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Title

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

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Abstract

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

Keywords

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

1. Introduction

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

Although the adoption of no tillage or minimum tillage has also increased globally for maize (Kassam et al., 2019), these techniques are still a matter of concern since they might negatively affect the soil environment in temperate growing areas with cooler temperatures and higher moisture contents in the early crop growth stages and greater root penetration resistance (Blanco-Canqui and Ruis, 2018; Guan et al., 2014). All these conditions may negatively influence the critical phase of crop establishment, and may result in a delay in emergence and slower early growth (Licht and Al-Kaisi, 2005). One strategy to overcome this problem is to apply tillage to only a part of the surface, which could help to reduce the adverse effects of no-tillage, and maintain most of its positive characteristics. With this technique, a narrow strip is tilled and prepared as a seedbed with a single passage that also includes the displacement of previous crop residues and the incorporation of fertilizers. The limited tilled areas in the strip tillage (ST) are associated with warmer soil temperatures and lower bulk densities than no-tillage (Celik et al., 2013). Licht and Al-Kaisi (2005) reported an increase of 1.2-1.4 °C when the soil was strip-tilled, compared to no-tillage, and the plant emergence rate was similar to a plowed treatment. Many studies have evaluated the effects of conventional, strip- and no-tillage techniques on maize yield. Liu and Wiatrak (2012) reported that the maize yield was similar under plowing and ST, but significantly lower under no-tillage, in a loamy sand soil in a dryland rain-fed environment. Conversely, Vyn and Raimbault (1992) recorded a 10-12% reduction in grain yield under ST in Ontario (Canada), compared to plowing, in both silty loam and clayey loam soils. Some authors have instead found an increase in yield under strip tillage, compared to a conventional system. In tropical growing areas, Mullins et al. (1998), for example, observed a yield increase of 16% of silage and 43% of grain in maize for silage and grain production cropping systems, respectively. Furthermore, in terms of economic costs and profitability, farmers who adopt ST have a 34% greater net return than those who apply conventional tillage (i.e. plowing followed by secondary tillage), mainly due to the reduced number of operations, and the consequent reduced time consumption, fuel consumption and machinery investment costs (Morris et al., 2010; Deleon et al., 2020). Moreover, the reduced number of preparatory operations could favor early sowing dates, thereby guaranteeing an optimal timeliness in crop establishment (Morris et al., 2010). However, the occurrence of previous crop residues on the soil surface as a result of reduced tillage is a critical key factor in the inoculum production of fungal species, and an accumulation of mycotoxins has been observed in wheat and other winter cereals (Blandino et al., 2012). Nevertheless, the role of reduced tillage on the content of such contaminants in maize grain is still unknown.

On the other hand, the initial growth of maize could benefit from localized fertilization. The precise placement of phosphorus (P) and nitrogen (N) fertilizers at sowing as a starter fertilization is a common practice used by maize farmers to improve the early-season nutrient uptake and early development of the plant, but also the total nutrient use efficiency, and to achieve adequate yields, in particular in regions where early temperatures are suboptimal for maize growth (Nkebiwe et al., 2016; Quinn et al., 2020). Many studies carried out under plowing conditions have reported benefits for the early growing of maize due to the synergism of P with N, especially as ammonium (NH_4^+), if supplied in sub-surface bands near the seed furrows. The main benefits include lateral and fine root proliferation (Jing et al., 2010; Ma et al., 2013), increased P and micronutrient availabilities, due to a lower rhizosphere pH following NH_4^+ uptake (Jing et al., 2012; Ma et al., 2014), and boosting of early crop development (Osborne, 2005; Ma et al., 2015). Positive effects have also been found in soils with a medium or high available P content (Blandino et al., submitted; Kaiser et al., 2016). In a context of sustainable agriculture, inspired by circular economy principles, i.e., nutrient recycling and recovery to avoid waste creation, P and N manufactured fertilizers could be replaced by animal manures or other organic materials. Animal manures are historically used for crop fertilization purposes in regions characterized by an integrated livestock-mixed agriculture, but their repeated supply often causes an enrichment of the soil P status, due to the lower N/P ratios of manures than that of plants, with possible threats to the quality of freshwater (Borda et al., 2011). Although the organic P fraction contained in manures is not immediately available for plant uptake, soluble organic phosphate compounds and organic acids are released after mineralization. These molecules prevent P fixation in soil and result in greater concentrations of available P (Laboski and Lamb, 2003). A literature review by Kratz et al. (2019) showed that farmyard manure and slurry contain a large amount of easily soluble P, thus displaying a medium-to-high P fertilizing value compared to mineral fertilizers. This was also observed for biogas digestates, which often contain animal manures as the main or co-substrate for digestion. Furthermore, Battaglia et al. (2021) demonstrated that manure injection is also compatible with both no- and minimum-tillage, if associated with precision auto-guidance systems which allow a simultaneous injection below the future seed rows with the tillage operation (Tauchnitz et al., 2018). The adoption of liquid manure injection along seed rows as a starter fertilization to replace mineral fertilizers can help increase the sustainability of agriculture and implement fertilization best management practices based on the 4Rs principles (right nutrient source, right rate, right time and right place).

The effects of the liquid manure injection in a band close to maize rows as an alternative N and P source to replace mineral fertilizers on the soil macro- and micronutrient availability and consequently on the nutrient use efficiency and on the final yield of maize silage have been tested in several studies carried out in Germany (Westerschulte et al., 2018, Federolf et al., 2016; Federolf et al., 2017), Denmark (Pedersen et al. 2020a), the Netherlands (Schröder et al. 2015), the USA (Battaglia et al., 2021) and Canada (Hunt and Bittman, 2021). However, only a few studies have evaluated the effects of manure injection as a starter fertilization and its combination with reduced tillage techniques on maize grain yield (Bittman et al., 2012; Preston et al., 2019). None of the aforementioned studies included measurements of the early growth of plants or the date of flowering, the associated grain yield or the qualitative benefits; the effects of starter fertilization, in a minimum tillage context, therefore still remain unclear.

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

2. Materials and methods

2.1. Study sites

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

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

2.2. Experimental set up

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

2.3. Tillage techniques

The strip tillage technique (ST) was compared with a reference plowed system (PLOW) for the seedbed preparation. ST was performed the day before maize sowing with combined equipment, which consisted of a four-row tiller (3 m width; Strip Hawk Easy, MOM, Villa Fontana, Verona, Italy) set at a depth of 25 cm that tilled 25 cm strips and left 50 cm of standing crop residues between the tilled rows. Each working unit consisted of a sequence of cutting discs followed by star-shape discs for the row cleaning and a C-shank with tines for a deeper soil tillage, plus a couple of side-closing discs that contained the clods within the strip. A final chain roll broke the clods and levelled and firmed the seedbed. The reference tillage technique in the PLOW treatment involved soil plowing at a depth of 30 cm, followed by disc harrowing and rotary tiller levelling, which represent the traditional sequence of operations commonly used by farmers in the area. The soil plowing and the following secondary operations were performed in spring just before maize sowing.

The previous crop was maize for grain at both sites, as maize continuous mono-cropping is typical in Northern Italy, and the maize stovers, after an autumn chopping, were left on the soil surface until tillage. No additional field operations were performed before maize sowing in the ST system.

2.4. Starter fertilization strategies and nutrients management

A deep-placed digestate fertilization (DIG) was compared with mineral fertilization (DAP) sub-surface placed at sowing and with a control (NT). The DIG and DAP treatments received the same - P starter fertilization at sowing, but from different sources. The DIG treatment used digestate originating from the anaerobic digestion of farmyard manure (75%) and maize (25%) to supply 202 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1}. The digestate was injected, concurrently with the strip tillage operation, or after rotary harrowing, as a deep band, at a depth of ~20 cm from the soil surface by a slurry tank equipped with injector tools. The digestate contained 66.5 g kg^{-1} of N (51% of which as N-NH_4), 24.1 g kg^{-1} of P_2O_5, and 75.4 g kg^{-1} of K_2O, all expressed on a dry matter basis, while its pH was 8.2.

Diammonium phosphate (18% N and 46% P_2O_5) was distributed in the DAP treatment to supply 27 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1} in sub-surface bands, 5 cm apart from the maize seed rows, using a calibrated granular dispenser mounted onto the precision seeder.

The control treatment NT did not receive any N or P fertilizer at sowing.

The pre-sowing fertilization management provided 100 kg ha^{-1} of K_2O as potassium chloride (60% K_2O *w/w*) broadcast applied, albeit only to the NT and DAP treatments.

In order to cope with the N crop needs, a top-dressing fertilization with urea was carried out at approximately GS 17 (according to the BBCH scale) without soil incorporation to maintain a homogeneous management between the tillage treatments. In order to minimize ammonia volatilization, urea was side-dressed using a pneumatic fertilizer spreader after a rainy day and in the late afternoon. The N requirement was calculated separately for each experimental site taking account the different soil fertility levels and local standards to achieve the full potential grain yield. The top-dressing N rates applied as urea were obtained as the difference between the crop needs and the amount of N supplied with the starter fertilization for the DAP and DIG treatments, while the NT treatment received the same N rate as the DAP treatment (Table S1).

2.5. Field operations and crop management practices

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

The weed control of the ST plots involved the use of glyphosate (1.92 kg Active Ingredient (AI) ha^{-1}) (Roundup® Platinum, Bayer CropScience S.r.l., Milan, Italy) before tillage. The weed control was performed at pre-emergence with mesotrione (0.15 kg AI ha^{-1}), S-metolachlor (1.25 kg AI ha^{-1}) and terbuthylazine (0.75 kg AI ha^{-1}) (Lumax®, Syngenta Crop Protection S.p.A., Milan, Italy) under both tillage conditions and in both locations. Furthermore, the ST plots in Poirino also required a post-emergence weed control, using Nicosulfuron (40.5 g AI ha^{-1}), Rimsulfuron (10.1 g AI ha^{-1}) and Dicamba (0.24 kg AI ha^{-1}) (Principal® Mais, Du Pont de Nemours Italiana S.r.l., Milan, Italy).

In order to avoid any drought stress until the physiological maturity, irrigation was performed with a drip irrigation hose system at Carmagnola and using the sprinkler method at Poirino, according to the management commonly used by farmers in the area.

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

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

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

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

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

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

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

2.7. Grain yield and yield components

Maize grain yield and components, fully developed ears, plant density and number of barren plants were assessed on 4.5 m^2 areas in the central part of each plot, at harvest maturity. The grain yield components included the cob length, as measured on a sub-sample of 15 ears, the number of kernel rows and the number of kernels per row, both of which were determined on 7 randomly selected ears. The number of kernels per ear was calculated by multiplying the number of kernel rows by the number of kernels per row, while the number of kernels per square meter (KSQ) was calculated by multiplying the number of kernels per ear by the number of ears per square meter (Testa et al., 2016). All the collected ears were shelled using an electric ear sheller and the grain moisture content was determined using a Dickey-John GAC100 grain analyzer (Auburn, IL, USA). Grain samples were dried in a forced-air oven at 60°C until constant weight to express the yield as dry matter and for the subsequent analyses regarding the qualitative and sanitary traits. A sub-sample of 200 kernels was weighed to obtain the thousand kernels weight (TKW). The test weight (TW) was determined on dry kernels, by means of a Dickey-John GAC2000 grain analysis meter (Dickey-John Corp).

2.8. Kernel quality and sanitary traits

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

The sanitary traits were assessed from the ECB severity, which was calculated as the percentage of kernels per ear characterized by injuries due to ECB larva activity, while ear rot severity was calculated as the percentage of kernels per ear with symptoms, according to Blandino et al. (2009a). The ECB and ear rot severity were both evaluated on a sub-sample of 20 ears. Furthermore, fumonisin B_1 and B_2 (FBs) and deoxynivalenol (DON) contaminations were analyzed using the ELISA method, by means of direct competitive immunoassays (RIDASCREEN® Fumonisin and RIDASCREEN® DON, R-Biopharm, Darmstadt, Germany), according to the manufacturer's instructions. Briefly, both mycotoxins were extracted for 15 min by mechanically shaking, at 100 rpm, (shaker model M102-OS, MOM Instruments, Milan, Italy) 20 g samples with 100 ml of methanol/distilled water, (70:30, v/v) for FBs and with distilled water for DON, respectively. After extraction and filtration through Whatman® n.1 299 filters, 50 µl of diluted filtrate was used for the ELISA test. The optical density was measured at 450 nm using an ELISA 96-well plate reader (Das srl, Rome, Italy), and all the standard and sample solutions were analyzed in duplicate wells. The limit of quantification (LOQ) of the analytical method was set at 250 $\mu g\ kg^{-1}$ and 37 $\mu g\ kg^{-1}$ for FBs and DON, respectively.

2.9. Data analysis

Data were analyzed using a mixed effects model where the experimental site, tillage technique and starter fertilization strategy were considered as fixed effects, while the maize hybrid and the year - nested effects, - were considered as random factors. A graphical method was used to verify the basic assumptions (Onofri et al., 2016). A logarithmic transformation was applied to the FBs and DON values. As the DON contamination at the Carmagnola site was smaller than the LOQ of the instrument for both experimental years, the mixed model was only applied at the Poirino site, considering the tillage technique and the starter fertilization strategy as fixed effects and the maize hybrid and the year - nested effects - as random factors. When single factors or their interaction determined significant effect, the means were compared using the Bonferroni post hoc test at $p \leq 0.05$. The statistical procedure was performed using the lmer function in the LME4 statistical package of the R software (R Core Team, 2019).

3. Results

3.1. Weather and soil conditions

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

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

3.2. Plant emergence and early crop vigor

The tillage technique for seedbed preparation and the experimental site clearly affected the plant emergence pattern, while the starter fertilization strategy did not (Table 4). The number of emerged plants was lower in the ST treatment than in the PLOW one for both measurement times. Moreover, in correspondence to the "50% emergence" measurement, the comparison of the density of emerged plants between the two tillage techniques showed more pronounced differences for Poirino (p <0.001; Figure S1), where the PLOW treatment had 4.4 plants m^{-2} and the ST treatment had 1.0 plants m^{-2}, while the crop density for Carmagnola was 5.5 and 3.3 plants m^{-2} for the PLOW and ST treatments, respectively. The tillage technique and the starter fertilization strategy at sowing clearly affected the maize plant development, as assessed by means of the NDVI measurements during the vegetative stages (Figure 2). The maize development during the early vegetative stages was different for the two growing seasons. The 2019 growing season recorded a slower plant development than 2020 for all the site-tillage technique combinations (the average NDVI increase per day was 0.0153 and 0.0183 units over 2019 and 2020, respectively). The adoption of the ST technique at Poirino resulted in a reduced plant growth, compared to the PLOW technique, in particular in 2019 (0.0140 *vs* 0.0161 NDVI units d^{-1}), while the differences were more limited (0.0142 *vs* 0.0151 NDVI units d^{-1}) in 2020. The gap in daily NDVI increases between the two tillage techniques was narrower at Carmagnola for both years (0.0150 *vs* 0.0161 and 0.0218 *vs* 0.0220 NDVI units d^{-1} over 2019 and 2020, respectively). The two starter fertilization strategies, that is, DAP and DIG, showed, on average, similar and faster daily crop developments (0.0173 NDVI units d^{-1}) than the NT treatment (0.0158 NDVI units d^{-1}). The NDVI values were summarized by the AUCDC index, thus allowing the differences in plant development during the early growing stages (Table 4) to be assessed. Generally, the PLOW treatment resulted in a significant (p <0.001) increase in the AUCDC, compared to the ST system, with a significantly more marked effect at Poirino (+20%) than at Carmagnola (+7%) (Figure 3a). On the other hand, the DAP treatment performed better than DIG and the NT control. The starter fertilization strategy differed between the two soil types (p <0.001), and between the two tillage techniques (p <0.001). In fact, the DIG treatment at Carmagnola showed an intermediate AUCDC value between the DAP and NT treatments (26.1 *vs* 29.0 and 24.8), while no differences were recorded at Poirino, compared to DAP (Figure 4a). The crop growth determined by the DIG injection at sowing did not show any differences from the NT when the soil was plowed, while it was higher than NT (+12%) when the seedbed was prepared by ST (Figure 5).

The results regarding the plant height during the vegetative growth stages (GS 15 and GS 33) confirmed those of the AUCDC index (Table 4). The plant height in the PLOW treatment increased significantly (p <0.001) by 34% and 56% at GS 15 and GS 33, respectively, compared to the ST. The starter fertilization with DAP recorded the highest plant height for both measurement times, followed by the DIG and the NT treatments. However, an interaction with the site was observed, with a more pronounced positive effect of PLOW than of ST at Poirino (+222%) than at Carmagnola (+12%), at GS 33 (Figures 3b and 3c). Another factor that affected the plant height was the starter fertilization strategy, which again showed a different pattern at the two sites, both at the GS 15 and GS 33 growing stages. During steam elongation, the plant height in the DIG treatment was 35% higher than in NT, and 28% lower than in DAP at Carmagnola, while it was higher than NT (+229%) but similar to DAP at Poirino (Figure 4b and 4c), thus enhancing the differences recorded during leaf emission.

The differences due to the tillage technique and to the starter fertilization strategy recorded during vegetative development were confirmed at flowering and harvest (Table 4). On average, maize anthesis in the PLOW system occurred 2.2 days before the ST system. However, this reduction in days from sowing to plant flowering was significantly (p <0.001; Figure S2) higher at Poirino (-3.4 days) than at Carmagnola (-1.1 days). The starter fertilization clearly affected the flowering date. When DIG was supplied, flowering occurred at 94.4 DAS, while it occurred at 92.4 and 97.0 DAS in the DAP and NT treatments. The earlier flowering resulted in a grain moisture content reduction at harvest. On average, the PLOW technique showed a decrease of 1.2% in the grain moisture content, compared to the ST system, while the DAP treatment resulted 0.8% drier than DIG, which was 1.1% drier than the NT treatment. Furthermore, the three-way interaction was significant. The DIG treatment showed a similar grain moisture content (24.6%) to the NT treatment (25.2%) for both tillage techniques at Carmagnola, while the DIG treatment at Poirino did not differ from the DAP treatment (Figure S3).

3.3. Plant morphological traits

The final crop density was affected by the experimental site and the tillage technique, but not by the fertilization strategy (Table S2). The crop density was higher at Poirino (7.9 plants m^{-2}) than at Carmagnola (7.4 plants m^{-2}). The tillage technique effect confirmed what was observed at emergence, i.e. PLOW resulted in 0.3 plants m^{-2} more than ST. The ear density exhibited the same trend as the crop density, while no effects of the analyzed factors were recorded on the percentage of barren plants. The stalk area, the plant height at maturity and the ear insertion height reported in Table S2 are proxies of plant stability. The ST system led to an increase (+3.4%) in this parameter, compared to the PLOW system, whereas the DAP and DIG similarly increased the stalk area in comparison to the NT treatment. The lowest plant and ear insertion height at maturity was recorded when DAP was supplied at sowing.

3.4. Grain yield and yield components

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

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

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

3.5. Grain qualitative and sanitary traits

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

Only the location affected the ECB and ear rot severities (*p* <0.001 and *p* =0.002, respectively; Table 6). Since all the experiments were treated with foliar insecticides, the injury caused by the ECB activity was generally low, with less damage observed at Poirino than at Carmagnola. Ear rot severity was significantly higher in Poirino than in the Carmagnola site, while tillage and starter fertilization did not affect the ear surface with mold symptoms at harvest at either site. The FBs contamination was higher (*p* =0.001) at Carmagnola than at Poirino, while DON was only detected in Poirino (Table 7). Although the starter fertilization did not affect ECB or the ear rot severity, the DIG injection on average lowered FBs contamination (*p* =0.002). However, differences were found between the two experimental sites: DIG and DAP behaved similarly in Carmagnola, while the DIG treatment in Poirino recorded the lowest FBs contamination, while DAP showed an intermediate FBs contamination compared to the NT and DIG treatments (Figure 6). The grain DON contamination in Poirino was negatively affected by the adoption of ST, which increased it by 118%, compared to PLOW, although no significant effect of starter fertilization was observed.

4. Discussion

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

4.1. Effect of the tillage technique

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

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

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

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

The content of mycotoxin in ST could be higher as a consequence of both a higher inoculum production (crop debris on soil surface) and higher infection and disease development (due to a later flowering and ripening). Tillage could affect the grain sanitary traits because of both later flowering and ripening or the different incorporations of the previous crop residues into the soil. The late development of maize cultivated in ST, with a late shift of ripening, could result in a higher severity of ECB in the absence of a direct control of this insect (Blandino et al., 2009a) or condition more favorable for fungal development, thus leading to a possible higher risk of mycotoxin contamination. As far as the role of debris is concerned, previous studies conducted on wheat and other small cereals (Blandino et al., 2010) reported remarkable effects of tillage on *Fusarium* head blight infection and the DON contamination of wheat. Debris is the primary reservoir of *Fusarium* inoculum (Koch et al., 2006), and DON contamination in wheat is more severe if the preceding crops is maize, particularly when minimum tillage or no-tillage is applied (Blandino et al., 2012). Conversely, only limited information has been reported for the role of previous crop management on soil tillage in maize

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

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

4.2. Effect of the starter fertilization

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

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

Poirino, while it caused a slower growth rate at Carmagnola, thus indicating an important effect of the type of soil, also regarding nutrient availability. The Carmagnola soil had a lower concentration of plant available-P and sub-alkaline pH, which may favor P sorption (Barrow, 2017), than the Poirino soil. Consequently, the more readily available mineral form of the sub-surface placed DAP was better utilized by small plants than the organic form of deep-injected DIG, which required a mineralization process and could have caused an additional local increase in soil pH due to its intrinsic alkalinity (Tampio et al., 2016). This suggested that supplying soluble P fertilizers in bands near the seed in situations where soil P reactions are dominated by sorption may improve the nutrient use efficiency (Battisti et al., submitted). An early availability of N and P provides an additional advantage to cereals, as it determines the potential grain number for physiological reasons (Angus et al., 2019).

The differences observed between the sub-surface placed DAP and the deep-injected DIG band, in terms of early crop growth enhancement, could also be linked to the depth of the fertilizer placement, that is, 5 cm apart from the seed in the case of DAP and ~20 cm below the seed in the case of DIG. These distances were chosen to avoid possible toxic effects of the fertilizer on the seedling, as young roots may encounter reducing conditions, high concentrations of ammonium and a high pH, which may cause plant death (Sawyer and Hoeft, 1990; Salvagiotti et al., 2013), when manure is placed too close to the seeds. The tested distances resulted in no negative effects on seed germination or the plant density, compared to NT. This was in agreement with the results of Pedersen et al (2020b), who suggested a distance of at least 5 cm between the manure band and the seed row to avoid root damage following slurry injection. However, nutrients placed in a deeper position as DIG band may be less plant-available during early stages of plant growth when root density is still low at high depths (Nkebiwe et al., 2016). Further investigations should be conducted to optimize the placement distance of digestate for the tested pedo-climatic situations.

The advantage, in terms of early development and earlier flowering of starter NP fertilization, led to a significant increase in grain yield and test weight. Although the type of fertilization, deep-injected DIG or sub-surface placed DAP, influenced the early growth of maize at sowing, it did not affect the yield or most of the grain quality traits. The only quality parameter that was influenced by the type of fertilization was the grain protein content, which was significantly increased when digestate was used. This was probably because only part of the total applied N in digestate is immediately available, while the organic fraction is slowly released through the mineralization process over the growing season, thus leading to an improved synchrony between N demand and N availability (Silva et al. (2005).

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

5. Conclusion

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

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

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

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Tables

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

Parameters	**Measurement units**	**Carmagnola**	**Poirino**
Soil type[1]		Typic Udifluvent	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

[1] Soil Surface Staff, 2014

Table 2. Cumulative monthly rainfall and air growing degree days (10°C base; GDDs) during the 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**

Table 3. Maize hybrids, relative maturity FAO class, sowing, trop-dressing fertilization and 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	04th April	30th May	25th September
Poirino - 2019	Korimbos (KWS Saat AG)	500	21st March	03rd June	24th September
Carmagnola - 2020	LG 30600 (LG Seeds, Limagrain)	600	08th April	22nd May	23rd September
Poirino - 2020	Sy Brabus (Syngenta)	700	31st March	27th May	24th September

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) and stem elongation stages (GS33), the area under the canopy development curve (AUCDC), the date of flowering, expressed as days after sowing (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
	p(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
	p(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
	p(F)	n.s.	n.s.	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Site x Tillage	*p*(F)	< 0.001	n.s.	< 0.001	< 0.01	< 0.001	< 0.001	< 0.001
Site x Starter	*p*(F)	n.s.	n.s.	< 0.001	< 0.001	0.001	n.s.	n.s.
Tillage x Starter	*p*(F)	n.s.	n.s.	n.s.	n.s.	0.001	n.s.	n.s.
Site x Tillage x Starter	*p*(F)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.027

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

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

[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 P_2O_5 ha^{-1} as digestate the day before sowing; DAP: 27 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1} as diammonium phosphate at sowing; NT: no NP fertilization at maize sowing.

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

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 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
	p(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
	p(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
	p(F)	< 0.001	n.s.	0,008	n.s.
Site x Tillage	*p*(F)	< 0.001	n.s.	0.008	0.039
Site x Starter	*p*(F)	n.s.	0.048	0.044	0.002
Tillage x Starter	*p*(F)	n.s.	n.s.	n.s.	n.s.
Site x Tillage x Starter	*p*(F)	n.s.	0.034	n.s.	n.s.

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

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

[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 P_2O_5 ha^{-1} as digestate the day before sowing; DAP: 27 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1} as diammonium phosphate at sowing; NT: no NP fertilization at maize sowing.

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

Table 6. Effect of site, tillage and starter fertilization on qualitative and sanitary traits, expressed as kernels test weight (TW), grain protein content (GPC), European Corn Borer (ECB) injuries and ear 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
	p(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
	p(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
	p(F)	0.002	< 0.001	n.s.	n.s.
Site x Tillage	*p*(F)	n.s.	n.s.	n.s.	n.s.
Site x Starter	*p*(F)	n.s.	n.s.	n.s.	n.s.
Tillage x Starter	*p*(F)	n.s.	n.s.	n.s.	n.s.
Site x Tillage x Starter	*p*(F)	n.s.	n.s.	n.s.	n.s.

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

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

[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 P_2O_5 ha^{-1} as digestate the day before sowing; DAP: 27 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1} as diammonium phosphate at sowing; NT: no NP fertilization at maize sowing.

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

Table 7. Effect of tillage and starter fertilization on fumonisins $B_1 + B_2$ (FBs) and deoxynivalenol (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
	p(F)	0.001		-	
Tillage[b]	ST	6.89	4457	5.11 a	275
	PLOW	6.81	4267	4.35 b	101
	p(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
	p(F)	0.002		n.s.	
Site x Tillage	*p*(F)	n.s.		-	
Site x Starter	*p*(F)	< 0.001		-	
Tillage x Starter	*p*(F)	n.s.		n.s.	
Site x Tillage x Starter	*p*(F)	n.s.		-	

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

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

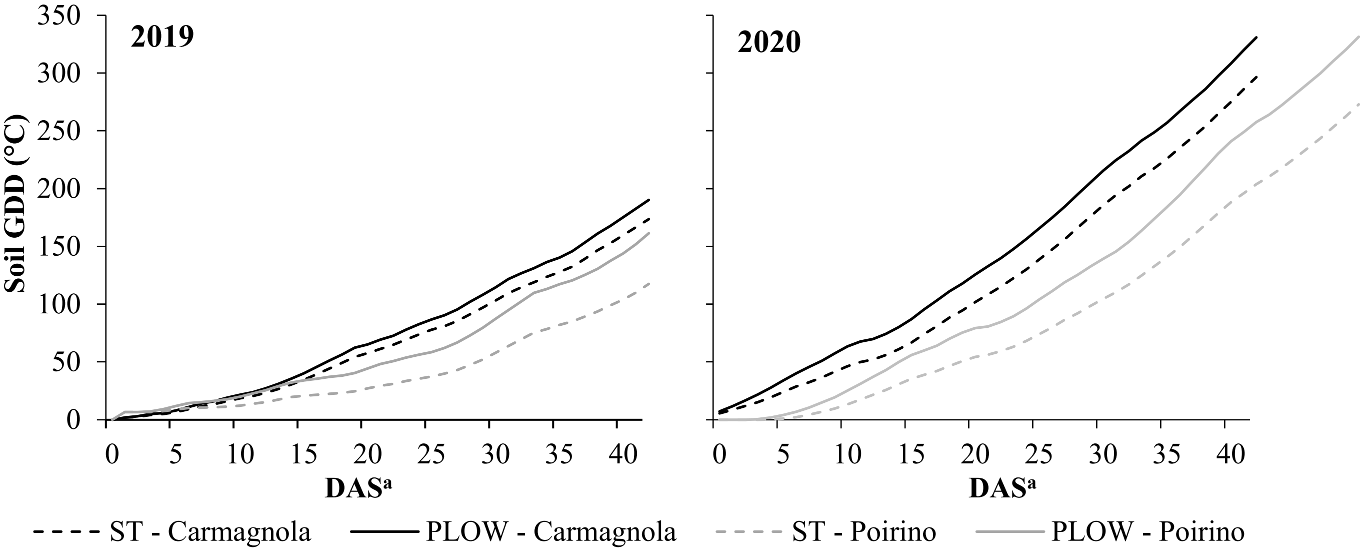
[b] The tillage values are based on 48 observations (2 years ×2 sites × 3 starter fertilizations × 4 repetitions). ST: strip tillage; PLOW: plowing.

[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 P_2O_5 ha^{-1} as digestate the day before sowing; DAP: 27 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1} as diammonium phosphate at sowing; NT: no NP fertilization at maize sowing.

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

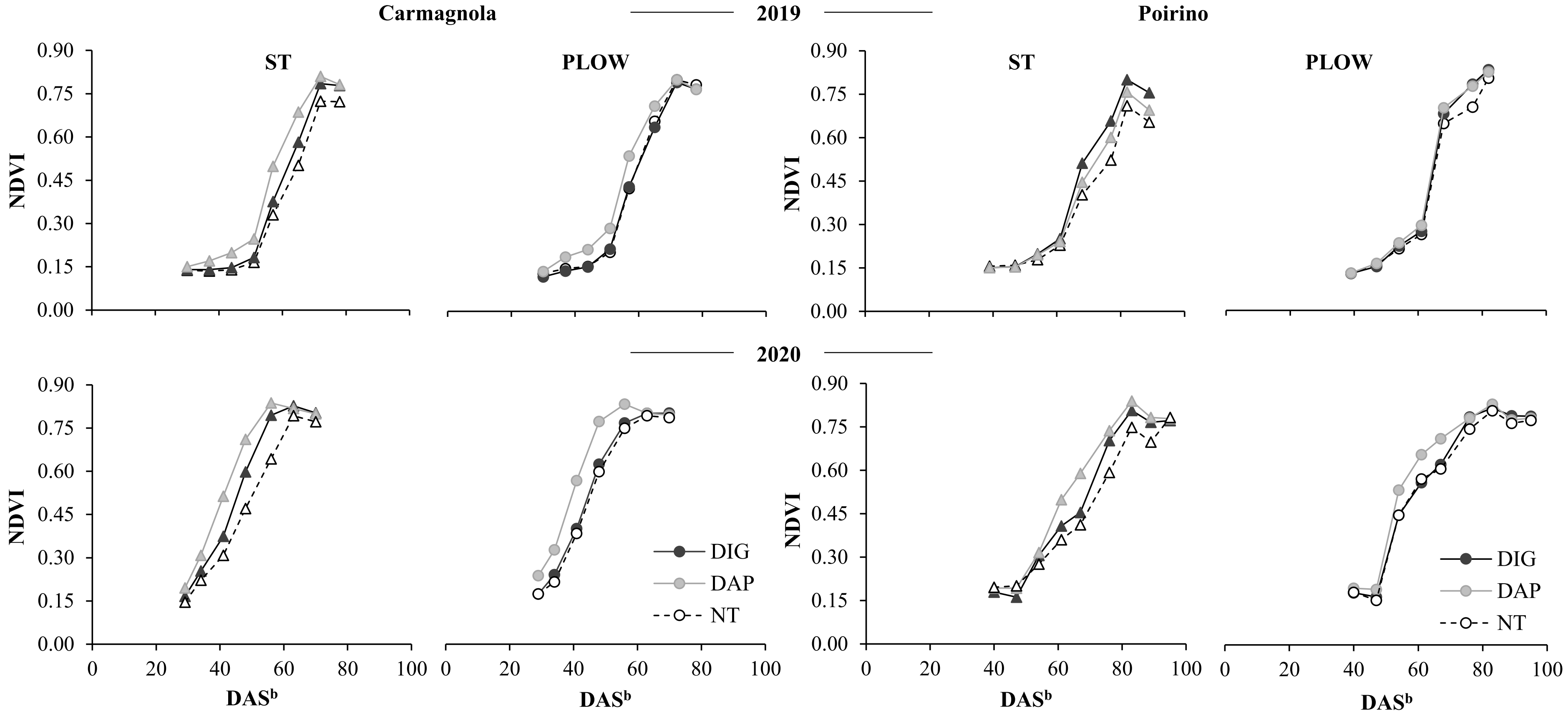
Figures

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



[a] DAS=days after sowing

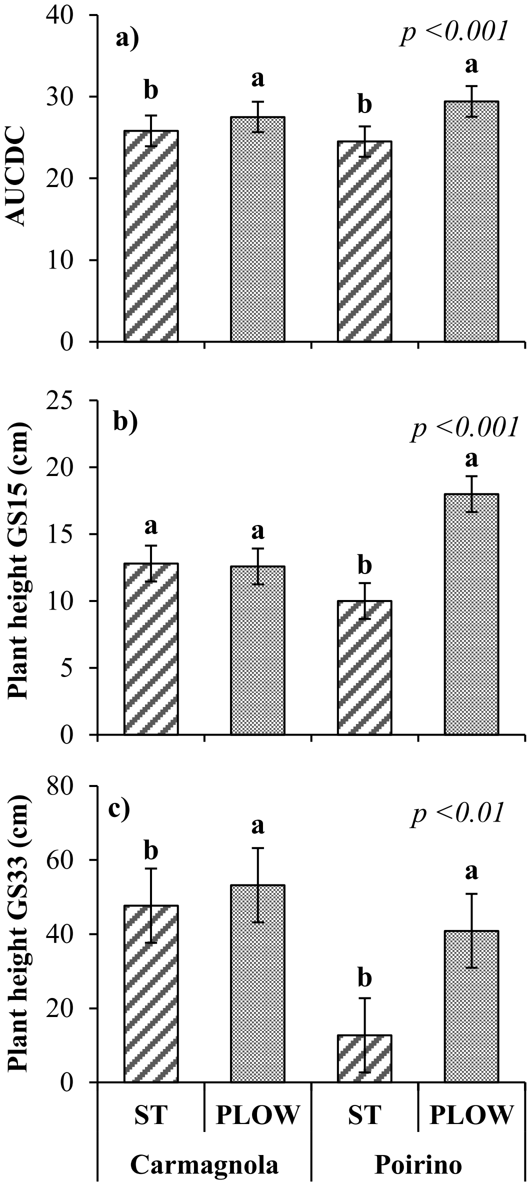
Figure 2. Effect of the starter fertilization strategies[a] on the maize canopy development, expressed as NDVI from the 3-leaf stage to tasseling for the two compared tillage systems: strip-tillage (ST; triangle symbols) and plowing (PLOW; circle symbols) at Carmagnola (left-hand side) and Poirino (right-hand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



[a] DIG: 202 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1} as digestate the day before sowing; DAP: 27 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1} as diammonium phosphate at sowing; NT: no NP fertilization at maize sowing.

[b] DAS=days after sowing

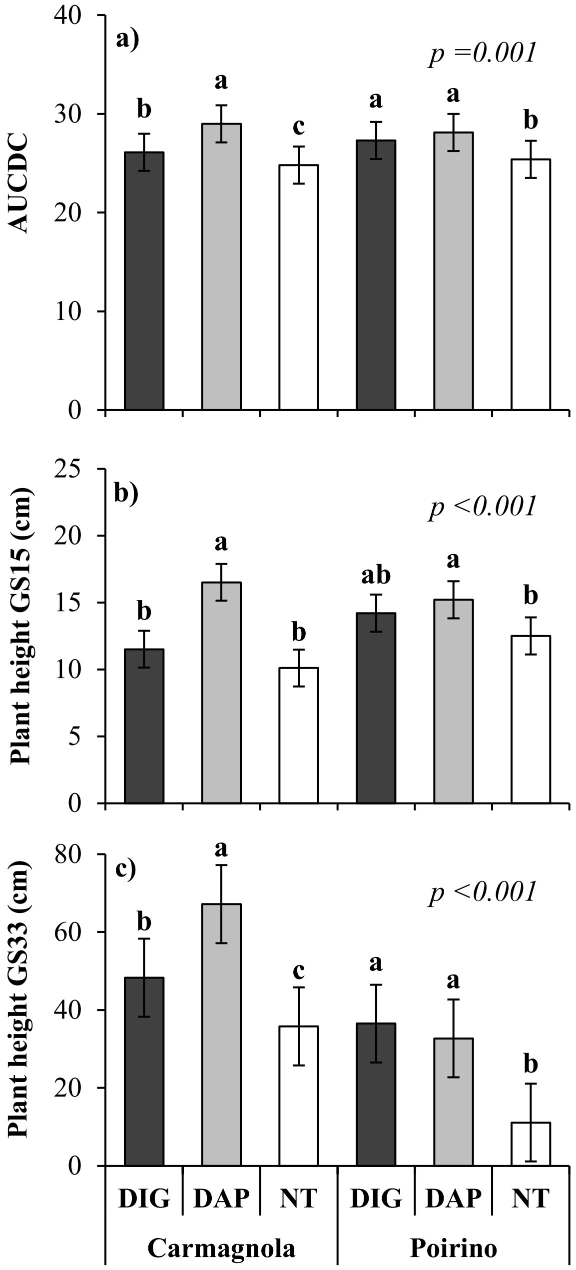
Figure 3. Effect of the tillage system, at each experimental site, on the early vigor of maize expressed as the area under the canopy development curve (AUCDC; a), the plant height at the leaf emission (GS15; b) and the stem elongation stages (GS33; c) for field experiments carried out in the 2019 and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



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

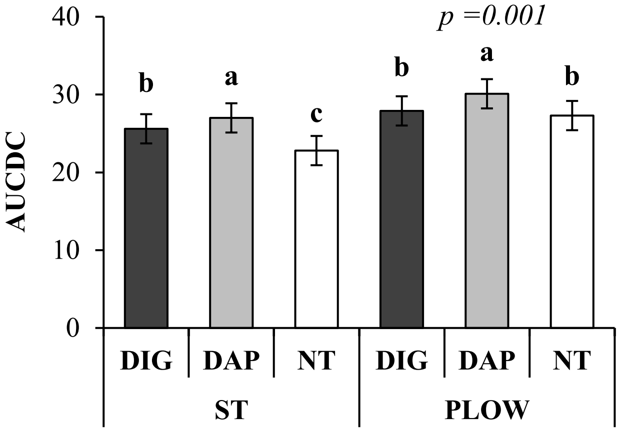
Means with different letters within each site are significantly different. Error bars show the ± standard error.

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



Starter fertilization: DIG, 202 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1} as digestate the day before sowing; DAP, 27 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1} as diammonium phosphate at sowing; NT, no NP fertilization at maize sowing. Means with different letters within each site are significantly different. Error bars show the ± standard error.

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

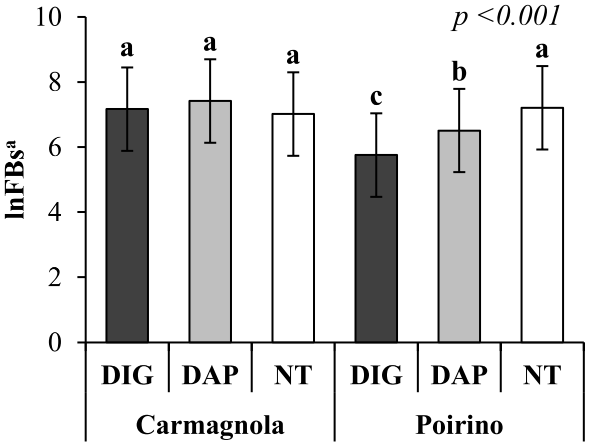


Soil tillage system: ST, strip tillage; PLOW, plowing.

Starter fertilization: DIG, 202 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1} as digestate the day before sowing; DAP, 27 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1} as diammonium phosphate at sowing; NT, no NP fertilization at maize sowing.

Means with different letters are significantly different. Error bars show the ± standard error.

Figure 6. Effect of the starter fertilization at each experimental site on fumonisins $B_1 + B_2$ (FBs) contamination for field experiments carried out in the 2019 and 2020 growing seasons.



Starter fertilization: DIG, 202 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1} as digestate the day before sowing; DAP, 27 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1} as diammonium phosphate at sowing; NT, no NP fertilization at maize sowing. Means with different letters within each site are significantly different. Error bars show the ± standard error.

[a] The reported FBs contamination means are transformed [y' = ln (x + 1)].