots were larger than for the MF treatment; however, in 1999, when
205 precipitation was low, no differences between PS and MF were found. In 2000, the PS
206 treatments did not differ significantly from C, whereas in 2001, which was characterized
207 by high precipitation in autumn and winter but low in spring, PS90 yielded better results
208 than C and MF. In 2002, with dry winter and rainy spring, no significant differences
209 were found between treatments. In 2003, exceptionally high temperatures and low
210 precipitation in spring led to very low grain yields for C, MF, and the treatments with
211 low PS rates; PS120 and PS150 resulted in significantly higher yields than C and MF.
212 In 2004, which was characterized by a slightly high precipitation in spring, grain yields

213 were higher for the PS treatments (except for PS30) than for C and MF.
 214 In 1999, the single applications of PS did not cause significant residual effects on grain
 215 yield with respect to the C treatment. In 2000 (i.e., third crop after the PS application),
 216 however, positive residual effects were observed for rates equal or higher than 60 m³ ha⁻¹,
 217 although not significant with respect to MF. In the next years, the residual effects of
 218 PS diminished and no significant differences were found compared to C.
 219 In general, the trends observed for AGB as affected by PS additions were similar to
 220 those observed for grain yields. From 1998 to 2000, no annual PS treatment was
 221 significantly different from MF. In 2001, only PS90 exhibited higher AGB than MF. In
 222 the last three years, PS120 and PS150 resulted in higher AGB than C and MF. With
 223 respect to C, some significant positive effects of the single applications of PS were
 224 observed on the AGB harvested in 1999 and 2000.
 225 Cumulative grain yield and AGB production after the seven years of experiment are
 226 shown in Table 2. Total grain yield and AGB were higher for the plots amended
 227 annually with PS than for the MF and C plots, and the differences increased
 228 significantly with increasing the PS rate up to 120 m³ ha⁻¹. By contrast, total grain yield
 229 and AGB production for the plots amended once with PS were similar to those obtained
 230 with the C treatment and significantly smaller than those for the MF plots.

231

232 3.3. Grain N content and plant N uptake

233

234 In general, N content of grain and N uptake by the plants were similar or higher for the
 235 annual PS treatments than for MF and C, and tended to increase with increasing the rate
 236 of PS (Fig. 5). Residual effects of the single applications of PS on grain N content and
 237 plant N uptake disappeared after 2000.

238 Highly significant positive linear relationships between the cumulative N uptake by the
239 barley crops harvested since 1998 until 2004 and the cumulative N applied were found
240 for MF and especially for the PS treatments (Fig. 6). The slope of the linear regression
241 (i.e., ratio between cumulative N uptake and N applied) was 0.946 kg N kg⁻¹ for MF and
242 ranged from 1.433 kg N kg⁻¹ for PS30 to 0.471 kg N kg⁻¹ for PS 150, tending to
243 decrease with increasing the PS rate. A slope lower than 1 indicates a smaller N uptake
244 by barley relative to the amount of N applied and thus a long-term over-fertilization.
245 Based on the N uptake by barley and the amount of N supplied, an excess of 778 and
246 1246 kg ha⁻¹ of N was estimated in the soils annually amended with 120 and 150 m³ ha⁻¹, respectively.
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248

249 *3.4. Parameters of N use efficiency*

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251 The NUE values of MF increased from 67% in 1998 to about 90% in 1999 and 2000,
252 and then decreased steadily to 40% in 2004 (Fig. 7). The NUE values were smaller for
253 the annual application of PS than for the MF treatment, but the differences tended to
254 disappear with the number of amendments. Except in 1998, NUE tended to decrease
255 with increasing the rate of PS applied. The values of NUE for the single additions of PS
256 were larger than the corresponding values for the annual applications (except in the case
257 of PS30).

258 The values of AE for the MF treatment increased markedly from 1998 to 2000, and then
259 gradually decreased (Fig. 7). In general, AE decreased with increasing the rate of PS
260 applied annually. With the exception of PS30 in 2003 and 2004, the AE values for the
261 single applications of PS were larger than the corresponding values for the annual PS
262 treatments.

263 In general, the MFE values tended to increase with increasing rate of PS annually
264 applied up to 120 m³ ha⁻¹ and with increasing number of applications, reaching values
265 much higher than 100% (Fig. 7). For the plots amended only once with PS, MFE tended
266 to increase with increasing the PS rate, up to about 100% for PS120 and PS150.

267

268

269 **4. Discussion**

270

271 Continuous cropping caused plant-available N to decrease over the years in the C and
272 MF soils, but not in the soils amended annually with high rates of PS. Differences
273 between the PS and the MF and C treatments in N accumulation in soil increased with
274 increasing the PS rate and appeared to be more evident in dry seasons, probably due to
275 water-limited processes of N losses by leaching and N uptake by the crop (Pardo et al.,
276 2009).

277 In general, barley response in terms of grain yield, biomass production, and grain N
278 content was positively affected by the annual application of PS. In agreement with
279 Thomsen and Sørensen (2006), moderate or high applications of PS enhance crop N
280 uptake compared with MF. Previous studies also suggest that that suitable PS rates can
281 result in satisfactory crop yields, similar to those obtained with mineral fertilizers
282 (Petersen, 1996; Nicholson et al., 1999; Mooleki et al., 2002; Daudén and Quílez, 2004;
283 Díez et al., 2004). In the literature, there are also data from long-term experiments
284 indicating that animal manures only influenced soil productivity when large inputs were
285 applied over many years (Edmeades, 2003).

286 In general agreement with previous results (Daudén and Quílez, 2004; Schröder et al.,
287 2007), PS exhibited a significant residual agronomic value, which was evident even two

288 years after the amendment and can be attributed to the slow release of N immobilized
289 shortly after the first PS application. According to Sørensen and Amato (2002), the
290 contribution of immobilized N released as mineral N to crops in PS-amended soils is
291 relatively low, but steady for 2-3 years after the amendment, which greatly determines
292 the residual fertilizer value of PS.

293 The crop response to PS was found to be affected by the temperature and precipitation
294 distribution during the crop season. When temperature and precipitation distribution
295 was adequate for crop development, differences in crop response between PS treatments
296 and C and MF treatments were not as evident as in years with unfavorable conditions.

297 When water supply is adequate at the end of the winter, high levels of N can over-
298 stimulate biomass production and favor cereal tillering (Pardo et al., 2009). However, if
299 water is in short supply at the end of the crop period, the grain may be not filled, which
300 may result low yields (Angás et al., 2006). High plant N uptake and low yield may be
301 indicative of luxury consumption (Angás et al., 2006).

302 In 2003, C, MF, and the residual PS treatments exhibited high weed (mainly *Lolium*
303 *rigidum* L.) emergence, while cumulative plots were hardly affected. Weed emergence
304 due to the continuous monoculture crop system is known to affect crop productivity,
305 especially in non-irrigated low-fertility soils (Díaz-Ambrona and Mínguez, 2001).

306 Petersen (2003) also found that barley was able to compete adequately with weeds for
307 nutrients in soils repeatedly amended with PS.

308 The results obtained indicated that cumulative N applied with the mineral fertilizer and
309 PS at rates of 30 and 60 t ha⁻¹ matched well the cumulative N uptake by the barley
310 crops. With increasing the rate and number of PS additions, the N applied exceeded
311 increasingly the barley N uptake. In agreement with Mooleki et al. (2002), increased
312 annual application rates of PS do not necessarily result in enhanced crop responses in

313 terms of N uptake and grain yield (i.e, high NUE and AE) because plant capacity to
314 assimilate N and produce grain is limited; also significant losses of N after PS
315 application may occur.

316 In our study, the mineral N content measured in the soils amended annually with high
317 PS rates after seven barley crops was relatively low. This suggests that relatively large
318 amounts of the mineral N applied with PS not taken up by the crops were immobilized
319 by soil microorganisms and/or lost by volatilization and leaching processes. Annual
320 application of high rates of PS was found to stimulate soil OM mineralization processes
321 in the experiment examined in this study (Plaza et al., 2002); N mineralization was also
322 found to occur at a greater rate than immobilization by means of an incubation
323 experiment (Plaza et al., 2005). Thus, our results suggest that large amounts of the
324 excess N in soils amended annually with high rates of PS were lost by volatilization
325 and/or leaching.

326 Consistently with the results obtained in this work, Gutser et al. (2005) reported values
327 of NUE for mineral fertilization of 50-80%, whereas the NUE values for PS were
328 generally smaller and more variable. Daudén and Quílez (2004) found levels of NUE of
329 30-40% for PS for an irrigated corn crop under Mediterranean conditions. Similarly,
330 Mooleki et al. (2002) reported values of NUE of 10-40% for liquid swine manure in a
331 field experiment with a canola-wheat-barley-canola rotation, and showed that the lower
332 the rate of liquid manure the lower the NUE. In a later work, Mooleki et al. (2004)
333 found values of NUE even lower for cattle manure (7-10%).

334 In conclusion, our data indicate that the annual application of $30 \text{ m}^3 \text{ ha}^{-1}$ of PS
335 (equivalent to an average of 67 kg N ha^{-1}) results in barley yields similar to those
336 obtained with the traditional mineral fertilization in the study area. The addition of 60
337 $\text{m}^3 \text{ ha}^{-1}$ yields slightly better results in terms of grain yield and permits to recycle larger

amounts of PS; however, the N applied exceeds slightly the N removed by the barley crop, albeit safely adheres to European regulations for the use of N in agriculture (175 kg N ha⁻¹ in zones draining into waters vulnerable to pollution from N compounds). The application of PS at rates higher than 60 m³ ha⁻¹ may result in better yields, but also in significantly lower N use efficiency and higher environmental impact. The high N relative to C content in PS and the Mediterranean climate may favor N mineralization over immobilization in PS-amended soils. Therefore, it is not advisable to apply PS at rates higher than those recommended for mineral N fertilizers. A good option could be to apply PS at low rates and, as suggested by Rogers et al. (2001) for other organic amendments, supply additional mineral N as needed based on fast soil N tests. The composition of PS depends on several factors such as type and physiological stage of the animals, time of sampling, and management conditions (Sánchez and González, 2005), and therefore should be analyzed prior to application.

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Research highlights

- Pig slurry affects positively barley yield under Mediterranean conditions
- Low rates of pig slurry give grain yields similar to traditional mineral fertilization
- Low rates of pig slurry result in better N use efficiency
- High pig slurry rates may result in better yields but higher environmental impact
- Residual effects are evident up to 3 years after pig slurry application

FIGURE CAPTIONS

Fig. 1. Monthly mean air temperature and monthly precipitation for the experimental period (1997-2003; bars). Historical patterns (1974-2004) are reported as solid lines.

Fig. 2. Scheme of the field plot experiment.

Fig. 3. Plant-available N content ($\text{NH}_4^+\text{-N}$ plus $\text{NO}_3^-\text{-N}$) after harvest in the control (C) soil, minerally fertilized (MF) soil, and soils amended annually (A) or once at the beginning of the experiment (O) with 30, 60, 90, 120, and 150 $\text{m}^3 \text{ha}^{-1}$ of pig slurry (PS30, PS60, PS90, PS120, and PS150, respectively). Error bars denote the standard error of the mean.

Fig. 4. Grain yield (a) and above-ground biomass (b) of barley grown on the control soil (C), minerally fertilized soil (MF), and soils amended with 30, 60, 90, 120, and 150 $\text{m}^3 \text{ha}^{-1}$ (PS30, PS60, PS90, PS120, and PS150, respectively) annually or once at the beginning of the experiment. Bars within the same chart with the same letter do not differ significantly according to the Tukey's HSD test at the 0.05 level.

Fig. 5. N content of grain (a) and N uptake (b) by barley grown on the control soil (C), minerally fertilized soil (MF) and soils amended with 30, 60, 90, 120, and 150 $\text{m}^3 \text{ha}^{-1}$ (PS30, PS60, PS90, PS120, and PS150, respectively) annually or once at the beginning of the experiment. Bars within the same chart with the same letter do not differ significantly according to the Tukey's HSD test at the 0.05 level.

Fig. 6. Cumulative N uptake by barley crops harvested since 1998 until 2004 as affected by the cumulative N applied with annual mineral fertilization (MF) and 30, 60, 90, 120, and 150 $\text{m}^3 \text{ha}^{-1} \text{y}^{-1}$ of pig slurry (PS30, PS60, PS90, PS120, and PS150, respectively).

Fig. 7. Cumulative N use efficiency (NUE), agronomic efficiency (AE), and mineral-fertilizer equivalents (MFE) of mineral fertilizer (MF) and 30, 60, 90, 120, and 150 $\text{m}^3 \text{ha}^{-1}$ of pig slurry (PS30, PS60, PS90, PS120, and PS150, respectively) applied annually

or once at the beginning of the experiment. Error bars denote the standard error of the mean.

- Control (C)
- Mineral fertilization (MF)
- 30 t ha⁻¹ of pig slurry applied every year or only once at the beginning of the experiment (PS30)
- 60 t ha⁻¹ of pig slurry applied every year or only once at the beginning of the experiment (PS60)
- 90 t ha⁻¹ of pig slurry applied every year or only once at the beginning of the experiment (PS90)
- 120 t ha⁻¹ of pig slurry applied every year or only once at the beginning of the experiment (PS120)
- 150 t ha⁻¹ of pig slurry applied every year or only once at the beginning of the experiment (PS150)

(a) Grain yield

(b) Above-ground biomass

(a) N content of grain

(b) N uptake by plants

× 1998 o 1999 ● 2000 □ 2001 ■ 2002 △ 2003 ▲ 2004 — Linear regression --- 1:1 Line

Main properties of the pig slurry applied each year

EC, electrical conductivity; TOC, total organic C.

Table 2

Total (1998-2004) above-ground biomass (AGB) and grain yield of barley grown on the control soil (C), soil amended with mineral fertilizer (MF), and soils amended with 30, 60, 90, 120, and 150 m³ ha⁻¹ (PS30, PS60, PS90, PS120, and PS150, respectively) annually (A) once at the beginning of the experiment (O)

Treatment	AGB (t ha ⁻¹)		Grain yield (t ha ⁻¹)	
	A	O	A	O
C	48.7 c	48.7 b	17.1 d	17.1 b
MF	64.8 b	64.8 a	22.1 cd	22.1 a
PS30	66.7 b	35.9 c	22.3 cd	14.0 b
PS60	79.0 ab	41.5 bc	25.2 bc	16.2 b
PS90	83.1 a	44.31 bc	28.4 ab	17.0 b
PS120	87.5 a	45.1 bc	31.7 a	15.4 b
PS150	82.4 a	46.5 b	30.6 ab	16.3 b

Values followed by the same letter in the same column do not differ significantly according to the Tukey's HSD test at the 0.05 level.