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Herbaceous plant species support soil microbial performance in deciduous temperate forests

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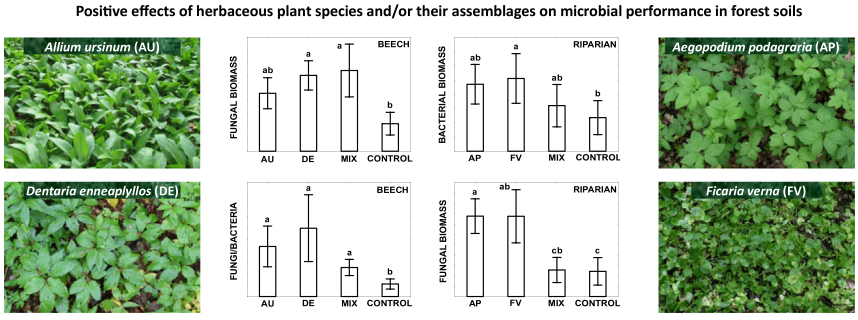
HIGHLIGHTS

- The effects of herbaceous plants on soil were assessed in beech and riparian forests.
- Herbaceous plants enhanced microbial parameters, mainly fungal and bacterial biomass.
- Mix of species did not have beneficial effect on microbes relative to single species.
- Soil physicochemical properties generally were not influenced by herbaceous plants.

GRAPHICAL ABSTRACT



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ABSTRACT

Although herbaceous plant layer may contribute significantly to plant diversity and nutrient turnover, its effects on the soil environment in forest ecosystems remain largely unexplored. In this study, we compared the effects of mono-dominant and multi-species assemblages of herb plants on soil physicochemical and microbial properties in two temperate deciduous (beech and riparian) forests. We hypothesized that the presence of herbaceous plants would increase microbial activity and biomass, and nutrient availability in soil when compared to bare soil. This increase would be the highest in multi-species assemblages as high plant diversity supports microbial performance and soil processes, and the expected patterns would be essentially similar in both forests. *Allium ursinum* L. and *Dentaria enneaphyllos* L. represented herb species forming mono-dominant patches in beech forest, while *Aegopodium podagraria* L. and *Ficaria verna* Huds. represented herb species forming mono-dominant patches in riparian forest. Our hypotheses were only partly supported by the data. We found that herb plant species affected soil microbial communities and processes, particularly in the riparian forest, but they generally did not influence soil physicochemical properties. In the beech forest, herbaceous plants increased saprotrophic fungi biomass, fungi/bacteria ratio, and arylsulfatase activity, with the highest values under *D. enneaphyllos*. In the riparian forest, a number of microbial parameters, namely bacteria, G+ bacteria, and saprotrophic fungi biomass, fungi/bacteria ratio, and soil respiration exhibited the lowest values in bare soil and the highest values in soil under *A. podagraria*. Contrary to expectations, soils under multi-species assemblages were characterized by intermediate values of microbial parameters. Concluding, herbaceous plant species largely supported soil microbial communities in deciduous temperate forests but did not affect soil chemical properties. The potential reasons for the positive influence of herb plants on soil microbes (litterfall, rhizodeposition) require further investigation.

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1. Introduction

Plant diversity has often been reported to support soil biota and positively affect the functioning and services of forest and grassland ecosystems (Eisenhauer et al., 2011, 2017; Hautier et al., 2018; Isbell et al., 2011). In forests, biodiversity is largely a function of herbaceous plant layer. Although herbaceous layer represents less than 1% of the forest biomass, it may contain more than 90% of forest plant species and contribute up to 20% of foliar litter (Gilliam, 2007). This layer also plays an important role in a wide range of ecosystem functions, including nutrient cycling and energy flow. Herb foliage may contain much more nutrient elements than that of trees, and thus its influence on the cycling of essential plant nutrients is disproportionate to its relative biomass in forest ecosystems (Gilliam, 2007; Roberts, 2004; Schulze et al., 2009). Although herbaceous vegetation is important for maintaining forest structure and function, and has higher extinction rates than vegetation in other strata, it remains an underappreciated component of forest ecosystems (Baldrian, 2017; Elliott et al., 2015; Gilliam, 2007). The effects of herbaceous plants on soil physicochemical properties, soil microorganisms and/or soil processes have rarely been investigated in forests. Previous studies suggested that the growth of herbaceous plants or deposition of their litter may increase microbial activity and the rate of soil processes (Chomel et al., 2016; Eisenhauer et al., 2011; Jandl et al., 1997; Rawlik et al., 2021). Rawlik et al. (2021) observed that the decomposition rate of herb plant litter was generally faster than that of trees and shrubs in oak-hornbeam forest. According to Chomel et al. (2016), the decomposition rate of tree litter and N release in boreal forest plantations were increased due to the addition of herbaceous litter to decomposing tree litter, and this phenomenon was mediated by higher abundance of detritivores and fungi. On the other hand, Cornwell et al. (2008) concluded that herbaceous litter did not decompose faster than woody species litter.

The influence of herb plants on forest soil and its processes depends on species identity and functional group (Cornwell et al., 2008; Gray et al., 2012; Rawlik et al., 2021). Litter of graminoids decomposed relatively slowly when compared to that of forbs (Cornwell et al., 2008). Moreover, litter decay rate in forest soil was faster in the case of spring ephemerals than in winter-green or summer green herb plants (Rawlik et al., 2021). Gray et al. (2012) showed that soil properties in pine barrens differed between ericaceous, graminoid, and lichen-moss-dominated ground vegetation cover. Soil under graminoid communities, in contrast to that under lichen-moss communities, had high values of extractable ammonium, while soil under ericaceous plants was characterized by low bulk density (Gray et al., 2012).

High plant diversity may support soil microorganisms and their activity (Chen et al., 2020; Eisenhauer et al., 2011; Lange et al., 2015). A global meta-analysis indicated that microbial, bacterial, and fungal biomass, fungi/bacteria ratio, and microbial respiration increased in soils under plant mixtures when compared to monocultures, and these effects were consistent across various ecosystem types including forests, grasslands, and croplands (Chen et al., 2019). Plant species richness correlated positively with basal respiration and microbial biomass in a deciduous forest dominated by *Populus tremuloides* Michx. (Eisenhauer et al., 2011). High plant diversity significantly increased the amount of root exudates, as well as bacterial and fungal biomass in experimental conditions. The effect of high plant diversity was the strongest in the case of fungi, resulting in the increase of fungi/bacteria ratio in soil (Eisenhauer et al., 2017). Conversely, Chodak et al. (2016) concluded that the number of plant species had little effect on the functional diversity of microbial communities, i.e., their ability to degrade various organic compounds, in temperate forests. Positive responses of microbial communities to plant diversity reported by most studies may be associated with reduced evaporation, enhanced plant productivity, and higher phylogenetic and root trait diversity, which in turn may result in larger amounts and diversity of rhizodeposits supporting microbial growth (Chen et al., 2018; Eisenhauer et al., 2017; Manzoni et al., 2012; Steinauer et al., 2016; Thakur et al., 2015).

The differential effects of plants on soil are connected with the variability in their functional traits, including traits related to the deposition of leaf litter and the release of root exudates (Chomel et al., 2016; Gilliam, 2007; Rawlik et al., 2021; Van Kleunen et al., 2010). Firstly, the quality of herbaceous foliar litter is generally higher than that of trees. Herb foliage often contains much more nutrients, for example K, Mg, N, P, and less phenolics, and have lower C/N ratio (Chomel et al., 2016; Gilliam, 2007). Secondly, herbaceous plant species may also vary widely in the quality and quantity of litter, which correlates with the rate of decomposition of organic matter and other soil processes. For example, litter decomposition rate of a number of forest plant species was correlated positively with specific leaf area and negatively with leaf dry matter content, and the rate of this parameter was the highest at intermediate values of leaf N content (Rawlik et al., 2021). The increase in decomposition rate, N release, and greater colonization of litter by decomposers was associated with the admixture of herbaceous litter of higher quality (lower C/N and phenolics content) to tree litter (Chomel et al., 2016). The addition of easily accessible C compounds, either with high quality litter or rhizodeposits, may result in a priming effect, which leads to stimulation of microbial decomposition of more recalcitrant organic matter (Blagodatsky et al., 2010; Chomel et al., 2016; Kuzyakov et al., 2000).

The aim of this study was to assess the effects of herbaceous vegetation on soil physicochemical and microbial properties in deciduous temperate forests. Specifically, the soil properties of two forest habitats, representing beech forest and riparian forest, were compared between plots with a mixture of herbaceous plant species, different mono-dominant (dominated by one herbaceous species) plots, and plots with negligible cover of herbaceous vegetation. We hypothesized that: 1) the presence of herbaceous plants would increase microbial activity and biomass, and nutrient availability in soil; 2) this increase would be the highest in plots with plant species mixtures as high plant diversity supports microbial performance and soil processes; 3) the expected patterns would be essentially similar in both types of forest, which would confirm their generality.

2. Materials and methods

2.1. Study sites and plant species

The study was conducted in two well-preserved unmanaged forests, a beech forest and a riparian forest, that were over 100 years old. The beech forest was located in the Pazurek Nature Reserve near Olkusz (50° 20′ 05″ N, 19° 37′ 43″ E) and the riparian forest in the Mogilski Forest in Kraków (50° 03′ 20″ N, 20° 03′ 26″ E), Poland. The beech forest belonged to *Dentario enneaphylli-Fagetum* Oberd. 1957 ex W. et A. Matuszkiewicz 1960 (beech forest) growing on Leptosols (rendzinas) developed on limestone bedrock and dominated by *Fagus sylvatica* L. with the admixture of *Acer pseudoplatanus* L. and *Abies alba* Mill. The riparian forest belonged to *Ficario vernae-Ulmetum minoris* Knap 1942 em. J. Matuszkiewicz 1976 (ash-elm riparian forest) occurring in lowland river valleys with periodic floods and fine-grained Fluvisols – genetically young soils developed on recent fluvial deposits and characterized by weak horizon differentiation (FAO, 2015), dominated by *Ulmus* spp. with the admixture of *Quercus robur* L. and *Fraxinus excelsior* L. In each forest, 10 study sites were established, each comprising of a group of four neighboring plots (1 m^2 each). The four plots were located close to each other, namely at 1–3 m distance between the borders of the plots to avoid differences in initial soil properties not related to the presence of herbaceous plant species. Within each group, the following plot types were selected: 1) mono-dominant stand (>70% cover) of a common herbaceous species (species 1); 2) mono-dominant stand (>70% cover) of another common herbaceous species (species 2); 3) mix of forest herbaceous plant species (>35% cover), and 4) bare soil, i.e., with negligible (<10%) cover of herbaceous plants (control). The selected plant species and the mixtures were typical for

each forest type. In the case of the beech forest, the species were *Allium ursinum* L. (Amaryllidaceae) and *Dentaria enneaphyllos* L. (syn. *Cardamine enneaphyllos* (L.) Crantz; Brassicaceae), while in the riparian forest these were *Aegopodium podagraria* L. (Apiaceae) and *Ficaria verna* Huds. (syn. *Ranunculus ficaria* L.; Ranunculaceae). These species were selected as they commonly occur in the respective forest types, form compact mono-dominant patches, and have contrasting functional traits. Moreover, the patches of particular species and species mixtures were located relatively close to each other within a particular forest, which was crucial for the purpose of this study. *Allium ursinum* (bear garlic, wild garlic) is a bulbiferous geophyte, growing up to 25 cm tall. Its blooming usually starts in April and ends in the first half of May. *Dentaria enneaphyllos* (nine-leaved toothwort) is a geophyte, growing up to 60 cm tall. It flowers from April to May and its foliage dies in late spring. *Aegopodium podagraria* (goutweed, ground elder, bishop's weed) is a hemicryptophyte, growing up to 1 m and spreading mainly by rhizomes. This species flowers from May to July and its biomass ages in the autumn. *Ficaria verna* (fig buttercup, lesser celandine) is a tuberous rooted geophyte, up to 10 cm tall. It starts growing in late winter and flowering in the early spring. *A. ursinum*, *D. enneaphyllos*, and *F. verna* are spring ephemerals that grow, bloom, and bear fruits before full development of tree canopy (Zubek et al., 2021 and references therein). The four plot types differed in plant species number and cover (Zubek et al., 2021). Plots with a mix of herbaceous plant species were characterized by significantly higher plant species number (on average 6.7 species in beech forest and 5.7 species in riparian forest) than mono-dominant plots (on average 3.9, 3.4, 2.9, and 3.0 species for *A. ursinum*, *D. enneaphyllos*, *A. podagraria*, and *F. verna* plots, respectively). Conversely, total plant cover was significantly higher in mono-dominant plots (on average 83, 84, 92, and 91% for *A. ursinum*, *D. enneaphyllos*, *A. podagraria*, and *F. verna* plots, respectively) than on plant mixture plots (on average 57% in beech forest and 71% in riparian forest). In mono-dominant plots, species other than the dominant ones made a negligible contribution to the total plant cover. More information on the study sites, sampling plots, and herbaceous plant species is provided by Zubek et al. (2021).

2.2. Soil sampling

In May 2019, three soil subsamples (10 cm × 10 cm each) were collected at each plot with a shovel to a depth of ca. 15 cm. Fragmented litter (soil organic horizon) was removed and the samples were pooled in a plastic bag to form one composite sample per plot. To prevent contamination, the shovel was cleaned from soil particles and disinfected with 70% ethanol before digging, and sterile gloves were used during sampling. Soils were transported in plastic bags to the laboratory. In total, 80 soil samples were collected: 2 forests × 4 plot types × 10 sites (replicates).

2.3. Laboratory work

2.3.1. Soil physicochemical analyses

Prior to physicochemical analyses, the soil samples were air-dried and sieved (2 mm mesh). Soil moisture was determined after drying the samples overnight at 105 °C (Ecocell; BMT). Maximum water-holding capacity (WHC) was determined according to Öhlinger (1996), with minor modifications. Soil texture, i.e., sand, silt, and clay contents, was determined in air-dried samples through a combination of sieving and sedimentation (ISO 11277, 1998). Soil pH was measured in air-dried samples in 1:5 (w:v) water suspensions with a HQ40d meter (Hach; ISO 10390, 1994). Organic C and total N were analyzed in soil samples dried at 105 °C and ground (Pulverisette 0; Fritsch). Organic C content was determined with a dry combustion analyzer RC-612 (Leco; ISO 10694, 1995). Total N was measured using the Kjeldahl method; samples were digested in H_2SO_4 with Kjeltabs ($K_2SO_4 + CuSO_4 \cdot 5H_2O$; Digestor 20 Auto; Foss Tecator) followed by distillation on a Kjeltec 2300 Analyzer Unit (Foss Tecator; Application Note AN 300). In order to determine total Ca, K, Mg, and P concentrations, ground soil samples were dried at 105 °C and digested in hot concentrated $HClO_4$ (Digestor 40 Auto; Foss Tecator). Exchangeable Ca, K, and Mg were extracted from air-dried soil samples by shaking in 0.1 M $BaCl_2$ three times for 1 h (PN-EN ISO 11260, 2011). Extracted metals were analyzed with flame atomic absorption spectrometry (AA280FS; Varian) and total P was measured colorimetrically (DR 3800; Hach Lange) using vanadate-molybdate method. To analyze water-extractable N-NH_4, N-NO_3, P-PO_4, and S-SO_4 concentrations, air-dried soil samples were shaken (Laboratory Shaker type 358S; elpan) in water for 1 h (1:10, w:v) and filtered through cellulose acetate membrane syringe filters. N-NO_3, P-PO_4, and S-SO_4 concentrations in the extracts were determined with an ion chromatograph ICS-1100 (Dionex), while N-NH_4 was determined with an ion chromatograph DX-100 (Dionex).

2.3.2. Soil microbiological analyses

Soil samples for microbiological analyses were kept frozen at −20 °C until use. Soil microbial activity was represented by basal respiration and the activities of four enzymes associated with N, P, and S cycling, namely urease, acid and alkaline phosphomonoesterases (phosphatases), and arylsulfatase. Microbial biomass was calculated on the basis of substrate-induced respiration (SIR) and phospholipid fatty acid (PLFA) analyses, and microbial community structure was characterized using PLFA analysis.

Soil respiration and substrate-induced respiration were determined in samples adjusted to 50% WHC. The soil samples were incubated in small glass ampoules at 22 °C in the dark for approx. 20 h (New Brunswick Innova 42R Shaker; Eppendorf). The concentration of CO_2 was measured with a gas chromatograph equipped with a methanizer and a flame ionization detector (GC-FID 450; Bruker). The PoraPLOT Q (27.5 m × 0.53 × 0.70) column (Agilent Technologies) was used. Helium was used as a carrier gas, and injections were made in splitless mode. After the soil basal respiration measurements, a mixture of talcum and glucose monohydrate (4:1; 10 mg glucose g^{-1} dw soil) was added and mixed into the soil samples in order to measure SIR after about 4 h incubation. The concentration of released CO_2 was determined as described above. Blank samples were included to estimate ambient CO_2 concentrations (Beck et al., 1996, modified; Rousk and Frey, 2015).

To measure acid and alkaline phosphatase activities, soil samples were incubated at 37 °C for 1 h after the addition of a buffered *p*-nitrophenyl phosphate solution. Released *p*-nitrophenyl was extracted and colored with NaOH after the addition of $CaCl_2$, and determined photometrically at 400 nm (Margesin, 1996) using a colorimeter DR 3800 (Hach Lange). To measure arylsulfatase activity, soil samples were incubated for 1 h at 37 °C after the addition of acetate buffer (pH = 5.8) and *p*-nitrophenylsulfate solution. Then distilled water and NaOH solution were added. The content of p-nitrophenol was determined photometrically at 420 nm (Strobl and Traunmuller, 1996) with a colorimeter DR 3800 (Hach Lange). To measure urease activity, soil samples were incubated for 2 h at 37 °C after the addition of urea solution. Released ammonium was extracted with KCl solution and determined by a modified Berthelot reaction. It is based on the reaction of sodium salicylate with NH_3 in the presence of sodium dichloroisocyanurate, which forms a green-colored complex under alkaline conditions. Sodium nitroprusside was used as a catalyst, which increases the sensitivity of the method about tenfold (Kandeler, 1996). The urease activity was measured photometrically at 690 nm with a colorimeter DR 3800 (Hach Lange).

PLFA analysis was performed according to Palojärvi (2006), with the exception of the lipid extraction which followed Macnaughton et al. (1997). Lipids were extracted from freeze-dried (Freeze Dry System; Labconco) soil with a mixture of methanol/chloroform/phosphate buffer (2/1/0.8, v/v/v) using accelerated solvent extractor ASE 200 (Dionex; two 15 min cycles, 80 °C, 1200 PSI). Following the extraction,

an appropriate volume of chloroform and deionized water was added to give the correct final ratio (chloroform/methanol/phosphate buffer/water; 1/1/0.9, v/v/v) and form two phases. The chloroform layer was evaporated under nitrogen at 40 °C. The lipids were separated into neutral-, glyco-, and phospholipids in Bakerbond silica gel SPE columns (500 mg; Baker) by eluting with chloroform, acetone, and methanol, respectively. The methanol fraction was reduced to dryness under nitrogen. The phospholipids were subjected to mild alkaline methanolysis and the resulting fatty acid methyl esters were separated and identified using a GC–MS system (Varian 3900 and Saturn 2100T) and NIST library. The Select FAME (100 m × 0.25 × 0.36) column (Agilent Technologies) was used. Helium was used as a carrier gas, and injections were made in split mode (1:10). Individual fatty acids were identified relative to several standards: 37-component FAME Mix (Supelco), Bacterial Acid Methyl Ester (BAME) Mix (Supelco), and a few additional one-component standards (Sigma-Aldrich, Matreya LLC). Methyl nonadecanoate (19:0; Fluka) was used as an internal standard.

2.3.3. Calculations and statistical analyses

SIR-biomass was calculated according to the equation: SIR-biomass ($\mu g\ g^{-1}$) = 40.04x + 0.37, where x is the respiration rate given in $\mu L\ CO_2\ h^{-1}g^{-1}$ (Anderson and Domsch, 1978). The sum of twenty PLFAs (those with >0.5% of the total relative abundance in most soil samples, namely 14:0, 14:1, 15:0, a15:0, i15:0, 16:0, i16:0, 16:1ω5, 16:1ω7, 17:0, a17:0, i17:0, cy17:0, 17:1, 18:0, 18:1ω7, 18:1ω9, 18:2ω6, cy19:0, 20:0) was calculated and used as an indicator of total microbial biomass. Bacteria were represented by the sum of a15:0, i15:0, i16:0, 16:1ω7, 17:0, a17:0, i17:0, cy17:0, 18:1ω7, and cy19:0. The sum of a15:0, i15:0, i16:0, a17:0, and i17:0 was an indicator of gram-positive (G+) bacteria, while the sum of 16:1ω7, cy17:0, 18:1ω7, and cy19:0 was an indicator of gram-negative (G−) bacteria. Saprotrophic fungi were represented by18:2ω6. Fungal/bacterial PLFA and G+/G− PLFA ratios were also calculated.

The types of plots in the beech forest were not the same as the types of plots in the riparian forest (mainly due to different herbaceous species in mono-dominant patches); therefore, statistical analyses were performed separately for both forest habitats. The data collected in this study had a hierarchical structure as each site contained four different plots. Typically, in such a situation, the site identifier (site ID) is taken as a random factor and the data are analyzed using mixed-effects models. However, we decided to treat the site ID as a fixed factor, which entailed the use of two-way analysis of variance without replication. The advantage of this approach is that it provides a measure of the variance component among the sites (Sokal and Rohlf, 2009), which is helpful in interpreting the results (sites turned out to be highly variable in terms of soil properties at the stage of data exploration). Prior to statistical analyses, variables were checked for normality using graphical tools (histogram, quantile-quantile plot) and transformed, if necessary, with a logarithmic or exponential function. The effects of the plot type and the site on the soil properties were determined using two-way permutational multivariate analysis of variance (PERMANOVA) without replication, which was based on the Euclidean resemblance matrices (Anderson et al., 2008). The routine was run with a posteriori pairwise comparisons among levels of the plot type factor. PERMANOVA was carried out separately for three soil datasets: 18 physicochemical parameters and 13 microbiological parameters representing microbial activity and biomass (Table S1) as well as 20 PLFAs (relative concentrations) representing microbial community structure (Table S3). For the purpose of interpreting the results of PERMANOVAs, the variables from Table 1 were subjected to univariate analysis: two-way ANOVA without replication followed by Tukey post-hoc comparisons. To visualize the PERMANOVA results, principal coordinates analysis (PCoA) was used; it was also based on Euclidean distances. To understand the main gradients in the soil data, factor analysis was performed separately for physicochemical and microbiological parameters. The number of factors was determined by parallel analysis. Factors were varimax-rotated to improve their interpretation. The degree of dependence between soil physicochemical and microbiological parameters was expressed by Pearson's correlation coefficients for pairs of factors extracted from both datasets. Statistical analyses were conducted in R 3.3.3 (R Core Team, 2017) and PRIMER 7 with the PERMANOVA+ package (Anderson et al., 2008).

Table 1
The effects of plot type (absence vs presence of different herbaceous plant cover) and site on compositional data – physicochemical properties, microbial activity and biomass, and microbial community structure (PLFA relative concentrations) as shown by F- and *p*-values derived from two-way PERMANOVA without replication.

Soil variables	Beech				Riparian			
	Plot type		Site		Plot type		Site	
	F	*p*	F	*p*	F	*p*	F	*p*
Physicochemical properties	1.16	0.30	2.24	**0.001**	0.97	0.52	6.41	**0.0001**
Microbial activity and biomass	1.69	0.07	3.27	**0.0001**	2.68	**0.003**	4.66	**0.0001**
Microbial community structure	1.30	0.22	6.10	**0.0001**	1.65	0.09	7.05	**0.0001**

Statistically significant ($p < 0.05$) effects are given in bold.

3. Results

The beech forest soil, in contrast to riparian forest soil, was characterized by relatively high content of sand and relatively low contents of fine particles, K, and Mg (Table S1). Loamy sand and sandy loam dominated in the beech forest, and silt loam in the riparian forest. Soil physicochemical properties hardly responded to the presence of herbaceous plant species (Table S2). The only soil physicochemical variable that differed significantly ($p < 0.05$) between the plot types was pH in the beech forest; it was the lowest in bare soil and the highest under *D. enneaphyllos* (Fig. 1). Soil microbial properties were more responsive to the presence of herb plant species, but mainly in the riparian forest (Table S2). In the beech forest, herbaceous plants clearly supported saprotrophic fungi. Fungal biomass and fungi/bacteria ratio were higher in soil under single- and multi-species plant cover than in bare soil (Fig. 1). Arylsulfatase activity followed a similar pattern, but the effect of plot type was marginally significant ($p = 0.059$). Other microbial parameters were not affected by plot type in the beech forest (Table S2). In the riparian forest, plot type significantly ($p < 0.05$), but differently, depending on the parameter, influenced soil respiration, the activity of phosphatases, bacterial (G−) and fungal biomass, and fungi/bacteria and G+/G− bacteria ratios (Table S2). Bare soil was characterized by lowest respiration, bacterial (G−) and fungal biomass, fungi/bacteria ratio, and highest acid phosphatase activity and G+/G− ratio compared to the other plot types (Fig. 2). Soil under *A. podagraria* was characterized by highest respiration, alkaline phosphatase activity, G− bacterial and fungal biomass, and fungi/bacteria ratio. Acid and alkaline phosphatases exhibited lowest values in soils under *F. verna* (Fig. 2). Generally, mono-dominant patches of herbaceous vegetation, especially those with *A. podagraria*, had beneficial effects on microbial parameters. In contrast, the patches with plant species mixtures did not differ in their effect from the plots with bare soil in the riparian forest. The effect of site was statistically significant ($p < 0.05$) in the case of many parameters in beech forest and almost all parameters in riparian forest (Table S2).

Absolute and relative amounts of phospholipid fatty acids are presented in Table S3. When the effect of plot type was analyzed using two-way PERMANOVA, i.e., taking into account many variables simultaneously, it turned out to be significant only in the case of the soil microbial activity and biomass in the riparian forest ($p = 0.003$; Table 1). According to post-hoc comparison tests, the bare riparian soil differed significantly ($p < 0.05$) from the *A. podagraria* and *F. verna* soil, and

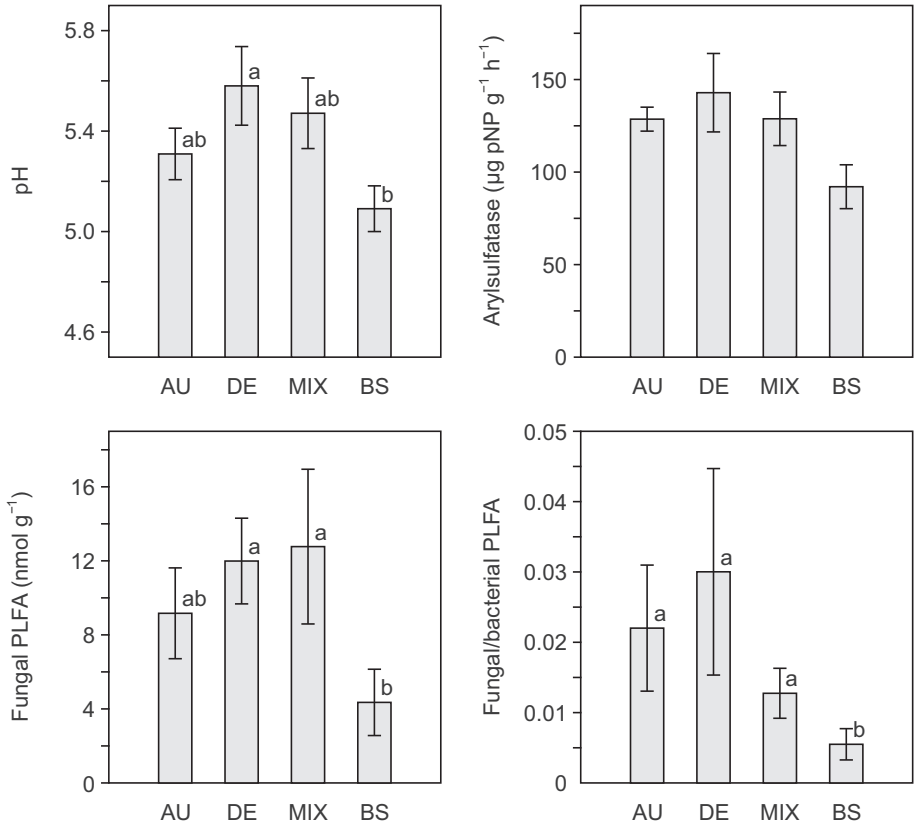

Fig. 1. Means (±standard errors) of selected soil properties calculated for four types of plots (AU – mono-dominant patch with *Allium ursinum*, DE – mono-dominant patch with *Dentaria enneaphyllos*, MIX – species mix, BS – bare soil) in the beech forest. Bars marked with different letters significantly differ from each other by Tukey's test ($p < 0.05$) (see the text and Table S2 for details).

the soil under species mix differed significantly from the *A. podagraria* soil. The PCoA diagram (Fig. 3) shows the nature of these differences; plots with *A. podagraria* and, to a lesser extent, those with *F. verna*, are shifted downwards and to the left of plots with bare soil and species mix, i.e., towards higher values of most microbial parameters, especially soil respiration. The effect of plot type on soil microbial activity and biomass in the beech forest and on the microbial community structure in the riparian forest were only marginally significant ($p < 0.1$), while in the case of soil physicochemical properties in both forests and the microbial community structure in the beech forest, the effect of plot type was not significant ($p > 0.1$). PERMANOVAs revealed that the effect of site was significant ($p < 0.01$) for all sets of variables in both forests Table 1.

Factor analysis reduced soil physicochemical variables to two factors in the beech forest and three factors in the riparian forest. These factors explained 64.2% and 66.6% of variance in physicochemical data in the beech and riparian forests, respectively (Table 2). In the case of the beech forest, factor 1 (F1pb) correlated positively with concentrations of base cations, contents of fine particles, organic C, N, moisture, and pH. Factor 2 (F2pb) was related with available S, N, and P concentrations. In the case of riparian forest, factor 1 (F1pr) was related to organic C, N, K, and Mg. Factor 2 (F2pr) was correlated positively with silt and N-NH_4 concentrations, and factor 3 (F3pr) correlated positively with soil alkalinity and Ca concentrations, and negatively with P and S availability.

Factor analysis reduced microbiological variables to three factors in the beech forest and three factors in the riparian forest. The factors explained 77.4% and 72.9% of variance in microbiological data in the beech and riparian forests, respectively (Table 3). In the case of the beech forest, factor 1 (F1mb) represented PLFA-derived total and bacterial biomass, and enzymatic activity, factor 2 (F2mb) represented SIR-biomass and soil respiration, and factor 3 (F3mb) correlated positively with fungal biomass and fungi/bacteria ratio. In the riparian forest, factor 1 (F1mr) was related to bacterial and total biomass, factor 2 (F2mr) represented fungal biomass and fungi/bacteria ratio, and factor 3 (F3mr) correlated with enzymatic activity and soil respiration.

Soil microbial parameters were significantly ($p < 0.05$) influenced by soil physicochemical parameters. Specifically, in the beech forest, F1pb (base cations, N, organic C, fine particles, and moisture) correlated positively with F1mb (bacterial and total microbial biomass, and enzymatic activity; $r = 0.77$, $p < 0.001$), and F2pb (S, N, and P availability) correlated positively with F3mb (fungal biomass and fungi/bacteria ratio; $r = 0.44$, $p = 0.005$). In the riparian forest, F1pr (organic C, N, K, and Mg) correlated positively with F1mr (bacterial and total microbial biomass; $r = 0.37$, $p = 0.018$) and F3mr (soil enzymatic activity and respiration; $r = 0.52$, $p < 0.001$). F2pr (fine soil particles and N-NH_4) correlated positively with F2mr (fungal biomass and fungi/bacteria ratio; $r = 0.42$, $p = 0.007$) and negatively with F1mr (bacterial and total microbial biomass; $r = -0.40$, $p = 0.011$).

4. Discussion

Although herbaceous plant layer comprises a large part of forest plant diversity and may play an important role in nutrient turnover due to higher quality of herbaceous litter relative to that of trees, this stratum and its effects on the soil environment remained largely unexplored (Baldrian, 2017; Elliott et al., 2015; Gilliam, 2007; Rawlik et al., 2021). Our study was designed to assess the effects of both mono-dominant patches and multi-species assemblages of herbaceous plant species in beech and riparian forests on soil physicochemical and

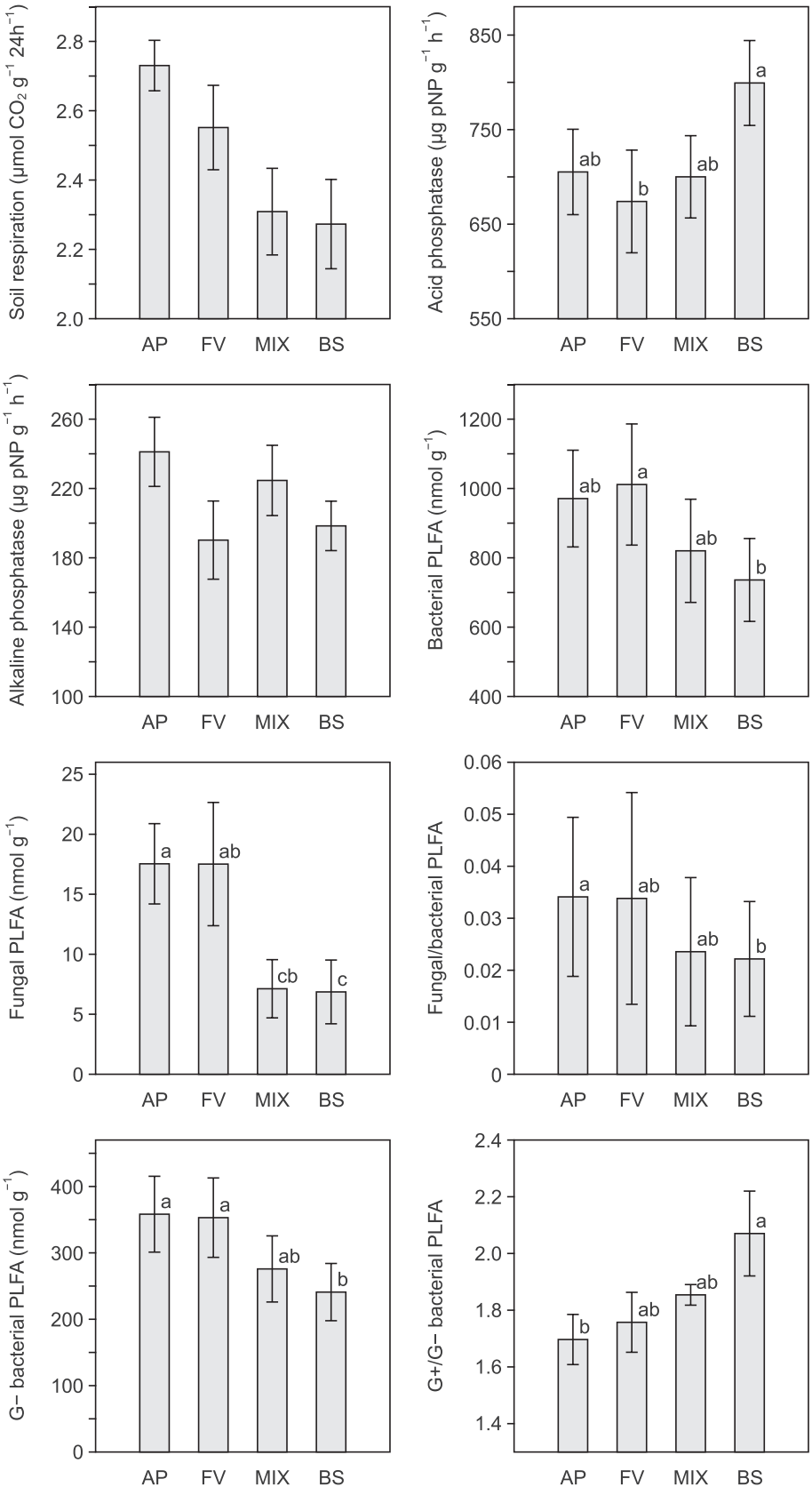

Fig. 2. Means (±standard errors) of selected soil properties calculated for four types of plots (AP – mono-dominant patch with *Aegopodium podagraria*, FV – mono-dominant patch with *Ficaria verna*, MIX – species mix, BS – bare soil) in the riparian forest. Bars marked with different letters significantly differ from each other by Tukey's test ($p < 0.05$) (see the text and Table S2 for details).

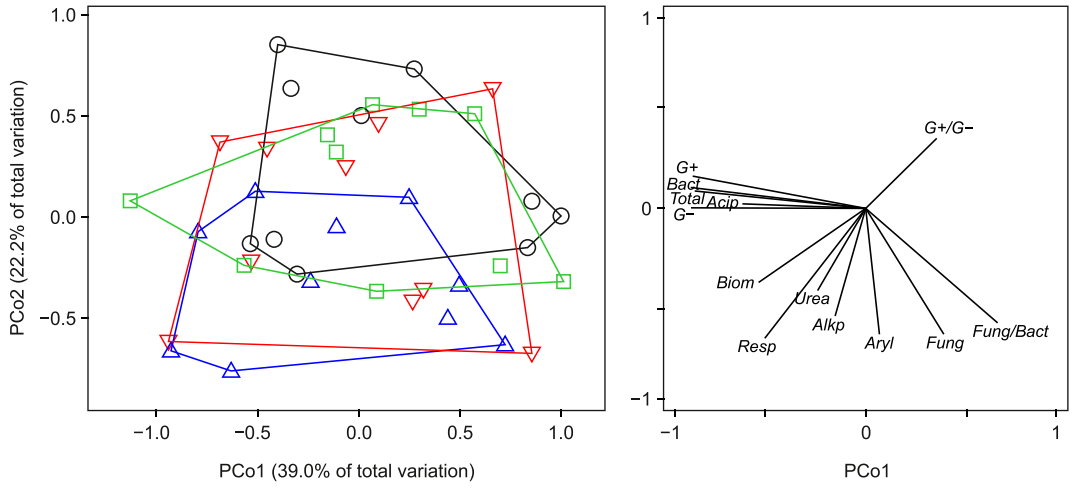

Fig. 3. The results of principal coordinates analysis (PCoA), based on Euclidean distances, for the 13 soil microbiological parameters measured in the riparian forest. The left diagram shows the position of four types of plots (up-pointing blue triangle – *Aegopodium podagraria*, down-pointing red triangle – *Ficaria verna*, green square – species mix, black circle – bare soil) in the ordination space; plots of the same type were enveloped. The right diagram shows the projection of microbiological parameters onto the ordination space.
Acip – acid phosphatase activity, Alkp – alkaline phosphatase activity, Aryl – Arylsulfatase activity, Urea – Urease activity, Resp – Respiration, Bact – bacterial PLFA, G+ – G+ bacterial PLFA, G−G − bacterial PLFA, G+/G− – G+/G− bacterial PLFA ratio, Fung – fungal PLFA, Fung/Bact – fungal/bacterial PLFA ratio, Total – total PLFA, Biom - SIR biomass.

microbial parameters relative to bare soil (i.e., soil with negligible cover of herbaceous species). The study showed that herbaceous vegetation significantly modified soil properties, which is in accordance with our hypothesis. However, in contrast to our expectations, the effects of herbaceous plants on soil were limited to microbiological parameters, while soil physicochemical variables generally did not depend on vegetation.

The presence of herb plants supported microbial performance in both beech and riparian forests, which was reflected in higher values of several microbial parameters, mainly microbial biomass. We suppose that the positive effect of herbaceous plants on soil microorganisms found in this study may be associated with high plant cover at the time of soil sampling, which was related to high density of plant roots in the topsoil. This in turn likely resulted in the release of rhizodeposits which contain, for example, sloughed-off cells and tissues, border cells, soluble lysates, mucilage, and root exudates rich organic acids, amino acids, sugars, and phenolics. They may act as a nutrient source for microbes and promote their growth and activity (Haichar et al., 2014; Tian et al., 2020). The rhizosphere may contain up to 10^{11} microbial cells per gram dry matter, which is hundreds to thousands fold higher than in the bulk soil (Tian et al., 2020). It is considered a microbial hotspot where the fraction of active microorganisms is 2–20 times higher than in the bulk soil, and microbial activities, for example respiration, microbial growth, mineralization potential, and enzyme activities may be much higher as well (Kuzyakov and Blagodatskaya, 2015). The root exudates may exhibit a rhizosphere priming effect towards the degraders of soil organic matter and influence C and N cycling (Haichar et al., 2014; Henneron et al., 2020; Hinsinger et al., 2009). However, as neither root exudates nor root biomass measurements were included in our study, further research is needed to gain insight into the role of rhizodeposition of herb plants in shaping microbial communities and processes in forest soil.

The quantity and quality of litter also play an important role in shaping soil microbial communities and other soil properties (Gilliam, 2007; Rawlik et al., 2021). Herbaceous plants have been suggested to increase nutrient availability and the rate of organic matter decomposition due to higher quality of their foliage. They contained 30% more N and P, while concentrations of Mg and K were twofold and threefold higher

Table 2
Results of factor analysis for soil physicochemical properties.

Factor	Variance explained (%)	Variables with the highest factor loadings
Beech forest		
F1pb	46.1	**Ca_{EX} (0.95)**, **Mg_{EX} (0.92)**, **Mg (0.91)**, **K (0.88)**, **N (0.86)**, **Sand (−0.83)**, **Moisture (0.81)**, **Silt (0.81)**, **C_{ORG} (0.75)**, K_{EX} (0.67), Ca (0.67), pH (0.65)
F2pb	18.1	**S-SO_4 (0.86)**, N-NH_4 (0.63), N-NO_3 (0.62), P-PO_4 (0.59), P (0.54), pH (−0.50)
Riparian forest		
F1pr	27.8	**N (0.82)**, **C_{ORG} (0.81)**, **K_{EX} (0.78)**, **K (0.77)**, **Mg (0.70)**, N-NO_3 (0.64), Mg_{EX} (0.60), Moisture (0.59), Clay (0.58), Ca_{EX} (0.57)
F2pr	19.9	**Sand (−0.84)**, **Silt (0.81)**, **N-NH_4 (0.74)**, Moisture (0.53)
F3pr	18.9	**pH (0.75)**, **P-PO_4 (−0.74)**, **Ca_{EX} (0.72)**, **Ca (0.70)**, S-SO_4 (−0.67)

Variables with factor loadings higher than 0.7 are given in bold.
EX – exchangeable, ORG – organic.

Table 3
Results of factor analysis for soil microbiological properties.

Factor	Variance explained (%)	Variables with the highest factor loadings
Beech forest		
F1mb	45.1	**Bact (0.97)**, **Total (0.97)**, **G+ (0.96)**, **G− (0.96)**, **Alkp (0.75)**, Acip (0.68), Aryl (0.62), Urea (0.58)
F2mb	16.3	**Biom (0.87)**, **Resp (0.86)**
F3mb	16.0	**Fungi (0.91)**, **Fung/Bact PLFA (0.90)**
Riparian forest		
F1mr	34.9	**G− (0.98)**, **Bact (0.96)**, **G+ (0.94)**, **Total (0.93)**, G+/G− (−0.53)
F2mr	19.9	**Fung (0.93)**, **Fung/Bact (0.82)**, Aryl (0.66)
F3mr	18.1	**Urea (0.74)**, Resp (0.69), Alkp (0.68), Biom (0.61), Acip (0.53)

Variables with factor loadings higher than 0.7 are given in bold.
Acip – acid phosphatase activity, Alkp – alkaline phosphatase activity, Aryl – Arylsulfatase activity, Urea – Urease activity, Resp – Respiration, Bact – bacterial PLFA, G+ – G+ bacterial PLFA, G− – G− bacterial PLFA, Fung – fungal PLFA, Fung/Bact – fungal/bacteria PLFA ratio, Total – total PLFA, Biom – SIR-biomass.

than that of trees, respectively (Gilliam, 2007; Muller, 2003). However, we suppose that in our study litter effects on soil were minor relative to those of rhizodeposition as soil sampling was done at the peak of herb plant biomass, prior to plant senescence and litterfall. The increase in microbial activity, biomass, functional diversity, and/or in the rate of soil processes due to the presence of herbaceous plant species has been reported earlier in both temperate forests (Rawlik et al., 2021) and in anthropogenic disturbed habitats (D'Hervilly et al., 2021; Stefanowicz et al., 2015). For example, Rawlik et al. (2021) observed that the decomposition rate of herb plant litter was generally faster than that of trees and shrubs. Soils under herbaceous strips had higher microbial biomass (per soil organic C) and microbial efficiency (lower metabolic quotient) when compared to soils without herb plants in an agroforestry system (D'Hervilly et al., 2021). Three herbaceous plant species growing in monocultures on coal mine spoils strongly increased soil basal respiration, bacterial activity, and bacterial functional richness when compared to bare spoils (Stefanowicz et al., 2015). In turn, microbial biomass and activity were either higher or lower, or did not differ between maquis trees and grasses, depending on management practices and soil type (Panico et al., 2018).

Plant species are expected to vary in their influence on soil microorganisms and processes as these species differ in their life strategies and functional traits, including the quality and quantity of their root exudates and litter (Henneron et al., 2020; Jagodziński et al., 2016; Orwin et al., 2010; Rawlik et al., 2021; Rothstein and Zak, 2001; Wardle et al., 2004). Rawlik et al. (2021) reported that the litter of spring ephemerals decomposed faster than those of winter-greens and summer-greens in oak-hornbeam forest. Bonanomi et al. (2021) analyzed decomposition of leaf and fine root litter of 43 plant species and found that litter decay rate was negatively correlated with litter lignin content, lignin/N and C/N ratios, and positively with N content, but these relationships were modified by temperature and litter type. Plant species that have N-rich, high-quality litter are thought to promote bacterial–based foodweb and accelerate the rate of soil processes (Liao et al., 2008; Orwin et al., 2010; Wardle et al., 2004). Similarly, the composition of root exudates depends on plant species identity and, to some extent, on whether a plant belongs to grasses or forbs (Herz et al., 2018). In our study, the effect of herb plant species on soil depended on plant species identity. In the beech forest, the highest values of all affected parameters, i.e., pH, arylsulfatase activity, fungal biomass, and fungi/bacteria ratio, were found under *D. enneaphyllos*, with lower values under *A. ursinum* and multi-species assemblage. To our knowledge, the effect of *D. enneaphyllos* on soil has not been studied so far and *A. ursinum* is known to produce phenolic compounds that may accumulate in the surface soil (Djurdjevic et al., 2004). These compounds may potentially affect soil microorganisms and processes, either positively or negatively, depending on their chemical characteristics (Bardon et al., 2014; Stanek et al., 2021; Zwetsloot et al., 2020). *Allium ursinum* has been reported to exhibit weak activity against bacteria and fungi of medicinal importance (Krstin et al., 2018); however, there is only limited information on its effect on soil microbial processes which suggests that *A. ursinum* may accelerate the decomposition of organic matter and nutrient release. Aboveground litter of *A. ursinum* has high nutrient contents and decomposes in favorable temperature conditions in early summer within a few weeks (Jandl et al., 1997). In the riparian forest, *A. podagraria* seemed to have the most beneficial effects on microbes as many soil microbial properties and processes, namely respiration, alkaline phosphatase activity, G− bacterial biomass, and fungal biomass were the highest under this species. In contrast to our study, Rawlik et al. (2021) reported that *A. podagraria* litter exhibited lower decomposition rate than that of *F. verna*, which was possibly related to its functional traits – lower specific leaf area and N content, and higher dry matter content. In our study, *A. podagraria* supported fungi to a greater extent than bacteria as indicated by its high soil fungi/bacteria ratio. Fungi, in contrast to bacteria, are associated with lower decomposition rates and nutrient availability and an increase in fungi/bacteria ratio should reflect reduced nutrient availability and slower growth rates (Orwin et al., 2018; Wardle et al., 2004). Additionally, G+/G− bacteria ratio was the lowest under *A. podagraria*. G+ bacteria may use more recalcitrant substrates than G− bacteria in conditions of high N availability, and they are also more drought tolerant (Orwin et al., 2018). Therefore, they may serve as indicators of changes in soil physicochemical properties. In our study, however, neither soil moisture nor N availability did not differ between plot types, which indicates that other factors not included in the analyses might have played a role in modifying the bacterial community structure.

Plant species diversity had less beneficial effect on soil than expected as soil microbial parameters reached intermediate values in plots with plant species mixtures. This contradicts results of many previous studies which suggest that species diversity/richness of herb plants in forest and grassland ecosystems is positively correlated with microbial activity, biomass, and/or the rate of soil processes (Chen et al., 2019; Eisenhauer et al., 2011, 2017; Lange et al., 2015; Thakur et al., 2015). For example, plant species richness of herbaceous layer influenced positively soil basal respiration and microbial biomass in a deciduous forest dominated by *Populus tremuloides* (Eisenhauer et al., 2011). Analysis of long-term data from a grassland diversity experiment showed that higher plant diversity resulted in increased microbial activity and C storage (Lange et al., 2015). A global meta-analysis showed that microbial biomass, bacterial biomass, fungal biomass, fungi/bacteria ratio, and microbial respiration increased in soils under plant mixtures when compared to monocultures, and these effects were consistent across various ecosystem types including forests, grasslands, and croplands (Chen et al., 2019). On the other hand, Chodak et al. (2016) concluded that the number of plant species had little effect on the functional diversity of microbial communities, i.e., their ability to degrade various organic compounds. Positive responses of microbial communities to plant diversity may be associated with several mechanisms. High plant diversity often results in enhanced plant productivity which in turn affects soil microorganisms and processes (Chen et al., 2018; Thakur et al., 2015). Diverse plant communities are characterized by higher phylogenetic and root trait diversity which may result in larger amounts and diversity of rhizodeposits supporting microbial growth (Eisenhauer et al., 2017; Lange et al., 2015; Steinauer et al., 2016; Thakur et al., 2015). Finally, dense vegetation reduces evaporation and higher soil moisture supports microbial performance (Manzoni et al., 2012). In our study, however, plots with a mix of herbaceous plants were characterized by lower plant cover than monodominant patches, which might have been responsible for the relatively weak effect of herb plants on soil.

In this study, the influence of herbaceous plant species on soil physicochemical properties was negligible. It is possible that soil conditions are shaped more by the decaying litter of herbaceous plants (Gilliam, 2007; Jandl et al., 1997; Rawlik et al., 2021) than by their root exudates. As nutrient fluxes from litter decomposition are seasonal, they might have not been relevant in our study in which soil sampling was performed at the peak of herb plant biomass. In other words, the inconsistent effects of herbaceous plant species on soil may result from the fact that soil microbes and other soil properties are either more rhizodeposition- or more litter-driven, depending on a plant growth stage. Moreover, soil chemistry is considered to be more stable in time when compared to soil microbial parameters such as the composition of microbial communities and the activity of microbial enzymes (Baldrian, 2014, 2017). The results of the statistical analyses showed that there is great variability in the data at the site level. This could be expected, taking into account the fact that the heterogeneity of the soil environment can be large even on a small spatial scale (Šťursová et al., 2016). Although efforts were made to ensure that the study areas were homogeneous in terms of tree species composition and soil type, the differences between sampling sites could not be avoided. The varied terrain was probably the main source of these differences. The beech forest was located on limestone hills, in slightly undulating terrain, which caused the variability of the surface slope and thus the

factors influencing the soil formation processes. The riparian forest, although located in a relatively flat area, contained depressions and elevations. They were small, but could generate considerable variation in soil moisture and the amount of accumulated river sediments. The fact that in statistical models the effect of site identity clearly exceeded the effect of the plot type may mean that the biological significance of differences in the herbaceous layer (its presence/absence and species composition) for the soil physicochemical and microbiological properties on the forest scale is relatively small.

Soil chemical and microbial parameters are largely controlled by soil physical properties such as texture and/or moisture (Baldrian, 2014; Chodak and Niklińska, 2010; Obayomi et al., 2021) and this was also observed in our study as higher contents of fine particles (silt and/or clay) in soil were typically associated with higher moisture, element concentrations, and/or microbial activity or biomass, though these relationships depended to some extent on the forest type. The strong effects of site on many soil parameters may indicate that the spatial soil heterogeneity related to texture and element content play a primary role in shaping soil microbial communities on a larger spatial scale, while the quantitative and qualitative differences in herbaceous layer species are more important for soil microbes on a smaller scale. Baldrian (2014) concluded that distribution of soil enzymatic activity is affected on a large scale (>1 km^2) by land use type, dominant vegetation, pH, nutrient contents, and long-term moisture, whereas on smaller scales by, among others, patches of available substrate, tree species and distance from trees, as well as the presence of plant roots.

Concluding, this study showed that the presence of herbaceous plant species affected soil microbial communities in beech and riparian forests. Many parameters, in particular microbial biomass, were significantly increased under herb plants when compared to bare soil, mainly in riparian forest. The effect of herb plants on soil also depended on plant species identity. Plant species richness had smaller influence on soil than expected as soil microbial parameters exhibited only intermediate values, possibly due to relatively low plant cover of multi-species assemblages. Soil physicochemical properties generally did not respond to the presence of herb plants. Further studies are required to fully understand the significance of herbaceous plant species for the soil environment. They should include the assessment of microbiome composition in both organic and mineral soil horizons, and seasonal changes in the effects of herb plants on soil in the context of rhizodeposition and litterfall.

CRediT authorship contribution statement

AM Stefanowicz: Conceptualization, Investigation, Writing – original draft; **P Kapusta:** Statistical analysis, Data curation, Writing – review and editing; **M Stanek:** Investigation, Writing – review and editing; **K Rola:** Conceptualization, Investigation, Writing – review and editing; **S Zubek:** Conceptualization, Investigation, Data curation, Writing – review and editing, Supervision, Project administration, Funding acquisition.

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Declaration of competing 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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.151313.

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