sciendo

Agriculture (Poľnohospodárstvo), 69, 2023 (2): 77 – 90

Original article

DOI: 10.2478/agri-2023-0007

SUPERABSORBENT POLYMER AND ITS EFFECT ON MAIZE GERMINATION, EMERGENCE AND GENETIC DIVERSITY OF RHIZOSPHERE MICROORGANISMS

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Ondreičková, K., Hrčková, K., Klčová, L., Cilík, P., and Gubiš, J. (2023). Superabsorbent polymer and its effect on maize germination, emergence and genetic diversity of rhizosphere microorganisms. *Agriculture (Poľnohospodárstvo), 69*(2), 77 – 90.

The application of superabsorbent polymer (SAP) for seed coating of agricultural crops is an innovative and promising method to effectively reduce plant stress during dry periods and thereby contribute to sustainable agriculture. However, due to the lack of experiments using SAP in field conditions in Central Europe, we focused on determining the effect of synthetic SAP, which was used for coating maize seeds, on the germination and emergence of these plants as well as on determining its impact on the genetic diversity of microbial communities in the maize rhizosphere for two consecutive years. The results showed that the growth parameters of coleoptiles and root systems of germinating maize plants at the very early development stage were biased in favour of SAP-coated seeds, and some values were statistically higher in the second year of evaluation. Similar observations were recorded in the assessment of maize emergence parameters. Also, these values were better when using SAP-coated seeds compared to the untreated controls. Plant height, dry weight, leaf tip number, and the Soil Plant Analysis Development (SPAD) value were statistically higher in the second year of evaluation. The rhizosphere bacterial and fungal genetic diversity results did not confirm statistically significant differences between the SAP-coated and control maize seeds. They concern both alpha diversity indices and the results from multivariate statistical methods. Principal coordinate analysis results with cluster analysis show that the main factor influencing the microbial diversity in the maize rhizosphere was the collection date, especially the year of sampling than the use of SAP-coated seeds.

Key words: bacterial diversity, fungal diversity, maize emergence, root system, seed germination, SAP-coating

Agricultural production depends heavily on water. It is increasingly exposed to water-related risks. It is also the sector with the largest use of water and 70% of global water withdrawal comes from irrigation (D'Odorico *et al*. 2018). Therefore, improving water management in agriculture is crucial for a sustainable and productive agri-food sector (Organisation for Economic Co-operation and Development, OECD). However, only 22% of global arable lands are irrigated and are at least twice as productive as non-irrigated agriculture (World Bank). Arable land that is not irrigated is entirely dependent on climatic conditions and is greatly affected by climate change. Global warming, causing prolonged droughts, tends to reduce agricultural productivity more and more (Oladosu *et al*. 2022; Rosa 2022). It is, therefore,

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necessary to make efforts to mitigate these negative impacts of climate change, for example, by using superabsorbent polymers.

Superabsorbent polymers (SAP) can also be called water-absorbing polymers or hydrogels. Their use in agriculture dates to the early 1980s (Ai *et al*. 2021; Oladosu *et al*. 2022). SAPs have a 3-D network and the ability to absorb and retain water 100 to 1,000 times more than their mass and thus can be used to effectively improve water use in agriculture (Chang *et al*. 2021). This absorption capacity is ensured by hydrophilic groups in the polymer chain (Patra *et al*. 2022) and this feature can be used to mitigate the adverse effects of water deficits and drought stress conditions on plant growth (Sayyari & Ghanbari 2012; Behera & Mahanwar 2020). Based on the source of origin, SAPs may be divided into natural (polysaccharide or polypeptide based), semisynthetic, and synthetic (petrochemical-based, such as polyacrylic or methacrylic acid, polyethylene glycol, vinyl acetate, and others) (Behera & Mahanwar 2020; Gutierrez *et al*. 2021; Skarpa *et al*. 2023). Unlike natural polymers, synthetic ones generally have better water absorption and retention abilities, as well as mechanical strength. Their better mechanical strength increases durability (Behera & Mahanwar 2020) but cannot be degraded in soil for a long time, which may cause potential pollution of agricultural soil (Chang *et al*. 2021). As another option, the production of polymers based together on natural with synthetic origins is often used when the disadvantages of alone using these types of origins are reduced (Ai *et al*. 2020; Chang *et al*. 2021; Patra *et al*. 2022).

In agriculture, SAPs are mainly used by incorporating them into the soil or for seed coating. In this way, it contributes to better seed germination and plant emergence of many crops (Malik *et al*. 2022). On the other hand, their effect on plants as well as on the surrounding soil ecosystem is currently poorly researched (Ai *et al*. 2020). For this reason, this study aimed to detect the impact of synthetic SAP, which seeds were coated with a newly patented superabsorbent-based suspension, on the seed germination and plant emergence of maize plants. It was also aimed at determining the influence of the polymer on the genetic diversity of bacterial and fungal communities in the maize rhizosphere for two consecutive years.

MATERIAL AND METHODS

Field trial

Field experiments were carried out in 2019 and 2020. They were established and treated by a stakeholder farm in local agricultural practice in the locality of Veselé (GPS 48.548111°N, 17.733067°E) in Slovakia. All the samplings, assessments and observations during the vegetation period were performed by the National Agricultural and Food Centre-Research Institute of Plant Production in Piešťany, Slovakia. The experimental site in both years was situated in the maize-growing region of Slovakia.

Maize (*Zea mays* L.) was a target crop. Hybrid NK Thermo (Syngenta, Switzerland) was used. Two seed treatments were involved and examined. An untreated control group of seeds (Control) was compared to seeds coated by superabsorbent polymer (SAP), which was performed by commercial SAP Aquaholder® Seed (PeWaS s.r.o., Bratislava, Slovakia). This SAP is based on acrylic acid, the potassium salt of acrylic acid and acrylamide, and the method of coating seeds with this superabsorbent-based suspension is under the Patent Application Number: PCT/SK2019/050008.

Large scale experiments were established in open field conditions in randomized block design in three repetitions. The size of the single plot was 18 m × 50 m. The long-term average annual amount of precipitation and an average annual air temperature for the period 1951 – 1980 in the area was 595 mm and 9.2°C per year, respectively. The soil type was Luvic Chernozem, characterised by a high content of available potassium and middle content of phosphorus. Agrochemical soil properties were analysed before sowing. N_{tot} and C_{tot} were determined by the Dumas method. Spectrophotometry was used to determine available forms of phosphorus and potassium (Mehlich III), $N\text{-}NH_4$ (Bertheloth reaction), $N\text{-}NO_3$ (KCl extraction). Additional data are shown in Table 1. Soil cultivation consisted of autumn ploughing to a depth of 0.3 m and spring seedbed preparation with a field cultivator. Mineral fertilisers

of NPK 15-15-15 and urea were incorporated into the soil shortly before the sowing with the following doses of pure elements: nitrogen 77.0 kg/ha, phosphorus 11.8 kg/ha and potassium 22.5 kg/ha. Postemergence herbicide treatment was performed by single application with active substances of foramsulfuron, iodosulfuron-methyl Na, thiencarbazone-methyl and cyprosulfamide. The sowing date and density are listed in Table 2. Seeds were placed into a depth of 0.05 m. The inter-row distance was 0.75 m. Additional pure nitrogen in doses of 50.0 kg/ha and 27.0 kg/ha was applied on the soil surface 10 and 70 days respectively after the sowing in the form of urea and LAD 27. Experiments were carried out without any irrigation. Meteorological parameters were recorded daily from the point of view of precipitation and air temperature (Figure 1).

Image analysis

Germinating maize plants from both experimental treatments were sampled in early growth stage BBCH 07 (2019) and BBCH 09 (2020) (Table 2). Ten plants in a row were gently excavated from soil and washed in tap water above the mesh to prevent root losses. The remaining soil, small stones and plant litter were removed as well as the plants with incomplete or damaged roots. Root system images were captured using a flat-bed scanner (HP Scanjet 3670) at a resolution of 600 dpi with black and opaque background to obtain strong contrast. Primary colour images were converted into grayscale in ImageJ software (version 1.53 j, National Institutes of Health, USA) and inverted with roots appearing darker than the background. In the next step, they were processed by ImageJ plug-in SmartRoot (version 3.42, Université Catholique de Louvain, France) where the tracing algorithm, vectorial representation of root objects and user interface were combined for data acquisition (Lobet *et al*. 2011). The diameter, macroscopic length, surface, and volume of radicle root and lateral seminal roots were obtained, as well as the coleoptile length and diameter by image analysis. Subsequently, plants were divided into coleoptile and root systems. Both parts were dried at 60°C for 48 hours and weighed to determine dry matter content. Quantitative parameters of the total root system were calculated from partial data of radicle root and lateral roots as the sum.

Plant analysis

During the growth stage of BBCH 12 (2019) and BBCH 15 (2020) plots were visually evaluated for

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Agrochemical soil properties

Year	$pH_{(KCl)}$	P [mg/kg]	K [mg/kg]	$N\text{-}NO_3$ [mg/kg]	$N\text{-}NH_4$ [mg/kg]	N_{min} [mg/kg]	N_{tot} [g/kg]	C_{tot} [g/kg]
2019	5.97	78.21	353.16	11.57	3.90	15.47	1.19	11.59
2020	6.12	66.36	322.95	19.31	4.04	23.35	1.38	12.79

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Sowing details, plant and rhizosphere sampling dates with corresponding maize growth stages

Year	Sowing date	Sowing rate	Plant sampling (S1)	Emergence assessment (S2)	First rhizosphere sampling for DNA analysis (R1)	Second rhizosphere sampling for DNA analysis (R2)
2019	04.04.2019	72,000	15.04.2019 (BBCH 7)	26.04.2019 (BBCH 12)	08.06.2019 (BBCH 18)	15.09.2019 (BBCH 75)
2020	08.04.2020	80,000	22.04.2020 (BBCH 9)	01.06.2020 (BBCH 15)	17.06.2020 (BBCH 18)	18.09.2020 (BBCH 75)

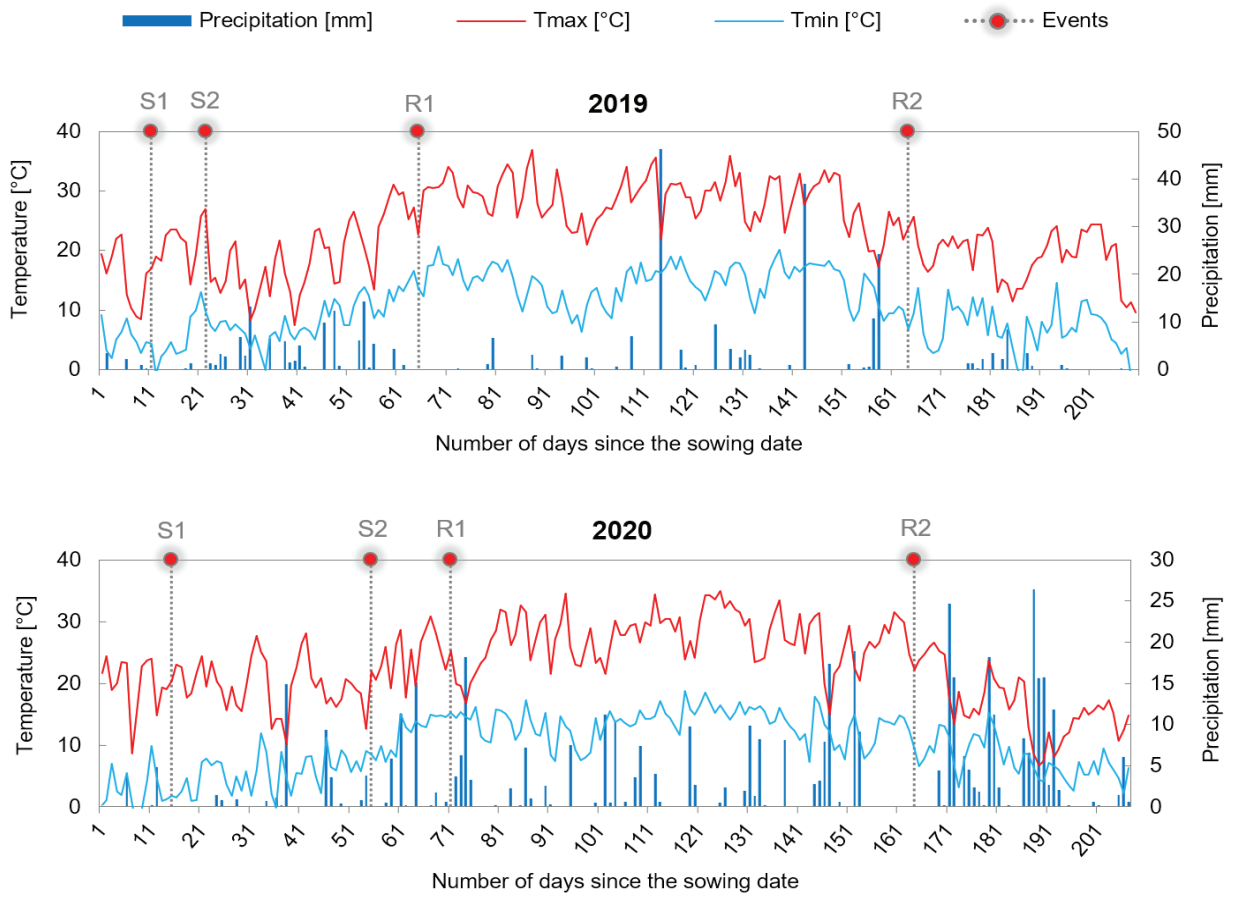


Figure 1. Meteorological parameters (maximum and minimum air temperature, precipitation) in daily steps measured from the sowing date.
Note: S1 – sampling of germinating plants; S2 – sampling of emerged plants in the leaf development stage; R1 – first rhizosphere sampling for DNA analysis; R2 – second rhizosphere sampling for DNA analysis.

emergence uniformity and plant spacing (Table 2). Four representative rows in length of 4 meters were marked to obtain an area of 12 m^2. All plants inside the determined area were counted, and an average number of emerged plants per hectare was calculated. Plant height was measured manually from the stem base to the leaf tip of the longest leaf. SPAD value of nitrogen status was measured non-destructively using a SPAD meter (Soil Plant Analysis Development meter) (SPAD 502 plus, Minolta, Japan) from the youngest fully expanded leaf. Leaves were fully intact, clean, dry, green, and free of signs of disease or damage. The mean SPAD value was derived from 20 individual measurements/plants in each experimental plot. SPAD index was calculated as follows: SPAD (SAP) / SPAD (Control) × 100%. The leaf tip number includes every visible leaf tip per single plant.

Rhizosphere sampling and DNA extraction

The rhizosphere from maize plants was collected in two stages of maize growth – the leaf/vegetative stage in June and the reproductive stage in September in 2019 and 2020 (altogether, four collection dates, Table 2, Figure 1). Three separate plots (3 individual samples, 3 independent plants/sample) were considered as control without SAP-coated seeds and 3 separate plots (3 individual samples, 3 independent plants/sample) were with SAP-coated seeds, marked as SAP. Thus, six samples in each collection date were sampled, and 24 were in total. The rhizosphere samples were collected according to Ondreičková *et al.* (2021) and stored before analysis at 4°C. On the same day of sampling, the rhizosphere samples were used for DNA isolation. The total metagenomic DNA (mgDNA) was extracted from 250 mg of rhizosphere samples using the PowerSoil™ DNA

Isolation kit (Qiagen, Hilden, Germany) according to instructions from the manufacturer. Isolated DNA was dissolved in 50 µL of nuclease-free water. The quantity and purity of DNA were spectrophotometrically measured with NanoDrop-1000 Spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). Subsequently, samples were diluted to the same final concentration (20 ng/µL), and stored at –20°C.

Molecular analysis of rhizosphere microbial diversity

Automated ribosomal intergenic spacer analysis (ARISA) was used to detect the bacterial and fungal genetic diversity in the rhizosphere of maize. The ITSF/ITSReub (Cardinale *et al*. 2004) primer set was used for amplification of the 16S-23S rRNA intergenic transcribed spacer (ITS) region from the bacterial rRNA operon and the 2234C/3126T (Sequerra *et al*. 1997) primer set was used for amplification of ITS1 – 5.8S rRNA – ITS2 region of fungal DNA. Primer set with 6-FAM fluorescent dye on the 5′ end of the reverse primer for bacteria and 6-FAM fluorescent dye on the 5′ end of the forward primer for fungi were used in PCR. DNA amplification of bacterial and fungal communities was carried out in 50 µL volume of reaction mixture containing 1 × PCR buffer (Invitrogen, Thermo Fisher Scientific Inc., Waltham, USA), 1.5 mmol Mg^{2+}, 0.25 µmol of both primers, 200 µmol of each dNTP (Invitrogen, Thermo Fisher Scientific Inc., Waltham, USA), 1 U Taq DNA polymerase (Invitrogen, Thermo Fisher Scientific Inc., Waltham, USA), and 20 ng of DNA isolated from the rhizosphere. The PCR was conducted in a GeneAmp PCR System 9700 (Applied Biosystems, Thermo Fisher Scientific, Inc., USA) using the following conditions: initial heat denaturation at 94°C for 3 min, followed by 35 cycles each consisting of a denaturation step at 94°C for 45 s, annealing at 60°C for 1 min, extension at 72°C for 2 min and a final extension step at 72°C for 10 min. PCR was confirmed by 1% (w/v) agarose gel electrophoresis using ethidium bromide and visualised using UV illumination. PCR products were subsequently precipitated with ethanol and dissolved in 15 µL of sterile water. One microliter of purified products was added to 9 µL formamide containing LIZ1200 size standard (Applied Biosystems, Thermo Fisher Scientific, Inc., USA), denatured at 95°C for 3 min and separated using capillary electrophoresis in ABI 3100 Prism Avant (Applied Biosystems, Thermo Fisher Scientific, Inc., USA). Data obtained from ARISA in the form of electrophoregrams were analysed by the Peak Scanner 2 (Applied Biosystems, Thermo Fisher Scientific Inc., Wilmington, USA), and operational taxonomic units (OTUs) in the range 200 – 1,000 bp were used for the evaluation. Only peaks above the threshold of 50 fluorescence units were considered.

Statistical evaluation

Plant analysis: the standardized skewness and standardized kurtosis were used with our obtained data to determine whether the sample came from a normal distribution. Their values were not outside the range of –2 to +2 and corresponded to normal distribution. So, data were statistically evaluated to find significant differences among samples by one-way analysis of variance (ANOVA) followed by the Fisher´s *LSD* (Least significant difference) *post-hoc* test, with a level of significance of 95% ($P \leq 0.05$), using the software Statgraphics Centurion XVI.

Molecular analysis: the Venn diagrams were constructed using Venny 2.1 online program (Oliveros 2016). The standardized skewness and standardized kurtosis were used to determine whether the sample data came from a normal distribution. Since the data came from a normal distribution, statistically significant differences between samples were calculated by a t-test or ANOVA at the 95% confidence limit for the means using Statgraphics x64 (Statpoint Technologies, Inc., Warrenton, USA). This software was also used to create frequency histograms. Alpha diversity indices such as Simpson, Shannon and Evenness were calculated from standardized profiles of individual samples using the peak number and heights in each profile as representations of the number and relative abundance of phylotypes. Microbial communities in diverse samples were compared from ARISA profiles using the fluorescence height in individual OTUs. These data were subsequently used for the Principal coordinate analysis (PCoA) with a correlation matrix using the scores of the first two principal coordinates in graphical form. Scores from all coordinates from PCoA were

used for ANOVA and to create histograms. ARISA profiles of bacterial and fungal communities were also compared using Cluster analysis with Unweighted pair-group average (UPGMA) algorithm and Bray-Curtis similarity index. Alpha diversity indices, PCoA, and Cluster analysis with cophenetic correlation coefficient were evaluated by using the PAST (PAleontological STatistics) software version 3.19 (Hammer *et al*. 2001).

RESULTS

Seed germination

The growth parameters of maize seedlings were evaluated in the very early development stage. Only the primary root system was developed at the time of plant sampling (S1) in both experimental years. It consisted of a radicle root and a few lateral seminal roots.

Plant sampling in 2019 was done 10 days after the sowing and plants matched BBCH 07. Soil temperature and volumetric soil moisture in the seedbed were 10.7°C and 13.2%, respectively. Plants from SAP-coated seeds were characterised by shorter coleoptiles with a wider diameter and higher dry matter content (Table 3). In the root system, the recorded differences were in favour of SAP-coated seedlings. Values of root length, root surface and root volume of radicle root and lateral seminal roots individually were slightly higher in comparison to the control group (Figure 2). Consequently, the same trend was visible in total length, surface, and volume of the whole root system (Table 4). Also, the number of the lateral seminal roots was higher than in case of the control group. All the previously men-

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Coleoptile parameters during the germination. The different letters denote statistically significant differences between control and SAP samples in each experimental year separately (*LSD, P* ≤ 0.05). The value after the ± sign represents the standard deviation.

Parameter	2019		2020	
	Control	SAP	Control	SAP
Coleoptile length [mm]	$6.21^{a} \pm 0.25$	$5.72^{a} \pm 0.87$	$26.13^{a} \pm 4.33$	$44.72^{b} \pm 10.81$
Coleoptile diameter [mm]	$1.61^{a} \pm 0.12$	$1.66^{a} \pm 0.10$	$2.13^{a} \pm 0.15$	$2.32^{a} \pm 0.19$
Coleoptile weight [mg]	$3.91^{a} \pm 1.75$	$4.39^{a} \pm 0.30$	$9.89^{a} \pm 2.07$	$19.02^{b} \pm 5.30$

Note: SAP – superabsorbent polymer; *LSD* – least significant difference.

T a b l e 4

Quantitative parameters of total root system during the germination. The different letters denote statistically significant differences between control and SAP samples in each experimental year separately (*LSD, P* ≤ 0.05). The value after the ± sign represents the standard deviation.

Parameter	2019		2020	
	Control	SAP	Control	SAP
Total root length [mm]	$55.34^{a} \pm 11.46$	$63.71^{a} \pm 11.81$	$150.05^{a} \pm 33.89$	$226.91^{b} \pm 52.01$
Total root surface [mm^2]	$194.50^{a} \pm 37.04$	$215.61^{a} \pm 60.48$	$580.44^{a} \pm 130.79$	$891.91^{b} \pm 180.41$
Total root volume [mm^3]	$61.95^{a} \pm 11.26$	$65.69^{a} \pm 17.35$	$187.36^{a} \pm 41.60$	$293.85^{b} \pm 51.38$
Total root weight [mg]	$10.85^{a} \pm 5.80$	$9.77^{a} \pm 4.26$	$30.20^{a} \pm 5.32$	$33.99^{a} \pm 4.94$
Lateral root number	$3.08^{a} \pm 0.42$	$3.34^{a} \pm 0.13$	$2.90^{a} \pm 0.32$	$3.15^{a} \pm 0.23$

Note: SAP – superabsorbent polymer; *LSD* – least significant difference.

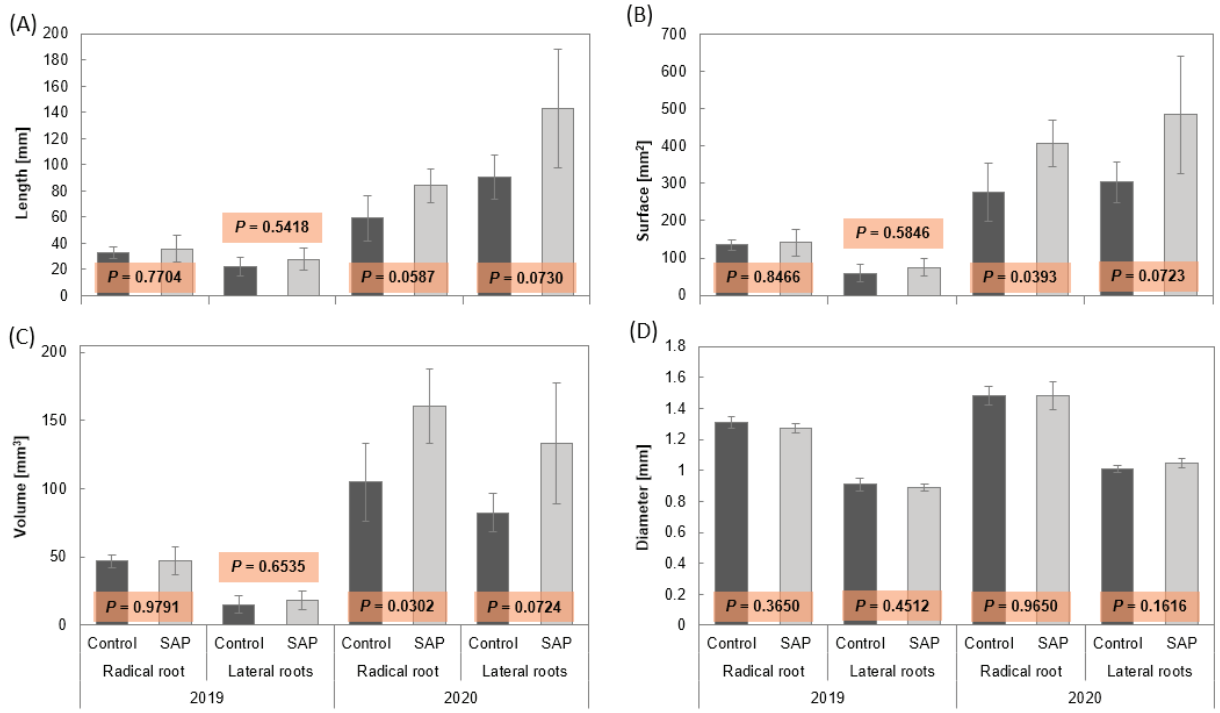


Figure 2. Length (A), surface (B), volume (C), and diameter (D) of radical and lateral maize roots in control and SAP-coated seeds. Bars represent standard deviations, *P*-values in orange boxes represent results from One-way ANOVA between control and SAP samples in each experimental year separately.
Note: SAP – superabsorbent polymer, ANOVA – analysis of variance.

tioned differences were not statistically significant. In general, the very first part of the vegetation period in 2019 showed slight increments in most monitored features.

In 2020 plants were sampled 14 days after the sowing in the stage of BBCH 09. Initial soil conditions of the second vegetation season were slightly different and more favourable for maize germination. Soil temperature and volumetric soil moisture in the seedbed were 14.6°C and 17.2%, respectively. Dry matter accumulation of coleoptile was significantly higher in the case of SAP-coated seedlings. Coleoptile was also significantly longer with a wider diameter in comparison to the untreated control group (Table 3). Radicle root as well as the seminal lateral roots of SAP-coated seedlings grew more intensively and showed important differences between treatments (Figure 2). Total root system length, surface and volume reached significant increments when compared to control variants (Table 4). Quantitative comparison of seminal lateral roots provided higher number in SAP-coated seedlings. All assessed features were positively influenced by SAP treatment in the early period of 2020.

Plant emergence

Plant number was evaluated in the stage of BBCH 12 in 2019. SAP coating provided a significantly higher number of correctly emerged maize plants per area unit (Table 5). Detail analysis showed that these plants from treated seeds were taller and produced more aboveground dry matter biomass, but the differences were nonsignificant. The development of both experimental groups was very similar to each other, and leaf tip numbers did not differ significantly. SPAD values showed higher leaf nitrogen levels of SAP-coated plants compared to the control group, and SPAD index reached 107.80%. The general plant fitness showed better parameters for the next development with SAP support.

Results of maize emergence in 2020 indicated a lower number of plants per area unit in the case of SAP coating (Table 5). However, the difference between experimental treatments was visually detectable in plant appearance. Correctly emerged plants of SAP treatment were characterised by significantly higher above ground dry matter accumulation, plant height and nitrogen level as well. The SPAD index

Table 5

Plant growth characteristics in the leaf development stage. The different letters denote statistically significant differences between control and SAP samples in each experimental year separately (*LSD, P* ≤ 0.05). The value after the ± sign represents the standard deviation.

Parameter	2019		2020	
	Control	SAP	Control	SAP
Plant number per ha [pcs]	60,250.00^{a} ± 1,060.66	64,750.00^{b} ± 1,060.66	78,750.00^{a} ± 4,500.00	73,250.00^{a} ± 1,717.83
Plant height [mm]	128.50^{a} ± 10.61	141.00^{a} ± 1.41	299.00^{a} ± 62.41	459.50^{b} ± 70.02
Dry matter [mg]	56.00^{a} ± 8.49	78.00^{a} ± 0.06	1,034.00^{a} ± 519.99	3,178.00^{b} ± 1,308.67
Leaf tip number [pcs]	2.90^{a} ± 0.14	3.00^{a} ± 0.02	6.95^{a} ± 0.66	8.75^{b} ± 0.41
SPAD – value	28.20^{a} ± 0.99	30.40^{a} ± 2.55	37.20^{a} ± 1.66	43.95^{b} ± 2.04

Note: SAP – superabsorbent polymer; *LSD* – least significant difference; SPAD – soil plant analysis development.

was 118.15%. Leaf tip number hinted at a delay in the development of the control group.

Microbial genetic diversity

In 2019, we detected a higher representation of diverse bacteria and fungi than in 2020. In the leaf (vegetative) stage (R1), 193 and 233 bacterial OTUs were observed in control and seeds coated with SAP in 2019 and 150 and 124 bacterial OTUs in 2020, respectively. In the maize reproductive stage (R2), the abundance of different bacterial OTUs in control and SAP-coated seeds was as follows: 230 and 349 OTUs in 2019; 144 and 168 OTUs in 2020, respectively. In the case of fungal communities, the abundance of different OTUs in both maize stages in control and SAP-coated seed was 384 and 266 fungal OTUs in the leaf stage in 2019; 101 and 98 OTUs in the leaf stage in 2020; 393 and 412 OTUs in the reproductive stage in 2019; 134 and 89 OTUs in the reproductive stage in 2020, respectively (data not shown). A more straightforward representation is shown in Figure 3 in the form of Venn diagrams, where the division into both growth stages of maize is omitted for better clarity. The Venn diagrams show that more bacterial than fungal OTUs were common in all treatments (12.1% *vs* 7.7%, respectively). However, in bacteria, the most represented OTUs were detected in SAP-coated seed in 2019, and in fungi, the most represented OTUs were between control and SAP in 2019 (Figure 3).

Simpson and Shannon diversity indices, espe-

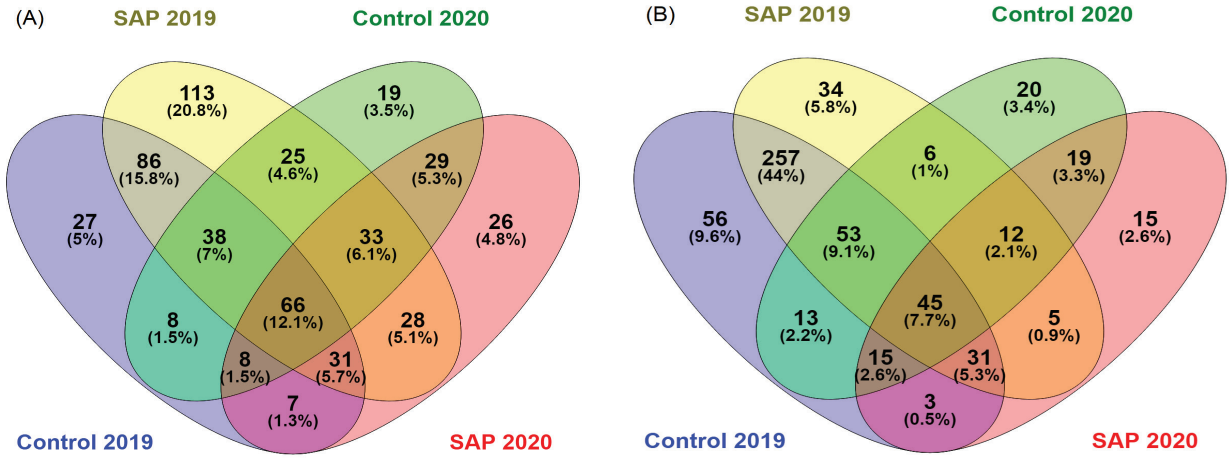


Figure 3. Venn diagrams showing the number of shared and unique OTUs between rhizosphere bacterial (A) and fungal (B) communities from control and maize seeds coated with SAP in 2019 and 2020.
Note: SAP –superabsorbent polymer.

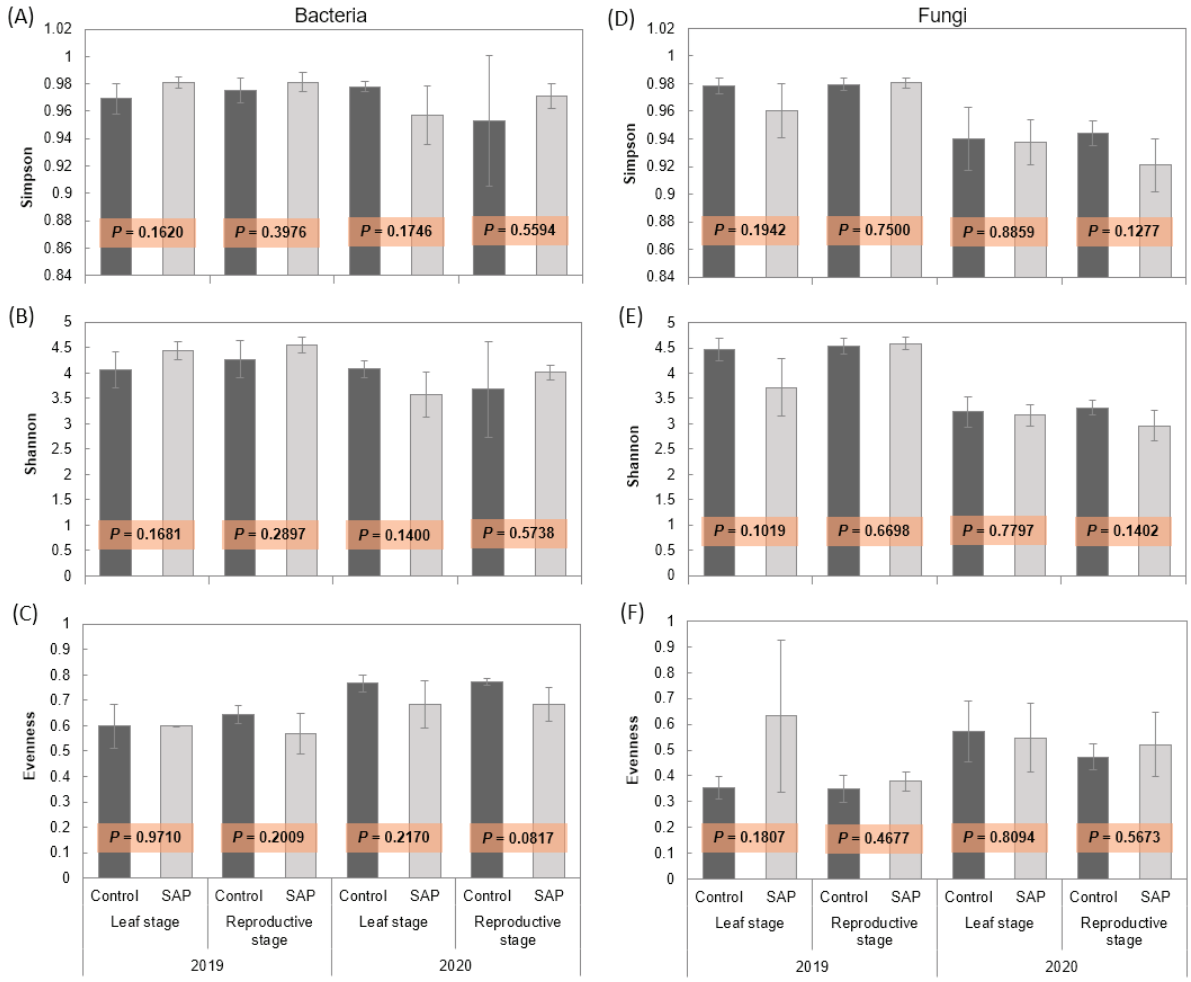


Figure 4. Alpha diversity indices of bacterial (A, B, C) and fungal (D, E, F) communities detected in the rhizosphere of maize in control and samples with SAP-coated seeds. Bars represent standard deviations. *P*-values in orange boxes represent results from the t-test between control and SAP samples in each collection date.
Note: SAP –superabsorbent polymer.

cially for fungal communities, showed lower values in 2020 than in 2019 (Figure 4). Although in some cases, a difference is seen in the mean values of diversity indices between control and SAP-coated seeds, this difference is not statistically significant in any case (t-test, $P > 0.05$).

Principal coordinate analysis (PCoA, Figure 5), especially the first coordinate except for three samples corresponding to fungal communities, divided the samples according to the collection year. Mainly in the case of bacterial communities, the first two principal coordinates divided the samples according to individual collection dates and according to the presence of SAP on the seeds (Figure 5A). This distribution is also noticeable for fungal communities in 2019 (Figure 5B). However, when statistically comparing all values from all generated coordinates from PCoA, no significant differences were detected between control and SAP, nor between different sampling dates (ANOVA, $P > 0.05$). At the same time, neglecting the collection dates and leaving only the influence of SAP alone, a statistical difference between the control and SAP-coated seeds was not recorded in the bacterial or fungal communities (t-test, $P > 0.05$, Figure 6).

The cluster analysis divided the samples preferentially according to the year of sampling and not according to the presence of SAP on the seed (Figure 7). The result from PCoA was confirmed, especially for fungal communities, where two samples with SAP-coated seeds from the maize leaf stage in 2019 formed a separate cluster, apart from the oth-

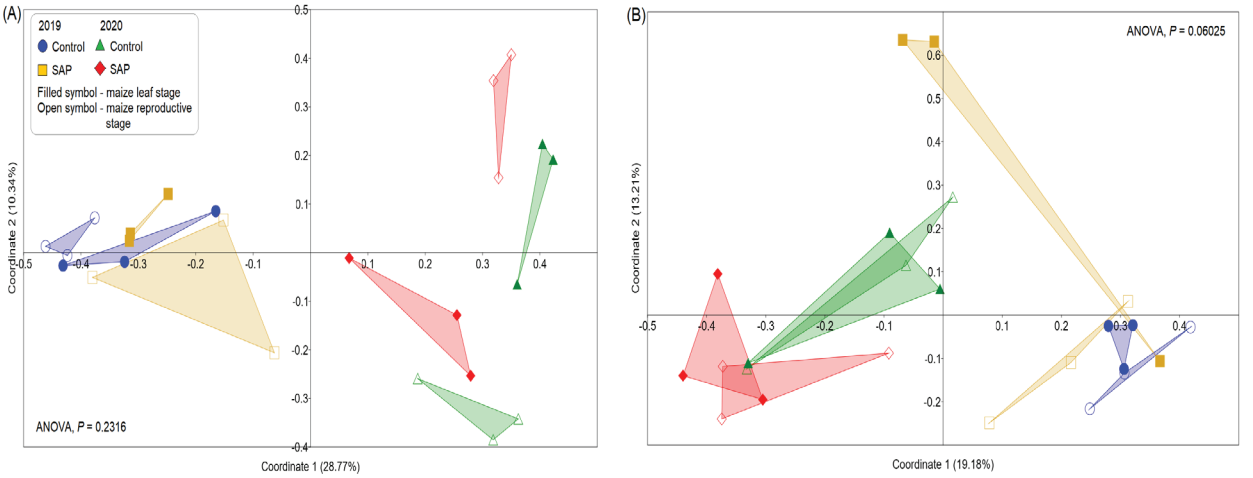


Figure 5. Principal coordinate analysis (PCoA) of bacterial (A) and fungal (B) communities detected in the rhizosphere of maize in control and samples with SAP-coated seeds. Analysis of variance (ANOVA) among eight subgroups (control and SAP in four collection dates) was calculated from all coordinate values from PCoA (not only from two principal coordinates presented graphically in this figure) and *P*-values were inserted into the plots.
Note: SAP – superabsorbent polymer.

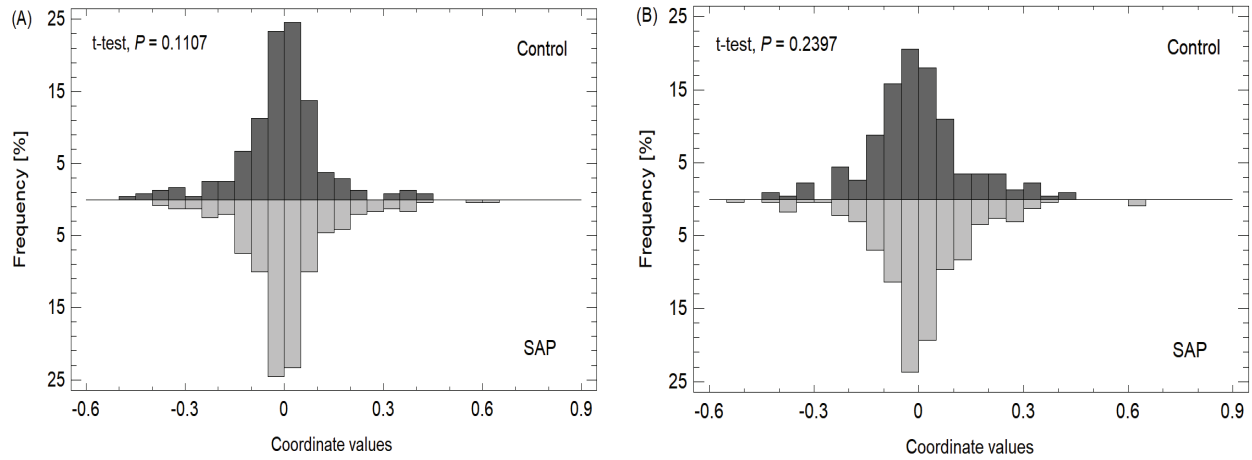


Figure 6. The frequency histogram displays the comparison of all coordinate values generated from PCoA corresponding to control and SAP-coated seeds regardless of the collection date from bacterial (A) and fungal (B) communities. The height of each bar in the plot represents the percentage of observations in a single interval. The inserted *P*-values represent the results of the t-test between control and SAP-coated seeds.
Note: SAP – superabsorbent polymer; PCoA – principal coordinate analysis.

er samples from 2019. This cluster is located at the bottom of the dendrogram (Figure 7B).

DISCUSSION

Studies dealing with superabsorbent polymer (SAP) are often focused on the development and improvement of SAP chemical compositions or technology of the application process (Amirkhami *et al*. 2023). From the point of view of plants, the most attention is paid to laboratory tests in controlled conditions, including the beginning of seed swelling and germination (Su *et al*. 2017; Afzal *et al*. 2020). SAP impact on final yield and yield components of various crops is usually assessed in connection with direct SAP application into the soil in doses of 50 – 300 kg per hectare. Research is very active in the field of drought stress and water shortage in arid and semiarid regions (Yazdani *et al*. 2007; Jahan & Mahallati 2020). But also, the humid regions of Europe suffer from drought periods and establishment delay, or early crop failure become more often, especially in segments of spring field crops.

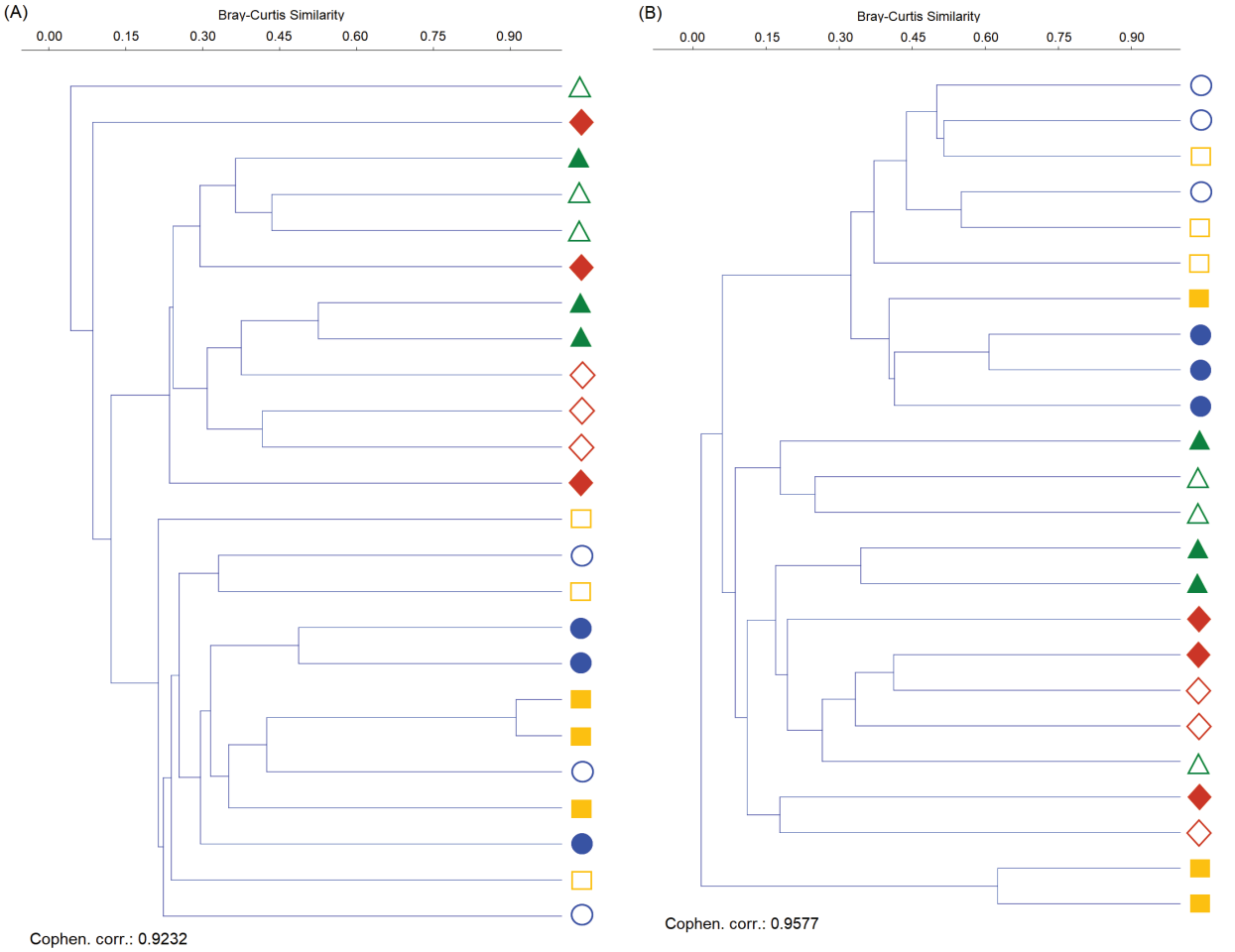


Figure 7. Cluster analysis using UPGMA clustering and Bray-Curtis similarity index of bacterial (A) and fungal (B) communities detected in the rhizosphere of maize in control and samples with SAP-coated seeds. The labelling of the samples is identical to Figure 5.
Note: Cophen. corr. – cophenetic correlation coefficient; SAP – superabsorbent polymer; UPGMA – unweighted pair group method with arithmetic mean.

De Barros *et al*. (2017) reported that sorghum (*Sorghum bicolor* L. Moench.) seedlings' emergence can be significantly highly reduced in the case of seed SAP coating and SAP application as a soil additive. However, both ways of SAP utilization increased seedling growth and dry matter of aerial plant parts in the early stages of 26 and 30 days after sowing. In our results, SAP treatment provided a higher level of field emergence in the first experimental year in comparison to the untreated control group. The presented decline in the average number of emerged plants during the second season was nonsignificant. The final plant number per hectare was enough for sufficient stand establishment in both cases. Another stand quality aspect is plant morphology. Nutrient absorption by roots depends on their length, surface area and diameter (Rout 2014). Seminal roots play an important role in nitrogen and phosphorus acquisition by maize seedlings. Approximately up to 35% is derived from topsoil by seminal roots in the first 25 days after sowing, especially in low-nitrogen environments. Various precipitation patterns, fertilisation rates, soil textures, and planting densities were examined and considered by Perkins and Lynch (2021). In this study, an increased number of seminal roots in SAP-coated treatment was indicated in both experimental years, together with the improvement of other root quantitative traits at the very beginning of the vegetation period (Table 4). These benefits were subsequently carried over into the next period of the leaf development stage. Evaluation of aboveground biomass parameters (Table 5)

indicates higher values for each one in SAP-coated treatment. They were able to create and store more assimilates under the given conditions. Metabolism was more effective, or they were able to obtain the necessary nutrients from available sources more efficiently than plants from the control group. The differences were obvious and significant, especially in the second experimental year.

The rhizosphere microbial genetic diversity results did not confirm a statistically significant difference between the SAP-coated and control maize seeds. They concern both alpha diversity indices (Figure 4) and the results from multivariate statistical methods (PCoA in Figure 5, subsequent frequency histograms in Figure 6, and cluster analysis in Figure 7). Our PCoA results with cluster analysis show that the main factor influencing the microbial diversity in the maize rhizosphere was the collection date, especially the year of sampling than the use of SAP-coated seeds. In particular, the results from the cluster analysis correspond to this (Figure 7). We can consider them reliable with the correctness of using the UPGMA algorithm and the Bray-Curtis similarity index due to the high values of cophenetic correlation coefficients. Our obtained coefficients were 0.9232 for bacterial and 0.9577 for fungal communities. These values for the cophenetic correlation coefficient can be from –1 to 1. The closer the values are to 1, the more appropriate the given choice of algorithm and the choice of distance measure or similarity index, as many different types of clustering are available (Saraçli *et al*. 2013). However, the SAP we used was based on acrylic acid, the potassium salt of acrylic acid and acrylamide, while the superabsorbent content was 25 to 40 wt. % of the suspension. At the same time, maize seeds were coated with this polymer, and studies dealing with the impact of SAP-coated seeds on the diversity of microbial communities are very limited. However, there are already published studies that detect soil microbial communities when different origin SAP was applied to the soil. Tian *et al*. (2020) used a modified polymer (polymer content was 2% and containing anionic polyacrylamide, polyvinyl alcohol, and manganese sulphate) with or without humic acid and modified polymer significantly decreased ACE species richness in soil with plants and significantly increased the ACEs in soil without plants. Also, their polymer material application significantly increased the Shannon index in soil with and without plants on at least one sampling date. But in their study, the soil with/without plants was a main factor affecting soil microbial diversity, not polymer application. At the same time, they did not observe that the application of the polymer adversely affected the soil microbial environment. Wang *et al*. (2018) observed using superabsorbent resin with boron (SARB) together with peat substrate or maize straw and based on PCoA and cluster analysis that bacterial communities in SARB + peat substrate samples clustered with each other, and bacteria in SARB + maize straw samples formed their group/cluster. Since in these groups, there were also control samples without the use of SARB from their experiment it follows that the addition of either a peat substrate or maize straw than a SARB had a greater impact on bacterial diversity. Li *et al*. (2020) detected that SAP (containing linear anionic polyacrylamide + humic acid + biochar), nine mixtures of different concentrations, affected soil bacterial communities. From the cluster analysis of the bacterial community composition at the OTU level, they found that the control samples showed the greatest similarity with treatments E1 (0.5 g/kg SAP + 3 g/kg humic acid + 5 g/kg biochar) and E5 (1 g/kg SAP + 6 g/kg humic acid + 5 g/kg biochar). They explain these changes in soil microbial diversity by improving soil chemical properties and physical structure after the addition of nine different treatments. These changes in soil physical and chemical properties after adding SAP to the soil were also reported in several studies (Bai *et al*. 2010; Caoa *et al*. 2017; Tian *et al*. 2020). Amina *et al*. (2021) even observed that the SAP (cross-linked copolymer of acrylamide and potassium acrylate) addition to the soil had a greater effect on some bacterial OTUs from the barley rhizosphere than the watering condition. They reasoned this also by changes in soil physicochemical properties that can only affect these SAP "specific" OTUs.

CONCLUSIONS

The superabsorbent polymer (SAP) application for seed coating is an innovative and promising

method for sustainable agriculture to increase the emergence of agricultural plants. Our data indicate that plants with SAP support in the form of seed treatment improved quantitative root and stem traits in germination and early leaf development stages and some of them were statistically significant. Furthermore, in our experiments, SAP-coated seeds did not show significant changes in the bacterial and fungal communities in the maize rhizosphere. Also, we did not observe any adverse effect of SAP-coated seeds on the soil microbiome. Variations in microbial diversity were mainly due to the sampling year, which we assess as a greater driver for these changes than SAP use.

Acknowledgement. This work was supported by the European Regional Development Fund under Programme Interreg V-A SK-CZ, project ITMS: 304011Y185 "Use of superabsorbent polymers (SAP) as an innovative tool to mitigate the effects of climate change in agriculture" and by the Operational Programme Integrated Infrastructure within the project "Innovative fertilizers with alternative natural sources and their implementation in agrotechnical practices", 313011BWL7, co-financed by the European Regional Development Fund.

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Received: June 5, 2023
Accepted: October 27, 2023