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Relationships between selected phenolic compounds and microbial abundance in grassland soils in the Obra River valley: a preliminary study

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Abstract

The aim of the study was to evaluate the relationships between selected phenolic compounds and microbial abundance in grassland soils. The objects of the study were topsoils (0–20 cm) from under grasslands located along the Obra River (Wielkopolska Lowland, central Poland). The field survey was conducted in September 2022. Five vegetation syntaxonomic units were selected in the study area*: Molinietum caeruleae, Alopecuretum pratensis, Arrhenatheretum elatioris, Lolio-Cynosuretum,* and community *Poa pratensis-Festuca rubra*. A one-way ANOVA test for pH showed statistically significant differences between grasslands (pH_{H2O} p=0.000 and pH_{KCl} p=0.000). The abundance of heterotrophic bacteria (p=0.000), actinobacteria (p=0.001), and fungi (p=0.014) were also traits that significantly differentiated grassland vegetation units. One-way ANOVA test showed that of all the phenolic compounds analyzed, only two were found to be significant: vanillic acid (p=0.003) and catechin (p=0.002). Our research indicates a positive correlation of actinobacteria with cinnamic and caffeic acid, heterotrophic bacteria with *p*-coumaric and ferulic acid and catechin, and fungi with gallic acid and catechin. In addition, taking into account statistically significant features, it can be concluded that *Molinietum caeruleae* shows a different structure compared to the other vegetation units, the *Arrhenatheretum elatioris* and *Lolio-Cynosuretum* group are similar, while com. *Poa pratensis-Festuca rubra* and *Alopecuretum pratensis* show different structures from the others. Environmental research is increasingly focusing on enhancing soil organic carbon accumulation. Understanding the relationship between phenolic compounds and microorganisms in grassland soils is crucial in this context. Proper grassland management is a key element of environmental protection.

1. Introduction

One of the main threats to soils is the decline in soil organic matter (SOM) content, at the same time SOM content is the most frequently cited indicator of soil quality. Grasslands are the richest in carbon of all agricultural ecosystems, storing approximately 34% of total global carbon stocks in terrestrial ecosystems. Grassland soils vary greatly in their organic matter content and quality. These soils have a higher organic matter content than cultivated soils. In Poland, the organic grassland soils organic carbon content is 10.42%, while in mineral soils, it is 3.81% (Pietrzak and Hołaj-Krzak, 2022). The organic carbon content in cultivated soils is about 2.2% (Kołacz, 2020). This is due to better conditions for immobilization and accumulation of SOM. These characteristics favor the sequestration of organic carbon in the soil. It is reported that global carbon sequestration in farmland soils is estimated at 0.3 t C ha^{-1} per year, while in grasslands, it is 0.5–0.7 t C ha per year (Eze et al., 2018; Pikuła, 2019; Ziółkowska, 2019). Valuable sources of SOM in grassland soils are dead above-ground parts of plants, plant roots, natural and organic fertilizers, soil-decayed microorganisms, and animal feces (Ziółkowska, 2014).

Plants produce a highly diverse set of primary and secondary metabolites. Cellulose, hemicelluloses, and lignins are important compounds of plant origin in the humification process. In addition, tannins, terpenes, and microorganism metabolites may also be involved in the process. Studies indicate that in grass communities, most lignins are contained in the roots of plants (up to 20%), while in the aboveground parts of grasses, they account for 2.1–9.1% d.m. (Ziółkowska et al., 2020a; Zwetsloot et al., 2020).

Among the phenolic compounds found in meadow soils, we can distinguish hydroxybenzoic acid and its derivatives (e.g., dihydroxybenzoic, protocatechuic (PA), syringic (SYR), and vanillic (VA) acids), hydroxycinnamic acid and its derivatives (e.g., cinnamic, ferulic (FEA), *p*-coumaric (*p*-CO), depsides of the core of the molecule, which contain an ester bond (e.g. chlorogenic acid (CHA)) (Ziółkowska et al., 2020b, 2020a).

Phenolic compounds are key in soil-plant interactions, affecting soil structure, chemical composition, and biological activity. Their effects can be both beneficial (e.g., by participating in the defense reactions of plants against pathogenic microorganisms: viruses, fungi or bacteria or by increasing plant resistance to stress) and negative (e.g., through toxicity or impact on nutrient availability) (Adom et al., 2003; Babenko et al., 2019; Golonko et al., 2015; Hättenschwiler and Vitousek, 2000; Kulbat, 2016; Moreira et al., 2020; Sies and Jones, 2020).

There is no doubt that phenolic compounds are an essential part of humification processes in the soil. These compounds are widely distributed in the environment and are one of the main components of soils (Clemensen et al., 2020; Horvat et al., 2020; Ziółkowska et al., 2020b). Many phenolic compounds dissolve well in water, including phenolic acids and tannins. In this form, they remain in solution between soil particles, where they can undergo reversible sorption due to hydrophobic, hydrogen, and ionic interactions. The processes of condensation and polymerization of these substances with amino acids and proteins in the soil lead to the formation of organic acids of high molecular weight, such as fulvic acids, humic acids, and humins, which changes the properties of the soil (Misra et al., 2023; Schmidt et al., 2011; Usha Rani and Jyothsna, 2010).

Phenolics play a vital role in the plant-soil relationship by modifying the growth and development of higher plants and soil microorganisms. They provide feedback to soil organic matter-degrading microorganisms by altering soil pH, nutrient availability, and enzyme activity (Macias-Benitez et al., 2020; Min et al., 2015; Sądej et al., 2016). For instance, phenols such as catechins can influence the mobility of phosphorus in the soil. When combined with organic acids, these compounds transform poorly soluble forms of soil phosphorus into more soluble forms, which is crucial for supplying phosphorus to plants (Hu et al., 2005; Sugiyama and Yazaki, 2012). Phenolic compounds also affect nitrogen availability by inhibiting the activity of nitrifying microorganisms. They can hinder the activity of enzymes involved in nitrogen metabolism, consequently reducing the rate of nitrogen transformation (Adamczyk et al., 2008; Chen et al., 2025; Ma et al., 2016; Thorpe and Callaway, 2011; Wang et al., 2013). On the other hand, at low concentrations, they can increase nitrogen mineralization (Chen et al., 2018). Therefore, phenolic compounds can potentially serve as biological nitrogen regulators.

Phenolic compounds regulate the response of plants caused by abiotic stress and other external stimuli (Golonko et al., 2015; Misra et al., 2023). Many phenolic allelochemical compounds inhibit the growth of other plants, affecting their germination and root development (Sugiyama and Yazaki, 2012). Microorganisms interacting with plants and soil secrete many allelochemicals, including lytic enzymes such as glucanases and chitinases, which affect the development of various plant diseases. For example, beta-1,3-glucanase destroys cell walls, which can lead to *Pythium phanidermatum* root rot and *Fusarium oxysporum* fusarium rot (Chatterton and Punja, 2009; Polyak and Sukcharevich, 2019). Plants also secrete phenolic compounds in response to stress from pathogens and insects (Golonko et al., 2015; Usha Rani and Jyothsna, 2010). For example, lignin protects plants from insect and pathogen attacks (Barakat et al., 2010; Johnson et al., 2009). Another example is quinones, which exhibit direct toxicity to insects and hinder herbivores' digestion of plant proteins (Bhonwong et al., 2009; Misra et al., 2023). Salicylic acid, on the other hand, is considered a secondary transmitter of information in the process of developing plant resistance to viruses and bacteria (Gałązka, 2013).

Environmental factors such as soil pH, temperature, and humidity can affect the degradation of phenols (Bell and Henry, 2011; Sinsabaugh, 2010; Xin et al., 2024; Ziółkowska et al., 2020a). Research indicates that predicting the direction of phenolic decomposition is difficult. The relationship between phenol oxidase activity and the concentration of phenols under natural conditions is unclear. Some authors indicate a positive correlation (Yao et al., 2009), while others show a negative one (White et al., 2011). In the case of peats, which have a high content of phenolic compounds, there is a correlation that the higher the phenolic oxidase content, the higher the phenolic content, resulting in a positive correlation. It is worth noting that drought increases the dynamics of decomposition, which is due to the higher activity of the described enzyme (Fenner et al., 2005).

Topsoil is the place where there is an accumulation of various interactions between microorganisms and plants. Soil microorganisms play an important role in grassland ecosystems through their influence on plant physiology. Root exudates secreted here improve the plant's interactions with soil microorganisms and accelerate the decomposition of SOM (Ma et al., 2022; Sun et al., 2024; Zwetsloot et al., 2020). Phenolic compounds are released from plants mainly during the decomposition process of mulch but can also be released as root exudates. Phenolic compounds induce the selection of soil microorganisms because they can be toxic to them at low concentrations. Due to their antibacterial properties, plant polyphenols can affect bacterial cells through various mechanisms, such as binding to proteins and cell walls, disturbing cytoplasmic functions and membrane permeability, inhibiting metabolic processes related to energy production, damaging DNA and blocking nucleic acid synthesis (Lobiuc et al., 2023). Gram-positive bacteria are most sensitive to phenolic compounds, which is due to the presence of peptidoglycans on their surface and the lack of an outer membrane. For example, gallic acid can affect the charge, hydrophobicity, and permeability of the membrane and, in the case of Gram-negative bacteria, lead to its destabilization (Cueva et al., 2010; Kahkeshani et al., 2019). Ferulic acid has antibacterial activity against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Listeria monocytogenes* at a minimum inhibitory concentration of 100 to 1250 µg/ml, while gallic acid has activity in the range of 500–2000 µg/ml (Borges et al., 2013). Phenolic acids can affect bacteria not only by affecting the cell membrane but also through other mechanisms. For example, *p*-coumaric acid has the ability to bind bacterial DNA (Lou et al., 2012).

However, phenol-rich soils contain groups of microorganisms that are resistant and capable of degrading phenols (Clocchiatti et al., 2021; Morones-Esquivel et al., 2022). Phenol-resistant organisms are, for example, Zygomycetes, Hypocreales, and Melanconiales (Mäkelä et al., 2015). Another example is *Burkholderia hospita* actively metabolizes benzoic acid but also reproduces in soil with high concentrations of this phenolic compound (100 μg of benzoic acid was dosed in the soil four times in this experiment) (Pumphrey and Madsen, 2008). Stimulation of microorganisms by phenolic compounds contained, for example, in root exudates, may occur from edaphic factors (Suseela et al., 2016).

The main objective of this study was to evaluate the relationships between selected phenolic compounds and microbial abundance in grassland soils. A better understanding of these relationships is crucial for further research on phenols and their effects on soil microorganisms. We address the following research hypotheses: 1. Vegetation stimulates the secretion of phenolic compounds, which affects the abundance of selected microorganisms; 2. The content of phenolic compounds in grassland soils depends on the content of organic matter and soil pH.

2. Materials and methods

2.1. Study area and sampling

The present study was conducted on semi-natural grasslands located along the Obra River (Wielkopolska Lowland, central Poland). The following grassland vegetation syntaxonomic units were recorded in the study area: *Molinietum caeruleae, Alopecuretum pratensis, Arrhenatheretum elatioris, Lolio-Cynosuretum and* community (com.) *Poa pratensis-Festuca rubra.* The releves were made at 20 survey points, represented areas of 100 m^2 and homogeneous species composition. The locations of the 20 sampling sites were marked in the field and described in a previous article (Mencel et al., 2024). Selected sites were sampled for soils in September 2022. Soil samples for laboratory analysis were taken from the uppermost soil horizons at 0–20 cm depth. In the area of each phytosociological releves, the soil was taken from three points and then spooled to one sample (to account for potential soil variability). Soil samples were collected in plastic bags and transported in a lightproof box to the laboratory. Soil was collected using Egner's Cane.

2.2. Chemical Analyses

Under laboratory conditions, the soils were dried and homogenized, then sieved through a 2 mm sieve. Chemical analyses included the following parameters: determination of total organic carbon (TOC) and total nitrogen (TN) using a Vario-Max CNS analyzer and determination of soil pH potentiometrically in 1 M KCl and in a suspension of distilled water in a 1:2.5 ratio.

2.3. Biological Analyses

Soil microorganisms count was measured using the plate method on adequate selective substrates with five replicates. The total count of colony-forming units (CFU) of heterotrophic bacteria, actinobacteria, and fungi was measured. Heterotrophic bacteria count was measured on ready-made Merck standard agar medium (26° for 5 days). Fungi count was measured on a Martin substrate (Martin, 1950) after a 5-day incubation at 24°. Actinobacteria count was measured on a selective Pochon substrate (Grabińska−Łoniewska, 1999), where the plates were incubated for 7 days at 26°.

2.4. Extraction of phenolic compounds

The phenolic compounds were extracted from dried soil according to Ziółkowska et al. (2020a) with some modifications. The soil samples were mixed with distilled water and 2M NaOH, were sonicated, and heated for 30 minutes at 90°C. After cooling the samples were neutralized with 6M HCl. Then, the extraction with diethyl ether was performed twice. The extract was transferred to vials. Next, the 6 M HCl was used for acid hydrolysis in a water bath at 80°C for 30 min. Then, the samples were extracted with diethyl ether (twice). The extracts were transferred to alkaline hydrolyzed extract and evaporated to dryness. Before ultra-performance liquid chromatography (UPLC) analyses, the samples were dissolved in 1mL methanol and filtrated.

2.5. UPLC analysis

High-performance liquid chromatography (ACQUITY UPLC H-Class System Waters Corporation, Milford, MA, USA) consisting of a quaternary pump solvent management system, online degasser, and autosampler was used to quantify phenolic compounds (Gąsecka et al., 2023; Kurasiak-Popowska et al., 2022). A Waters Acquity UPLC BEH C18 column (150 × 2.1 mm, 153 1.7 μm) thermostated at 35°C was used to separate phenolic compounds. The gradient elution with water and acetonitrile (both containing 0.1% formic acid, pH=2) with flow 0.4 ml min^{-1} was according to the gradient program: 5% B (2 min), 5–16% B (5 min), 16% B (3 min), 16–20% B (7 min), 20–28% B (11 min), 28% (1 min), 28–60% B (3 min) 60–95% B (1 min), 65% B (1 min), 95–5% B (0.1 min) min−1 5% B (1.9 min). The injection of the extracts, filtered through a 0.22 mm syringe filter, was 5 μl. Identification of the compounds was based on comparing the retention times of the peaks with the retention times of chemical standards. Detection was performed using an external detector on a Waters Photodiode Array Detector (Waters Corporation, Milford, MA, USA) at the following wavelengths λ=280 nm (catechin, gallic acid, 4-hydroxybenzoic acid (4-HBA), syringic acid, cinnamic acid, vanillic acid) and λ=320 nm (2,5-dihydroxybenzoic acid (2,5-DHBA), caffeic acid, chlorogenic acid, *p*-coumaric acid, ferulic acid, sinapic acid). The detection limits (DL) were calculated based on a signal-to-noise ratio 3:1. The recovery rates of the phenolics as follows: gallic acid – 92 ± 4.4%, vanillic acid – 79 ± 8.5%, catechin – 89 ± 5.7%, 4-HBA – 96 ± 3.78%, chlorogenic acid – 92 ± 2.8%, caffeic acid – 86 ± 6.7%, syringic acid – 94 ± 3.9%, *p*-coumaric acid – 89 ± 3.6%, ferulic acid – 91 ± 4.9%, sinapic acid – 94 ± 5.1%, and cinnamic acid – 97 ± 2.9% (Kurasiak-Popowska et al., 2022). Raw data were acquired and processed using Empower 3 software.

The standards of phenolic compounds (4-HBA ≥ 99%, 2,5–DHBA ≥98%, gallic acid ≥98%, vanillic acid ≥ 97%, syringic acid

≥ 98%, catechin ≥ 98%, *p*-coumaric acid ≥ 98%, ferulic acid ≥ 98%, chlorogenic acid ≥ 95%, sinapic acid ≥ 97, cinnamic acid ≥ 99%, were obtained from Sigma–Aldrich (Steinheim, Germany). Caffeic acid (certified reference material TraceCERT®) was purchased in Sigma–Aldrich (Basel, Switzerland). Ethanol (absolute for EMSURE® ACS, ISO, Reag. Ph Eur, ≥ 99.9 %).

2.6. Statistical Analysis

For statistical analysis of the mean levels of the analyzed soil parameters, a single one-way ANOVA test was used, with a prior check of the conformity of the analyzed variables to a normal distribution in all subgroups based on the Kolmogorov-Smirnov test. Tukey's HSD post-hoc test was used to evaluate homogeneous groups. The correlation analysis of traits was based on the Student's t-test for the r-Pearson linear correlation coefficient. We used $\alpha = 0.05$ as the level of statistical significance. Cluster analysis is a multivariate method that searches for patterns in a data set by grouping the observations into clusters (Polowy and Molińska-Glura, 2023). The distance between the data determines the level of data similarity. The small distance between the data indicates a high similarity level of the data. Euclidean metric was used as a measure of similarity.

3. Results and discussion

3.1. Chemical soil properties

Grassland soils are an extremely important element in the carbon sequestration process, as they provide suitable conditions for the accumulation and storage of organic matter. These stores should be protected. It cannot be denied that phenolic compounds play a very important role in the transformation of soil organic matter. These compounds are one of the most common components in the soil, which affect the circulation of nutrients and the abundance of soil microorganisms (Wiesmeier et al., 2019; Ziółkowska et al., 2020a). According to Min et al. (2015) and Ziółkowska et al. (2020a), the high content of phenolic compounds in the soil solution reduces the intensity of soil organic matter decomposition.

The total organic carbon (TOC) content in the grassland soils analyzed differed among selected syntaxonomic units of grassland vegetation (Table 1). The highest TOC content was recorded in *Alopecuretum pratensis* (148.23 g kg^{-1}) and the lowest in *Molinietum caeruleae* (69.40 g kg^{-1}). The contents were distributed similarly for TN, with the highest recorded in *Alopecuretum pratensis* and the lowest in *Molinietum caeruleae* (Table 1). The pH values indicated slightly acidic soils in *Alopecuretum pratensis*, *Molinietum caeruleae*, *Lolio-Cynosuretum*, com. *Poa pratensis-Festuca rubra* and slightly alkaline soils in *Arrhenatheretum elatioris*. A one-way ANOVA test for pH showed statistically significant differences between grasslands (for pH_{H2O} p=0.000 and for pH_{KCl} p=0.000). Tukey's post-hoc tests revealed that *Arrhenatheretum elatioris* differed from other vegetation units (Table 1).

3.2. Abundance of microorganisms in soils

The soil of grassland ecosystems contains huge amounts of microorganisms, including bacteria, fungi, and other life forms. Environmental factors play a key role in shaping microbial communities. Soil microorganisms are uniquely susceptible to conditions in their environment. They are able to respond quickly to changes in the soil, which in turn affects plant species diversity and soil structure (Ma et al., 2023). Microorganisms perform key functions such as decomposing organic matter, maintaining soil fertility, improving soil structure and drainage, sequestering carbon, and regulating greenhouse gas emissions. High levels of microbial abundance and diversity lead to significant improvements in the resilience of the ecosystem, which is soil (Grządziel, 2017; Maron et al., 2018; Roux et al., 2011).

Soil bacteria are the most numerous and widespread microorganisms in the soil, accounting for 70% to 90% of the total soil microbial population (Chi et al., 2023; Lu et al., 2022; Sui et al., 2019). Our study noted that heterotrophic bacteria had the highest abundance among the analyzed microorganisms (Table 1). The highest abundance was observed in *Alopecuretum pratensis* and the lowest in *Molinietum caeruleae*. The one-way ANOVA test indicates that individual grassland units differ significantly for the analyzed trait (p=0.000). Tukey's post-hoc tests showed that *Alopecuretum pratensis* stands out significantly from other grassland units (Table 1). For actinobacteria, the highest abundance values were recorded in *Lolio-Cynosuretum* and the lowest in *Molinietum caeruleae* (Table 1). As with heterotrophic bacteria, individual grassland units differed significantly due to actinobacteria (p=0.001). Due to actinobacteria, individual grassland units form three homogeneous groups (Table 1). Another trait analyzed was the number of fungi. Their abundance in all grassland units was most equal. However, the greatest number of fungi was recorded in *Alopecuretum pratensis* and the least in com. *Poa pratensis-Festuca rubra*. One-way ANOVA test indicates that fungi significantly differentiated grassland units (p=0.014). Due to fungi, individual vegetation units form two similar groups (Table 1).

3.3. Phenolic compound content

There are many phenolic compounds in grassland soils. However, according to Kovaleva and Kovalev (2009), a characteristic of these soils is the predominance of *p*-coumaric and ferulic acids compared to the content of other phenolic compounds, which is due to the species composition of the sward. The chemical composition of plants affects the chemical composition of soil organic matter and humus.

The phenolic profile of the analyzed plants was very diverse. Within *Molinietum caeruleae*, catechin had the highest content (3.66 μg g^{-1} d.m. soil) and cinnamic acid the lowest (0.18 μg g^{-1} d.m. soil). 2,5-dihydroxybenzoic acid and sinapic acid were detected but at concentrations below the detection limit. *Alopecuretum pratensis* had a very high syringic acid content compared to other grassland units (Table 1). In the rim of this syntaxonomic unit, the lowest value was re-

Table 1
Characterization of the chemical and microbial soil properties (mean±SD). Statistically significant differences in the tested parameters between grassland vegetation syntaxonomic units are marked by different letters (n=4)[1]

Variable	*Molinietum caeruleae*	*Alopecuretum pratensis*	*Arrhenatheretum elatioris*	*Lolio-Cynosuretum*	com. *Poa pratensis-Festuca rubra*
pH_{H2O} *p=0.000*[2]	6.42±0.28[a]	6.27±0.20[a]	7.71±0.18[b]	6.58±0.47[a]	6.62±0.24[a]
pH_{KCl} *p=0.000*[2]	6.05±0.30[a]	6.00±0.18[a]	7.29±0.17[b]	6.13±0.59[a]	6.31±0.24[a]
			10^5 cfu g^{-1} d.m. soil		
Heterotrophic bacteria *p=0.000*[2]	30.44±7.59[a]	162.17±60.08[b]	71.66±22.62[a]	57.77±1.83[a]	46.85±7.24[a]
Actinobacteria *p=0.001*[2]	11.97±6.07[a]	60.43±24.29[bc]	55.69±20.30[abc]	84.19±31.07[c]	26.61±6.24[ab]
			10^3 cfu g^{-1} d.m. soil		
Fungi *p=0.014*[2]	37.26±5.02[ab]	45.21±11.82[b]	29.05±6.41[ab]	27.74±3.62[a]	24.87±5.24[a]
			g kg^{-1}		
TOC[3] *p=0.578*[2]	69.40±10.29	148.23±60.72	87.67±59.30	98.12±33.88	121.82±10.24
TN[4] *p=0.594*[2]	5.74±1.35	11.67±4.04	7.71±4.57	8.60±2.60	10.35±1.24
			μg g^{-1} d.m. soil		
2,5-DHBA[5] *p=0.162*[2]	BDL[7]	BDL[7]	7.72±10.15	BDL[7]	5.69±0.24
4-HBA[6] *p=0.937*[2]	2.86±3.02	3.91±4.82	2.98±3.58	4.73±2.41	3.81±3.24
Caffeic acid *p=0.637*[2]	0.72±0.86	0.70±0.28	0.82±0.69	1.02±0.50	0.38±0.24
Chlorogenic acid *p=0.389*[2]	0.55±0.88	0.71±1.42	0.45±0.59	0.62±0.80	1.66±0.24
Cinnamic acid *p=0.623*[2]	0.18±0.37	0.46±0.91	1.13±1.33	1.23±1.55	0.73±0.24
Ferulic acid *p=0.122*[2]	BDL[7]	0.44±0.35	BDL[7]	0.12±0.25	0.39±0.24
Gallic acid *p=0.097*[2]	1.64±1.30	9.11±9.17	1.27±1.16	2.14±1.40	2.48±1.24
p-Coumaric acid *p=0.163*[2]	1.07±0.52	2.21±0.90	1.37±0.73	0.65±1.30	1.29±0.24
Sinapic acid p^2*=0.073*[2]	BDL[7]	0.14±0.28	0.32±0.42	0.50±0.39	0.77±0.24
Syringic acid *p=0.440*[2]	0.20±0.41	71.57±142.01	0.84±0.60	0.24±0.49	1.51±0.24
Vanillic acid *p=0.003*[2]	0.49±0.65[a]	4.81±2.44[ab]	3.15±1.65[a]	3.34±1.10[a]	12.87±0.24[b]
Catechin *p=0.002*[2]	3.66±4.59[a]	15.25±5.77[b]	5.86±3.94[a]	5.56±2.57[a]	1.43±4.24[a]

[1]sample size for each syntaxonomic units; [2]p-value for one-way ANOVA; [3]total content of nitrogen; [4]total content of organic carbon; [5]2,5-dihydroxybenzoic acid; [6]4-hydroxybenzoic acid; [7]below detection limit

corded for sinapic acid (0.14 μg g^{-1} d.m. soil). The presence of 2,5-DHBA was not recorded. *Arrhenatheretum elatioris* soils had the highest 2,5-dihydroxybenzoic acid content among all analyzed vegetation units (7.72 μg g^{-1} d.m. soil), while no ferulic acid was recorded. *Lolio-Cynosuretum* was characterized by the absence of 2,5-DHBA, low ferulic acid content (0.12 μg g^{-1} d.m. soil), and 5.56 μg g^{-1} d.m. soil of catechin. Community *Poa pratensis-Festuca rubra* had high vanillic acid content (12.87 μg g^{-1} d.m. soil) and low caffeic acid (0.38 μg g^{-1} d.m. soil). In Ziółkowska et al. (2020a) study, high levels of chlorogenic acid (88.82–135.56 μg g^{-1} d.w.), ferulic acid (52.66–103.46 μg g^{-1} d.w.) and caffeic acid (36.16–81.08 μg g^{-1} d.w.) were recorded in the grassland soil of the Czerskie Meadows. However, the values in the previously mentioned research were much higher than in our study. It is worth noting that the content of phenolic compounds decreased with soil depth. These relationships were noted by Ziółkowska et al. (2020a) and Dębska and Banach-Szott (2010) in their study. Another factor affecting the content of phenolic compounds is, for example, the sorption capacity of the soil. Soils with higher sorption capacity can bind more of these compounds, which affects their availability and biological activity. In addition, environmental factors such as soil pH, temperature, oxygen availability, and the presence of substrates can affect the distribution of phenolic compounds found in the soil (Cecchi et al., 2004; Min et al., 2015).

One-way ANOVA test showed that of all the phenolic compounds analyzed, only two were found to be significant: vanillic acid (p=0.003) and catechin (p=0.002). With vanillic acid, individual grassland units form two homogeneous groups due to the analyzed trait (Table 1). With catechin, the analyzed units form two homogeneous groups, in which the second group contains only *Alopecuretum pratensis*, distinguishing it from the others (Table 1).

The results of Bao et al. (2022) indicate a positive correlation of ferulic acid and vanillic acid (VA) with most of the bacteria studied. In addition, the authors indicate that VA also correlated with most fungi. Adding VA to the soil significantly increased the abundance of *Ruminiclostridium* and *Lachnospirae* Group, while a decrease in the abundance of *Haliangium* was noted. The results showed that phenolic acids can promote the growth of pathogenic bacteria, and the interaction between soil rhizosphere microorganisms and phenolic acids was the main reason for the disruption of the rhizosphere microbiota.

Catechin, a polyphenolic compound found in plants such as *Centaurea stoebe* Lam. (spotted knapweed), has been studied for its allelopathic effects on soil microorganisms in grassland ecosystems. Research indicates that catechin can suppress total culturable bacterial counts and inhibit the growth of specific soil bacterial populations, demonstrating a reversible bacteriostatic mechanism in various bacterial strains. Understanding how catechin influences microbial communities in grassland soils is essential for grasping its ecological role (Pollock et al., 2011).

3.4. Relationship between selected chemical properties of soil and the content of phenolic compounds

Our analyses indicated a positive correlation between actinobacteria and cinnamic and caffeic acids, heterotrophic bacteria and *p*-coumaric acid, ferulic acid and catechin, and fungi and gallic acid and catechin (Table 2). Our hypotheses have been partially confirmed. There was no correlation between pH and phenolic compounds.

Phenolic compounds in the soil play a significant role in shaping the microbial community and its functions. Studies have shown that phenolic acids like *p*-hydroxyphenylacetic acid (HPA) and *p*-hydroxybenzoic acid (HBA) can influence soil bacterial and fungal community structures, enriching specific bacterial genera like *Bacillus* or *Actinobacteria*, as well as fungal species such as *Penicillium* and *Aspergillus* (Li et al., 2023).

Additionally, phenolics like benzoic acid, caffeic acid, and catechin have been shown to inhibit microbial activity while also promoting the decomposition of soil organic matter, especially in the presence of glucose, which leads to shifts in microbial communities (Zwetsloot et al., 2020). Furthermore, the presence of polyphenols in soil is associated with changes in microbial metabolism under anoxic conditions, challenging the assumption that polyphenols are not bioavailable in such environments (McGivern et al., 2021). These findings highlight the intricate relationship between phenolic compounds and soil microorganisms, demonstrating their role in modulating microbial communities and functions in various soil ecosystems.

A correlation between TOC and, ferulic acid and cinnamic acid was observed, and the same applies to TN (Table 2). This confirms the associations noted by other authors, who indicate the link between organic matter and phenolic compounds (Kovaleva and Kovalev, 2009). The authors emphasize that the level of lignin decomposition increases with the humification of organic matter. According to these authors, the quantitative composition of phenolic compounds relies on the extent of decomposition of SOM. The organic matter decomposition process in grassland soils correlates with an increase in the ratio of vanillyl and syringyl compounds while decreasing the ratio of cinnamyl compounds.

The Fig. 1 shows the differentiation of vegetation syntaxonomic units based on the structure of the analyzed phenolic compounds based on the average content of these compounds in the studied soils. Cluster analysis showed that considering statistically significant features, it can be concluded that *Molinietum caeruleae* shows a different structure compared to the other vegetation units. In contrast, *Arrhenatheretum elatioris* and *Lolio-Cynosuretum* are similar, while the com. *Poa pratensis-Festuca rubra* and *Alopecuretum pratensis* show different structures from the others (Fig. 1).

One limitation of the conducted studies may be the difficulty in discussing them due to the lack of available research results that consider three factors simultaneously: the vegetation units, the abundance of microorganisms, and the content of phenolic compounds in the soil under these vegetation units. As our preliminary studies indicate, distinguishing between types of plant communities is significant in this context.

Table 2

Values of r-Pearson correlation coefficients. Only statistically significant correlation coefficients are included (n=20)[1]

Var.	pH_{H2O}	pH_{KCl}	HB[2]	AB[3]	F[4]	TOC[5]	TN[6]	CA[7]	p-CO[8]	CHA[9]	FEA[10]	SIN[11]	GA[12]	VA[13]	CT[14]	CIA[15]
pH_{H2O}		0.98 *p=0.000*														
pH_{KCl}																
HB[2]					0.55 *p=0.011*				0.60 *p=0.005*		0.47 p=0.036				0.80 p=0.000	
AB[3]								0.49 *p=0.027*								0.47 *p=0.036*
F[4]						0.46 *p=0.042*	0.44 *p=0.049*						0.49 *p=0.029*		0.69 *p=0.001*	
TOC[5]							0.99 *p=0.000*				0.55 *p=0.012*					0.46 *p=0.039*
TN[6]											0.56 *p=0.010*					0.47 *p=0.036*
CA[7]																
p-CO[8]																
CHA[9]											0.66 *p=0.001*	0.54 *p=0.015*		0.53 *p=0.017*		
FEA1[0]														0.48 *p=0.033*		
SIN[11]																
GA[12]																
VA[13]																
CT[14]																
CIA[15]																

[1] sample size; [2]heterotrophic bacteria; [3]actinobacteria; [4]fungi; [5]total content of organic carbon; [6]total content of nitrogen; [7]caffeic acid; [8]*p*-coumaric acid; [9]chlorogenic acid; [10]ferulic acid; [11]sinapic acid; [12]gallic acid; [13]vanillic acid; [14]catechin; [15]cinnamic acid;

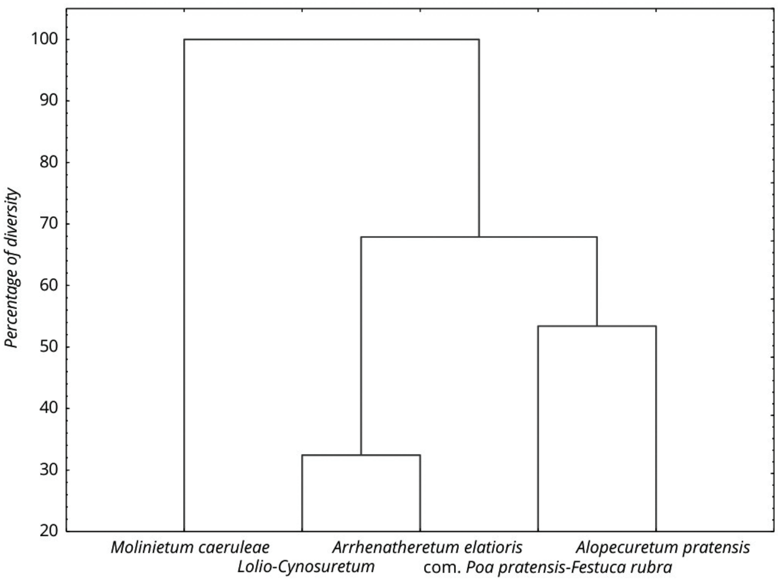

Fig. 1. Cluster analysis for grassland vegetation units

4. Conclusions

1. The study shows a positive correlation between actinobacteria and cinnamic and caffeic acids, heterotrophic bacteria and p-coumaric acid, ferulic acid and catechin, and fungi with gallic acid and catechin.
2. A correlation was observed between TOC and TN and ferulic acid and cinnamic acid.
3. Catechin was the dominant phenolic compound in soils under *Molinietum caeruleae* and *Lolio-Cynosuretum*. Syringic acid was dominant in the soil under *Alopecuretum pratensis*. 2,5-dihydroxybenzoic acid had the highest content in the soil under *Arrhenatheretum elatioris*. Soils under com. *Poa pratensis-Festuca rubra* was dominated by vanillic acid.
4. Soil from under *Molinietum caeruleae* shows a different structure of analyzed phenolic compounds compared to soils from other plant units.
5. Studies of phenolic compounds and microbial abundance in grassland soils are important in the context of climate change in terms of organic carbon sequestration.
6. In conclusion, the study indicates that the levels of TOC, TN, the abundance of microorganisms, and the content of phenolic compounds in the soil vary across different vegetation units. The compounds examined undoubtedly play a significant role in the plant-soil relationship, influencing the growth and development of higher plants as well as soil microorganisms. This research is pioneering in the context of grassland soils and should be further pursued.

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Conflict of interest

The authors declare no conflict of interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. This research did not involve human or animal subjects.

Author Contributions

Justyna Mencel – *Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Validation, Writing – original draft.* **Monika Gąsecka** – *Investigation, Validation, Writing – review & editing.* **Marta Molińska-Glura** – *Investigation, Validation, Writing – review & editing.* **Agnieszka Mocek-Płóciniak** – *Conceptualization, Funding acquisition, Supervision, Validation, Writing – review & editing.* All authors read and approved the final manuscript.

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Zależności między wybranymi związkami fenolowymi a liczebnością mikroorganizmów w glebach użytków zielonych w dolinie rzeki Obry: Badania wstępne

Słowa kluczowe

Mikroorganizmy glebowe
Użytki zielone
Jednostki wegetacyjne
Fenole
Glebowa materia organiczna

Streszczenie

Celem pracy była ocena zależności pomiędzy wybranymi związkami fenolowymi, a liczebnością mikroorganizmów w glebach użytków zielonych. Obiektem badań były wierzchnie warstwy gleby (0–20 cm) użytków zielonych położonych wzdłuż rzeki Obry (Nizina Wielkopolska, centralna Polska). Badania terenowe przeprowadzono we wrześniu 2022 roku. Na badanym obszarze wybrano pięć jednostek syntaksonomicznych roślinności: *Molinietum caeruleae, Alopecuretum pratensis, Arrhenatheretum elatioris, Lolio-Cynosuretum* oraz zbiorowisko *Poa pratensis-Festuca rubra*. Jednokierunkowy test ANOVA dla pH wykazał statystycznie istotne różnice między użytkami zielonymi (dla pH_{H2O} p=0,000, a dla pH_{KCl} p=0,000). Liczebność bakterii heterotroficznych (p=0,000), promieniowców (p=0,001) i grzybów (p=0,014) była również cechą istotnie różnicującą jednostki roślinności użytków zielonych. Jednokierunkowy test ANOVA wykazał, że spośród wszystkich analizowanych związków fenolowych tylko dwa były się istotne: kwas wanilinowy (p=0,003 i katechina (p=0,002). Nasze badania wskazują na dodatnią korelację promieniowców z kwasem cynamonowym i kawowym, bakterii heterotroficznych z kwasem *p*-kumarowym i ferulowym oraz katechiną, a grzybów z kwasem galusowym i katechiną. Ponadto, biorąc pod uwagę cechy istotne statystycznie, można stwierdzić, że *Molinietum caeruleae* wykazuje inną strukturę w porównaniu z innymi jednostkami wegetacyjnymi, *Arrhenatheretum elatioris* i *Lolio-Cynosuretum* są podobne, podczas gdy zbiorowiska *Poa pratensis-Festuca rubra* i *Alopecuretum pratensis* wykazują odmienną strukturę od pozostałych. Badania środowiskowe coraz częściej koncentrują się na potrzebie zwiększenia akumulacji węgla organicznego w glebie. Poznanie relacji między związkami fenolowymi i mikroorganizmami w glebach użytków zielonych jest niezwykle ważne w tym aspekcie. Właściwe zarządzanie użytkami zielonymi jest ważnym elementem ochrony środowiska.