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Maize response to localized mineral or organic NP starter fertilization under different soil tillage methods

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1 **Title**

2 **Maize response to localized starter fertilization with digestate under different soil tillage**

3 **methods**

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17 **Abstract**

18 An early maize (*Zea mays* L.) sowing date plays a key role in increasing the grain yield, especially in
19 a temperate climate. The adoption of reduced tillage techniques, such as strip tillage (ST), can
20 decrease cultivation costs, compared to plowing (PLOW), but might also reduce the crop early
21 development rate as a result of lower soil temperatures. Therefore, farmers often adopt starter
22 fertilizations when sowing maize, in order to improve the early-season nutrient uptake, nutrient use
23 efficiency and plant development. The aims of this study were to assess the effects of the adoption of
24 ST, compared to PLOW, and a starter fertilization with digestate (DIG) or diammonium phosphate
25 (DAP), compared to an unfertilized treatment (NT) on the early growth, grain yield and grain
26 qualitative and sanitary traits of maize. Field experiments were carried out in NW Italy over the 2019
27 and 2020 growing seasons on two different soils. ST determined cooler soil conditions at both sites,
28 compared to PLOW, thus resulting in lower NDVI values due to a lower final crop density. The starter
29 fertilization with DAP recorded the highest early vigor and canopy development, expressed as plant
30 height in both sites and for both tillage techniques, while the DIG values were intermediate between
31 the DAP and NT treatments. Although the effects on the early vigor of maize differed between the
32 experimental sites, displaying more pronounced differences in the finer-textured soil, ST on average
33 delayed the flowering (+2.2 days) and increased the grain moisture content at harvesting (+1.2%),
34 compared to PLOW. The starter fertilization with DIG or DAP led to an earlier flowering, compared
35 to NT (-2.6 and -4.6 days, respectively). The grain yield was not affected by the tillage system, as the
36 plants in ST compensated for the lower plant density with an increased ear length (+1.5%). Instead,
37 DIG and DAP recorded similar and higher grain yields (+1.8 and +1.6 Mg ha⁻¹) than NT. DIG
38 application led to a higher grain protein content and lower level of fumonisin contamination than the
39 other treatments, while ST determined higher deoxynivalenol values than PLOW. The combination
40 of ST and DIG as a starter fertilization can help maintain the production level and the grain quality,
41 compared to conventional techniques that involve the use of mineral fertilizers and plowing, thereby
42 leading to a higher sustainability of the maize cropping system.

43

44 **Keywords**

45 reduced tillage, phosphorus, nitrogen, yield quality, mycotoxins, early growth

46

47 **1. Introduction**

48 Maize (*Zea mais* L.) is the crop with the largest global production, and it is used for both food and
49 feeds as well as for industrial or energy purposes. Early planting plays a key role in achieving the full
50 potential yield in temperate growing areas as it leads to an increase in the length of both the vegetative
51 period and the reproductive phase (Waqas et al., 2021), thus promoting higher yields and higher
52 resource use efficiencies. Farmers sow maize early to escape heat and drought stress during the
53 reproductive phase (Waqas et al., 2021) obtaining an early development and an earlier flowering,
54 which in turn leads to a higher radiation interception and a greater number of kernels per surface unit
55 and a faster growth rate during ripening (Sacks and Kucharik, 2011; Islam et al., 2015), an increase
56 in grain yield (Salvagiotti et al., 2013) and a lower mycotoxin content (Blandino et al., 2009a). In
57 addition to the sowing time, other crop practices, such as soil tillage and starter fertilization, could
58 also have a direct influence on the capacity of maize to overcome a late development in the early
59 stages, when low-temperature stress may occur, thus leading to clear flowering earliness benefits.

60 Although the adoption of no tillage or minimum tillage has also increased globally for maize (Kassam
61 et al., 2019), these techniques are still a matter of concern since they might negatively affect the soil
62 environment in temperate growing areas with cooler temperatures and higher moisture contents in
63 the early crop growth stages and greater root penetration resistance (Blanco-Canqui and Ruis, 2018;
64 Guan et al., 2014). All these conditions may negatively influence the critical phase of crop
65 establishment, and may result in a delay in emergence and slower early growth (Licht and Al-Kaisi,
66 2005). One strategy to overcome this problem is to apply tillage to only a part of the surface, which
67 could help to reduce the adverse effects of no-tillage, and maintain most of its positive characteristics.

68 With this technique, a narrow strip is tilled and prepared as a seedbed with a single passage that also
69 includes the displacement of previous crop residues and the incorporation of fertilizers. The limited
70 tilled areas in the strip tillage (ST) are associated with warmer soil temperatures and lower bulk
71 densities than no-tillage (Celik et al., 2013). Licht and Al-Kaisi (2005) reported an increase of 1.2-
72 1.4 °C when the soil was strip-tilled, compared to no-tillage, and the plant emergence rate was similar

73 to a plowed treatment. Many studies have evaluated the effects of conventional, strip- and no-tillage
74 techniques on maize yield. Liu and Wiatrak (2012) reported that the maize yield was similar under
75 plowing and ST, but significantly lower under no-tillage, in a loamy sand soil in a dryland rain-fed
76 environment. Conversely, Vyn and Raimbault (1992) recorded a 10-12% reduction in grain yield
77 under ST in Ontario (Canada), compared to plowing, in both silty loam and clayey loam soils. Some
78 authors have instead found an increase in yield under strip tillage, compared to a conventional system.
79 In tropical growing areas, Mullins et al. (1998), for example, observed a yield increase of 16% of
80 silage and 43% of grain in maize for silage and grain production cropping systems, respectively.
81 Furthermore, in terms of economic costs and profitability, farmers who adopt ST have a 34% greater
82 net return than those who apply conventional tillage (i.e. plowing followed by secondary tillage),
83 mainly due to the reduced number of operations, and the consequent reduced time consumption, fuel
84 consumption and machinery investment costs (Morris et al., 2010; Deleon et al., 2020). Moreover,
85 the reduced number of preparatory operations could favor early sowing dates, thereby guaranteeing
86 an optimal timeliness in crop establishment (Morris et al., 2010). However, the occurrence of previous
87 crop residues on the soil surface as a result of reduced tillage is a critical key factor in the inoculum
88 production of fungal species, and an accumulation of mycotoxins has been observed in wheat and
89 other winter cereals (Blandino et al., 2012). Nevertheless, the role of reduced tillage on the content
90 of such contaminants in maize grain is still unknown.

91 On the other hand, the initial growth of maize could benefit from localized fertilization. The precise
92 placement of phosphorus (P) and nitrogen (N) fertilizers at sowing as a starter fertilization is a
93 common practice used by maize farmers to improve the early-season nutrient uptake and early
94 development of the plant, but also the total nutrient use efficiency, and to achieve adequate yields, in
95 particular in regions where early temperatures are suboptimal for maize growth (Nkebiwe et al., 2016;
96 Quinn et al., 2020). Many studies carried out under plowing conditions have reported benefits for the
97 early growing of maize due to the synergism of P with N, especially as ammonium (NH_4^+), if supplied
98 in sub-surface bands near the seed furrows. The main benefits include lateral and fine root

99 proliferation (Jing et al., 2010; Ma et al., 2013), increased P and micronutrient availabilities, due to a
100 lower rhizosphere pH following NH_4^+ uptake (Jing et al., 2012; Ma et al., 2014), and boosting of early
101 crop development (Osborne, 2005; Ma et al., 2015). Positive effects have also been found in soils
102 with a medium or high available P content (Blandino et al., submitted; Kaiser et al., 2016). In a context
103 of sustainable agriculture, inspired by circular economy principles, i.e., nutrient recycling and
104 recovery to avoid waste creation, P and N manufactured fertilizers could be replaced by animal
105 manures or other organic materials. Animal manures are historically used for crop fertilization
106 purposes in regions characterized by an integrated livestock-mixed agriculture, but their repeated
107 supply often causes an enrichment of the soil P status, due to the lower N/P ratios of manures than
108 that of plants, with possible threats to the quality of freshwater (Borda et al., 2011). Although the
109 organic P fraction contained in manures is not immediately available for plant uptake, soluble organic
110 phosphate compounds and organic acids are released after mineralization. These molecules prevent
111 P fixation in soil and result in greater concentrations of available P (Laboski and Lamb, 2003). A
112 literature review by Kratz et al. (2019) showed that farmyard manure and slurry contain a large
113 amount of easily soluble P, thus displaying a medium-to-high P fertilizing value compared to mineral
114 fertilizers. This was also observed for biogas digestates, which often contain animal manures as the
115 main or co-substrate for digestion. Furthermore, Battaglia et al. (2021) demonstrated that manure
116 injection is also compatible with both no- and minimum-tillage, if associated with precision auto-
117 guidance systems which allow a simultaneous injection below the future seed rows with the tillage
118 operation (Tauchnitz et al., 2018). The adoption of liquid manure injection along seed rows as a starter
119 fertilization to replace mineral fertilizers can help increase the sustainability of agriculture and
120 implement fertilization best management practices based on the 4Rs principles (right nutrient source,
121 right rate, right time and right place).

122 The effects of the liquid manure injection in a band close to maize rows as an alternative N and P
123 source to replace mineral fertilizers on the soil macro- and micronutrient availability and
124 consequently on the nutrient use efficiency and on the final yield of maize silage have been tested in

125 several studies carried out in Germany (Westerschulte et al., 2018, Federolf et al., 2016; Federolf et
126 al., 2017), Denmark (Pedersen et al. 2020a), the Netherlands (Schröder et al. 2015), the USA
127 (Battaglia et al., 2021) and Canada (Hunt and Bittman, 2021). However, only a few studies have
128 evaluated the effects of manure injection as a starter fertilization and its combination with reduced
129 tillage techniques on maize grain yield (Bittman et al., 2012; Preston et al., 2019). None of the
130 aforementioned studies included measurements of the early growth of plants or the date of flowering,
131 the associated grain yield or the qualitative benefits; the effects of starter fertilization, in a minimum
132 tillage context, therefore still remain unclear.

133 The objective of the present study has been to quantify the effects of the adoption of ST, compared
134 to plowing, and of localized fertilization at sowing with biogas raw digestate or mineral NP fertilizer,
135 on the early development, growth, yield and sanitary traits of maize, in two different kinds of soils,
136 under the hypothesis that a localized manure distribution could efficiently surrogate mineral NP
137 fertilizers in promoting the early growth of maize, in particular in a context of reduced tillage
138 techniques.

139 **2. Materials and methods**

140 **2.1. Study sites**

141 The study was performed in field experiments at Carmagnola (44°23'N, 7°40'E) and Poirino
142 (44°56'N, 7°51'E), in North-West Italy, during the 2019 and 2020 growing seasons. Both soils lie in
143 a plain area which is intensively cultivated. The soil texture was loamy silt at both locations, but the
144 soil at Carmagnola was richer in sand and poorer in clay than the Poirino soil (Tab. 1). The soil pH
145 was medium alkaline (8.1) at Carmagnola and slightly acid (6.3) at Poirino. The available P content
146 (Olsen et al., 1954) was medium in both plots used in the two growing seasons (14 and 21 mg kg⁻¹)
147 at Carmagnola, while it was medium (18 mg kg⁻¹) in 2019 and high (58 mg kg⁻¹) in 2020 at Poirino.
148 The total N in the soil was medium at Carmagnola in 2019 and low for all the other site-year
149 combinations. The amount of organic matter was medium at both sites (2.3 and 1.5% at Carmagnola
150 and Poirino, respectively) and so was the cation exchange capacity.

151 The climate was temperate, sub-continental at both sites, with two main rainy periods concentrated
152 in spring and autumn. Daily temperatures and precipitation were measured by meteorological stations
153 located near the experimental sites. Table 2 reports the monthly rainfall and the growing degree days
154 (GDDs) relative to the two experimental seasons.

155

156 **2.2. Experimental set up**

157 Different soil tillage and starter fertilization strategies were compared in each site and for each year
158 according to a full factorial combination. The field experiments utilized a factorial arrangement
159 within a split-plot design, where the tillage system represented the main plot and the starter
160 fertilization was the subplot. Subplots were 30 × 3 m, replicated four times. In 2020, the experiment
161 was performed in a new area adjacent to the one used in 2019 at both sites, to avoid carry-over effects
162 due to treatments.

163

164 **2.3. Tillage techniques**

165 The strip tillage technique (ST) was compared with a reference plowed system (PLOW) for the
166 seedbed preparation. ST was performed the day before maize sowing with combined equipment,
167 which consisted of a four-row tiller (3 m width; Strip Hawk Easy, MOM, Villa Fontana, Verona,
168 Italy) set at a depth of 25 cm that tilled 25 cm strips and left 50 cm of standing crop residues between
169 the tilled rows. Each working unit consisted of a sequence of cutting discs followed by star-shape
170 discs for the row cleaning and a C-shank with tines for a deeper soil tillage, plus a couple of side-
171 closing discs that contained the clods within the strip. A final chain roll broke the clods and levelled
172 and firmed the seedbed. The reference tillage technique in the PLOW treatment involved soil plowing
173 at a depth of 30 cm, followed by disc harrowing and rotary tiller levelling, which represent the
174 traditional sequence of operations commonly used by farmers in the area. The soil plowing and the
175 following secondary operations were performed in spring just before maize sowing.
176 The previous crop was maize for grain at both sites, as maize continuous mono-cropping is typical in
177 Northern Italy, and the maize stovers, after an autumn chopping, were left on the soil surface until
178 tillage. No additional field operations were performed before maize sowing in the ST system.

179

180 **2.4. Starter fertilization strategies and nutrients management**

181 A deep-placed digestate fertilization (DIG) was compared with mineral fertilization (DAP) sub-
182 surface placed at sowing and with a control (NT). The DIG and DAP treatments received the same -
183 P starter fertilization at sowing, but from different sources. The DIG treatment used digestate
184 originating from the anaerobic digestion of farmyard manure (75%) and maize (25%) to supply 202
185 kg N ha⁻¹ and 69 kg P₂O₅ ha⁻¹. The digestate was injected, concurrently with the strip tillage operation,
186 or after rotary harrowing, as a deep band, at a depth of ~20 cm from the soil surface by a slurry tank
187 equipped with injector tools. The digestate contained 66.5 g kg⁻¹ of N (51% of which as N-NH₄), 24.1
188 g kg⁻¹ of P₂O₅, and 75.4 g kg⁻¹ of K₂O, all expressed on a dry matter basis, while its pH was 8.2.

189 Diammonium phosphate (18% N and 46% P₂O₅) was distributed in the DAP treatment to supply 27
190 kg N ha⁻¹ and 69 kg P₂O₅ ha⁻¹ in sub-surface bands, 5 cm apart from the maize seed rows, using a
191 calibrated granular dispenser mounted onto the precision seeder.
192 The control treatment NT did not receive any N or P fertilizer at sowing.
193 The pre-sowing fertilization management provided 100 kg ha⁻¹ of K₂O as potassium chloride (60%
194 K₂O w/w) broadcast applied, albeit only to the NT and DAP treatments.
195 In order to cope with the N crop needs, a top-dressing fertilization with urea was carried out at
196 approximately GS 17 (according to the BBCH scale) without soil incorporation to maintain a
197 homogeneous management between the tillage treatments. In order to minimize ammonia
198 volatilization, urea was side-dressed using a pneumatic fertilizer spreader after a rainy day and in the
199 late afternoon. The N requirement was calculated separately for each experimental site taking account
200 the different soil fertility levels and local standards to achieve the full potential grain yield. The top-
201 dressing N rates applied as urea were obtained as the difference between the crop needs and the
202 amount of N supplied with the starter fertilization for the DAP and DIG treatments, while the NT
203 treatment received the same N rate as the DAP treatment (Table S1).

204

205 **2.5. Field operations and crop management practices**

206 The maize hybrids, the relative maturity FAO classes, and the sowing and harvesting dates are
207 reported in Table 3. The distance between the plants was 16 cm, thereby providing a theoretical crop
208 density of 8.3 plant m⁻². An RTK GPS-based guidance was employed to obtain an accurate seed
209 placement along the central line of either the ST or the digestate bands. The planter was also carefully
210 calibrated in order to obtain the same sowing depth in both tillage systems.

211 The weed control of the ST plots involved the use of glyphosate (1.92 kg Active Ingredient (AI) ha⁻¹
212 ¹) (Roundup® Platinum, Bayer CropScience S.r.l., Milan, Italy) before tillage. The weed control was
213 performed at pre-emergence with mesotrione (0.15 kg AI ha⁻¹), S-metolachlor (1.25 kg AI ha⁻¹) and
214 terbuthylazine (0.75 kg AI ha⁻¹) (Lumax®, Syngenta Crop Protection S.p.A., Milan, Italy) under both

215 tillage conditions and in both locations. Furthermore, the ST plots in Poirino also required a post-
216 emergence weed control, using Nicosulfuron (40.5 g AI ha⁻¹), Rimsulfuron (10.1 g AI ha⁻¹) and
217 Dicamba (0.24 kg AI ha⁻¹) (Principal® Mais, Du Pont de Nemours Italiana S.r.l., Milan, Italy).
218 In order to avoid any drought stress until the physiological maturity, irrigation was performed with a
219 drip irrigation hose system at Carmagnola and using the sprinkler method at Poirino, according to the
220 management commonly used by farmers in the area.

221 In order to evaluate the role of the presence of maize residues on the soil surface as ear rot inoculum,
222 the control of European Corn Borer (*Ostrinia nubilalis* Hübner; ECB) was conducted by applying
223 Chlorantraniliprole (0.03 kg AI ha⁻¹) and Lambda-cialotrina (0.01 kg AI ha⁻¹) (Ampligo®, Syngenta
224 Crop Protection S.p.A., Milan, Italy) as a foliar insecticide at the maize milk ripening stage (GS75).

225

226 **2.6. Soil temperature, plant emergence and early crop vigor measurements**

227 A series of measurements on the soil and the crop were made on the two central rows of each plot.
228 The soil temperature was measured during the crop emergence and seedling stages using a 10K NTC
229 Thermistor sensor (Tinytag® TGP-4017 temperature logger devices with hourly record frequency)
230 placed at a depth of 7 cm along the seed rows. Maize emergence was assessed twice by measuring
231 the number of emerged plants per square meter, when approximately 50% and 100% of the potential
232 plants had emerged in the PLOW reference treatment.

233 The Normalized Difference Vegetation Index (NDVI) was measured during the vegetative stages by
234 means of a hand-held optical sensing device GreenSeekerTM® (Trimble©, Sunnyvale, California,
235 USA). An operator held the instrument approximately 60 cm above the maize canopy and walked at
236 a constant speed along the entire length of the plot. This assessment was performed every week from
237 the three-leaf stage (GS 13) until tassel emission (GS 55). The plant growth rate was calculated as the
238 average daily NDVI increase during the vegetative period, from the 3-leaf emission stage to the
239 middle of stem elongation. As detailed by Capo et al. (2020), the NDVI observations were used to
240 calculate the Area Under Canopy Development Curve (AUCDC) using the following formula:

$$AUCDC = \sum_i^{n-1} \{[(R_i + R_{i+1})/2] (t_{i+1} - t_i)\}$$

242 where R is the NDVI value, t is the time of observation expressed in days and n is the number of
243 measurements.

244 The plant height was recorded between the leaf emission (~GS 15) and the stalk elongation (~GS 33)
245 stages on 20 plants, from the ground level up to the collar of the uppermost fully-developed leaf or
246 up to the uppermost detectable node, respectively. The anthesis date was determined when >50% of
247 the plants in each plot showed the stigmata tips (GS 63) and was expressed as days after sowing
248 (DAS). The stalk diameter was measured in correspondence of the first internode during the ripening
249 stage with a caliper to calculate its area, and the plant height with a folding ruler, as a proxy of plant
250 stability. At maturity plant height was measured from the ground up the collar of the uppermost leaf.

251

252 **2.7. Grain yield and yield components**

253 Maize grain yield and components, fully developed ears, plant density and number of barren plants
254 were assessed on 4.5 m² areas in the central part of each plot, at harvest maturity. The grain yield
255 components included the cob length, as measured on a sub-sample of 15 ears, the number of kernel
256 rows and the number of kernels per row, both of which were determined on 7 randomly selected ears.
257 The number of kernels per ear was calculated by multiplying the number of kernel rows by the number
258 of kernels per row, while the number of kernels per square meter (KSQ) was calculated by multiplying
259 the number of kernels per ear by the number of ears per square meter (Testa et al., 2016). All the
260 collected ears were shelled using an electric ear sheller and the grain moisture content was determined
261 using a Dickey-John GAC100 grain analyzer (Auburn, IL, USA). Grain samples were dried in a
262 forced-air oven at 60°C until constant weight to express the yield as dry matter and for the subsequent
263 analyses regarding the qualitative and sanitary traits. A sub-sample of 200 kernels was weighed to
264 obtain the thousand kernels weight (TKW). The test weight (TW) was determined on dry kernels, by
265 means of a Dickey-John GAC2000 grain analysis meter (Dickey-John Corp).

266

267 **2.8. Kernel quality and sanitary traits**

268 The grain quality traits were assessed through the kernel test weight (TW), as calculated on dry
269 kernels using a Dickey-John GAC100 grain analyzer (Auburn, IL, USA) and the grain protein content
270 (GPC), which was determined according to AACC (2000).

271 The sanitary traits were assessed from the ECB severity, which was calculated as the percentage of
272 kernels per ear characterized by injuries due to ECB larva activity, while ear rot severity was
273 calculated as the percentage of kernels per ear with symptoms, according to Blandino et al. (2009a).

274 The ECB and ear rot severity were both evaluated on a sub-sample of 20 ears. Furthermore, fumonisin
275 B₁ and B₂ (FBs) and deoxynivalenol (DON) contaminations were analyzed using the ELISA method,
276 by means of direct competitive immunoassays (RIDASCREEN® Fumonisin and RIDASCREEN®
277 DON, R-Biopharm, Darmstadt, Germany), according to the manufacturer's instructions. Briefly, both
278 mycotoxins were extracted for 15 min by mechanically shaking, at 100 rpm, (shaker model M102-
279 OS, MOM Instruments, Milan, Italy) 20 g samples with 100 ml of methanol/distilled water, (70:30,
280 v/v) for FBs and with distilled water for DON, respectively. After extraction and filtration through
281 Whatman® n.1 299 filters, 50 µl of diluted filtrate was used for the ELISA test. The optical density
282 was measured at 450 nm using an ELISA 96-well plate reader (Das srl, Rome, Italy), and all the
283 standard and sample solutions were analyzed in duplicate wells. The limit of quantification (LOQ) of
284 the analytical method was set at 250 µg kg⁻¹ and 37 µg kg⁻¹ for FBs and DON, respectively.

285

286 **2.9. Data analysis**

287 Data were analyzed using a mixed effects model where the experimental site, tillage technique and
288 starter fertilization strategy were considered as fixed effects, while the maize hybrid and the year -
289 nested effects, - were considered as random factors. A graphical method was used to verify the basic
290 assumptions (Onofri et al., 2016). A logarithmic transformation was applied to the FBs and DON
291 values. As the DON contamination at the Carmagnola site was smaller than the LOQ of the instrument

292 for both experimental years, the mixed model was only applied at the Poirino site, considering the
293 tillage technique and the starter fertilization strategy as fixed effects and the maize hybrid and the
294 year - nested effects - as random factors. When single factors or their interaction determined
295 significant effect, the means were compared using the Bonferroni post hoc test at $p \leq 0.05$. The
296 statistical procedure was performed using the lmer function in the LME4 statistical package of the R
297 software (R Core Team, 2019).

298 **3. Results**

299 **3.1. Weather and soil conditions**

300 The two growing seasons showed slightly different meteorological trends for both rainfall and
301 temperature (expressed as GDDs in Table 2). June 2019 had less rainfall than June 2020, which was
302 the wettest month of the 2020 growing season. The wettest month in 2019 was July and was
303 concurrent with the maize anthesis stage. The 2019 growing season was characterized by cooler
304 temperatures during April and May, while June was warmer, thus resulting in similar cumulative
305 GDDs for the two growing seasons at both experimental sites.

306 The soil temperatures measured during the seedling stages are reported in Figure 1 and are expressed
307 as GDDs. The soil temperatures confirmed the air temperature trends of the two experimental years.
308 If we consider the plowed soil as a reference, the GDDs reached at 42 DAS were 140°C and 100°C
309 higher in 2020 than in 2019 at Carmagnola and Poirino, respectively. The PLOW treatment showed
310 higher temperatures than the ST treatment at both locations, but with differences between the two
311 soils; the greatest differences between treatments were observed at Poirino. For example, in 2019, the
312 differences in GDDs at 42 DAS between the PLOW and ST tillage techniques were 17°C and 44°C
313 at Carmagnola and Poirino, respectively, while they were 34°C and 54°C, respectively, in 2020.

314

315 **3.2. Plant emergence and early crop vigor**

316 The tillage technique for seedbed preparation and the experimental site clearly affected the plant
317 emergence pattern, while the starter fertilization strategy did not (Table 4). The number of emerged
318 plants was lower in the ST treatment than in the PLOW one for both measurement times. Moreover,
319 in correspondence to the “50% emergence” measurement, the comparison of the density of emerged
320 plants between the two tillage techniques showed more pronounced differences for Poirino ($p < 0.001$;
321 Figure S1), where the PLOW treatment had 4.4 plants m^{-2} and the ST treatment had 1.0 plants m^{-2} ,
322 while the crop density for Carmagnola was 5.5 and 3.3 plants m^{-2} for the PLOW and ST treatments,
323 respectively. The tillage technique and the starter fertilization strategy at sowing clearly affected the

324 maize plant development, as assessed by means of the NDVI measurements during the vegetative
325 stages (Figure 2). The maize development during the early vegetative stages was different for the two
326 growing seasons. The 2019 growing season recorded a slower plant development than 2020 for all
327 the site-tillage technique combinations (the average NDVI increase per day was 0.0153 and 0.0183
328 units over 2019 and 2020, respectively). The adoption of the ST technique at Poirino resulted in a
329 reduced plant growth, compared to the PLOW technique, in particular in 2019 (0.0140 vs 0.0161
330 NDVI units d⁻¹), while the differences were more limited (0.0142 vs 0.0151 NDVI units d⁻¹) in 2020.
331 The gap in daily NDVI increases between the two tillage techniques was narrower at Carmagnola for
332 both years (0.0150 vs 0.0161 and 0.0218 vs 0.0220 NDVI units d⁻¹ over 2019 and 2020, respectively).
333 The two starter fertilization strategies, that is, DAP and DIG, showed, on average, similar and faster
334 daily crop developments (0.0173 NDVI units d⁻¹) than the NT treatment (0.0158 NDVI units d⁻¹).
335 The NDVI values were summarized by the AUCDC index, thus allowing the differences in plant
336 development during the early growing stages (Table 4) to be assessed. Generally, the PLOW
337 treatment resulted in a significant ($p < 0.001$) increase in the AUCDC, compared to the ST system,
338 with a significantly more marked effect at Poirino (+20%) than at Carmagnola (+7%) (Figure 3a). On
339 the other hand, the DAP treatment performed better than DIG and the NT control. The starter
340 fertilization strategy differed between the two soil types ($p < 0.001$), and between the two tillage
341 techniques ($p < 0.001$). In fact, the DIG treatment at Carmagnola showed an intermediate AUCDC
342 value between the DAP and NT treatments (26.1 vs 29.0 and 24.8), while no differences were
343 recorded at Poirino, compared to DAP (Figure 4a). The crop growth determined by the DIG injection
344 at sowing did not show any differences from the NT when the soil was plowed, while it was higher
345 than NT (+12%) when the seedbed was prepared by ST (Figure 5).
346 The results regarding the plant height during the vegetative growth stages (GS 15 and GS 33)
347 confirmed those of the AUCDC index (Table 4). The plant height in the PLOW treatment increased
348 significantly ($p < 0.001$) by 34% and 56% at GS 15 and GS 33, respectively, compared to the ST. The
349 starter fertilization with DAP recorded the highest plant height for both measurement times, followed

350 by the DIG and the NT treatments. However, an interaction with the site was observed, with a more
351 pronounced positive effect of PLOW than of ST at Poirino (+222%) than at Carmagnola (+12%), at
352 GS 33 (Figures 3b and 3c). Another factor that affected the plant height was the starter fertilization
353 strategy, which again showed a different pattern at the two sites, both at the GS 15 and GS 33 growing
354 stages. During stem elongation, the plant height in the DIG treatment was 35% higher than in NT,
355 and 28% lower than in DAP at Carmagnola, while it was higher than NT (+229%) but similar to DAP
356 at Poirino (Figure 4b and 4c), thus enhancing the differences recorded during leaf emission.
357 The differences due to the tillage technique and to the starter fertilization strategy recorded during
358 vegetative development were confirmed at flowering and harvest (Table 4). On average, maize
359 anthesis in the PLOW system occurred 2.2 days before the ST system. However, this reduction in
360 days from sowing to plant flowering was significantly ($p < 0.001$; Figure S2) higher at Poirino (-3.4
361 days) than at Carmagnola (-1.1 days). The starter fertilization clearly affected the flowering date.
362 When DIG was supplied, flowering occurred at 94.4 DAS, while it occurred at 92.4 and 97.0 DAS in
363 the DAP and NT treatments. The earlier flowering resulted in a grain moisture content reduction at
364 harvest. On average, the PLOW technique showed a decrease of 1.2% in the grain moisture content,
365 compared to the ST system, while the DAP treatment resulted 0.8% drier than DIG, which was 1.1%
366 drier than the NT treatment. Furthermore, the three-way interaction was significant. The DIG
367 treatment showed a similar grain moisture content (24.6%) to the NT treatment (25.2%) for both
368 tillage techniques at Carmagnola, while the DIG treatment at Poirino did not differ from the DAP
369 treatment (Figure S3).

370

371 **3.3. Plant morphological traits**

372 The final crop density was affected by the experimental site and the tillage technique, but not by the
373 fertilization strategy (Table S2). The crop density was higher at Poirino (7.9 plants m⁻²) than at
374 Carmagnola (7.4 plants m⁻²). The tillage technique effect confirmed what was observed at emergence,

375 i.e. PLOW resulted in 0.3 plants m⁻² more than ST. The ear density exhibited the same trend as the
376 crop density, while no effects of the analyzed factors were recorded on the percentage of barren plants.
377 The stalk area, the plant height at maturity and the ear insertion height reported in Table S2 are proxies
378 of plant stability. The ST system led to an increase (+3.4%) in this parameter, compared to the PLOW
379 system, whereas the DAP and DIG similarly increased the stalk area in comparison to the NT
380 treatment. The lowest plant and ear insertion height at maturity was recorded when DAP was supplied
381 at sowing.

382

383 **3.4. Grain yield and yield components**

384 The grain yield was affected by the site and the starter fertilization strategy, while no effects were
385 recorded for the tillage technique as a single factor (Table 5). The highest yield was registered at
386 Carmagnola (16.4 Mg ha⁻¹), where 6% more was produced than at Poirino. The DAP and the DIG
387 applications at sowing led to a similar significant grain yield increase, compared to the NT treatment
388 (+11 and +12%, respectively). The significant interaction site × tillage indicated that the grain yield
389 was decreased by the ST adoption of 0.9 Mg ha⁻¹ at Poirino. On the contrary, ST recorded higher
390 grain yield (+ 1 Mg ha⁻¹) than PLOW at Carmagnola (Figure S4).

391 The significant three-way interaction showed the same ear density regardless the tillage technique
392 and the starter fertilization at Carmagnola site, while the ST treatment recorded lower values than
393 PLOW at Poirino site (Figure S5). Although, the ear length was 1.5% higher for the ST treatment
394 than for the PLOW one and the number of rows was 2.5% higher for PLOW than for ST, no
395 differences were observed for the number of kernels per row (Table S3) or for the number of kernels
396 per ear as a result of the tillage technique (Table 5). In contrast, the number of kernels per ear was
397 differently affected by the starter fertilization in the two sites. Compared to the NT treatment, the
398 DAP treatment increased the number of kernels per ear by 5.5 and 8.9% at Carmagnola and Poirino
399 sites, respectively, while the DIG injection led a similar enhancement (~ +6%) in the two sites (Figure
400 S6). Owing to the higher crop density observed in the PLOW treatment, KSM was also enhanced by

401 6%, compared to ST (Table S3), although TKW was not affected by the tillage system as single factor
402 (Table 5). However, the significant interaction site \times tillage ($p=0.039$; Figure S7a) showed that TKW
403 in the ST system was higher (+2.4%) than in the PLOW system at the Carmagnola site. In contrast,
404 the tillage system did not affect the TKW at Poirino. All the analyzed yield components, except TKW,
405 were significantly affected by the starter fertilization. The DAP fertilization determined an increase
406 in the cob length (+2.5%), compared to NT, while the number of rows per ear decreased (-2.4%),
407 compared to DIG. Furthermore, the DAP and DIG treatments led to a similar increase in the number
408 of kernels per row, the number of kernels per ear and the KSM to the untreated control. The starter
409 fertilization only affected the TKW at Carmagnola ($p < 0.001$; Figure S7b), where DAP and DIG were
410 characterized by similar kernels weights (+4 and +6% than NT, respectively).

411

412 **3.5. Grain qualitative and sanitary traits**

413 The qualitative parameters of the grain were affected by the experimental site and the starter
414 fertilization, while the tillage system did not show any significant effects (Table 6). The highest grain
415 hardness value, expressed as TW, was recorded at Poirino (78.1 kg hl⁻¹). The starter fertilization
416 performed with DAP and DIG equally increased TW (+1.2%), compared to NT. On the other hand,
417 GPC was higher at Carmagnola, while the starter fertilization improved GPC when DIG was
418 compared with NT, while DAP was in between.

419 Only the location affected the ECB and ear rot severities ($p < 0.001$ and $p = 0.002$, respectively; Table
420 6). Since all the experiments were treated with foliar insecticides, the injury caused by the ECB
421 activity was generally low, with less damage observed at Poirino than at Carmagnola. Ear rot severity
422 was significantly higher in Poirino than in the Carmagnola site, while tillage and starter fertilization
423 did not affect the ear surface with mold symptoms at harvest at either site. The FBs contamination
424 was higher ($p = 0.001$) at Carmagnola than at Poirino, while DON was only detected in Poirino (Table
425 7). Although the starter fertilization did not affect ECB or the ear rot severity, the DIG injection on
426 average lowered FBs contamination ($p = 0.002$). However, differences were found between the two

427 experimental sites: DIG and DAP behaved similarly in Carmagnola, while the DIG treatment in
428 Poirino recorded the lowest FBs contamination, while DAP showed an intermediate FBs
429 contamination compared to the NT and DIG treatments (Figure 6). The grain DON contamination in
430 Poirino was negatively affected by the adoption of ST, which increased it by 118%, compared to
431 PLOW, although no significant effect of starter fertilization was observed.

432 **4. Discussion**

433 The adoption of the two tested tillage techniques and starter fertilization with deep-injected DIG or
434 sub-surface placed DAP, did not show on average any differences in terms of grain yield. However,
435 important differences were recorded for the early plant development.

436 **4.1. Effect of the tillage technique**

437 The ST technique determined a significant delay in the early crop growth, probably because it
438 hampered the soil heat from building-up during the emergence and seedling stages, compared to
439 PLOW. The reduction in soil temperatures with ST could be due to the presence of crop residues on
440 the soil surface in the no-tilled areas (Griffith et al., 1973; Gauer et al., 1982). The overall effect of
441 tillage techniques on maize production depends to a great extent on their effect on the initial crop
442 establishment, starting from crop emergence (Trevini et al., 2013). Cooler soil temperatures (<12.5
443 °C), combined with a higher soil water content during the emergence period, increase the time
444 required for seedling emergence, decrease the emergence rate due to longer seed exposure to soil
445 pathogens, and determine a limited root surface area that could reduce the nutrient uptakes (Cutforth
446 et al., 1986; Imran et al., 2013), in particular in soils with a low drainage capacity (Dwyer et al.,
447 2000). The soil at the Poirino site, which is characterized by a slightly finer texture and lower drainage
448 capacity than that of the Carmagnola site, showed a limited heat build-up that slowed down the
449 germination, emergence and early growth of maize for both tillage techniques, but in particular when
450 ST was adopted. The Carmagnola soil, with a coarser texture and better drainage, showed higher soil
451 temperatures and a general faster emergence and early growth of maize, but also limited differences
452 between the strip-tilled and the plowed treatments. Apart from low temperatures, the type of tillage
453 also influences crop emergence through a uniformity of the depth and spacing of seed placement. An
454 inaccurate seed placement when adopting minimum tillage or ST techniques can be caused on one
455 hand by the hampering of crop residues in the seed-furrow, and on the other hand by the large soil
456 clods that result from the action of soil tillage machines.

457 Despite the delay in early development of ST, the final yield was similar for the two tillage systems.
458 The absence of an overall effect of tillage on yield could have been masked by the favorable late
459 summer weather recorded for both growing seasons, which may have allowed complete maturation,
460 even for strip tillage.

461 Among the yield components, tillage influenced the crop density and cob length, and caused a smaller
462 number of KSM in the ST treatment. These findings are in agreement with the study of Rashidi and
463 Keshavarzpour (2007), who recorded a lower crop density for a variety of reduced tillage techniques.
464 The same authors also reported a reduced cob length and a higher number of grain rows per ear for
465 minimum tillage, while our study showed opposite trends, like those of Ali et al. (2012).

466 Among the yield quality traits, GPC and TW were similar in the two tillage systems, while the grain
467 moisture content at harvest was different. The grain moisture increased by 1.2% for the ST treatment,
468 thus implying an additional grain drying process in autumn, probably because anthesis was postponed
469 by 2.2 days, compared to PLOW. Similar results were reported by Hudon et al. (1990).

470 The content of mycotoxin in ST could be higher as a consequence of both a higher inoculum
471 production (crop debris on soil surface) and higher infection and disease development (due to a later
472 flowering and ripening). Tillage could affect the grain sanitary traits because of both later flowering
473 and ripening or the different incorporations of the previous crop residues into the soil. The late
474 development of maize cultivated in ST, with a late shift of ripening, could result in a higher severity
475 of ECB in the absence of a direct control of this insect (Blandino et al., 2009a) or condition more
476 favorable for fungal development, thus leading to a possible higher risk of mycotoxin contamination.

477 As far as the role of debris is concerned, previous studies conducted on wheat and other small cereals
478 (Blandino et al., 2010) reported remarkable effects of tillage on *Fusarium* head blight infection and
479 the DON contamination of wheat. Debris is the primary reservoir of *Fusarium* inoculum (Koch et al.,
480 2006), and DON contamination in wheat is more severe if the preceding crops is maize, particularly
481 when minimum tillage or no-tillage is applied (Blandino et al., 2012). Conversely, only limited
482 information has been reported for the role of previous crop management on soil tillage in maize

483 (Palumbo et al., 2020). The present work, carried out by limiting the contamination linked to ECB
484 injuries through a direct control of the vectors, has highlighted that the occurrence of debris on the
485 soil surface with ST did not increase the risk of contamination by FBs, the most frequently detected
486 mycotoxins in temperate maize growing areas. Although the present experiment was carried out in
487 growing seasons with a low DON contamination, the ST treatment resulted in a significant increase
488 in this mycotoxin, compared to PLOW. This finding confirms the hypothesis advanced by Munkvold
489 (2014) who suggested that the tillage technique of maize has a greater influence on the fungi that
490 produce DON (e.g., *F. graminearum* or *F. culmorum*, which are characterized by an inoculum splash
491 dispersal), than on FBs-producing fungi (e.g. *F. verticillioides* or *F. proliferatum*). In fact, although
492 crop residues are also the most important source of inoculum for FBs-producing fungi, these species
493 produce large numbers of aerielly dispersed microconidia, and the effects of debris burying, as a result
494 of plowing, could be negligible in areas with high prevalence of maize, because of their long-distance
495 air dispersal (Munkvold, 2014).

496 In order to limit the occurrence of DON in maize, as a consequence of the additive effect of the
497 agronomic factors involved in the cropping system, it would be preferable not to apply ST in
498 environments in which there is cool and prolonged ripening, or where late maturity hybrids, high
499 plant populations or nitrogen fertilization are adopted (Blandino et al., 2009b).

500

501 **4.2. Effect of the starter fertilization**

502 The organic or mineral NP starter fertilization allowed the early development gap between the ST
503 and PLOW treatments to be compensated for, compared to NT (Fig. 5). As extensively reported in
504 literature, our results confirm the synergistic effect of combined applications of N and P on early crop
505 vigor, as assessed by the plant height and the AUCDC index growing curves.

506 The type of fertilization, which supplied equal amounts of P at sowing and equal total supplies of N
507 in deep-injected DIG and sub-surface placed DAP, influenced early maize development, as shown by
508 the plant height and AUCDC index. The DIG treatment equaled the crop development of DAP at

509 Poirino, while it caused a slower growth rate at Carmagnola, thus indicating an important effect of
510 the type of soil, also regarding nutrient availability. The Carmagnola soil had a lower concentration
511 of plant available-P and sub-alkaline pH, which may favor P sorption (Barrow, 2017), than the Poirino
512 soil. Consequently, the more readily available mineral form of the sub-surface placed DAP was better
513 utilized by small plants than the organic form of deep-injected DIG, which required a mineralization
514 process and could have caused an additional local increase in soil pH due to its intrinsic alkalinity
515 (Tampio et al., 2016). This suggested that supplying soluble P fertilizers in bands near the seed in
516 situations where soil P reactions are dominated by sorption may improve the nutrient use efficiency
517 (Battisti et al., submitted). An early availability of N and P provides an additional advantage to
518 cereals, as it determines the potential grain number for physiological reasons (Angus et al., 2019).
519 The differences observed between the sub-surface placed DAP and the deep-injected DIG band, in
520 terms of early crop growth enhancement, could also be linked to the depth of the fertilizer placement,
521 that is, 5 cm apart from the seed in the case of DAP and ~20 cm below the seed in the case of DIG.
522 These distances were chosen to avoid possible toxic effects of the fertilizer on the seedling, as young
523 roots may encounter reducing conditions, high concentrations of ammonium and a high pH, which
524 may cause plant death (Sawyer and Hoef, 1990; Salvagiotti et al., 2013), when manure is placed too
525 close to the seeds. The tested distances resulted in no negative effects on seed germination or the plant
526 density, compared to NT. This was in agreement with the results of Pedersen et al (2020b), who
527 suggested a distance of at least 5 cm between the manure band and the seed row to avoid root damage
528 following slurry injection. However, nutrients placed in a deeper position as DIG band may be less
529 plant-available during early stages of plant growth when root density is still low at high depths
530 (Nkebiwe et al., 2016). Further investigations should be conducted to optimize the placement distance
531 of digestate for the tested pedo-climatic situations.

532 The advantage, in terms of early development and earlier flowering of starter NP fertilization, led to
533 a significant increase in grain yield and test weight. Although the type of fertilization, deep-injected
534 DIG or sub-surface placed DAP, influenced the early growth of maize at sowing, it did not affect the

535 yield or most of the grain quality traits. The only quality parameter that was influenced by the type
536 of fertilization was the grain protein content, which was significantly increased when digestate was
537 used. This was probably because only part of the total applied N in digestate is immediately available,
538 while the organic fraction is slowly released through the mineralization process over the growing
539 season, thus leading to an improved synchrony between N demand and N availability (Silva et al.
540 (2005).

541 In the environment where this study was conducted, the application of mineral NP fertilizer as a
542 starter fertilization, especially when combined with an early sowing date, could result in an overall
543 lower mycotoxin contamination, since maize flowering and kernel development occur for weather
544 conditions that are less favorable for fungal infection and, for FBs, the ripening can partially skip the
545 attack of ECB (Blandino et al., submitted). In the present study, in which a foliar insecticide was
546 applied to minimize the role of injuries caused by the insect vector that favors mycotoxin
547 contamination, a starter fertilization with both deep-injected DIG and sub-surface placed DAP was
548 also effective in reducing the FBs contamination at Poirino. This effect is probably related to the
549 earlier flowering and the earlier maturity of the fertilized treatments than the control, although a role
550 could also be played by the observed increase in ear length and grain yield, which contribute to
551 diluting the proportion of ears affected by ear rot (Blandino et al., 2008).

552 **5. Conclusion**

553 This study has provided useful information on how tillage techniques and starter fertilization
554 strategies, based on the placement of N and P in bands at specific distances from seed rows, affect
555 maize emergence, early plant growth, yield components, as well as qualitative and sanitary grain
556 traits. Our findings highlighted the suitability of the adoption of the ST technique in different soils,
557 especially when conjugated with a proper starter fertilization management performed with digestate
558 or DAP. The deep injection of digestate showed moderate advantages on the early development of
559 maize and allowed the same yield to be achieved as the mineral starter fertilization. Furthermore,
560 digestate injection, together with strip tillage, enables farmers to prepare their seedbeds and distribute
561 manure in a single operation, thus saving time and reducing oil consumption. In addition, ammonia
562 emissions after manure spreading are reduced after an injection, compared to a broadcast application,
563 and the farm N use efficiency is therefore increased (Federolf et al, 2016).

564 The combination of these two good practices can therefore increase the environmental and economic
565 sustainability of farms, without significant impairments of the production level, grain quality or
566 sanitary aspects, compared to more conventional techniques that involve the use of mineral fertilizers
567 and plowing.

568 This study focused on the first-year effects of both practices. Future research should address the
569 medium and long-term effects, and also explore a wider annual variability, as several effects on grain
570 traits could be magnified over years with adverse ripening conditions.

571

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848 **Tables**

849

850 Table 1. Main physical and chemical characteristics of the topsoil (0-30cm) of the Carmagnola and
851 Poirino soils.

| Parameters | Measurement units | Carmagnola | Poirino |
|-----------------------------------|--------------------------|-------------------|-----------------------|
| Soil type ¹ | | Typic Udifluent | Fluventic Haplustepts |
| Sand (2 -0.05 mm) | % | 35.5 | 25.7 |
| Silt (0.05 - 0.002 mm) | % | 57.9 | 61.8 |
| Clay (< 0.002 mm) | % | 6.6 | 12.5 |
| pH | | 8.1 | 6.3 |
| Total CaCO ₃ | % | 1.2 | - |
| Organic matter | % | 2.3 | 1.5 |
| C/N | | 8.0 | 9.6 |
| Cation Exchange Capacity (C.E.C.) | meq/100g | 1.2 | 13.9 |
| Exchangeable K | mg kg ⁻¹ | 174.0 | 82.0 |
| Total Nitrogen | | | |
| 2019 | % | 0.163 | 0.089 |
| 2020 | % | 0.079 | 0.085 |
| Olsen P | | | |
| 2019 | mg kg ⁻¹ | 14 | 18 |
| 2020 | mg kg ⁻¹ | 21 | 58 |

852 ¹ Soil Surface Staff, 2014

853

854 Table 2. Cumulative monthly rainfall and air growing degree days (10°C base; GDDs) during the
 855 2019 and 2020 growing seasons at the Carmagnola and Poirino experimental sites.

| | GDDs (°C- day) | | | | Rainfall (mm) | | | |
|--------------|----------------|-------------|-------------|-------------|---------------|------------|------------|------------|
| | Carmagnola | | Poirino | | Carmagnola | | Poirino | |
| | 2019 | 2020 | 2019 | 2020 | 2019 | 2020 | 2019 | 2020 |
| March | 115 | 54 | 120 | 64 | 3 | 13 | 6 | 26 |
| April | 114 | 146 | 123 | 160 | 86 | 91 | 85 | 86 |
| May | 167 | 248 | 185 | 266 | 72 | 93 | 103 | 98 |
| June | 344 | 295 | 370 | 318 | 25 | 129 | 25 | 167 |
| July | 412 | 395 | 432 | 420 | 119 | 37 | 120 | 36 |
| August | 400 | 402 | 426 | 415 | 79 | 115 | 86 | 91 |
| September | 265 | 281 | 287 | 232 | 92 | 29 | 63 | 5 |
| Total | 1817 | 1821 | 1943 | 1874 | 476 | 508 | 488 | 509 |

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857

858 Table 3. Maize hybrids, relative maturity FAO class, sowing, top-dressing fertilization and
 859 harvesting dates for each site-year combination.

| Site - Year | Hybrid | FAO Class | Sowing date | Top-dressing fertilization date | Harvesting date |
|--------------------|--------------------------------|------------------|------------------------|--|----------------------------|
| Carmagnola - 2019 | Korimbos (KWS Saat AG) | 500 | 04 th April | 30 th May | 25 th September |
| Poirino - 2019 | Korimbos (KWS Saat AG) | 500 | 21 st March | 03 rd June | 24 th September |
| Carmagnola - 2020 | LG 30600 (LG Seeds, Limagrain) | 600 | 08 th April | 22 nd May | 23 rd September |
| Poirino - 2020 | Sy Brabus (Syngenta) | 700 | 31 st March | 27 th May | 24 th September |

860

861 Table 4. Effect of site, tillage and starter fertilization on maize emergence, early plant vigor, expressed as the plant height at the leaf emission (GS17)
 862 and stem elongation stages (GS33), the area under the canopy development curve (AUCDC), the date of flowering, expressed as days after sowing
 863 (DAS), and grain moisture content at harvest for 2019 and 2020.

| Factor | Source of Variation | 50% Emergence (plants m ⁻²) | 100% Emergence (plants m ⁻²) | Plant height GS 15 (cm) | Plant height GS 33 (cm) | AUCDC | Date of flowering (DAS) | Grain moisture (%) |
|------------------------------------|---------------------|---|--|-------------------------|-------------------------|---------|-------------------------|--------------------|
| Site ^a | Carmagnola | 4.4 a | 7.2 b | 12.7 b | 50.4 a | 26.6 | 87.5 b | 24.4 b |
| | Poirino | 2.7 b | 7.6 a | 14.0 a | 26.8 b | 26.9 | 101.7 a | 25.6 a |
| | <i>p</i> (F) | < 0.001 | 0.048 | 0.045 | < 0.001 | n.s. | < 0.001 | < 0.001 |
| Tillage ^b | ST | 2.2 b | 7.2 b | 11.4 b | 30.2 b | 25.1 b | 95.7 a | 25.6 a |
| | PLOW | 4.9 a | 7.6 a | 15.3 a | 47.0 a | 28.4 a | 93.5 b | 24.4 b |
| | <i>p</i> (F) | < 0.001 | 0.002 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 |
| Starter fertilization ^c | DIG | 3.6 | 7.6 | 12.9 b | 42.4 b | 26.7 b | 94.4 b | 24.9 b |
| | DAP | 3.6 | 7.2 | 15.8 a | 50.0 a | 28.5 a | 92.4 c | 24.1 c |
| | NT | 3.4 | 7.3 | 11.3 c | 23.5 c | 25.1 c | 97.0 a | 26.0 a |
| | <i>p</i> (F) | n.s. | n.s. | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 |
| Site x Tillage | <i>p</i> (F) | < 0.001 | n.s. | < 0.001 | < 0.01 | < 0.001 | < 0.001 | < 0.001 |
| Site x Starter | <i>p</i> (F) | n.s. | n.s. | < 0.001 | < 0.001 | 0.001 | n.s. | n.s. |
| Tillage x Starter | <i>p</i> (F) | n.s. | n.s. | n.s. | n.s. | 0.001 | n.s. | n.s. |
| Site x Tillage x Starter | <i>p</i> (F) | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | 0.027 |

864 ^a The site values are based on 48 observations (2 years × 2 tillage systems × 3 starter fertilizations × 4 repetitions).

865 ^b The tillage values are based on 48 observations (2 years × 2 sites × 3 starter fertilizations × 4 repetitions). ST: strip tillage; PLOW: plowing.

866 ^c The starter fertilization values are based on 32 observations (2 years × 2 sites × 2 tillage systems × 4 repetitions). DIG: 202 kg N ha⁻¹ and 69 kg P₂O₅ ha⁻¹ as
 867 digestate the day before sowing; DAP: 27 kg N ha⁻¹ and 69 kg P₂O₅ ha⁻¹ as diammonium phosphate at sowing; NT: no NP fertilization at maize sowing.

868 Means followed by different letters are significantly different at *p* < 0.05

869

870 Table 5. Effect of site, tillage and starter fertilization on maize grain yield and yield components: ear density, number of kernels (K) per ear and
 871 thousand kernels weight (TKW) for 2019 and 2020. DM: dry matter.

| Factor | Source of Variation | Grain yield (Mg ha ⁻¹ DM) | Ear density (ear m ⁻²) | K ear ⁻¹ | TKW (g) |
|------------------------------------|---------------------|--------------------------------------|------------------------------------|---------------------|---------|
| Site ^a | Carmagnola | 16.4 a | 7.2 b | 623 a | 381 a |
| | Poirino | 15.5 b | 7.7 a | 594 b | 362 b |
| | <i>p</i> (F) | 0.013 | 0.006 | < 0.001 | < 0.001 |
| Tillage ^b | ST | 15.9 | 7.3 b | 603 | 373 |
| | PLOW | 15.9 | 7.6 a | 614 | 371 |
| | <i>p</i> (F) | n.s. | 0.016 | n.s. | n.s. |
| Starter fertilization ^c | DIG | 16.6 a | 7.6 | 619 a | 374 |
| | DAP | 16.4 a | 7.4 | 624 a | 375 |
| | NT | 14.8 b | 7.4 | 582 b | 366 |
| | <i>p</i> (F) | < 0.001 | n.s. | 0,008 | n.s. |
| Site x Tillage | <i>p</i> (F) | < 0.001 | n.s. | 0.008 | 0.039 |
| Site x Starter | <i>p</i> (F) | n.s. | 0.048 | 0.044 | 0.002 |
| Tillage x Starter | <i>p</i> (F) | n.s. | n.s. | n.s. | n.s. |
| Site x Tillage x Starter | <i>p</i> (F) | n.s. | 0.034 | n.s. | n.s. |

872 ^a The site values are based on 48 observations (2 years × 2 tillage systems × 3 starter fertilizations × 4 repetitions).

873 ^b The tillage values are based on 48 observations (2 years × 2 sites × 3 starter fertilizations × 4 repetitions). ST: strip tillage; PLOW: plowing.

874 ^c The starter fertilization values are based on 32 observations (2 years × 2 sites × 2 tillage systems × 4 repetitions). DIG: 202 kg N ha⁻¹ and 69 kg P₂O₅ ha⁻¹ as
 875 digestate the day before sowing; DAP: 27 kg N ha⁻¹ and 69 kg P₂O₅ ha⁻¹ as diammonium phosphate at sowing; NT: no NP fertilization at maize sowing.

876 Means followed by different letters are significantly different at *p* < 0.05

877 Table 6. Effect of site, tillage and starter fertilization on qualitative and sanitary traits, expressed as
 878 kernels test weight (TW), grain protein content (GPC), European Corn Borer (ECB) injuries and ear
 879 rot severity on maize ears at harvest for 2019 and 2020.

| Factor | Source of Variation | TW (kg hl ⁻¹) | GPC (%) | ECB severity (%) | Ear rot severity (%) |
|------------------------------------|---------------------|---------------------------|---------|------------------|----------------------|
| Site ^a | Carmagnola | 76.5 b | 9.3 a | 3.7 a | 2.3 b |
| | Poirino | 78.1 a | 8.9 b | 2.1 b | 6.1 a |
| | <i>p</i> (F) | < 0.001 | 0.004 | < 0.001 | 0.002 |
| Tillage ^b | ST | 77.2 | 9.2 | 2.6 | 4.4 |
| | PLOW | 77.5 | 9.0 | 3.1 | 4.7 |
| | <i>p</i> (F) | n.s. | n.s. | n.s. | n.s. |
| Starter fertilization ^c | DIG | 77.6 a | 9.3 a | 2.9 | 4.2 |
| | DAP | 77.6 a | 9.1 ab | 3.0 | 5.1 |
| | NT | 76.7 b | 8.9 b | 2.8 | 4.3 |
| | <i>p</i> (F) | 0.002 | < 0.001 | n.s. | n.s. |
| Site x Tillage | <i>p</i> (F) | n.s. | n.s. | n.s. | n.s. |
| Site x Starter | <i>p</i> (F) | n.s. | n.s. | n.s. | n.s. |
| Tillage x Starter | <i>p</i> (F) | n.s. | n.s. | n.s. | n.s. |
| Site x Tillage x Starter | <i>p</i> (F) | n.s. | n.s. | n.s. | n.s. |

880 ^a The site values are based on 48 observations (2 years × 2 tillage systems × 3 starter fertilizations × 4
 881 repetitions).

882 ^b The tillage values are based on 48 observations (2 years × 2 sites × 3 starter fertilizations × 4 repetitions). ST:
 883 strip tillage; PLOW: plowing.

884 ^c The starter fertilization values are based on 32 observations (2 years × 2 sites × 2 tillage systems × 4
 885 repetitions). DIG: 202 kg N ha⁻¹ and 69 kg P₂O₅ ha⁻¹ as digestate the day before sowing; DAP: 27 kg N ha⁻¹
 886 and 69 kg P₂O₅ ha⁻¹ as diammonium phosphate at sowing; NT: no NP fertilization at maize sowing.

887 Means followed by different letters are significantly different at *p* < 0.05.

888

889 Table 7. Effect of tillage and starter fertilization on fumonisins B₁ + B₂ (FBs) and deoxynivalenol
 890 (DON) contaminations for 2019 and 2020.

| Factor | Source of Variation | FBs | | DON | |
|------------------------------------|---------------------|---------|--------------------------|--------|--------------------------|
| | | T | N (µg kg ⁻¹) | T | N (µg kg ⁻¹) |
| Site ^a | Carmagnola | 7.21 a | 4699 | - | < 37 |
| | Poirino | 6.49 b | 3998 | - | 188 |
| | <i>p</i> (F) | 0.001 | | - | |
| Tillage ^b | ST | 6.89 | 4457 | 5.11 a | 275 |
| | PLOW | 6.81 | 4267 | 4.35 b | 101 |
| | <i>p</i> (F) | n.s. | | 0.002 | |
| Starter fertilization ^c | DIG | 6.47 b | 3449 | 4.57 | 114 |
| | DAP | 6.97 a | 5209 | 4.55 | 150 |
| | NT | 7.12 a | 4466 | 5.07 | 301 |
| | <i>p</i> (F) | 0.002 | | n.s. | |
| Site x Tillage | <i>p</i> (F) | n.s. | | - | |
| Site x Starter | <i>p</i> (F) | < 0.001 | | - | |
| Tillage x Starter | <i>p</i> (F) | n.s. | | n.s. | |
| Site x Tillage x Starter | <i>p</i> (F) | n.s. | | - | |

891

892 The FBs and DON contamination means reported are transformed [$T; y' = \ln(x + 1)$] and non-transformed
 893 (N) values. The DON content in the Carmagnola site was always below the Limit of Quantification (LOQ) of
 894 the ELISA analysis, 37 µg kg⁻¹. The reported data only refer to the Poirino experiment in 2019 and 2020.

895 ^a The site values are based on 48 observations (2 years × 2 tillage systems × 3 starter fertilizations × 4
 896 repetitions).

897 ^b The tillage values are based on 48 observations (2 years × 2 sites × 3 starter fertilizations × 4 repetitions). ST:
 898 strip tillage; PLOW: plowing.

899 ^c The starter fertilization values are based on 32 observations (2 years × 2 sites × 2 tillage systems × 4
 900 repetitions). DIG: 202 kg N ha⁻¹ and 69 kg P₂O₅ ha⁻¹ as digestate the day before sowing; DAP: 27 kg N ha⁻¹
 901 and 69 kg P₂O₅ ha⁻¹ as diammonium phosphate at sowing; NT: no NP fertilization at maize sowing.

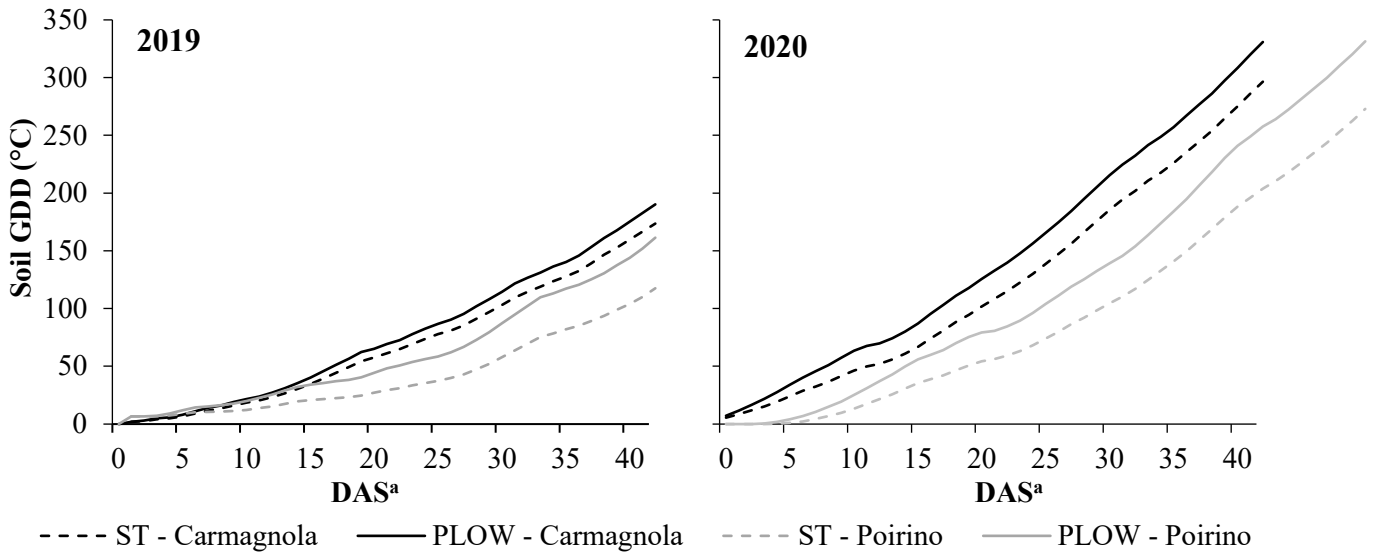
902 Means followed by different letters are significantly different at $p < 0.05$.

903

904 **Figures**

905

906 Figure 1. Cumulative soil growing degree days (10°C base; GDDs, at a depth of 7 cm) over the first
907 42 days after sowing (DAS) of the maize crop cycle in the 2019 and 2020 growing seasons at
908 Carmagnola and Poirino for the plowing (PLOW) and strip-tillage (ST) systems.

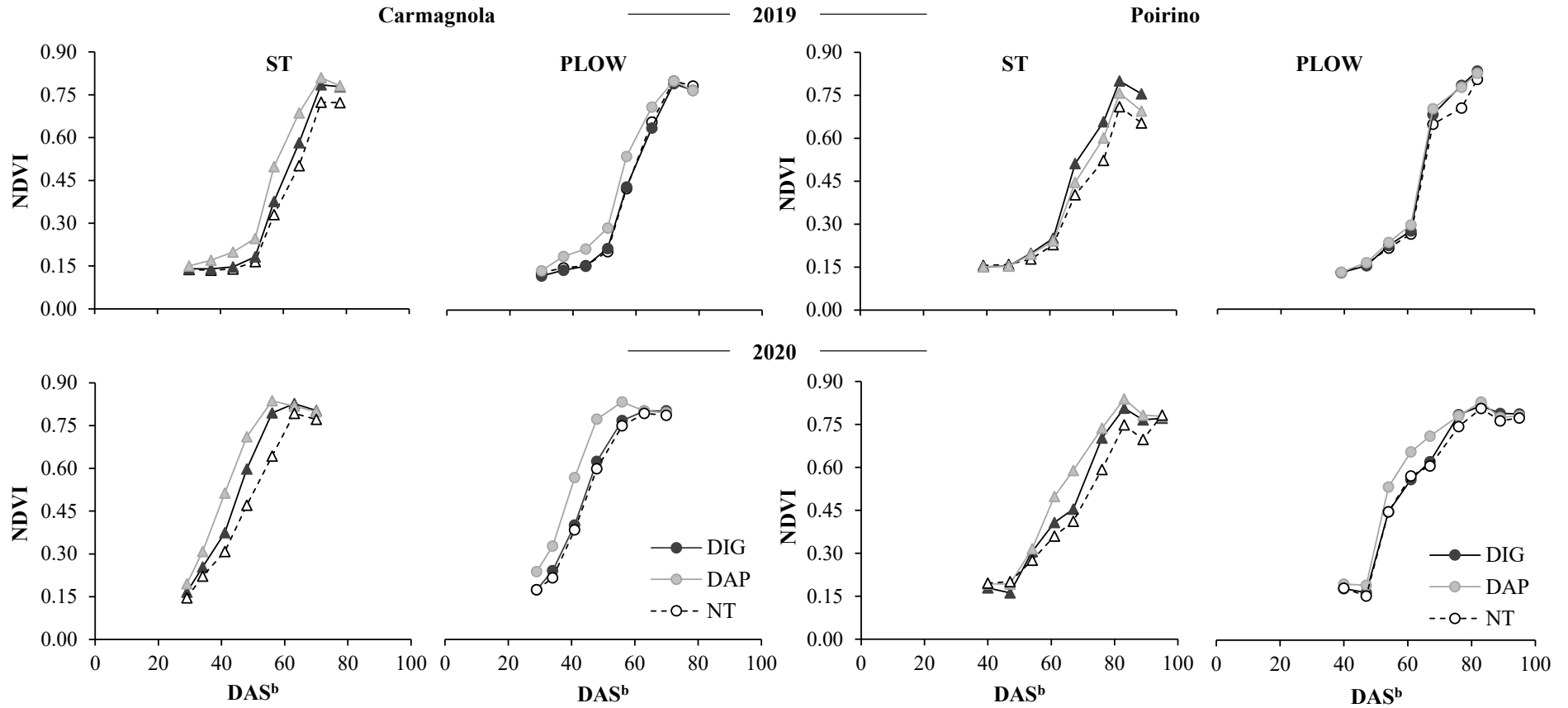


909

910 ^a DAS=days after sowing

911

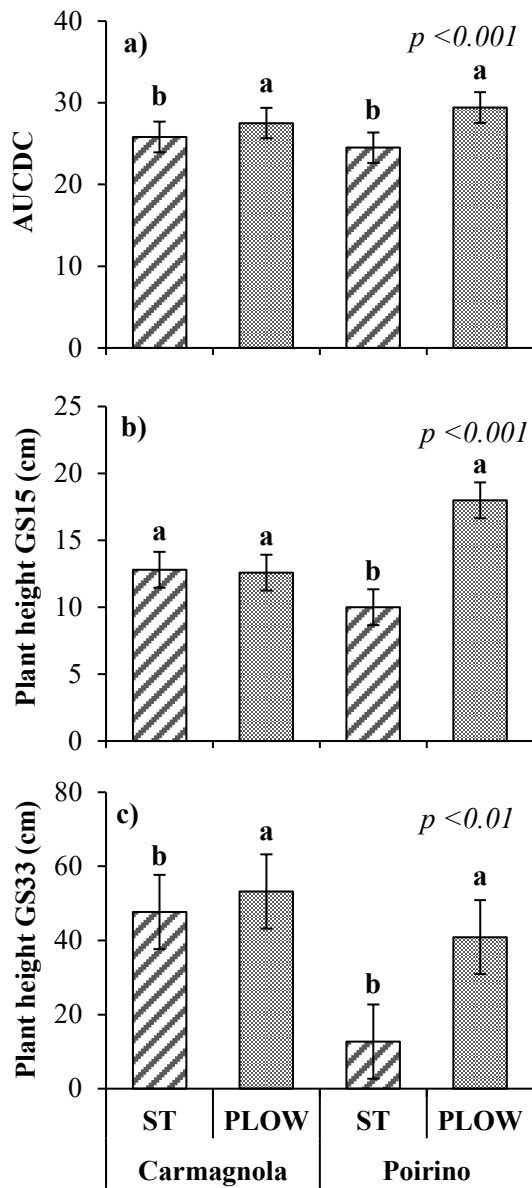
912 Figure 2. Effect of the starter fertilization strategies^a on the maize canopy development, expressed as NDVI from the 3-leaf stage to tasseling for the
 913 two compared tillage systems: strip-tillage (ST; triangle symbols) and plowing (PLOW; circle symbols) at Carmagnola (left-hand side) and Poirino
 914 (right-hand side) experimental sites for the 2019 (upper row) and 2020 (lower row) growing seasons.
 915



916
 917 ^a DIG: 202 kg N ha⁻¹ and 69 kg P₂O₅ ha⁻¹ as digestate the day before sowing; DAP: 27 kg N ha⁻¹ and 69 kg P₂O₅ ha⁻¹ as diammonium phosphate at sowing; NT: no
 918 NP fertilization at maize sowing.

919 ^b DAS=days after sowing

921 Figure 3. Effect of the tillage system, at each experimental site, on the early vigor of maize expressed
 922 as the area under the canopy development curve (AUCDC; a), the plant height at the leaf emission
 923 (GS15; b) and the stem elongation stages (GS33; c) for field experiments carried out in the 2019 and
 924 2020 growing seasons.



925 Soil tillage system: ST, strip tillage; PLOW, plowing.

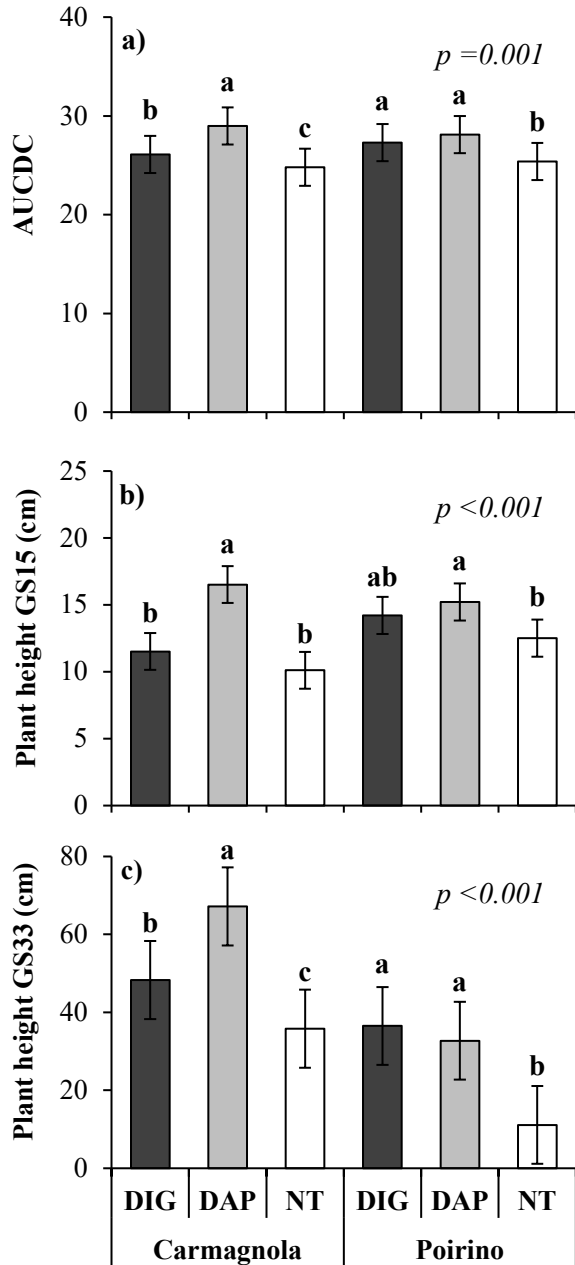
926 Means with different letters within each site are significantly different. Error bars show the \pm standard error.

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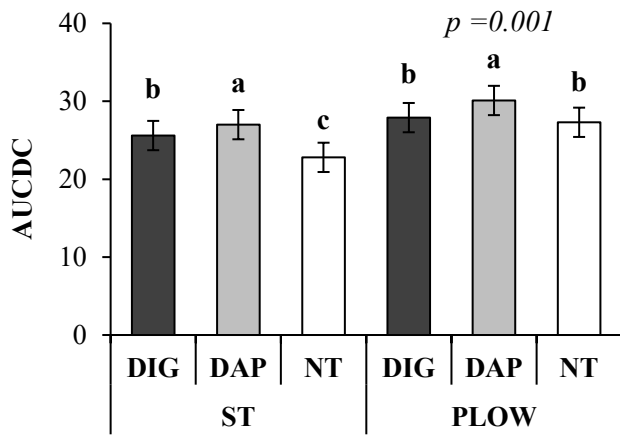
929

930 Figure 4. Effect of the starter fertilization at each experimental site on the early vigor of maize
 931 expressed as the area under the canopy development curve (AUCDC; a), the plant height at the leaf
 932 emission (GS15; b) and the stem elongation stages (GS33; c) for field experiments carried out in the
 933 2019 and 2020 growing seasons.
 934



935 Starter fertilization: DIG, 202 kg N ha⁻¹ and 69 kg P₂O₅ ha⁻¹ as digestate the day before sowing; DAP, 27 kg
 936 N ha⁻¹ and 69 kg P₂O₅ ha⁻¹ as diammonium phosphate at sowing; NT, no NP fertilization at maize sowing.
 937 Means with different letters within each site are significantly different. Error bars show the ± standard error.
 938

939 Figure 5. Effect of the starter fertilization within each tillage system on the early vigor of maize
940 expressed as the area under canopy development curve (AUCDC) for field experiments carried out
941 in the 2019 and 2020 growing seasons.



942

943 Soil tillage system: ST, strip tillage; PLOW, plowing.

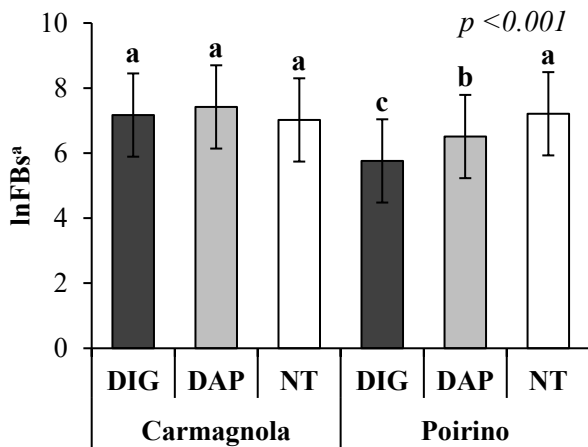
944 Starter fertilization: DIG, 202 kg N ha⁻¹ and 69 kg P₂O₅ ha⁻¹ as digestate the day before sowing; DAP, 27 kg

945 N ha⁻¹ and 69 kg P₂O₅ ha⁻¹ as diammonium phosphate at sowing; NT, no NP fertilization at maize sowing.

946 Means with different letters are significantly different. Error bars show the \pm standard error.

947

948 Figure 6. Effect of the starter fertilization at each experimental site on fumonisins B₁ + B₂ (FBs)
 949 contamination for field experiments carried out in the 2019 and 2020 growing seasons.



950

951 Starter fertilization: DIG, 202 kg N ha⁻¹ and 69 kg P₂O₅ ha⁻¹ as digestate the day before sowing; DAP, 27 kg
 952 N ha⁻¹ and 69 kg P₂O₅ ha⁻¹ as diammonium phosphate at sowing; NT, no NP fertilization at maize sowing.

953 Means with different letters within each site are significantly different. Error bars show the ± standard error.

954 ^aThe reported FBs contamination means are transformed [$y' = \ln(x + 1)$].

955