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Long-term application of biogas digestate improves soil physical properties

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ABSTRACT

Rapid recent increase in biogas plants has inspired heightened interest in agricultural digestate use. This paper investigates the long-term effect of digestate application on soil physical, chemical and mineralogical properties, and compares it with the results of mineral fertiliser and compost use. The research was initiated in 2011 as a long-term field experiment at three Czech Republic sites. The field management comprised conventional tillage with 6-year crop rotation. The following five treatments were evaluated; unfertilised control, mineral fertiliser with 27% nitrogen, digestate I comprising corn silage and cattle slurry, digestate II composed of corn silage, pig slurry, farmyard manure and hay, and finally compost. Each treatment was replicated four times in the three sites, and soil samples were collected for analysis twice a year from 2018 to 2021. Statistically significant correlations were established between the stability of 1–2 mm soil aggregates and soil characteristics. The correlation coefficient was 0.37 for total organic carbon; 0.45 for total organic nitrogen; 0.36 for hot water extractable carbon; −0.54 for bulk density; 0.57 for porosity and 0.38 for water infiltration. Although treatments did not affect mineralogical properties, the soil aggregate stability was significantly increased by compost and digestate organic fertilisers; with the highest 36.47% average on plots with digestate II and the lowest 26.22% on the control and 26.1% on mineral fertilised plots. Organic fertiliser addition also significantly decreased soil bulk density and increased porosity, with a larger proportion of capillary pores. Finally, although digestate application did not improve soil organic matter as significantly as compost, its long-term use positively affected soil physical properties and water infiltration.

1. Introduction

Accelerating global warming and climate change are urgent challenges for humanity, and a "green-deal" has therefore been adopted in European Union countries. The main green-deal aim is to achieve climate neutrality by 2050, and one of its key principles is to develop a power sector based on renewable resources. The agricultural biogas sector provides acceptable potential as a low-carbon electricity source, and the European Union policy now promotes energy independence by financial incentives for new biogas plant construction (European Commission, 2019). More than 18,000 biogas plants were registered by October 2020 and these provide 13,520 mega-watts electric storage (Pastorelli et al., 2021). The current geopolitical situation ensures that their input will increase, because the European Commission has set an RE Power EU target of 35 billion cubic metres of biomethane by 2030 (European Biogas Association, 2022).

Czekała (2019) records that digestate is classified as whole digestate, liquid and solid fractions depending on the applied separation techniques. However, there is considerable variation in anaerobic digestate biochemical properties, and this reflects the diversity of biomass input, operating conditions and digestate processing techniques. Additional current research provides information on the most beneficial ranges for dry matter and organic matter content, nutrient status, pH and other digestate characteristics (Möller and Müller, 2012; Nkoa, 2014).

Liquid digestates are referred to as fugates, and their dry weight normally contains 5–6% mineral nitrogen and less organic carbon than the non-digested input materials (Johansen et al., 2013). Additional comparisons include that the digestate carbon/nitrogen ratio (C/N) can be ten times lower than that contained in farmyard manure (Alburquerque et al., 2012a) and it also has higher ammonium total nitrogen ratios, decreased organic matter content, elevated pH values and reduced viscosities compared to undigested animal manures (Möller and

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Müller, 2012).

Despite digestate practical use as fertilisers, there was limited scientific knowledge of their actual functions in soil fertility. Researchers therefore initiated studies to fully monitor digestate's impact on the growth and yields of different crops, and thus compare digestate effects with other organic and mineral fertilisers (Tambone et al., 2010; Nkoa, 2014).

Many authors have indicated that digestates have similar or greater crop performance than corresponding undigested animal manures and slurries (Alburquerque et al., 2012b; Bachmann, and Lee et al., 2011, 2021). A number of other studies have shown that anaerobic digestates are at least as effective as mineral fertilisers (Cristina et al., 2020; Haraldsen et al., 2011; Tiwari et al., 2000). For example, Witorożec-Piechnik et al. (2021) reported that digestate fertilisers produced higher dry matter yield of maize, sorghum and triticale than mineral fertilisers in a field experiment. Barłóg et al. (2020) found increased N, S, Na, Zn and Fe grain content after digestate application compared to mineral nitrogen, phosphate and potassium use. Moreover, Barłóg et al. (2020) and Slepetiene et al. (2020) reported the digestate effect on soil fertility expressed by the content of plant-available P, K and mineral N in soil. Additional authors have assessed the organic C and N dynamics (Galvez et al., 2012; Šimon et al., 2015); soil microbial communities (Coelho et al., 2020) and CO_2 and N_2O emissions (Johansen et al., 2013).

However, studies into the effect of long-term digestate application on soil physical properties have sadly been neglected, and recent studies on the impact of agricultural food-industry sludges on soil properties have returned conflicting results (Skic et al., 2020). The conflicting results included those on digestate research, and we were therefore confronted by the question 'do all organic inputs have similar impact on soil properties regardless of their source?'.

Bhogal et al. (2018) showed that the application of low-loading organic matter, such as digestates and livestock slurries, had limited ability on improving soil biological and physical functioning. The soil bulk density increased and porosity decreased when organic materials with low dry matter content were applied. Simeckova and Jandak (2016) then examined the effect of digestate and mineral fertiliser on bulk density, total porosity, field capacity and minimum air capacity in field experiments on maize. The authors reported that two years digestate application led to deterioration in these soil physical properties compared to mineral fertilisers application. In contrast, Garg et al. (2005) recorded that the application of liquid digestate from agricultural waste reduced soil bulk density and increased saturated hydraulic conductivity and moisture retention capacity.

Skic et al. (2020) reported that the application of exogenous organic materials influenced soil porosity, while digestates decreased the number of soil transmission pores in some instances and increased structural pore number and radius above 15 μm. Badagliacca et al. (2020) recorded that 30 t ha^{-1} solid digestate incorporation in olive and citrus orchards increased the soil's aggregate stability index. And Pastorelli et al. (2021) added that although soil bulk density was not affected by digestate application in their three-year maize-triticale rotation experiment, the aggregate stability had transient improvement. There was also decrement in the proportion of transmission pores and increased fissures in the treated soils. Finally, Beni et al. (2012) examined the effect of anaerobic digestate administration on soil physical and mechanical behaviour, and they found lower topsoil porosity and penetration resistance in this treatment compared to compost application.

Moreover, scientific studies on biogas digestate have especially increased since 2000, and this continues (Baştabak and Koçar, 2020). While these studies indicate that digestate has a wide range of applications and can partly replace fossil resources, further work is required on fertiliser treatment methods and the influence of digestate on soil and plants in long-term applications.

Therefore, herein we assessed the long-term effect of digestate application on soil properties compared to compost and mineral fertiliser. We investigated the soil physical and chemical properties and their interactions, and found that (1) digestate application affects chemical properties and soil organic matter and (2) the long-term effects of digestates potentially improve soil physical properties, such as bulk density, soil aggregate stability and water infiltration.

Our work was enabled by The Czech Republic Central Institute for Supervising and Testing in Agriculture. This institute initiated a field experiment with digestate application as part of the pan-European support for biogas plants, and it is conducted at the highest standard. The field experiment compares digestate effectiveness with different types of fertilisers, and the most important part of the experiment assesses digestate impact on soil properties and crop yields. Our study presents the results of this experiment.

2. Material and methods

2.1. Field experiment; site description and experimental design

The field experiment was established in 2011 at the Jaroměřice nad Rokytnou, Lípa and Hradec nad Svitavou sites at the Czech Republic Central Institute of Supervising and Testing. All sites are located in the potato growing region and their soil and climate characteristics are presented in Table 1. Field trial management included conventional tillage and a 6-year crop rotation in the following sequence; potatoes/winter wheat/silage corn/spring barley/oilseed rape/winter wheat. The 2018–2021 experimental period was in this crop-order; winter wheat (2018) – silage corn (2019) – spring barley (2020) – oilseed rape (2021). The design was based on 10.82 × 3.74 m plots, with four replicates at each of the three sites.

Five treatments at each site were selected for soil sampling: (1) the control unfertilised treatment; (2) a mineral fertilised treatment with a mixture of ammonium nitrate and finely ground limestone – LAV with 27% N; organic fertilised treatments - (3) digestate I; (4) digestate II and (5) compost. Fertiliser rates were applied according to cultivated crop growing requirements. The basic dose of N fertiliser was 120–150 kg N ha^{-1} for LAV and digestates, from which N is released rapidly, and 300 kg N ha^{-1} for compost, with slow N release. Barley was not fertilised with either LAV or organic fertilisers to comply with its growth requirements. Finally, the organic fertilisers came from the same sources in all sites.

Digestates were produced as a by-product of anaerobic biomass fermentation at the biogas plant. The biogas production substrates were corn silage and cattle slurry (digestate I) and corn silage, pig slurry, farmyard manure and hay (digestate II). Registered compost is produced by homogenisation and composting of biodegradable substances, from urban greenery, distillery and forestry waste and sewage sludge. The compost and digestates compositions are listed in Table 2.

Table 1
Climate and soil characteristics of the experimental sites.

Characteristics	Jaroměřice nad Rokytnou	Lípa	Hradec nad Svitavou
Coordinates	49.0997961 N, 15.8762206E	49.5632089 N, 15.5381272E	49.7312900 N, 16.5038400E
Altitude (m)	425	505	460
Average annual temperature (°C)	8.0	7.5	7.4
Average annual rainfall total (mm)	481	594	616
Soil type	haplic Luvisols	Cambisols	haplic Luvisols
Soil texture	Silt loam	Sandy loam	Silt loam
pH (KCl)	6.1	5.6	7.1
pH (H_2O)	6.9	6.2	7.4
P_{avail} (mg kg^{-1})	62.5	44.8	77.5
K_{avail} (mg kg^{-1})	169	80	132
Ca_{avail} (mg kg^{-1})	2653	1543.3	2204.5
Mg_{avail} (mg kg^{-1})	223.5	92.5	46.8

Table 2
The composition of used organic fertilisers - average values for 2018–2021.

Organic fertiliser	Dry biomass (%)	pH	C: N	Total N (%)	Combustible substances (%)	P_2O_5 (%)	K_2O (%)
Compost	36.8	8.2	17.5	0.8	26.4	0.8	1.6
Digestate I	7.1	8.0	6.1	6.1	73.7	2.5	5.5
Digestate II	5.6	8.4	4.2	8.5	72.3	2.7	10.2

2.2. Soil sampling and sample processing

Soil samples were taken by field-shovel from the 0–7 cm upper soil layer in 2018–2021 spring and summer soon after crop harvests. A total of 60 samples were collected - 12 samples of approximately 2 kg soil for each of 12 replicates in each treatment. The soil samples were air-dried, homogenised and divided into two portions. The first was fine soil obtained by sieving through a 2 mm sieve. This was used for pH; hot water extractable carbon (C_{hwl}); N, C and S concentrations and measurement and spectroscopic characteristics of soil organic matter. A 1–2 mm grain-size soil fraction was obtained by sieving the soil through a system of sieves with mesh sizes of 1 and 2 mm. This was employed for total glomalins and assessing soil aggregate water stability.

Total soil organic carbon (C_{tot}) and total organic nitrogen (N_{tot}) content was evaluated by Vario/CNS analyzer (Elementar Analysensysteme GmbH, Langenselbold, Germany). Hot water extractable labile carbon (C_{hwl}) was determined as in Körschens et al. (1990). FTIR spectra were measured by Thermo Nicolet Avatar 320 FTIR spectrometer (Nicolet, Madison, USA) in a homogeneous mixture of bulk soil and FTIR grade KBr (Sigma-Aldrich, Darmstadt, Germany). This was then analysed at the following functional group absorption bands; aliphatic hydrophobic CH_2 and CH_3 (3000–2800 cm^{-1}), aromatic COO− , C═C (1660–1580 cm^{-1}) and hydrophilic (1740–1600 cm^{-1}) (Demyan et al., 2012). The following were then calculated; the decomposition index (DI) which determines the intensity of FTIR spectra for C═C/C-H functional groups, and the hydrophobicity index (HI) which establishes the intensity of FTIR spectra for ratio of hydrophobic to hydrophilic functional groups (Ellerbrock et al., 2005; Margenot et al., 2015).

pH was measured using a glass electrode in a 1:5 (volume fraction) suspension of soil in water (pH in H_2O) and in 1 mol/l potassium chloride solution (pH in KCl) according to the ISO (1039)0 (2005) standard. The available nutrients were measured by Mehlich III extraction as in Carter and Gregorich (2007).

Soil aggregate stability, measured as the proportion of water stable aggregates, was assessed using Kandeler's (1996) wet–sieving method and HERZOG laboratory equipment (Adolf Herzog GmbH, Vienna, AT). The sieving time was 5 min with 3 repetitions for each sample.

Glomalins were extracted from the soil as in Wright and Upadhyaya (1996), by autoclaving in neutral or alkaline citrate solution to yield easily extractable and total fractions. These were quantified according to Bradford's (1976) non-specific colorimetric assay.

Determination of the hydro–physical soil properties was initiated in summer 2018–2021 by collecting undisturbed samples to 7 cm soil depth in 100 cm^3 steel cylinders. Three samples were collected for each trial plot, so that 12 samples were taken for each treatment. Determinations of the following parameters followed Zbíral et al. (2011) and Pospíšilová et al. (2016); (1) bulk density (BD); (2) capillary porosity (CP); (3) non–capillary porosity (NP);(4) semi-capillary porosity (SP) and (5) the maximum content of non–gravitational soil water. The soil moisture reduced from full saturation after 30 min free drainage is θ30, and this denotes the amount of water held in the capillary and semi–capillary pores.

Water infiltration was measured after harvesting in the 2018–2021 summers. Saturated hydraulic conductivity (Kfs) was determined using the ponded infiltration method in 150 mm diameter cylinders (Bagarello et al., 2006) with 8 repetitions. The cylinders penetrated to 10 cm soil depth, and the soil moisture was recorded using the ML3 Theta Probe sensor in the surrounding soil (θ-sensor). The cylinder was then gently filled with 1000 ml of water in 20 s using the perforated bowl. The water volume corresponded to 56 mm precipitation, and the time required to soak the water was recorded. The soil moisture inside the cylinder was measured after complete water infiltration, and soil texture was assessed with parameter α set to 12. Finally, the hydro-physical soil properties and water infiltration were evaluated without "digestate I" treatment to circumvent additional time and labour intensity.

2.3. Geochemical and mineralogical analysis

Samples sieved under 2 mm underwent following analyses; (1) the cation exchange capacity (CEC) was determined by the barium chloride extract method ($BaCl_2$) under ISO (1353)6 (1995) standard procedure, where the concentration of the main exchangeable cations Na^+, K^+, Mg^{2+} and Ca^{2+} was analysed in solution by flame atomic absorption spectroscopy (AAS, Mg^{2+}, Ca^{2+}) and atomic emission spectroscopy (AAS, Na^+, K^+); (2) oxalate extraction of homogenised samples was performed according to Buurman et al. (1996).

The Fe, Al, Mn and Si concentrations in solution were measured by AAS and (3) semiquantitative mineralogical composition was studied by Xray diffraction analysis. Random powder mounts were prepared from bulk samples, sieved under 2 mm, milled by McCrone mill using the back side filling method (PMMA holder). X-ray diffraction analysis (XRD) was performed with Bruker D8 Advance diffractometer (CuKα, primary and secondary Soller slits 2.5°, detector Lynxeye XE) with automatic divergence slit (ADS, 10 mm).

X-ray diffraction patterns of random powder bulk-rock mounts were recorded from 4° to 80°2th, with 0.015°2th step and 0.8 s per step.

The minerals were identified using the ZDS-WX software (Ondruš, 2004), and PDF-2 database (McClune, 2004). The semiquantitative phase analysis was performed by the Rietveld method (Snyder and Bish, 1989), using the Topas 5. software (Coelho, 2014), with models of crystal structures from the ICSD (FIZ, 2020) database.

The samples sieved under 0.25 mm were boiled in sodium hexametaphosphate solution and the grain-size distribution was then determined by pipetting under ISO (1127)7 (1998) and ÖNORM L (, 1061, 1988) standard procedures.

The clay fraction under 2 μm was separated by sedimentation in distilled water (Tanner and Jackson, 1948). Samples were sonified in distilled water for 2 min, wet-sieved to less than 0.063 mm and sonified for 8 min (modified method by Balesdent et al., 1998). Oriented preparations were obtained by pipetting 2.5 ml of the sample suspension onto a Si-slides. The ethylene-glycol solvation was achieved by exposing of Si-slides with clay fraction to ethylene-glycol vapour for 8 h at 60 °C. The clay fractions were saturated with K^+ using five 24-hour saturation cycles with 1 M KCl, and excess salt was removed by washing in distilled water until there was a negative reaction with $AgNO_3$. Oriented preparations of K-saturated clay fraction were analysed by X-ray diffraction in the air dry state, heated for 5 h at 110 °C and 2 h at 330 °C.

X-ray diffraction analysis (XRD) was performed with Bruker D8 Advance diffractometer (CuKα, primary and secondary Soller slits 2.5°, detector Lynxeye XE) with automatic divergence slit (ADS, 10 mm). X-ray diffraction patterns of oriented preparations were recorded within range 3–50°2th with 0.019°2th step and 0.8 s per step. The XRD patterns were modelled by the Chevron ETC proprietary Sybilla Version 2.2.2 software (Aplin et al., 2006) developed by Drits and Sakharov (1976) and Drits and Tchoubar (1990). Finally, the individual mixed-layered minerals were identified, fitted as in Viennet et al. (2015) and Bakker

et al., (2018, 2019), and calculated to relative proportions of different phyllosilicate layer-types: S – smectite, I – illite, C – chlorite, K – kaolinite.

2.4. Statistical analyses

The basic statistical values comprised averages, standard deviations, and Pearson's correlation coefficients (R). These were calculated by Microsoft Excel (Microsoft Corporation, Redmund, WA, USA) and STATISTICA 14.0.0.15 software (TIBCO Software Inc., USA). The analysis of variance was from STATISTICA 14.0.0.15 software, and Scheffe's multiple comparison and Tukey HSD tests at $\alpha = 0.05$ were then employed to determine homogenous groups.

3. Results

3.1. Mineralogical characteristics

Clay minerals in unfertilised plots did not change over the long-term experiments compared to minerals in the fertilised plots (Bakker et al., 2018 and 2019). This highlights the minimum effect of agronomic practices on soil mineralogy, and the subtle variations in X-rays patterns indicate internal sample variability rather than systematic mineralogical differences. Therefore, the geochemical and mineralogical analyses were performed only on the 2018 control and the compost fertilised treatment, and these confirmed only non-significant differences in mineralogy (Tables S1, S2).

3.2. Soil Chemical Properties

Tables 3 and 4 show the soil characteristics in the different fertiliser treatments and sites. The pH/H_2O active soil reaction differed in the sites, with the highest pH in Hradec and the lowest in Lípa. Fertiliser treatments also varied, with lower 6.73 pH on the fertilised control and 6.8 on the LAV fertilised treatment. These were lower than the pH on the organic fertilised treatments, where the highest 7.26 pH was recorded for the compost fertilised treatment.

The statistically significant influence of compost application was observed in the content of C_{tot} (1.59%), N_{tot} (0.15%) and C_{hwl} (0.47 mgC g^{-1}), and addition of digestates to the soil increased the hot water extractable carbon content, but not total organic carbon and nitrogen (Table 3). The soil organic matter (SOM) components were affected in all sites and treatments (Table 4, S3). While aliphatic SOM components predominated in Hradec at 1.71, the 6.88 hydrophilic and 3.6 aromatic values predominated in Jaroměřice. In addition, the aliphatic, more labile SOM components significantly increased in the compost fertilised plots compared to other plots, but there was no difference in the hydrophilic components between treatments. Finally, the 1.9 decomposition index was significantly lower in the compost fertilised plots, and the hydrophobicity index was significantly higher at 0.33.

Table 3
The average values of soil chemical properties (mean ± standard error) in the experimental sites and different fertiliser treatments for 2018–2021. Different letters indicate significant differences between the means at $\alpha = 0.05$ in the given variables (Tukey HSD test).

Independent variable		pH (H_2O)	C_{tot}	N_{tot}	C_{hwl}
Site	Lípa	$6.47^a \pm 0.06$	$1.23^b \pm 0.03$	$0.11^a \pm 0.00$	$0.37^b \pm 0.01$
	Jaroměřice	$6.93^b \pm 0.04$	$1.35^c \pm 0.04$	$0.13^b \pm 0.00$	$0.39^c \pm 0.01$
	Hradec	$7.47^c \pm 0.04$	$1.07^a \pm 0.02$	$0.10^a \pm 0.00$	$0.35^a \pm 0.01$
Treatment	LAV	$6.73^a \pm 0.1$	$1.1^a \pm 0.02$	$0.11^a \pm 0.00$	$0.32^a \pm 0.01$
	Control	$6.80^{ab} \pm 0.09$	$1.09^a \pm 0.02$	$0.10^a \pm 0.00$	$0.34^a \pm 0.01$
	Digestate I	$6.95^{bc} \pm 0.09$	$1.13^a \pm 0.02$	$0.11^a \pm 0.00$	$0.36^b \pm 0.01$
	Digestate II	$7.05^c \pm 0.08$	$1.17^a \pm 0.02$	$0.11^a \pm 0.00$	$0.37^b \pm 0.00$
	Compost	$7.26^d \pm 0.06$	$1.59^b \pm 0.07$	$0.15^b \pm 0.01$	$0.47^c \pm 0.02$

C_{tot} – total organic carbon (%), N_{tot} – total organic nitrogen (%), C_{hwl} – hot water extractable carbon (mgC g^{-1}).

3.2.1. Total glomalin

The total glomalin values were significantly affected by site, treatment, year and their interactions (Table 5). The highest average glomalin value was 2.4 mg g^{-1} in Hradec nad Svitavou and the lowest 1.88 mg g^{-1} in Lípa. Table 6 and Fig. 1 highlight the significant differences in fertiliser treatments. The digestate fertilised plots had lower 1.61 and 1.73 mg g^{-1} total glomalin values compared to other treatments, and the compost fertilised plots had the highest average 3.22 mg g^{-1} value. Finally, there were significant differences in experimental years, with lower 1.68 mg g^{-1}average total glomalin in 2018 compared to 2.45 mg g^{-1} in 2019.

3.3. Soil physical and hydro-physical properties

3.3.1. Soil aggregate stability

Soil aggregate stability (% SAS) assessment was performed for 2018–21with 8 samples for each treatment. SAS was statistically significantly affected by treatments, site and year. There were also significant interactions between site and treatment, site and year and treatment and year (Table 5).

Table 7 highlights the average SAS values in the experimental sites, years and for different fertiliser treatments. The average 37.75% SAS value was significantly higher in Jaroměřice than in the other two sites, and the organic fertilised treatments differ from the unfertilised control and the LAV mineral fertilised treatment in all sites (Fig. 2).

The highest average 36.47% SAS value was recorded for the digestate II treatment compared to the lowest 26.22% for the control and 26.21% for LAV treatment. There was also significant difference in the experimental years, with the lowest 22.6% average SAS value in 2021 and the highest 37.6%. in 2018.

3.3.2. Soil bulk density and porosity

Soil bulk density was significantly influenced by site, treatment, year and their interactions (Table 5). There was difference between sites and treatments. The lowest 1.35 g cm^{-3} bulk density value was in Jaroměřice and the highest 1.49 g cm^{-3} in Lípa. Lower bulk density values were at treated plots, where both organic fertilised treatments recorded lower 1.39 g cm^{-3} than the control's 1.41 g cm^{-3}, and the LAV treatment recorded 1.42 g cm^{-3}. Bulk density was also significantly lower in 2019 than in the other sampling years (Table 7).

Table 5 shows that porosity percentage and distribution were significantly affected by site, treatment, year and interaction, and Table 7 highlights its distribution differences between sites. Hradec recorded a relatively high proportion of non-capillary pores, with 30.1% of total porosity and a low proportion of capillary pores at 50.5% of total porosity, while Lípa has a relatively low proportion of non-capillary pores with 18.5% of total porosity and a high proportion of capillary pores with 65.4%. The porosity distribution was also partly affected by treatment. Although differences in non-capillary pores were not statistically significant, there was a higher proportion of capillary pores on organic fertilised treatments (compost – 59%; diagestate II – 58,1%) than on the control (57%) and on the mineral fertilised treatment (57.2%). Meanwhile, site and treatment interaction indicated that Jaroměřice had the largest differences in pore distribution, and Lípa had the least differences between semi-capillary and non-capillary pores (Fig. 3). Finally, the porosity distribution differed in sampling years, and this was particularly noticeable in 2021 because it differed from other

Table 4
Components of soil organic matter (SOM) by intensity of FTIR spectra (mean ± standard error) in the experimental sites and fertiliser treatments for 2018–2021. Different letters indicate significant differences between the means at α = 0.05 in the given variables (Tukey HSD test).

Independent variable		SOM components			DI	HI
		aliphatic	aromatic	hydrophilic		
Site	Jaroměřice	$0.80^{a} \pm 0.04$	$3.60^{b} \pm 0.03$	$6.88^{c} \pm 0.08$	$5.45^{b} \pm 0.25$	$0.12^{a} \pm 0.01$
	Lípa	$1.48^{b} \pm 0.04$	$2.81^{a} \pm 0.05$	$5.56^{b} \pm 0.12$	$2.06^{a} \pm 0.08$	$0.27^{b} \pm 0.01$
	Hradec	$1.71^{c} \pm 0.04$	$2.70^{a} \pm 0.05$	$4.87^{a} \pm 0.10$	$1.71^{a} \pm 0.07$	$0.37^{c} \pm 0.01$
Treatment	LAV	$1.22^{a} \pm 0.06$	$3.10^{b} \pm 0.08$	$5.68^{a} \pm 0.16$	$3.59^{b} \pm 0.33$	$0.22^{a} \pm 0.02$
	Control	$1.27^{ab} \pm 0.06$	$3.04^{b} \pm 0.07$	$5.50^{a} \pm 0.16$	$2.93^{b} \pm 0.26$	$0.25^{a} \pm 0.02$
	Digestate I	$1.25^{ab} \pm 0.08$	$3.16^{b} \pm 0.09$	$5.86^{a} \pm 0.17$	$3.51^{b} \pm 0.37$	$0.23^{a} \pm 0.02$
	Digestate II	$1.28^{b} \pm 0.08$	$3.08^{b} \pm 0.08$	$5.93^{a} \pm 0.17$	$3.46^{b} \pm 0.40$	$0.24^{a} \pm 0.02$
	Compost	$1.74^{c} \pm 0.08$	$2.80^{a} \pm 0.08$	$5.88^{a} \pm 0.21$	$1.90^{a} \pm 0\ 15$	$0.33^{b} \pm 0.02$

DI–Decomposition index; HI–Hydrophobicity index.

Table 5
Significance of the effects of site, treatment, year and their interaction on followed soil properties revealed by multi- factorial ANOVA.

Dependent variable	Statistic	Site	Treatment	Year	Site x treatment	Site x year	Treatment x year
Total glomalin	**P**	0.0000	0.0000	0.0000	0.00000	0.00004	0.0000
	F – value	30.84	116.14	254.68	12.51	10.41	45.51
SAS	**P**	0.0000	0.0000	0.0000	0.00000	0.0000	0.00000
	F – value	756.72	303.07	687.84	11.6	210	4.63
Soil bulk density	**P**	0.0000	0.00064	0.0000	0.11 ns	0.00012	0.00242
	F – value	138.98	7.47	58.21	1.9	5.89	3.038
Porosity	**P**	0.0000	0.01061	0.0000	0.02098	0.00015	0.00209
	F – value	198.43	4.59	58.19	2.92	5.77	3.01
CP, SP, NP	**P**	0.0000	0.00000	0.0000	0.02979	0.00000	0.0000
	F – value	154.06	14.14	129.49	1.9	7.57	4.33
Water infiltration	**P**	0.0025	0.0967	0.0000	0.40737	0.00016	0.95086
	F – value	6.11	2.13	13.74	1.0286	5.8836	0.26922

SAS–soil aggregate stability, CP–capillary, SP–semi-capillary and NP–non-capillary pores.

Table 6
Average total glomalin (mean ± standard error, mg g^{-1}) in the experimental sites, years and different fertiliser treatments. Different letters indicate significant differences between the means at α = 0.05 for the given variables (Tukey HSD test).

Independent variable		**Glomalin (mg g^{-1})**
Site	Lípa	$1.88^{a} \pm 0.08$
	Jaroměřice	$2.18^{b} \pm 0.07$
	Hradec	$2.40^{c} \pm 0.13$
Treatment	LAV	$2.26^{c} \pm 0.1$
	control	$1.91^{b} \pm 0.09$
	Digestate I	$1.61^{a} \pm 0.05$
	Digestate II	$1.73^{ab} \pm 0.04$
	Compost	$3.22^{d} \pm 0.16$
Year	2018	$1.68^{a} \pm 0.05$
	2019	$2.45^{b} \pm 0.08$

years with high average capillary pore value and small average semi-capillary porosity.

3.3.3. Water infiltration

Water infiltration was significantly influenced by experimental site and year (Table 5). The most rapid infiltration was recorded in Hradec with 19.67 mm $hour^{-1}$ and the slowest in Lípa at 10.07 mm $hour^{-1}$. The results show higher infiltration in the organic fertilised treatments, but the variations are relatively high and the differences between treatments are therefore not significant (Table 7). The results are better interpreted by individual site depicted in Fig. 4. The sampling year also significantly affected infiltration, where very low 4.19 mm $hour^{-1}$ infiltration values were recorded for 2021. The infiltration rate therefore followed the annual trend noted in other soil physical properties.

3.4. Relationships between soil characteristics

Table 8 shows the correlation coefficients (r) for evaluated soil characteristics. The analysis revealed significant dependence of soil aggregate stability (SAS) on C_{tot}, N_{tot}, C_{hwl} and SOM components. The strength of the correlation between SAS and chemical properties varied between treatments, with the strongest dependence noted for digestate fertilised treatments.

There was a significantly strong − 0.54 negative correlation between SAS and soil bulk density, and strong 0.57 positive correlation between SAS and total porosity in all sites and treatments. The relationship between SAS and infiltration was also significant at 0.38, but this was less tight. Correlation coefficient values varied with treatment (Table 9).

There was correlation between glomalin and SAS only in Lípa, at 0.56, and correlation between treatments was unproven. Total glomalin was positively associated with $C_{tot,}$ at 0.44 and N_{tot} and C_{hwl} at 0.46, but glomalin association with pH was not proven (Table 10).

4. Discussion

4.1. Effects of local soil condition on chemical and physical properties

Significant differences between sites were determined for soil aggregate stability. This was due to mineralogical and geochemical soil properties, and Kraemer et al. (2019) support this by highlighting the importance of inherent soil characteristics such as mineralogy and soil texture.

The Jaroměřice site recorded the following: (1) the highest 37.75% soil aggregate stability in its silt loam soil; (2) approximately twice the clay fraction content of other sites at 24.5%; (3) the highest cation exchange capacity at 19 mmol 100 g^{-1}; (4) 13.6 mmol 100 g^{-1}exchangeable Ca^{2+} and (5) the pH values in most treatments were above 6.8 when exchangeable Ca^{2+} content was the most important factor in SOM stability (Rasmussen et al., 2018).

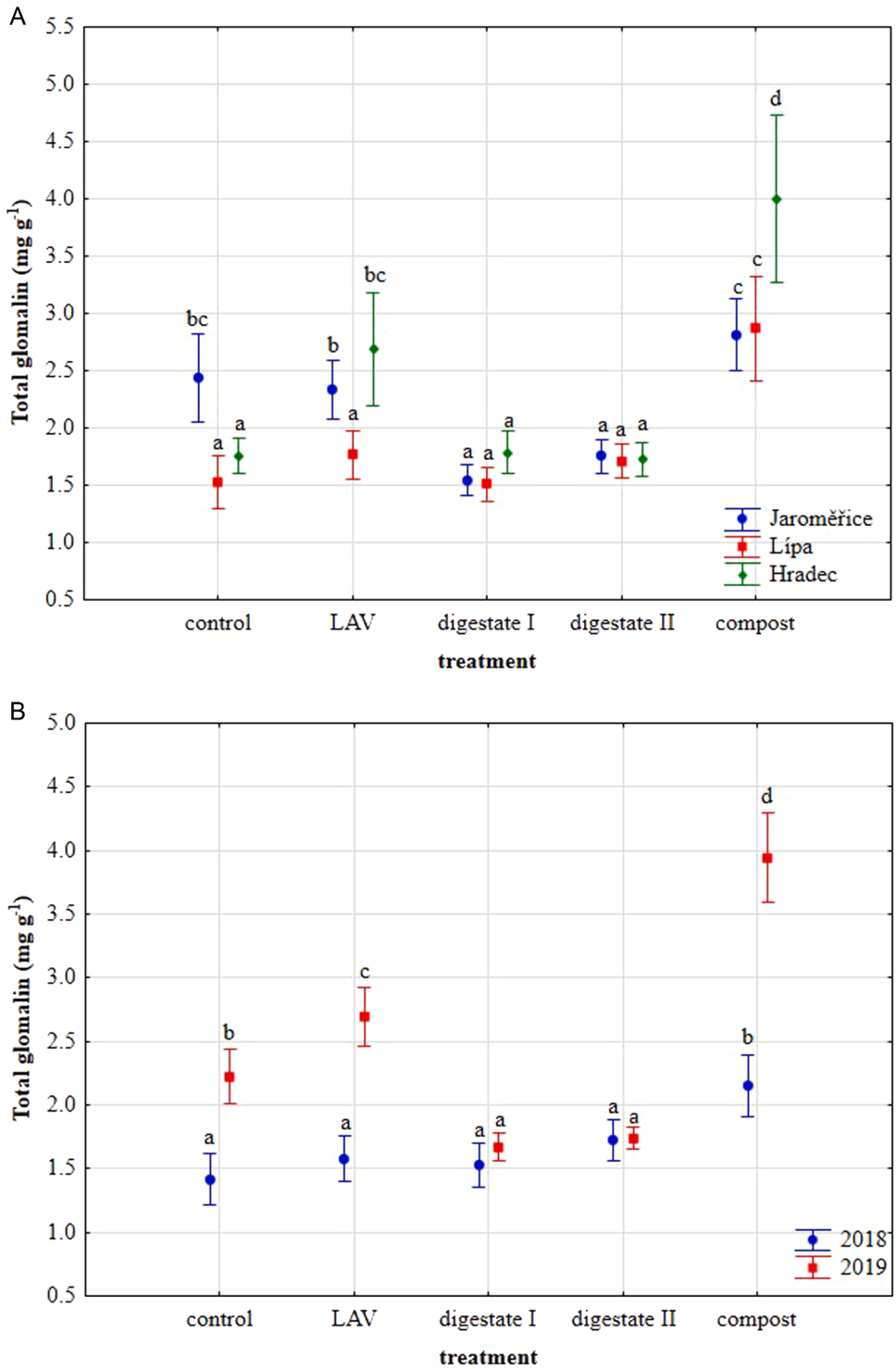

Fig. 1. The effects of treatment and site interaction (Fig. 1 A) and treatment and year interaction (Fig. 1B) on total glomalin. Different letters indicate significant differences at α = 0.05 by Tukey HSD test, and the vertical columns show 0.95 confidence interval.

In comparison, the Hradec site has the highest proportion of smectite layers in the clay fraction at 30 wt%. However, there was approximately 15 wt% clay-fraction with a high quartz content which caused the lowest 12.3 mmol 100 g^{-1} total cation-exchange capacity. The pH values were above 7 in all treatments. The SAS is affected especially by exchangeable Ca^{2+} content when pH is over 7 (Rasmussen et al., 2018), and this site recorded 10.9 mmol 100 g^{-1} exchangeable Ca^{2+} content.

Rasmussen et al. (2018) also reported that the effect of short-range-ordered (SRO) minerals prevails over Ca^{2+} effect between pH 5.5 and 6.5. Here, the Lípa site recorded the lowest 6.4 average pH and had low 28% average SAS. This was despite the high 5.415 ppm Fe_{ox}-SRO phases and organically complexed Fe, low 12.3 mmol 100 g^{-1} cation exchange capacity and 11 mmol 100 g^{-1} exchangeable Ca^{2+} content. In addition, Muggler et al. (1999) consider that the Fe_{ox} effect on aggregation depends on Fe-oxyhydroxide micro-morphology. Regelink et al. (2015) added that the soil micro-porosity and micro + meso-porosity increase with increasing aggregate abundance, and this correlation is significant for the water stable aggregate fraction. Finally,

Table 7
Soil physical and hydro-physical properties (mean ± standard error) in the experimental sites, years and different fertiliser treatments. Different letters indicate significant differences between the means at α = 0.05 for the given variables (Tukey HSD test and Scheffe test for infiltration).

Independent variable		Soil aggregate stability (%)	Soil bulk density (g cm^{-3})	Capillary pores (%)	Semi capillary pores (%)	Non-capillary pores (%)	Infiltration (mm hour^{-1})
Site	**Jaroměřice**	$37.75^{b} \pm 0.43$	$1.34^{a} \pm 0.01$	$29.06^{c} \pm 0.25$	$8.07^{b} \pm 0.13$	$12.84^{b} \pm 0.43$	$12.80^{a} \pm 1.8$
	Hradec	$27.51^{a} \pm 0.43$	$1.38^{b} \pm 0.01$	$23.72^{a} \pm 0.2$	$9.11^{c} \pm 0.22$	$14.15^{c} \pm 0.37$	$19.67^{b} \pm 1.55$
	Lípa	$28.0^{a} \pm 0.42$	$1.49^{c} \pm 0.01$	$28.10^{b} \pm 0.19$	$6.90^{a} \pm 0.14$	$7.97^{a} \pm 0.28$	$10.07^{a} \pm 1.47$
Treatment	**Control**	$26.22^{a} \pm 0.51$	$1.41^{bc} \pm 0.01$	$26.47^{a} \pm 0.28$	$8.05^{ab} \pm 0.23$	$11.19^{a} \pm 0.61$	$10.35^{a} \pm 1.65$
	LAV	$26.1^{a} \pm 0.53$	$1.42^{c} \pm 0.01$	$26.32^{a} \pm 0.32$	$8.41^{b} \pm 0.22$	$11.22^{a} \pm 0.42$	$13.33^{a} \pm 2.13$
	Digestate I	$32.35^{b} \pm 0.6$	n.d.	n.d.	n.d.	n.d.	n.d
	Digestate II	$36.47^{d} \pm 0.63$	$1.39^{a} \pm 0.01$	$27.32^{b} \pm 0.4$	$7.81^{a} \pm 0.22$	$11.86^{a} \pm 0.61$	$15.11^{a} \pm 2.77$
	Compost	$34.64^{c} \pm 0.6$	$1.39^{a} \pm 0{,}01$	$27.75^{b} \pm 0.29$	$7.80^{a} \pm 0.18$	$11.69^{a} \pm 0.49$	$18.22^{a} \pm 2.74$
Year	**2018**	$37.6^{d} \pm 0.41$	$1.41^{b} \pm 0.01$	$25.14^{a} \pm 0.34$	$8.67^{c} \pm 0.29$	$12.70^{b} \pm 0.46$	$18.3^{bc} \pm 2.4$
	2019	$30.36^{b} \pm 0.7$	$1.36^{a} \pm 0.01$	$25.05^{a} \pm 0.24$	$7.70^{b} \pm 0.16$	$15.64^{b} \pm 0.54$	$12.04^{ab} \pm 1.71$
	2020	$33.76^{c} \pm 0.33$	$1.4^{b} \pm 0.01$	$27.78^{b} \pm 0.28$	$9.71^{d} \pm 0.15$	$9.23^{b} \pm 0.31$	$23.28^{c} \pm 3.34$
	2021	$22.6^{a} \pm 0.41$	$1.45^{c} \pm 0.01$	$29.32^{c} \pm 0.27$	$6.27^{a} \pm 0.13$	$9.31^{b} \pm 0.33$	$4.19^{a} \pm 0.75$

Lado and Ben-Hur (2004) reported that a certain proportion of smectite layers in the clay fraction helps to disperse and reduce aggregates.

Change in pH caused by fertiliser application depends on site soil type. The addition of both organic fertilisers, compost and digestate, significantly increased pH in Lípa's sandy loam soil compared to the unfertilised control. In contrast, digestate addition increased pH only slightly, or even decreased it, in the other two sites with their silt loam soil (Table 11). This is supported by Voelkner et al.'s (2015) explanation that the digestate effect on pH depends on soil type and physical-chemical properties. While digestate application acidified pH in loamy soil from initially higher pH values, its application in acid sandy loam caused alkalinity. The initial acidification processes in the loamy soil are considered to be associated with the transition of organic acids to the soil matrix. These are developed in the digestate during anaerobic fermentation and microbial oxidation of the applied digestate organic matter. In contrast, the ammonification effect predominated in sandy soils. Large amounts of NH_4^+-N contained in the organic wastes then caused hydroxyl ion production in ammonification, and this increased pH. A further influential factor is that the digestate applied herein was strongly alkaline.

4.2. Fertiliser effects on soil mineralogical, chemical, physical and hydro-physical characteristics

4.2.1. Mineralogical properties

Mineralogy of the smectite, illite, chlorite and kaolinite layers in the clay fraction was very similar in the Jaroměřice and Lípa areas. The small changes were due to statistically non-significant inhomogeneity. In contrast, the Hradec site had a higher proportion of illite layers and less smectite layers in the compost fertilised plots than in the control. Finally, the overall low clay content and high clay quartz content in individual phases and the proportion of phyllosilicate layers are due to sample inhomogeneity.

4.2.2. Soil organic matter quality and quantity

Zhou et al. (2020) recorded that soil organic carbon influences aggregate stability and soil structure, and its stability differs in different size aggregates. In addition, the organic carbon in micro-aggregates is less susceptible to change than organic carbon in macro-aggregates. Our study highlighted that soil 1–2 mm aggregate stability was influenced by soil carbon content and the composition of organic matter bound to the aggregate surface.

The soil organic matter (SOM) consists of amphiphilic compounds that have both hydrophilic and hydrophobic properties (Milanovskiy et al., 2013), and their ratio determines soil solubility, spatial organisation and diverse functional properties. Urbanek et al. (2007) studied the distribution of functional carbon groups in soil aggregates and they found that the content of C═O hydrophilic groups was three times greater than the C–H hydrophobic groups. The authors added that more hydrophilic groups were present in the exterior of the aggregates than in their interior.

Herein, we established that hydrophobic SOM components correlated negatively with soil aggregation, and the aromatic and the hydrophilic components correlated positively with its aggregation. Therefore, the assumption that higher hydrophobic content in SOM repelled more water, and thus increased aggregate stability, does not always hold. It is actually the distribution of hydrophilic and hydrophobic groups and their mutual orientation in the soil aggregates that determine the ratio of soil water-repellence and wettability in the organic matter.

Work by Voelkner et al. (2015) supports our finding that differing sensitivity to digestate depends on soil texture. For example, digestate application to Lípa sandy-loam soil caused increased hydrophobic SOM components and hydrophobicity index compared to the untreated control. In contrast, there was a decrease, or a small increase, in hydrophobicity in the Hradec and Jaroměřice silt loam soil following digestate application (Table S3). These differences are explained by the sand particles' smaller surface area, and this smaller area is covered more rapidly by the amphiphilic organic molecules in the applied digestate (Voelkner et al., 2015).

Hydrophobicity results in reduced water infiltration to the soil. This effect is apparent in Lípa's sandy loam soil, where we found small difference in infiltration rate between the organic fertilised plots and the control (Fig. 4). However, the Hradec site recorded the highest infiltration rate despite its naturally higher content of hydrophobic SOM components. In addition, the high infiltration rate at the Hradec area is also influenced by lower bulk density and the higher proportion of non-capillary pores.

The distribution of the hydrophilic and hydrophobic SOM components in the soil aggregate is especially important when soil moisture decreases below the critical water level. (Graber et al., 2009). For example, in periods of drought, the organic components are rearranged, so that the hydrophilic polar heads are attached to the soil mineral surface and the hydrophobic components increase to fill the pore spaces (Doerr and Thomas, 2000).

This is one cause of the seasonal variability in soil aggregation observed in our experiment. In addition, aggregate stability correlates with the soil's ability to repel water, and this correlation increases following dry periods (Kraemer et al., 2019). Therefore, the site's soil moisture and wet/dry history must be known when assessing soil aggregate stability. In our work, the average soil aggregation stability at our experimental sites reached the highest value in 2018. This is because the longest drought period during our experiment was recorded in 2018 (Table S4). In addition, our digestate applications increased aggregation compared to the control in that year. The percentage increases in aggregation were 19.3% for digestate I and 35% for digestate II application (Fig. 2).

The benefits for soil quality and organic C content from applying

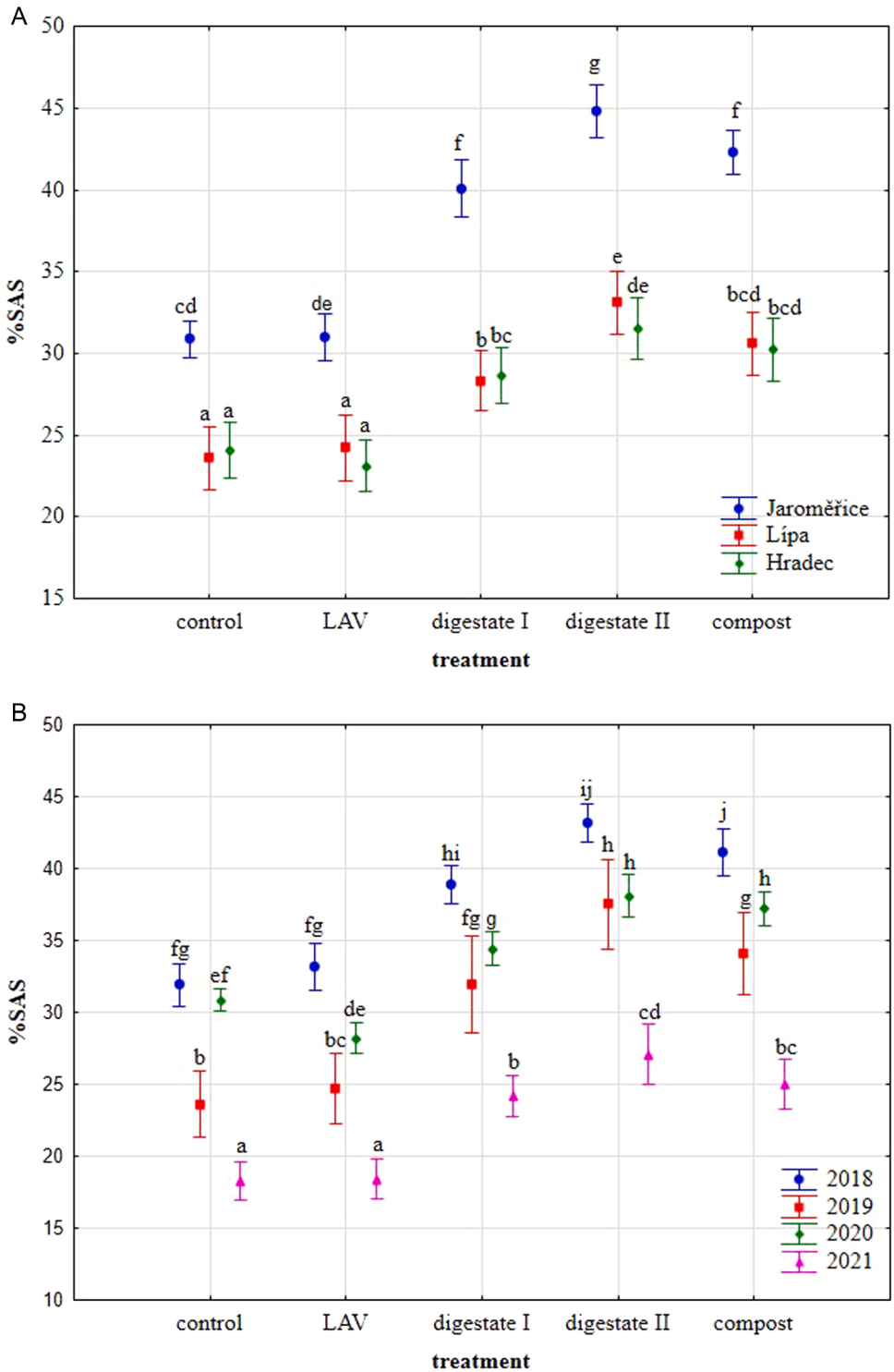

Fig. 2. The effect of treatment and site interaction (Fig. 2 A) and treatment and year interaction (Fig. 2B) on soil aggregate stability (% SAS). Different letters indicate significant differences at α = 0.05 by Tukey HSD test, and the vertical columns show 0.95 confidence interval.

organic materials (farmyard manures, composts, cattle or pig slurries and digestates) depends on the C and N content in organic material, dry weight, the C/N ratio and total organic-matter-loading (Bhogal et al., 2018). Both compost and digestate addition increased total C content (C_{tot}) compared to the unfertilised treatment in our experiment, but the compost increased C_{tot} 38% more than digestate. However, there was a slight increase by 4.5% in the total and soluble C content after digestate treatment compared to the unfertilised and mineral N fertilised soils.

Our previous results recorded that the effect of digestate with 6.2% dry weight in the liquid phase and 6.1 nitrogen content in the dry weight is close to those of cattle slurry, and it does not significantly improve the organic matter level in the soil (Šimon et al., 2015). Organic matter from other sources must be added if further organic carbon increase is needed in the soil.

This is especially true for crop production without livestock farming, and Tiwari et al. (2000) proposed adding cereal straw to maintain adequate organic carbon in the soil during long-term digestate application. However, Barłóg et al. (2020) failed to confirm any positive

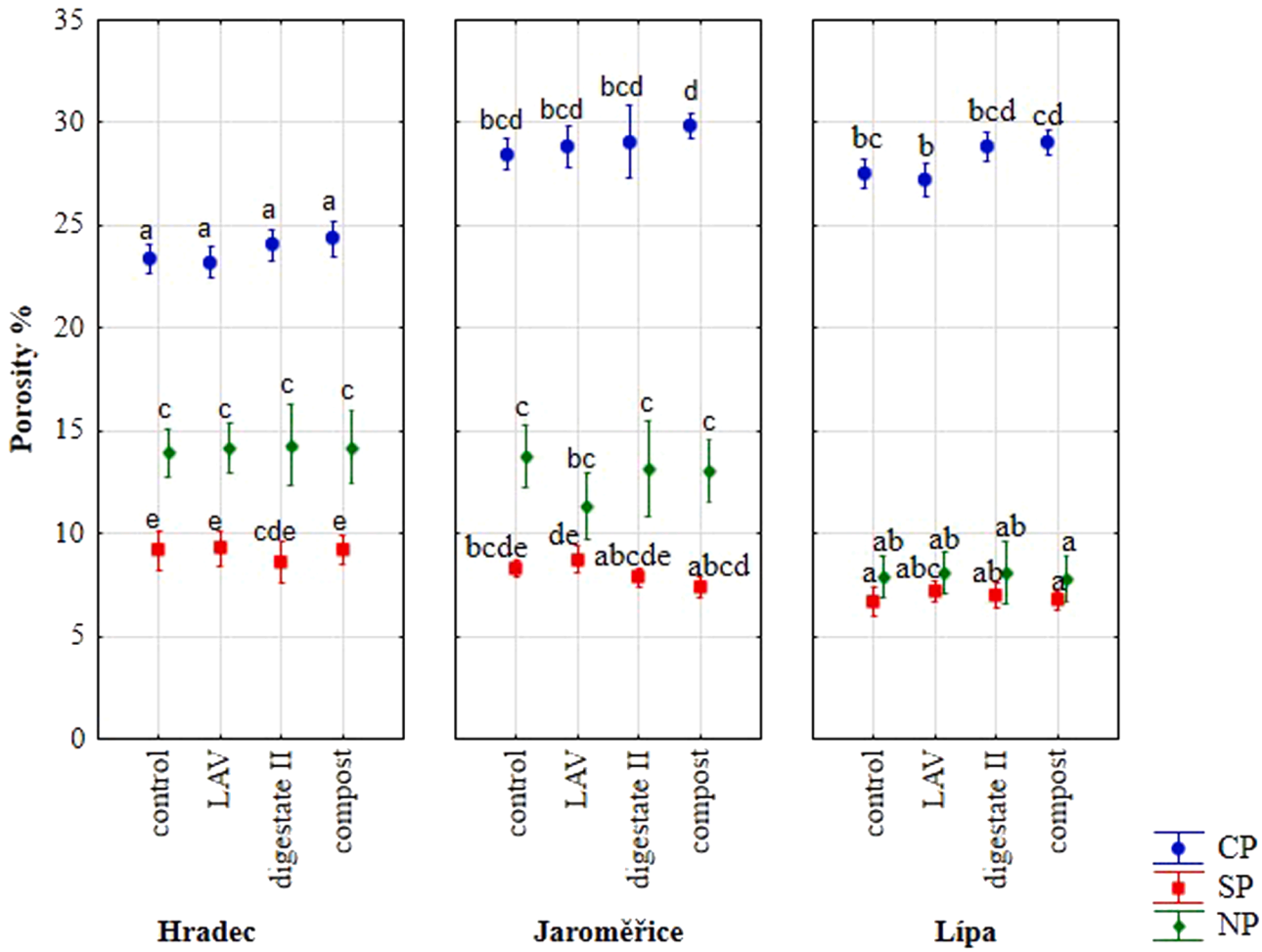

Fig. 3. The influence of treatment and experimental site interaction on pore distribution in the three pore types (CP–capillary, SP–semi-capillary and NP–non–capillary). The different letters indicate significant differences at $\alpha = 0.05$ by Scheffe's test for CP, SP and NP, and the vertical columns indicate 0.95 confidence interval.

result from the application of digestate and straw on soil organic carbon content. Therefore, farmyard manure and compost remain the best organic sources for maintaining or increasing the soil carbon level.

4.2.3. Total glomalin

Glomalins are glycoproteins produced especially in arbuscular mycorrhiza fungi mycelium (AMF), and some authors consider that these are particularly important in soil aggregation (Rillig, 2004). Wuest et al. (2005) add that there is strong correlation between glomalin and C_{tot} and N_{tot} in intensively tilled silt-loam soil. The phosphorus availability (P) and pH are the most important abiotic determinants of AFM colonisation and abundance (Davison et al., 2021; Konvalinková et al., 2017). However, the critical 11–30.7 mg kg^{-1} P-availability inhibits AMF colonisation, depending on the plant's developmental stage (Deng et al., 2017). Davison et al. (2021) also proved optimum niche pH 4–6 depending on the individual AMF taxa.

Some glomalin content was detected in our experiment. This was despite the unfavourable pH > 6 and P-available content above 40 mg kg^{-1} conditions for AMF colonisation. In addition, we had significantly different glomalin results in fertiliser treatments only in 2019 (Fig. 1). The most glomalin was found on plots fertilised by compost, and this result is supported in other authors' work. Yang et al. (2018) established that compost addition stimulated the host plant's AMF growth, spore density and root colonisation. This compost addition, however, did not affect the AMF community composition or richness.

Although digestate fertiliser adversely influenced AMF, the highest P-available content found on compost-fertilised plots had no detrimental effects. This can be attributed to the gradual P release from compost, as reported by Joner (2000) for farm-yard manure use. Finally, Susana Grigera et al. (2007) reported that maize cultivation increased the carbon allocation to AMF in the plant's reproductive stages, and the AMF facilitated P uptake in these highly productive crops. These processes most likely contributed to the higher glomalin content recorded in our 2019 maize cultivation compared to the content found in the 2018 winter wheat cultivation.

4.2.4. Physical properties

Soil bulk density (BD) and porosity are accepted physical indicators of soil compaction, and changes in these soil properties can influence crop yield. The normal bulk density ranges from 1.0 to 1.6 mg m^{-3} for clay soils and 1.2–1.8 mg m^{-3} for sandy soils, and those with a high proportion of solids-to-pore-space have higher BD. Critical BD can begin at 1.3 mg m^{-3}, depending on the soil textural class (Reichert et al., 2009) and a potential reduction in plant root growth occurs at those values.

The addition of organic digestate and compost fertilisers significantly decreased our soil bulk density and increased porosity compared to the control and mineral fertilised treatment. For example, there was greater obvious improvement from organic fertiliser use in Lípa's sandy-soil physical properties than in the Hradec and Jaroměřice silt loams (Fig, 3). Rivenshield and Bassuk (2007) support this in their conclusion that organic fertiliser treatment induced changes in soil BD and porosity.

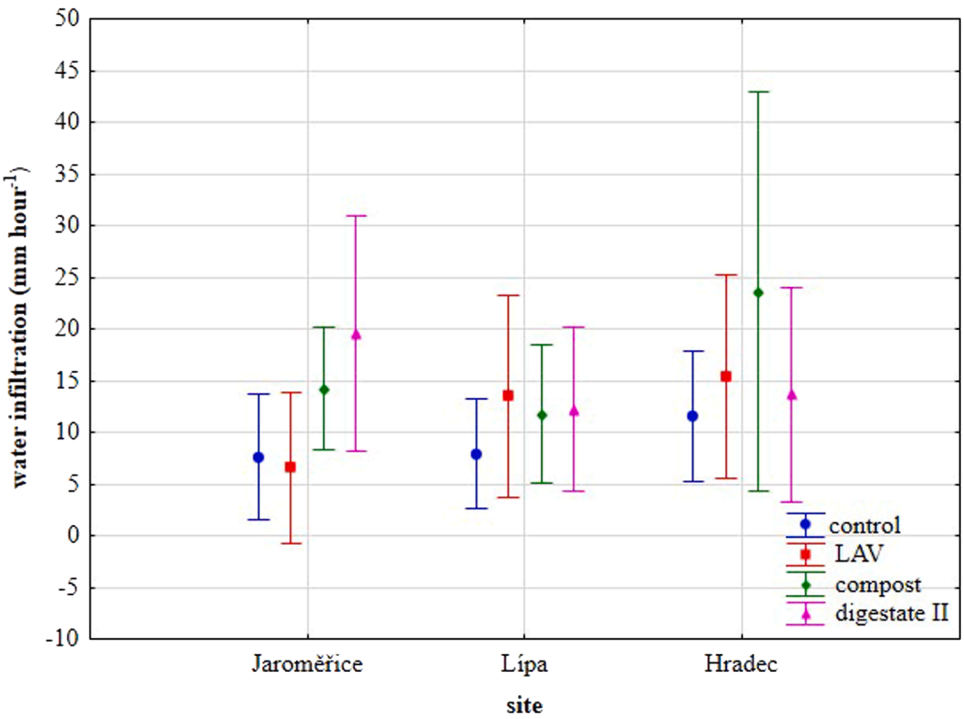

Fig. 4. Effect of treatment and site interaction on water infiltration in mm hour^{-1}. The vertical columns indicate 0.95 confidence interval. Differences are not significant ($p = 0.407$).

Table 8
Chemical soil properties and soil aggregate stability relationships for 2018–2021 (correlation coefficients *r*). Statistical significance at $p < 0.05$ is in bold.

Variable	pH (H_2O)	N_{tot}	C_{tot}	C_{hwl}	aliphatic	aromatic	hydrophylic	DI	HI
N_{tot}	0.14								
C_{tot}	0.11	**0.98**							
C_{hwl}	**0.21**	**0.82**	**0.87**						
Aliphatic	0.12	-0.01	0.09	0.09					
Aromatic	-0.08	**0.29**	0.16	0.01	**-0.50**				
Hydrophylic	-0.07	**0.55**	**0.47**	**0.24**	**-0.33**	**0.88**			
DI	0.10	-0.07	-0.15	-0.02	**-0.77**	**0.35**	**0.18**		
HI	0.12	**-0.21**	-0.11	-0.04	**0.90**	**-0.72**	**-0.633**	**-0.67**	
SAS	-0.04	**0.45**	**0.37**	**0.36**	**-0.38**	**0.41**	**0.45**	**0.31**	**-0.39**

SAS – soil aggregate stability; C_{tot} – total organic carbon; N_{tot} – total organic nitrogen; C_{hwl} – hot water extractable carbon, DI–Decomposition index; HI–Hydrophobicity index.

Table 9
Correlation coefficients (r) for soil aggregate stability and hydro-physical soil properties in sites and fertiliser treatments for 2018–2021. Statistical significance at $p < 0.05$ is in bold.

Independent variable		Soil bulk density	Porosity	CP	SP	NP	Infiltration
All groups		**-0.54**	**0.57**	0.13	**0.34**	0.19	**0.38**
Site	**Jaroměřice**	**-0.61**	**0.56**	-0.28	0.05	**0.5**	**0.62**
	Hradec	**-0.42**	**0.44**	0.34	**0.57**	0.1	**0.47**
	Lípa	**-0.56**	**0.54**	-0.08	**0.55**	0.17	**0.60**
Treatment	**Control**	**-0.59**	**0.67**	0.04	0.26	0.24	**0.48**
	LAV	**-0.51**	**0.61**	0.20	0.12	0.13	0.32
	Digestate II	**-0.49**	**0.56**	0.08	**0.71**	0.21	**0.67**
	Compost	-0.43	**0.48**	0.21	**0.78**	-0.09	0.15

CP–capillary, SP–semi-capillary and NP–non-capillary pores.

Their results suggest that treatments on clay loam soil may not produce as much change in physical soil properties as on sandy loam soil, and greater organic fertiliser amounts are therefore required to improve clay loam soil.

Pastorelli et al. (2021) reported that digestate had no influence on soil bulk density. That finding was strongly disputed, because the experiments lasted only three years and the soil tillage management had contrasting effects on aggregate formation and stability. Bhogal et al. (2018) found that soil bulk density increased after short-term application of organic materials with low dry matter content, such as food-based digestate and livestock slurry. They had also applied livestock slurry for 20 years and found noticeable increases in the soil's organic carbon and biological and physical attributes. Finally, they suggested that repeated long-term digestate application should have the

Table 10
Correlation between total glomalin and soil aggregate stability (SAS) and chemical properties (correlation coefficients *r*) for 2018 and 2019. Statistical significance at $p < 0.05$ is in bold.

Independent variable		SAS	pH (H_2O)	C_{tot}	N_{tot}	C_{hwl}	aliph.	arom.	hydr.	DI	HI
All groups		**0.32**	0.31	**0.44**	**0.46**	**0.46**	**0.27**	-0.06	0.19	-0.06	0.25
Site	Jaroměřice	0.03	0.2	0.4	0.41	0.42	0.43	-0.35	0.14	-0.41	0.43
	Hradec	0.21	0.17	**0.83**	**0.76**	**0.80**	0.43	-0.38	0.33	-0.14	0.28
	Lípa	**0.56**	0.36	0.52	**0.53**	**0.39**	0.23	**-0.64**	0.19	-0.06	0.22
Treatment	Control	0.23	0.54	0.41	0.47	-0.15	-0.06	0.06	0.06	0.20	-0.07
	LAV	0.24	-0.27	**0.61**	**0.62**	0.20	-0.35	0.33	0.32	0.25	-0.44
	Digestate I	-0.38	-0.08	0.04	0.09	0.34	-0.28	0.05	-0.06	0.34	-0.37
	Digestate II	0.06	-0.26	0.37	0.30	0.54	0.43	0.05	0.13	-0.29	0.46
	Compost	0.28	0.39	-0.25	-0.18	-0.09	-0.02	0.26	-0.08	-0.03	-0.02

C_{tot} – total organic carbon, N_{tot} – total organic nitrogen, C_{hwl} – hot water extractable carbon, aliph. – aliphatic, arom.– aromatic and hydr. –hydrophilic SOM components, DI–Decomposition index, HI–Hydrophobicity index

Table 11
Impact of fertilisers on pH in the experimental sites (mean ± standard error). Different letters indicate significant differences between means at $\alpha = 0.05$ for the given variables (Tukey HSD test).

Site	Treatment	pH (H_2O)
Jaroměřice	Control	$6.88^{cd} \pm 0.05$
	LAV	$6.66^{cd} \pm 0.07$
	Digestate I	$6.79^{cd} \pm 0.11$
	Digestate II	$6.99^{de} \pm 0.05$
	Compost	$7.32^{ef} \pm 0.04$
Lípa	Control	$6.17^{ab} \pm 0.09$
	LAV	$6.12^{a} \pm 0.07$
	Digestate I	$6.52^{abc} \pm 0.1$
	Digestate II	$6.57^{bc} \pm 0.09$
	Compost	$7.00^{de} \pm 0\ 08$
Hradec	control	$7.36^{ef} \pm 0.1$
	LAV	$7.42^{f} \pm 0.11$
	Digestate I	$7.53^{f} \pm 0.06$
	Digestate II	$7.58^{f} \pm 0.05$
	Compost	$7.45^{f} \pm 0.15$

same effect, and this is confirmed by our long-term experiment with digestate application which began in 2011. Garg et al. (2005) also reported reduced bulk density and increased soil moisture retention following their short-term application of biogas slurry on sandy loam soil.

The application of organic fertilisers had some influence on pore-distribution, with more capillary pores at the expense of semi capillary pores. Rivier et al. (2022) confirmed that compost addition to sandy and loam soils affected pore-size distribution, increased water holding capacity and reduced bulk density in their pot-plant experiments. They also observed a significant increase in the stability of 1–2 mm aggregates when they applied organic fertiliser to sandy soil. Finally, digestate treatment generated the greatest aggregate stability increase up to 9%, and vermicomposted treatment only provided 4% extra. In contrast to our experiment, they did not find statistically significant differences in macro-aggregate stability between the fertilised treatments and controls in the loamy soil, except for the digestate treatment which had the lowest stability of all.

However, our experiment established increased soil aggregate stability compared to controls for organic fertilisers on both sandy and silt loam soil texture. The addition of digestate I increased aggregation by an average 23%, and digestate II increased it by 38%. The greatest increase was recorded in the Jaroměřice silt loam soil, and this site also had the greatest aggregate increase of 37% in compost fertilised plots (Fig. 5 A). This improved aggregate stability following organic fertiliser treatment is attributed to increased $C_{tot,}$ N_{tot} and C_{hwl} content. In addition, some of the studies cited in Abiven et al.'s review confirmed strong correlation between aggregate stability and the organic product's decomposable fraction (Abiven et al., 2009). This indicates that the increased aggregate stability was related to microbial activity, and it corresponds with our previous work where there was increased eubacterial abundance (Řezáčová et al., 2021).

Pastorelli at al. (2021) reported the positive effect of digestate treatment on soil aggregate stability soon after digestate application in the first experimental years. The differences between digestate fertilised treatments and the urea fertilised control were not significant after two years, but this contrasts sharply with our findings. The soil aggregate stability in our experiment increased significantly on digestate and compost fertilised plots compared to the control. This was evident even in 2020 when no fertiliser was applied to our spring barley. The effect of long-term organic fertiliser therefore persisted for at least a year.

Frøseth et al. (2014) also highlighted the increased benefits of herbage-based digestate for aggregate stability over mulched herbage effects. Moreover, the use of digestate from field residues provides greater benefits than those from mulching, and this promotes soil aggregation and limits nitrous oxide emission and the risk of N leaching (Möller and Müller, 2012).

Long-term mineral fertiliser use had minimum effect on soil physical properties at our three experimental sites in our experiment. The bulk density, porosity and aggregate stability values were close to control values. This is consistent with other works (Zhang et al., 2006; Zhou et al., 2016). In addition, LAV fertiliser application in the Hradec site reduced aggregates slightly by 4% compared to controls. This result is supported by Zhou et al. (2016) and Blanco-Canqui et al. (2014). These latter authors also considered that it was essential not to exceed 80 kg ha^{-1} nitrogen fertiliser, and thus avoid deteriorated soil aggregation. but Marinari et al. (2000) gained improved soil porosity with short-term NH_4NO_3 mineral fertiliser. This increased both regular and irregular pores, and primed the native soil organic matter.

4.2.5. *Water infiltration*

The positive organic fertiliser effects on water infiltration vary according to site and year. This was especially noted in the organic-fertilised Jaroměřice silt loam soil, with significant 156% improvement with digestate use, and also 87% extra compost (Fig. 5B).

The infiltration also strongly correlated with aggregate stability. These high values are attributed to more rapid organic matter decomposition and penetration to the soil in Jaroměřice's warmer climate than in our other sites, where colder weather induced lower values than the controls. Finally, although Liu et al. (2018) failed to find any significant effect on water infiltration in their long-term mineral nitrogen use in their Fluvo-aquic soil, we recorded 70% infiltration increase in our Lípa sandy-loam soil.

The plots at our Hradec location had the lowest 18% increase in water infiltration after digestate application compared to the control value. This may be due to either the area's higher proportion of smectite layers in the phyllosilicates of the clay fraction, or the highest initial infiltration at this location. This initial infiltration value is most likely due to Hradec's lowest C_{tot} and Feox+Alox contents and small clay fraction. The clay fraction there also contains the highest proportion of

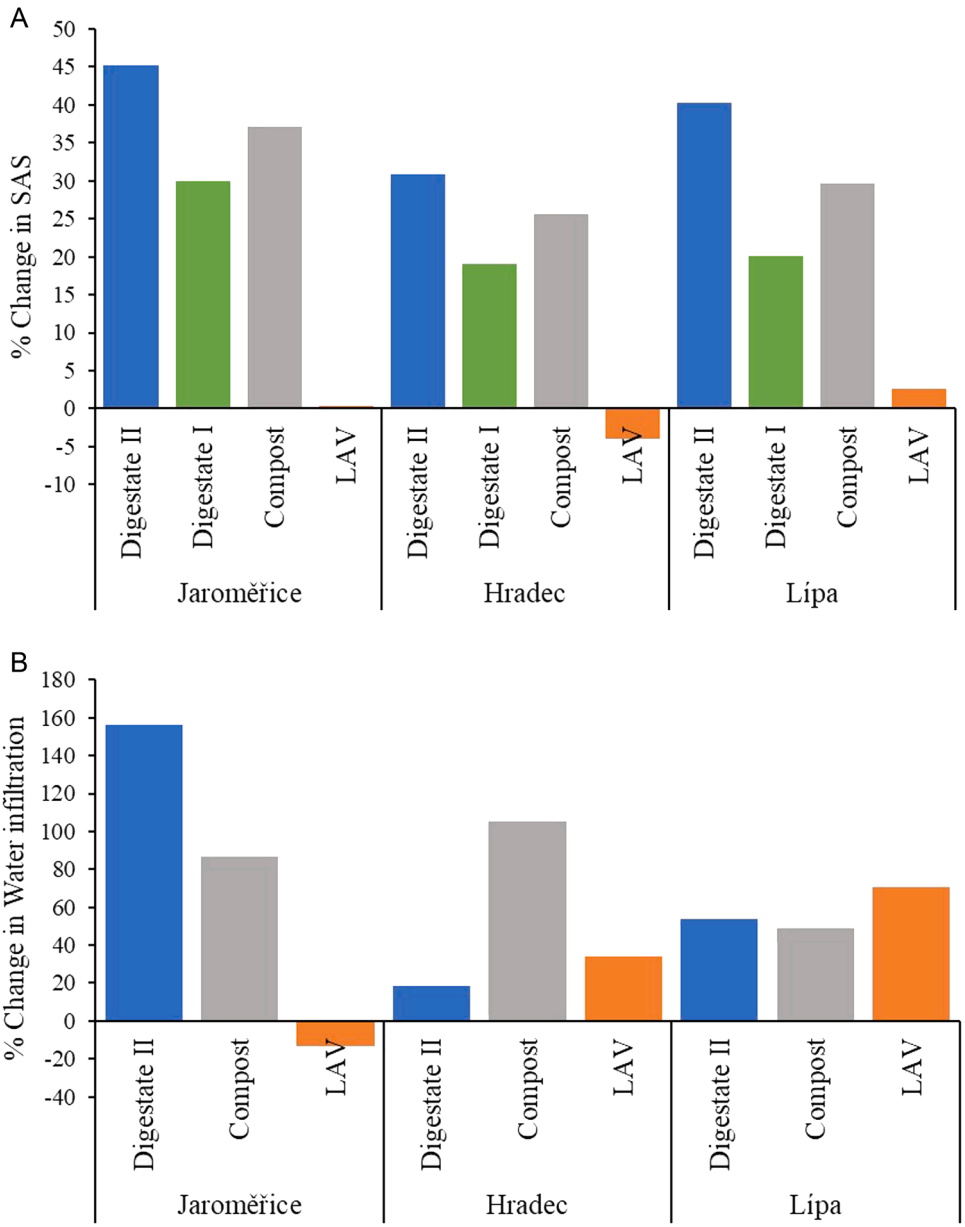

Fig. 5. Changes compare to control in soil aggregate stability (SAS), (Fig. 5 A) and water infiltration (Fig. 5B) at the three experimental sites after repeated treatment with organic materials and LAV over10 years.

quartz.

In addition, Lado and Ben-Hur (2004) consider that smectite's effect on soil aggregation is most likely increased by colloidal particle dispersion from digestate, with consequent lower infiltration. The authors attribute the decreased infiltration to these dispersion colloids, and Unterfrauner et al. (2010) agreed in their work on Austrian loam-sand cambisol. In contrast, Garg et al. (2005) and Singh et al. (2007) registered the positive digestate effect of 27% higher water infiltration on unfertilised loamy sand soils with approximate 7.7 pH, and Singh et al. (2007) recorded greater aggregate diameter in fertilised plants, and consequent positive effect on water infiltration.

In summary, we confirmed significant seasonal variability in soil physical properties, including soil bulk density, porosity and aggregate stability. These results are supported in the studies by Zhang et al. (2006) and Wuest et al. (2005). Measurements of water infiltration exhibited significant temporal variability between years, with infiltration decreased at higher soil moisture (Table S5). The relationship between infiltration and soil moisture content was confirmed by the work of Wei et al. (2022). Moreover, the different time since the last soil cultivation for cultivated crops must be considered. The soil was without cultivation for 11 months in 2021, when winter rape was cultivated. Soil was than quite compacted and it was reflected in a higher bulk density (Table 7). On the contrary, spring barley was grown in 2020 and soil was without cultivation only 5 months. Finally, existing local field conditions can have significant impacts on water infiltration, and this variability must be considered when fertiliser effects on soil physical properties are assessed. The knowledge that organic fertiliser effects alter with seasonal weather in different sites is one of the most important of these variations that must be considered.

5. Conclusion

The organic fertilisers have minimum effect on soil mineralogical characteristics.

The long-term application of compost and digestate organic fertilisers positively affected the physical soil properties on sandy loam and

silt loam soils. However, the effect on organic matter varied with fertiliser type. Herein, compost application significantly increased labile and total organic C and N_{tot} and simultaneously raised soil hydrophobicity index compared to digestate application. Although digestate treatment was less effective than compost on soil organic matter, its long-term application increased aggregate stability and thus water infiltration. Our results highlight that achieving the potential of digestate use requires initial assessment of soil and climate conditions, tillage systems and individual requirements of the cultivated crop.

In conclusion, our results are especially important for development of the agricultural biogas sector and increase in the amount of produced digestate. Digestate has great potential to substitute for both mineral and organic fertilisers in maintaining soil fertility, and this is essential because of the limited resources of mineral fertilisers and the lack of organic fertilisers such as farmyard manure. Finally, digestate use is also appropriate to maintain environmental sustainability.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.still.2023.105715.

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