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MANAGEMENT PRACTICES INFLUENCE THE NATURAL ARBUSCULAR MYCORRHIZAE COMMUNITY OF MAIZE

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Abstract

The productivity of the agroecosystems is affected by the presence and the biodiversity of arbuscular mycorrhizal fungi communities. Understanding how mycorrhizal communities respond to various factors is crucial for promoting sustainable agriculture. This study examines how four cropping systems affect the natural arbuscular mycorrhizal community associated with maize. The trial involved three corn hybrids: DKC4949 (FAO 390), P8523 (FAO 260), and P9537 (FAO 390). The experiment was conducted using a randomized complete block design with three replications in a plot size of 25 m^2. For the tested period the highest number of spores (348 in 2022 and 370 in 2023) has been recorded by the hybrid P8523 when cultivated according to the no-till technology combined with mulching. Hybrid P9537, the highest yielding for the region, also exhibits the greatest rate of mycorrhizal colonization. Although no-till and its combination with mulching enhanced the colonization potential of mycorrhiza by all hybrids, the positive result has no quantitative manifestation and the yields remain the lowest by those variants.

***Key words**: arbuscular mycorrhiza, colonization rate, cropping system, maize.*

INTRODUCTION

Nowadays, conventional intensive agriculture raises many concerns (Ujvári et al., 2023). Although inorganic fertilizers positively affect the productivity of crops, they may negatively impact soil health, fertility, and microbial biodiversity (Sun et al., 2015). The scientific community is trying to boost the transition toward sustainability (Foley et al., 2011) and focuses on beneficial traits, improved nutrient-use efficiency, soil health, and biodiversity of soil microbial communities (Menendez & Garcia-Fraile 2017; Ke et al., 2021). The utilization of plant growth-promoting bacteria or arbuscular mycorrhizal fungi is a successful strategy aiming to achieve sustainable crop production (Ujvári et al., 2023). Arbuscular mycorrhizal fungi (AMFs) are beneficial non-host-specific soil microbiomes and highly effective partners in our ecosystem. As plant symbionts, they colonize more than 80% of our land plants, including most cultivated plants such as maize. They support their development by promoting increased nutrient and water uptake, increased drought tolerance, and improved soil structure (Smith & Read 2008). Soil and crop management practices modulate biological soil fertility (Ortas, 2015). Establishing functional arbuscular mycorrhizal (AM) symbioses could improve soil nutrient availability (Ortas, 2015). Management strategies have a strong positive or negative influence on AM communities (Brito et al., 2012; Bedini et al., 2013). Additionally, low-input cropping systems impact population density and species composition (Harikumar, 2015). AM spore abundance is higher in organically maintained fields, compared to conventional ones (Gosling et al., 2010). AMFs are key factors in plant health and the productivity of agroecosystems is affected by their presence and biodiversity in soil (Lekberg & Koide, 2005). AMFs are obligated biotrophs and can infect various crop species, depending on soil conditions and soil fertility (Ortas, 2015). The knowledge of the influence of different factors on AMF's population is essential and supports sustainable agriculture (Brito et al., 2012). According to Dodd et al. (2000), *Glomus* spp. can survive upsets and predominate in disturbed agricultural systems. Jansa et al. (2003) suggested that variations in AMF community structure of maize roots could result from differences in AMF species tolerance to tillage-induced disruption of the hyphae. The properties of biofertilizers to improve the physicochemical properties of the soil, and stimulate root growth and vegetative mass, while supporting the resistance of plants to abiotic environmental factors, gives them key importance in the process of soil phytoremediation (Rasouli et al., 2022). Nowadays, arbuscular mycorrhiza is among the most widely used biological fertilizers that favour the resistance of plant and soil systems (Habeeb et al., 2020). Global demand for maize is increasing each year due to its versatile applications. (Ranum et al., 2017). In conventional farming systems maize productivity is supported and enhanced through higher inputs of mineral fertilizers (Tilman et al., 2002). Excessive application of these products reduces soil microbial biodiversity and causes loss of soil fertility (Thiele-Bruhn et al., 2012). Maize associates with native AMF communities (Alvarado-Herrejón et al., 2019). The common phenotypic reaction of maize to inoculation with AMF is growth promotion (Sawers et al., 2017). However, the strength of this expression depends on the genotype, crop rotation, tillage, and fertilization (Sarabia et al., 2017; López-Carmona et al., 2019). The current study investigates how four different cropping systems affect the density and species composition of native arbuscular mycorrhiza populations of three maize hybrids.

MATERIALS AND METHODS

Field experiment

A field trial was conducted in the village of Kapinovo (43.745003° n. w. 27.991992° e. l.), located in North-Eastern Bulgaria over two consecutive years (2022-2023). The following maize hybrids were included in the experiment: DKC4949 (FAO 390), P8523 (FAO 260), and P9537 (FAO 390). The study was set using a randomized complete block design in three replications with a plot size of 25 m^2.

The sowing was carried out in the period 1-10 of April with row spacing of 70 cm after wheat as a predecessor. The maize hybrids were planted using four different cropping systems: classical conventional tillage (T) with the removal of all residues; conventional tillage + mulching (T+M) crop residues remain on the soil surface as a cover; no-till (NT) performed with a double-disk opener assembly following a ripple coulter and no-till+mulching (NT+M) without removal of the crop residues.

Soil conditions

The dominant soil type in the region is represented by the chernozems. The area's soil is loamy, with physical clay constituting 50%. Chemical analysis revealed a significant amount of carbonates, and it has a neutral to alkaline pH of 7 to 7.2. The humus content is high, ranging from 5.2% to 6%. The total nitrogen content in the soil ranged from 55 to 58 mg per 1000 g, according to Gurov. Phosphorus levels were reported to be between 0.5 and 0.8 mg per 1000 g /Egner and Rheen/. Potassium content was between 26 to 30 g per 1000 g of soil. The presence of nitrates in larger quantities contributed positively to the physical and mechanical properties of the soil. Additionally, the humic content gradually decreased with soil depth. While the amounts of nitrogen, phosphorus, and potassium were significant, their available forms were limited. Soil nutritional regime was improved through fertilization. Phosphorus and nitrogen fertilizers were applied before sowing at rates of 50 kg per hectare of triple superphosphate and 30 kg per hectare of ammonium nitrate.

Weather conditions

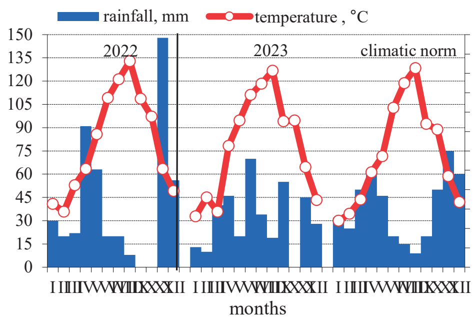

Figure 1. Meteorological data for the period

AMF colonization rate

To determine the impact of cropping systems on AMF population density, soil samples have been taken from the roots of 10 plants per plot. The samples were then mixed for statistical data processing. Sampling occurred at a depth of 0 to 27 cm at maize development stage R1. Fine roots were carefully separated from the soil, and 2 cm segments of these roots were used in the staining procedure to assess the density of the arbuscular mycorrhizal community. The plants were randomly selected. The cleaned root segments were placed in 15 ml falcon tubes filled with a 30% alcohol solution. The prepared root segments underwent a staining procedure. This process began with immersing the segments in a 10% potassium hydroxide (KOH) solution for one day to bleach them. Following this, the segments were boiled in a water bath at 90 °C for four minutes. After boiling, they were rinsed three times with tap water and stained using a 5% solution of ink and vinegar, following the method outlined by Vierheilig et al. (1998). Once stained, the roots were rinsed again with tap water and then stored in 30% ethanol. The colonization rate of the arbuscular mycorrhizal roots was determined using the gridline intersection method (Newman, 1966; Giovanetti & Mosse, 1980). The extent of root colonization was measured using the following formula:

$$Root\ Colonization\ Percentage = \frac{Number\ of\ AMF\ Positive\ Intersections}{Total\ Number\ of\ Intersections} x\ 100\%$$

Spores were counted using the wet sieving and decanting method developed by Gedemann and Nicolson (1963), followed by sucrose density centrifugation (Ianson & Allen, 1986). First, 100 g of soil from each variant was dispersed in 1 liter of water and centrifuged. The resulting suspension was then decanted through sieves with mesh diameters ranging from 500 to 40 µm, after which a 40% sucrose solution was added. All residues were filtered, and the intact spores were counted under a stereomicroscope.

Statistics

The results were statistically processed using analysis of variance (ANOVA), and differences between the groups were determined through the multi-rank LSD test.

RESULTS AND DISCUSSIONS

During the years of the investigation, the spore density in the soil varies depending mostly on the cropping system (Figure 2), as the values for the second year are slightly increased. For the tested period the highest number of spores (348 in 2022 and 370 in 2023) has been recorded by the hybrid P8523 when cultivated according to the no-till technology combined with mulching. On second place is the variant cultivated under no-till technology applied individually (between 320 and 340 spores, respectively), followed by the variant sown after classical tillage with mulching with a spore density of 107 in the first and 135 in the second year. By all hybrids, classical plowing significantly reduces the number of spores of the arbuscular mycorrhizal societies.

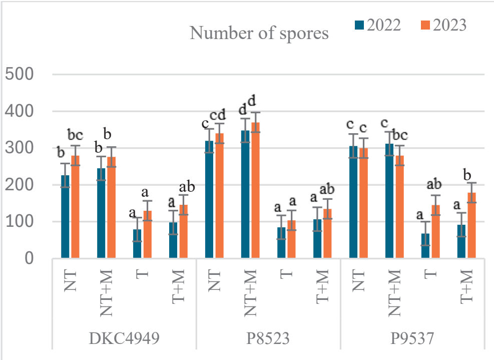

Figure 2. AMF spores in $10g^{-1}$ soil

The differences between the variants grown using classical tillage technology and the combination of plowing with mulching by hybrid P9537 were statistically proven and significant in both years of the experiment, while by the other hybrids, the differences were proven only in the second year. By the hybrids DCC4949 and P9537, in the first year, the number of spores is highest in the variants sown according to the cropping system NT+M. In the second year, no-till farming produced slightly better results. Soil cultivation is a major factor in the breakage of the extraradical mycelium (Evans & Miller, 1990). Mixing surface residues with the soil profile stimulates or inhibits certain species of mycorrhizal societies (Klironomos & Hart, 2002). Brito et al. (2012) suggested that AMFs that depend mainly on extraradical mycelium to colonize the roots of newly sown plants would be more frequent in no-till systems, whereas those relying mostly on spores for colonization would be less affected by soil disturbance. Additionally, mulching improves the survival of AM fungal propagules by releasing water-soluble carbon, which enhances root colonization by AM fungi (Nyamwange et al., 2018).

During the study period fluctuations in the mycorrhizal colonization rate have been observed depending on the cropping system (Tables 1, 2). Although for the test period in all three hybrids, no-till and its combination with mulching contribute to increasing the colonization potential of mycorrhiza, the positive result has no quantitative manifestation and the yields remain the lowest by those variants.

Table 1. Mycorrhiza colonization rate depending on the cropping system during 2022

Variety	Cropping system	root colonization (%)	grain yield (t ha-1)
DKC4949	NT	25.2[b]	8.4[a]
	NT+Mulch	27.4[b]	8.5[a]
	Tillage	12.2[a]	9.1[a]
	Tillage+Mulch	15.6[a]	8.9[a]
P8523	NT	31.3[c]	9.7[b]
	NT+Mulch	30.1[c]	9.5[a]
	Tillage	16.2[a]	10.2[c]
	Tillage+Mulch	23.8[b]	10[c]
P9537	NT	38.5[d]	10.2[c]
	NT+Mulch	34.2[c]	10[c]
	Tillage	20.3[b]	11.4[e]
	Tillage+Mulch	25.8[b]	11.3[e]
LSD<5%		*7.6*	*0.65*

[a]Means within columns followed by different lowercase letters are significantly different (P<0.05)

Table 2. Mycorrhiza colonization rate depending on the cropping system during 2023

Variety	Cropping system	root colonization (%)	grain yield (t ha-1)
DKC4949	NT	38.2[c]	8.6[c]
	NT+Mulch	32.7[b]	8.5[b]
	Tillage	19.5[a]	9.0[c]
	Tillage+Mulch	20.2[a]	9.0[c]
P8523	NT	43.5[d]	7.7[a]
	NT+Mulch	40.2[d]	7.5[a]
	Tillage	28.8[b]	8.2[b]
	Tillage+Mulch	29.2[b]	8.8[c]
P9537	NT	40.1[d]	8.1[a]
	NT+Mulch	42.8[d]	8.4[b]
	Tillage	33.5[c]	9.2[c]
	Tillage+Mulch	36.7[c]	9.0[c]
LSD<5%		*6.7*	*0.52*

[a]Means within columns followed by different lowercase letters are significantly different (P<0.05)

However, the results make it possible to establish genotypic differences between hybrids, expressed as differences in the percentage of mycorrhizal colonization. The trend that is found is that the P9537 hybrid, which is the highest yielding for the region, also has the highest rate of mycorrhizal colonization. By hybrid DKC4949 yields are the lowest and, accordingly, the degree of mycorrhization is also at the lowest value. Hybrid P8523 occupies an intermediate position in terms of test performance. The productivity of agricultural plants depends on many factors, among which are the genotypic capabilities and potential of the respective hybrid. Although for the tested period the hybrid DKC4949 distinguished as the lowest yielding for the region, its yields are not affected by the cultivation technology, which is evident from the absence of statistically significant differences between the different variants.

Differences in climatic terms do not lead to sharp variations in productivity, from which it can be concluded that the hybrid exhibits great ecological plasticity. For the other two hybrids, classical plowing and its combination with mulching lead to the highest productivity, and the differences between the two technologies are statistically insignificant and unproven. Opinions about the impact of tillage on yield are contradictory. While Mathers et al. (2023) and Gaudin et al. (2015) found a positive effect of no-till or reduced tillage on maize productivity, Daigh et al. (2018) did not observe any effect on yield.

During the study, 9 mycorrhizal species were identified, reflected in Table 3.

Table 3. Frequency of identified AMF communities

Identified species	Cropping system NT	NT+M	T	T+M
Funneliformis geosporum	C	D	R	
Funneliformis mosseae	D	D	R	R
Glomus macrocarpum	D	D	C	C
Glomus glomerulatum	C	D	C	C
Glomus ambisporum	C	C	C	C
Glomus pansihalos	D	D	C	C
Rhizophagus irregularis	D	D	D	D
Rhizophagus claris	C	C		R
Rhiizaphagus fasciculatus	D	D		

*D-dominant (FO>66%), C-common (33%≤FO≤66%), R-rare (FO≤33%)

Concerning the composition of the mycorrhizal society, no differences were found between the individual hybrids, but only between the applied cropping systems. Our results align with those of Brito et al. (2012) and Mathimaran et al.

(2005), who found that *R. irregulare* spores were predominant in a soil community associated with cereals as the host plant. Several studies have reported a reduction in the diversity of AM fungal communities in roots from different agroecosystems specifically caused by conventional tillage (Alguacil et al., 2008; Schnoor et al., 2011).

Reduced tillage can enhance the diversity of arbuscular mycorrhizal (AM) fungi and lead to higher colonization rates compared to conventionally maintained soils (Bowles et al., 2017). Understanding how cropping systems affect various soil chemical, biological, and agronomic aspects is crucial for effective management and decision-making in farming (Mhlanga et al., 2022) Even more essential is grasping the causal relationships within cropping systems, maize production, and other related variables, as this knowledge can enhance our understanding of the multifunctionality of agroecosystems (Mhlanga et al., 2022). The choice of appropriate tillage practices is of great importance for the sustainability of the AMF communities.

CONCLUSIONS

Soil cropping system influences the population density and richness of the natural arbuscular communities of the tested maize hybrids. Genotypic differences between hybrids, expressed as differences in the percentage of mycorrhizal colonization. Hybrid P9537, which is the highest yielding for the region, also has the highest rate of mycorrhizal colonization. For the tested period hybrid DKC4949 exhibits the greatest ecological plasticity because its yields are neither affected by the cultivation technology nor by the climatic conditions of the year. No-till had a positive impact on natural mycorrhizal societies but not on the productivity of corn hybrids.

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