



Experimental assessment of forest floor geophyte and hemicryptophyte impact on arbuscular mycorrhizal fungi communities

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Abstract

Purpose Herbaceous plants are important components of temperate forest structure and its functioning, however, their impacts on arbuscular mycorrhizal fungi (AMF) remain largely unexplored. We studied the influence of forest herbaceous plant species on AMF abundance, morphospecies richness, and community composition in soil.

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Methods We tested the influence of plant species identity in an outdoor mesocosm experiment, using two soils, differing in physicochemical properties, planted with four plant species of contrasting traits related to morphology, phenology, reproduction, and ecology; the hemicryptophyte, summer-green *Aegopodium podagraria*, and spring ephemeral geophytes comprising *Allium ursinum*, *Anemone nemorosa*, and *Ficaria verna*. The plants were grown on both soils in four monocultures, in a combination of *A. podagraria* and *A. ursinum*, and a mixture of all four species.

Results *Aegopodium podagraria* and *A. ursinum* promoted AMF abundance and diversity the most. Higher AMF root colonization and/or soil concentrations of AMF structural and storage markers 16:1ω5 PLFA and NLFA, as well as higher AMF spore and morphospecies numbers were found in the *A. podagraria* and *A. ursinum* monocultures and mixture. The short period of photosynthetic activity of *A. ursinum* due to rapid leaf decay does not negatively affect the symbiosis with AMF. Although *A. nemorosa* and *F. verna* are mycorrhizal, their effect on AMF in soil was weak.

Conclusions The plant impact on AMF may be related to the differences in plant coverage and the character of their interactions with AMF. The herbaceous plants can form niches in soil differing in AMF abundance and diversity.

Keywords Temperate forest plants · Glomeromycota · Spring ephemerals · Soil chemical properties

Abbreviations

A_{AMF}	Relative arbuscular richness
AM	Arbuscular mycorrhiza
AMF	Arbuscular mycorrhizal fungi
F_{AMF}	Mycorrhizal frequency
M_{AMF}	Relative mycorrhizal root length
NLFA	Neutral lipid fatty acid
PLFA	Phospholipid fatty acid

Introduction

Numerous abiotic and biotic factors affect the structure and functioning of soil microbial communities in temperate forests. Among the latter, the identity of both overstorey and understorey plant species may have an impact on soil microorganisms (Bainard et al. 2011; Malý et al. 2014; Bahnmann et al. 2018; Rožek et al. 2021; Stefanowicz et al. 2021, 2022). Although the herbaceous layer is characterized by higher species richness than any other forest layer and significantly influences nutrient cycling, it is a particularly underappreciated aspect of these ecosystems (Nilsson and Wardle 2005; Gilliam 2007), and its effects on soil microbiota is yet little explored (Eisenhauer et al. 2011; Elliott et al. 2015; Zubek et al. 2021; Stefanowicz et al. 2022).

Herbaceous plant species of temperate forests are characterized by specific traits, being adapted to the conditions of the forest floor. Among them are spring ephemerals that grow, assimilate and bloom before the full development of tree leaves and canopy closure; their shoots die off shortly afterwards. They are geophytes, storing carbohydrates in belowground organs, allowing them to quickly develop foliage and efficiently use the period of increased light availability (Raunkiaer 1934; Jagodziński et al. 2016; Rawlik and Jagodziński 2020). After tree leaf expansion, plant species that are less light-demanding dominate forest floor (Elemans 2004). Among them are hemicryptophytes, whose shoots stay vital for the whole growing season (Raunkiaer 1934; Jagodziński et al. 2016). Many species of both groups of plants are able to effectively propagate by seeds or vegetative organs and they form compact patches on the forest

floor, in which other species may be sparsely present (Witkowska-Żuk 2008). Geophytes account for 90% of understory plant community biomass production during spring, whereas hemicryptophytes account for over 90% of biomass during summer (Jagodziński et al. 2013).

The different effects of herbaceous plant species on microbial soil biota are thought to relate to different traits (Zechmaister-Boltenstern et al. 2002; Gilliam 2007; Rawlik et al. 2021). It was shown that the rate of litter decomposition in forest soil was faster for spring ephemerals than winter-green or summer-green herbs (Rawlik et al. 2021). Jandl et al. (1997) found that *Allium ursinum* caused seasonal increases in organic matter mineralization rate and a considerable influx of nutrients to the soil in a beech forest due to its readily degradable litter of high chemical quality, decomposing in spring and summer. The biomass of *A. ursinum* was also shown to contain organic sulfur compounds with an inhibitory effect on microorganisms (Puxbaum and König 1997; Sobolewska et al. 2015). Moreover, the traits related to preferences of symbiotic partners, including the ability of a plant species to form a certain type of mycorrhiza, the degree of fungal root colonization, and the responsiveness of symbiotic partners to this relationship, may also impact the abundance and diversity of soil microorganisms in forest soils (van Aarle et al. 2003; Grünfeld et al. 2019; Rožek et al. 2019, 2021; Zubek et al. 2021).

Although the biology and ecology of herbaceous plant species of temperate forests have been a focus of numerous studies (Djurdjevic et al. 2004; Phartyal et al. 2009; Orav et al. 2010; D'Hertefeldt et al. 2014; Popović et al. 2016; Jagodziński et al. 2016; Rawlik and Jagodziński 2020; Vanneste et al. 2020), their interactions with soil microorganisms have been studied less frequently (Zechmaister-Boltenstern et al. 2002). Moreover, investigations focused specifically on the interactions of temperate forest herbaceous plants with the most common plant symbionts, arbuscular mycorrhizal fungi (AMF), are limited (Patreze et al. 2012), although glomeromycotan fungi may be an important component of temperate forest ecosystems (Helgason et al. 1998, 2002, 2007; Hewins et al. 2015).

The literature suggests that AMF abundance, species richness, and composition in roots and soils may be determined by plant species identity and plant

community structure (Merryweather and Fitter 1998; Helgason et al. 2002; Vandenkoornhuyse et al. 2003; Johnson et al. 2004). Burke (2008) observed differences in AMF root colonization among three herbaceous forest species, *Arisaema triphyllum*, *Maianthemum racemosum*, and *Trillium grandiflorum*, and suggested that these differences may in part be due to their different traits. Further, in a forest spring ephemeral, *Allium tricoccum*, seasonal changes in the degree of AMF colonization coincided with plant phenological changes; however, no differences in AMF community structure in the roots were observed (Hewins et al. 2015). Zubek et al. (2021) found based on a field survey that the herbaceous plants *Aegopodium podagraria*, *A. ursinum*, *Dentaria enneaphyllos*, and *Ficaria verna* had little impact on the abundance and diversity of AMF in beech and riparian forests. Different qualities and quantities of biomass produced by different plant species influence nutrient cycling and chemical properties of forest soils (Jandl et al. 1997; Gilliam 2007). These differences in the chemical properties of the soil can have an impact on the AMF communities of the forest soil. Burke et al. (2011) found that the occurrence and abundance of some AMF species were positively related to P and others were related to N concentration in soil, suggesting that different AMF species can acquire different resources in forest soil. Koorem et al. (2014) found that the abundance of AMF negatively correlated with P and positively correlated with the concentration of N in boreonemoral forest soils. In temperate forest, it was also shown that high concentrations of C, Ca, K, Mg, and N have a positive effect on AMF abundance (Zubek et al. 2021). Rožek et al. (2021) found that AMF abundance in soils and roots increased along with increasing soil alkalinity and macronutrient levels in tree monocultures of the temperate zone. Göransson et al. (2008) reported low AMF colonization of woodland grasses growing in acidic soils in an oak forest and suggested that this may be related to a sensitivity of AMF to Al toxicity. A similar relationship with pH was suggested by Postma et al. (2007) in beech forest forbs. Herbaceous plant species richness and community composition may also play a role in determining AMF diversity (Smith and Read 2008). The co-existence of trees and herbaceous species, which differ in formation of AM, ectomycorrhiza, or are non-mycorrhizal, affected the abundance of AMF propagules in soils, and thus influenced the

degree of AMF root colonization (Hewins et al. 2015; Veresoglou et al. 2017; Zubek et al. 2021). Zubek et al. (2021) showed that higher vascular plant cover increased AMF abundance in temperate forest soils. Chen et al. (2019), in a meta-analysis, indicated that microbial, bacterial, and fungal biomass, fungi/bacteria ratio, and microbial respiration rate increased in soils under plant mixtures, but not monocultures, consistently across various ecosystems.

Although these aforementioned studies have revealed the occurrence of AMF in roots and soils of temperate forests, there is insufficient information on how herbaceous forest plant species with different traits, namely morphology, phenology, reproduction, and ecology, influence AMF abundance and shape AMF soil communities in this ecosystem. Moreover, there are no data on the impact of such plants on AMF communities resulting from an experimental study. Therefore, we conducted an extensive outdoor pot experiment for two consecutive growing seasons, which aimed at assessing the impact of forest herbaceous plant species on AMF abundance, morphospecies richness, and composition in the soil of two forest types. We established the experiment under uniform environmental conditions to address issues of heterogeneity in the field in testing plant and soil effects (Štursová et al. 2016; Bahnmann et al. 2018). We expected that due to trait differences of herbaceous plant species, the abundance and diversity of AMF in soils would vary between plant species and mixtures of those. We additionally expected that plant-AMF relations would depend on soil origin. Knowledge of plant-AMF interactions seems a prerequisite for developing ecologically sound strategies of forest management, biodiversity conservation, and restoration (Bainard et al. 2011; Bahnmann et al. 2018).

Materials and methods

Forest plants

This pot experiment was carried out using four herbaceous plant species of deciduous forests: 1) *Aegopodium podagraria* L. (Apiaceae), 2) *Allium ursinum* L. (Amaryllidaceae), 3) *Anemone nemorosa* L. (Ranunculaceae), and 4) *Ficaria verna* Huds. (syn. *Ranunculus ficaria* L.; Ranunculaceae) (Fig. 1). These species have contrasting traits with respect to

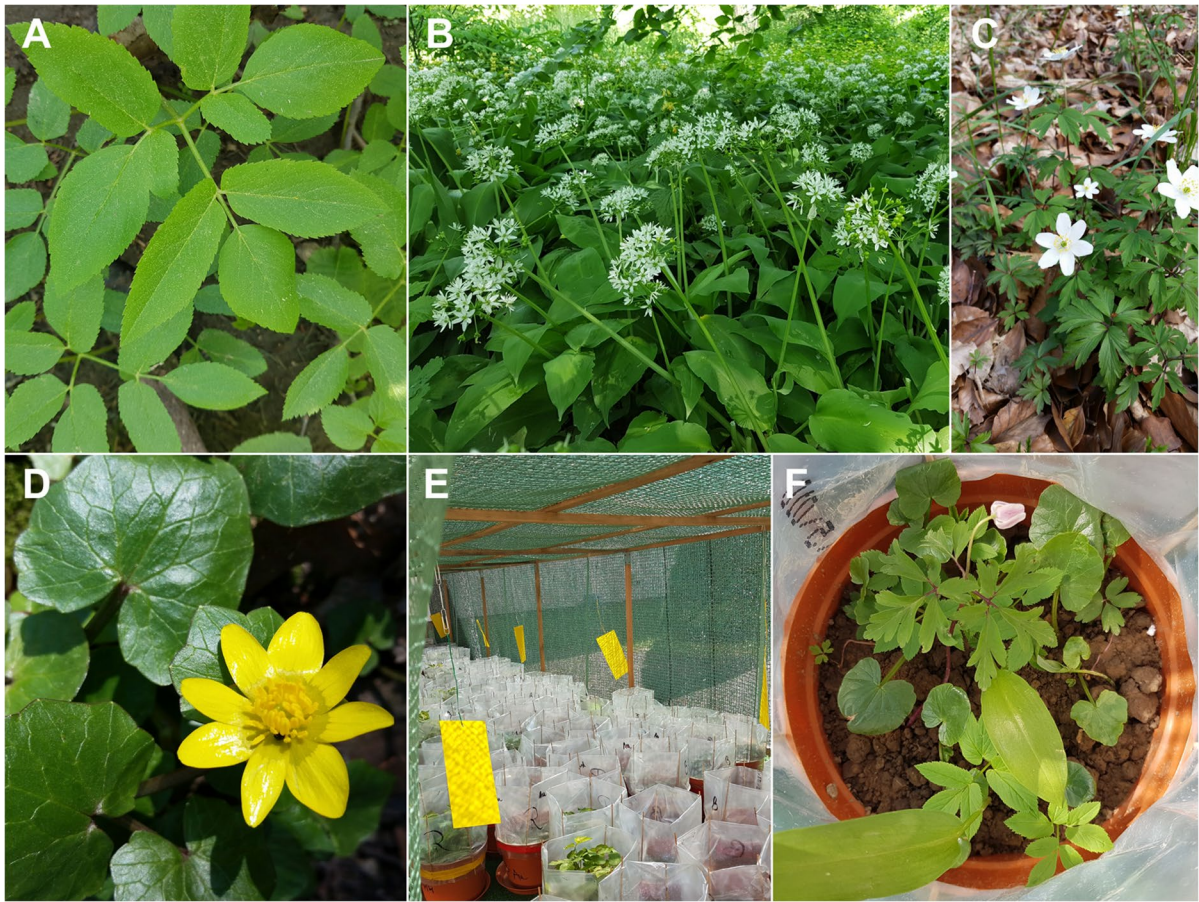


Fig. 1 Studied herbaceous plant species of forest floor: *Aegopodium podagraria* L. (A), *Allium ursinum* L. (B), *Anemone nemorosa* L. (C), and *Ficaria verna* Huds. (D), and the exemplary experimental pots (E, F)

morphology, phenology, reproduction, and ecology, and can form compact, nearly monospecific patches on the forest floor.

Aegopodium podagraria (commonly known as goutweed, ground elder, bishop's weed) is a hemicryptophyte, spreading mainly by rhizomes, in which carbohydrates are stored for the rapid development of shoots after winter (D'Hertefeldt et al. 2014). The species can grow up to 1 m tall. It flowers from May to July and its biomass ages in the autumn (Witkowska-Żuk 2008; Jagodziński et al. 2016). *Aegopodium podagraria* inhabits open and shaded habitats of beech, riparian, oak-hornbeam, and oakwood forests, as well as anthropogenic habitats (Witkowska-Żuk 2008), and its natural range is Europe and Eastern Asia (Meusel et al. 1978).

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. It is characterized by a well-degradable litter of high chemical quality, decomposing at the end of spring and early summer, after which the plant enters a dormancy phase (Jandl et al. 1997; Witkowska-Żuk 2008; Sobolewska et al. 2015). It grows in beech, oak hornbeam, and alder forests (Witkowska-Żuk 2008), and its natural range is Europe (Stearn 1980). It is under partial legal protection in Poland (Regulation of the Minister of the Environment 2014).

Anemone nemorosa (wood anemone) is a rhizomatous geophyte that grows up to 30 cm tall. It flowers from March to May and its foliage dies in late spring, after which the plant enters a dormancy phase

(Witkowska-Żuk 2008). Its natural range is Europe (Meusel et al. 1978), where it occurs in beech, riparian, oak-hornbeam, and oakwood forests (Witkowska-Żuk 2008).

Ficaria verna (fig buttercup, lesser celandine) is a low-growing (up to 10 cm tall) tuberous geophyte. It starts to grow at the end of winter and begins to bloom in early spring. By late spring, the plant quickly ages, its foliage dies, and it enters a dormancy phase (Witkowska-Żuk 2008; Jagodziński et al. 2016). It occurs in riparian, oak-hornbeam, and beech forests in Europe (Meusel et al. 1965; Witkowska-Żuk 2008).

Allium ursinum, *A. nemorosa*, and *F. verna* are considered spring ephemerals, that is, plant species that grow, assimilate, bloom, and bear fruits before full canopy closure. Their shoots die within a short period, but their belowground organs store assimilates for early development in the coming spring (Jagodziński et al. 2016; Rawlik and Jagodziński 2020). All four plant species form arbuscular mycorrhiza (AM) (Rožek et al. 2019; Zubek et al. 2021).

In this experiment, we used *A. podagraria* and *A. nemorosa* rhizomes and tubers of *F. verna* due to the rarity or absence of seeds of these species and importance of vegetative spreading (Witkowska-Żuk 2008; Phartyal et al. 2009; Jagodziński et al. 2016). In case of *A. ursinum*, we used bulbs due to difficulties in seed germination of this species under laboratory conditions. Plant materials were collected two days before the setup of the experiment, in forests where also soils were collected (see Section [Forest soils](#)), but not from the same stands/soils where the soils were excavated. *Anemone nemorosa* and *A. ursinum* were collected from the beech forest, whereas *A. podagraria* and *F. verna* from the riparian forest. The rhizomes, tubers, and bulbs were cleaned of soil particles in tap water prior to planting.

Forest soils

The soils of two deciduous forests were selected to compare the forest soils with different characteristics resulting from different soil genesis: 1) a beech forest soil formed on limestone bedrock and 2) a riparian forest soil occurring along the lowland river valleys with characteristic periodic floods and fine-grained Fluvisols. The soils were collected from two locations in southern Poland: the Las Zawale forest in

Grabie near Kraków (49°54'59.2" N, 19°44'52.7" E) and the Las Łęgowski forest in Kraków (50°03'06.0" N, 20°02'15.0" E), respectively. Two days before the experiment setup, the soils were collected to a depth of about 20 cm after organic matter removal by a garden rake and transported to the experimental garden.

The soils were mixed and sieved, using a 1-cm mesh, to remove larger inorganic and organic residues. Ten randomly chosen samples of each soil were analyzed for physicochemical properties (see Section [Soil chemical analyses](#)). The soils differed significantly in terms of all measured parameters. All measured parameters, with the exception of the silt content, were significantly higher in the riparian forest soil than in the beech forest soil (Table S1).

Setup and maintenance of a common garden-like pot trial

The experiment was carried out over two growing seasons, from 15 March 2019 to 15 June 2020, in the experimental garden of the Institute of Botany of the Jagiellonian University in Kraków (50°01'39"N, 19°54'12.9"E). The garden is situated in a temperate oceanic (Cfb) climate zone (Kottek et al. 2006). During the 15-month period of the experiment, the following meteorological conditions occurred: average air temperature 11.44 °C, average relative humidity 75.39%, average monthly rainfall 50.69 mm, precipitation 709.7 mm, number of days with rainfall 133, average general cloudiness 4.91 octas (IMGW meteorological station – code '350,190,566', data obtained from the Institute of Meteorology and Water Management, National Research Institute).

Planting treatments in the two soil types comprised (1) bare soil, that is, soil without any vascular plant species, (2) *A. podagraria*, (3) *A. ursinum*, (4) *A. nemorosa*, (5) *F. verna*, (6) a mixture of two species (*A. podagraria* and *A. ursinum*), and (7) a mixture of four species. Only these two mixtures were established to stay within a manageable number of samples for analysis. In total, the experiment consisted of 14 treatments. Brown plastic pots (19×15 cm; 3 000 ml in volume) were filled with 2 700 ml of one of the two soils. Fragments of rhizomes (*A. podagraria*, *A. nemorosa*), tubers (*F. verna*), and bulbs (*A. ursinum*) were placed in each pot. Eight fragments of rhizomes, tubers, or bulbs were planted in pots of the single-species treatments, four of each species in the mixture

of two-species treatment, and two of each species in the mixture of four-species treatment. Each pot was supplied with the same amount of washings of the belowground organs of all plant species to potentially allow soil biota of the other soil to colonize the respective other soil. We established 12 pot replicates of each treatment, in total, 168 pots. At harvest, due to lack of plant growth or accidental destruction of some pots, the final number of pots (replicates) was 12 pots for bare riparian soil, 11 for the *A. ursinum* and *Allium-Aegopodium* mixture on riparian soil, 10 for bare beech soil and *A. podagraria*, *A. nemorosa*, *F. verna*, *Allium-Aegopodium* mixture on riparian soil, and 9 pots for the remaining of the treatments.

The pots were arranged randomly in the experimental garden and kept in an open space under natural sunlight. The pots had drainage holes and saucer trays. The saucer plates had drainage holes at the edges to remove excess water. Above the edges of the pots, plastic foil was installed to a height of 20 cm to avoid contamination between treatments. The plants were watered with 500 ml of tap water per pot from April to October, depending on the need. If any undesirable plant species appeared in the pots, it was immediately removed. To mimic light conditions on the forest floor during the vegetation season, the pots were shaded in two stages using garden mesh. The first shading garden mesh was established on 01 April and the second one on 01 June. They were removed on 15 October. During the autumn and winter periods, from 18 November 2019 to 01 March 2020, the plants were protected from the cold using non-woven fabric. The sides of every single pot were covered to 10 cm above the pot edge, then randomly grouped into blocks, and wrapped with the same non-woven fabric. In spring, the cover was removed and pots were re-randomized.

Plant harvesting and soil sampling

On 15 June 2020, after two growing seasons, the experiment was completed. Vascular plant cover was estimated as a percentage for each pot using digital photographs. We used a Nikon D5300 digital camera attached to a portable camera tripod to take these photographs. The pictures were taken 35 cm above the pot at a downward angle of 90° with the same field of view, resolution and other settings. Bubble levels were used to ensure that tripod, camera and resulting

images were accurately vertical. Subsequently, the plant cover was estimated manually using the Motic Images Plus 2.0 software (Hong Kong, Asia) and converted into a percentage of the pot surface (cf Rožek et al. 2020).

After harvesting the shoots, the total cover of bryophytes was estimated in the same way (Rola et al. 2021). Samples of the bryophytes were taken and identified under the microscope. The nomenclature of bryophytes follows Hill et al. (2006). The bryophyte specimens were deposited in the KRA herbarium (Jagiellonian University in Kraków).

After removing a 1 cm-layer of soil from the top, sides and bottom, the remaining soil was subsampled after homogenization. Shoot and root samples were rinsed before drying for dry weight determination. Fine roots of less than 2 mm in diameter were collected for AMF colonization measurements after staining. The soil from each pot was homogenized and then divided into portions: 1) approximately 50 g of soil was taken to determine AMF spore density, morphospecies richness, and composition, 2) approximately 50 g of soil was freeze-dried to quantify the concentration of the AMF marker PLFA and NLFA, and 3) approximately 100 g of soil for physicochemical analyses. As samples for soil PLFA and NLFA analyses could not be processed immediately, they were frozen at −20 °C (Öhlinger 1996). The frozen soil samples were stored in a freezer for approximately 4 weeks.

Root staining and assessment of AMF colonization

Roots were processed according to the method of Phillips and Hayman (1970) with modifications. They were rinsed with tap water to remove any soil particles, placed in plastic containers, and cleared and softened in 10% KOH in water for 24 h. The roots were then washed in tap water and placed in 5% lactic acid in water for 24 h. The solution was removed and the roots were stained with 0.05% aniline blue in lactic acid until microscopic assessment. Thirty root fragments, approximately 1 cm long, per sample were analyzed, using a Nikon Eclipse 80i light microscope, using a lens with a magnification of 40. Colonization by AMF was determined following the procedure of Trouvelot et al. (1986). The following parameters describing the abundance of AMF were calculated: mycorrhizal frequency (F_{AMF}), relative mycorrhizal

root length (M_{AMF}), and relative arbuscular richness (A_{AMF}) (Trouvelot et al. 1986).

AMF spore density, morphospecies richness and community composition

The spores were isolated by centrifuging 50 g of soil in a 50% solution of sucrose and filtering (Brunnett et al. 1996). They were counted in Petri dishes under a dissecting microscope and then placed on a slide in a drop of polyvinyl alcohol/lactic acid/glycerol (PVLG) and a mixture of PVLG/Melzer's reagent (1:1, v:v) (Omar et al. 1979). The intact as well as crushed spores were examined. Their generic and species affiliation was determined based on the mode of spore formation, size and color, subcellular structure, as well as the phenotypic and histochemical properties of components of the spore wall(s) (Błaskowski 2012). Morphospecies were determined using an Olympus BX51 light microscope.

Lipid fatty acid concentration in soil

The phospholipid (PLFA) and neutral lipid (NLFA) 16:1 ω 5 concentrations were quantified as indicators of AMF biomass (Olsson et al. 1997; Olsson 1999). PLFA 16:1 ω 5 was considered as a marker of structural lipids and NLFA 16:1 ω 5 as a marker of storage lipids (Olsson et al. 1997; Olsson 1999; Sharma and Buyer 2015). Lipids were extracted from the freeze-dried soil (Freeze Dry System Labconco), using an accelerated solvent extractor (ASE 200, Dionex) with a mixture of methanol:chloroform:phosphate buffer (2:1:0.8; v:v:v) (Macnaughton et al. 1997). Lipids were separated into neutral- (NLFA), glyco- (GLFA) and phospholipids (PLFA) (Palojärvi 2006) on Bakerbond silica gel SPE columns (500 mg, Baker) by elution with chloroform, acetone, and methanol, respectively. The chloroform and methanol fractions were collected and dried under nitrogen flow. Then NLFAs and PLFAs were subjected to mild alkaline methanolysis. Fatty acid methyl esters (FAMES) were separated on a Select FAME (100 m $\times$ 0.25 $\times$ 0.25) column (Agilent Technologies) and identified by GC–MS chromatograph (Varian 3900 and Saturn 2100 T, Varian) with the NIST library. Fatty acids were identified by comparison to the Matreya LLC standards.

Soil chemical analyses

Soil pH was measured in a 1:5 suspension (w:v) with H_2O , using a Hach HQ40d multimeter (ISO 10390 1994). The total C (C_T), and organic C (C_{ORG}) contents were analysed with a LECO RC-612 analyzer (ISO 10694 1995) and total N (N_T), using a Foss Tecator 2300 Kjeltac analyzer after digestion in H_2SO_4 with Kjeltabs ($K_2SO_4 + CuSO_4 \cdot 5H_2O$), according to the application AN 300 Ver. 4.0 (Foss Tecator). Total Ca (Ca_T), K (K_T), and P (P_T) measurements were made after digesting the soil in hot concentrated $HClO_4$, using a Foss Tecator Digester 40 Auto. The exchangeable Ca^{2+} (Ca_{EX}) and K^+ (K_{EX}) concentrations were measured after shaking the soil three times for 1 h in 0.1 M $BaCl_2$, using Laboratory Shaker type 358S, elpan (PN-EN ISO 11260 2011). The concentrations of the elements in the extracts were measured using a fast sequential atomic absorption spectrometer (Varian AA280 FS), with the exception of total P, which was analyzed colorimetrically (Hach Lange DR 3800), using the vanadate-molybdate method. $N-NH_4$, $N-NO_3$, and $P-PO_4$ were determined in a 1:10 suspension in H_2O (w:v) on a Dionex DX-100 for $N-NH_4$, and Dionex ICS-1100 for $N-NO_3$ and $P-PO_4$ ion chromatographs. Six Cation Standards II and Seven Anion Standards II (Dionex) were used for calibration. The accuracy of the soil analyses was assured by inclusion of two certified reference materials: CRM048-050 (RTC; total element concentrations) and ISE-912 (WEPAL; exchangeable element concentrations). The accuracy of the organic C, total C, N, and P analyses, was guaranteed with the certified materials ISE-859 and ISE-995.

Statistical analysis

Student's t-tests ($p < 0.05$) were performed to test for physicochemical differences between the beech and riparian forest soils. Before analysis, normal distribution was verified, using the Kolmogorov–Smirnov test and the assumption of variance homogeneity was tested with the Brown–Forsythe test. Box-Cox transformation was used if necessary. The data transformation was performed for the following variables: pH (H_2O), K_T , Ca_{EX} , and K_{EX} . If the test assumptions were not met, a nonparametric Mann–Whitney U test was applied at $p < 0.05$.

Two-way analysis of variance followed by a *post-hoc* Tukey's (HSD) test for unequal sample size ($p < 0.05$) was used to test the effect of planting treatment and soil origin and their interaction on AMF parameters (M_{AMF} , A_{AMF} , PLFA 16:1 ω 5, NLFA 16:1 ω 5, AMF spore density, AMF morphospecies richness), and plant parameters (ground cover by vascular plants and bryophytes, bryophyte species richness, vascular plant shoot mass, vascular plant belowground mass). Before analysis, the normality of data distribution was verified using the Kolmogorov–Smirnov test, and Levene's test was performed to assess the homogeneity of variances. The variables that did not meet the assumptions were Box-Cox-transformed. The parameter F_{AMF} was excluded from the analysis due to a lack of variance homogeneity even after transformations. Due to the early biomass decomposition of *F. verna* shoots in beech soil treatments and the lack of data on AMF colonization for this species, the samples of *F. verna* treatment from both soils were excluded from the analysis of M_{AMF} , A_{AMF} , and vascular plant shoot mass parameters. The bare soil treatment was excluded from the analysis of the parameters M_{AMF} , A_{AMF} , vascular plant shoot mass, vascular plant belowground mass, and vascular plant cover.

Correlations between plant, fungal, and soil chemical parameters were quantified with Pearson's correlation coefficients. The analysis was done for the whole data matrix and for beech and riparian forest soil, separately. The relationships between vascular plant cover, vascular plant shoot mass, belowground mass of vascular plants and PLFA 16:1 ω 5 as well as NLFA 16:1 ω 5 were subjected to simple regression analysis to test the effect of plant size parameters on AMF abundance in soil.

The AMF soil parameters (PLFA 16:1 ω 5, NLFA 16:1 ω 5, AMF spore density, AMF morphospecies richness) and basic soil chemical parameters (pH in H_2O , C_{ORG} , N_T , $N-NO_3$, $N-NH_4$, P_T , $P-PO_4$, K_{EX} , Ca_{EX}) were used to run a discriminant analysis to identify which variables were the most influential for treatment separation. Due to large differences in the chemical properties of the two soils, this analysis was applied separately for each soil. The analysis involved a stepwise forward procedure in which the variables were evaluated and added to the model if they contributed significantly to the discrimination of the treatments. Discriminatory

power was expressed with the Wilks' lambda statistic. Canonical discriminant analysis was conducted to determine the successive functions and canonical roots and to analyze the factor structure coefficients that represent correlations between the variables included in the model and the discriminant functions.

A two-way permutational multivariate analysis of variance (PERMANOVA) was performed to test for differences in AMF community composition due to planting treatment and soil origin and their interaction. The algorithm follows Anderson (2001) with a modification for unbalanced designs. The III Sum of Squares (SS) was used for partitioning the multivariate variance due to the unbalanced design. Pairwise comparisons between levels of the factor plant species identity within each level of the factor soil origin and between levels of the factor soil origin within each level of the factor plant species identity were calculated as multivariate pseudo-t statistics and p-values obtained using a permutation procedure. The analysis was based on a matrix of the presence/absence of AMF morphospecies using the Jaccard coefficient with 9999 permutations for each test. The seriation of AMF morphospecies occurrence (presence/absence) was performed using an unconstrained algorithm (Brower and Kile 1988). We used seriation as a tool to visualize the distribution pattern of AMF morphospecies across particular treatments. The method attempts to reorganize the data matrix so that the presence of species is concentrated along diagonal. Pots without AMF morphospecies were excluded from the analysis. Detrended correspondence analysis (DCA) was used to examine AMF morphospecies occurrence within particular treatments. The analysis was done for the whole data matrix and for beech and riparian forest soil, separately. The analysis was based on the matrix of morphospecies frequencies calculated for particular treatments that represent relative morphospecies occurrence based on the 9–12 replicate pots. The frequency of morphospecies was calculated as the percentage of samples of a given treatment in which a given morphospecies occurred.

Statistical calculations were performed using STATISTICA 13 (StatSoft, Tulsa, OK), PRIMER 7 statistical software (Primer-E, Plymouth, UK; Anderson et al. 2008), and PAST 4.06 (Hammer et al. 2001).

Results

Plant characteristics

Vascular plant cover, vascular plant shoot mass, and vascular plant belowground mass were influenced by both planting treatment and soil origin and their interaction (Table 1, Fig. 2a, b, c). A significantly higher vascular plant cover was observed for the riparian forest soil of *A. podagraria*, *Allium-Aegopodium*

mixture, and the mixture of four species. The same applies to vascular plant shoot mass, where, additionally, all treatments with beech forest soil yielded less biomass. All planting treatments representing riparian forest soil, except that with *F. verna*, yielded higher vascular plant belowground mass than the remaining treatments.

Bryophyte cover was influenced by planting treatment (Table 1, Fig. 2d) and was significantly higher in the *A. ursinum*, *A. nemorosa*, and *F. verna*

Table 1 The results of two-way ANOVA ($p < 0.05$) on the effects of planting treatment, soil origin, and their interaction on plant and arbuscular mycorrhizal fungi (AMF) parameters. Coefficients of determination (R^2) of the whole model are also provided

Source of variation	Factors	Df	F	<i>p</i>	R^2
Vascular plant cover (%)	Planting treatment	5	59.36	<0.001	0.91
	Soil origin	1	814.89	<0.001	
	Planting treatment × Soil origin	5	7.98	<0.001	
Vascular plant shoot mass (g)	Planting treatment	4	26.47	<0.001	0.89
	Soil origin	1	613.46	<0.001	
	Planting treatment × Soil origin	4	9.46	<0.001	
Vascular plant belowground mass (g)	Planting treatment	5	43.70	<0.001	0.89
	Soil origin	1	712.09	<0.001	
	Planting treatment × Soil origin	5	3.38	0.007	
Bryophyte cover (%)	Planting treatment	6	13.34	<0.001	0.45
	Soil origin	1	35.88	<0.001	
	Planting treatment × Soil origin	6	0.77	0.594	
Bryophyte species richness	Planting treatment	6	3.26	0.005	0.57
	Soil origin	1	164.97	<0.001	
	Planting treatment × Soil origin	6	1.24	0.290	
Relative mycorrhizal root length— $M_{AMF}\%$	Planting treatment	4	30.78	<0.001	0.73
	Soil origin	1	65.97	<0.001	
	Planting treatment × Soil origin	4	10.23	<0.001	
Relative arbuscular richness— $A_{AMF}\%$	Planting treatment	4	29.57	<0.001	0.75
	Soil origin	1	84.63	<0.001	
	Planting treatment × Soil origin	4	11.67	<0.001	
PLFA 16:1 ω 5 (nmol g ⁻¹ dw)	Planting treatment	6	78.26	<0.001	0.86
	Soil origin	1	38.66	<0.001	
	Planting treatment × Soil origin	6	41.85	<0.001	
NLFA 16:1 ω 5 (nmol g ⁻¹ dw)	Planting treatment	6	36.35	<0.001	0.91
	Soil origin	1	1126.51	<0.001	
	Planting treatment × Soil origin	6	7.38	<0.001	
AMF spore density	Planting treatment	6	15.73	<0.001	0.73
	Soil origin	1	222.12	<0.001	
	Planting treatment × Soil origin	6	13.91	<0.001	
AMF morphospecies richness	Planting treatment	6	14.28	<0.001	0.58
	Soil origin	1	67.26	<0.001	
	Planting treatment × Soil origin	6	8.58	<0.001	

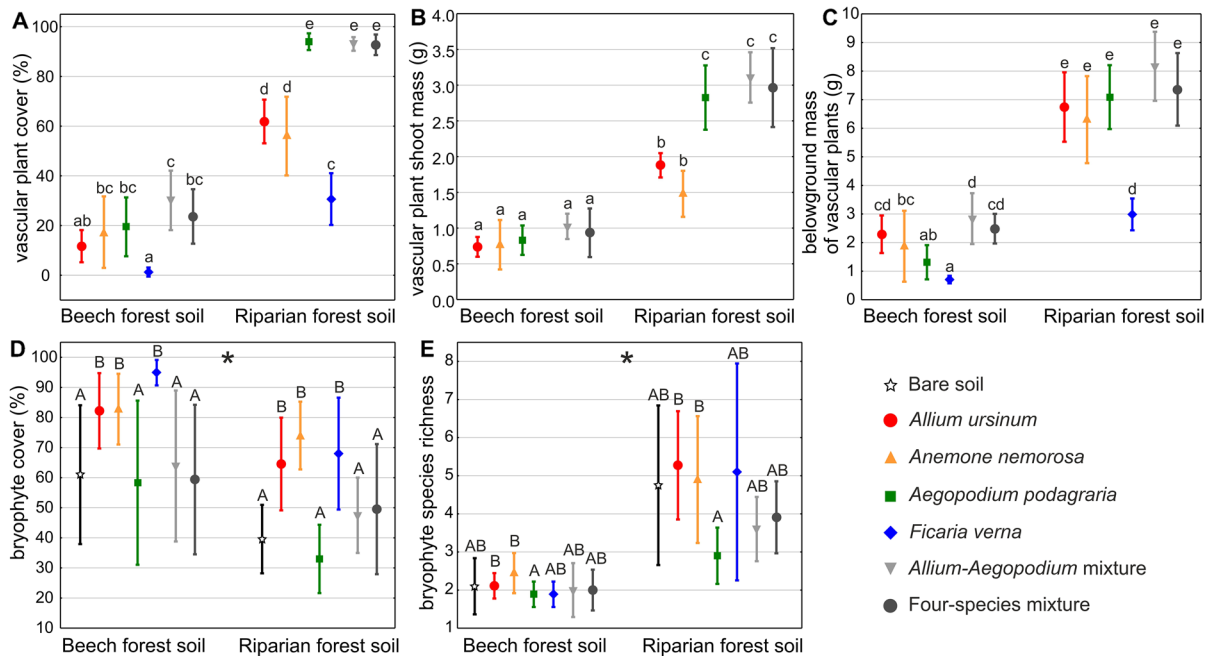


Fig. 2 Plant characteristics (mean $\pm$ SD) in the experimental treatments: (A) vascular plant cover, (B) vascular plant shoot dry mass, (C) belowground dry mass of vascular plant, (D) bryophyte cover, and (E) bryophyte species richness. Lowercase letters above the whiskers indicate a statistically significant interaction between the planting treatment and soil origin

planting treatments in comparison to the remaining planting treatments. The effect of soil origin was also significant and pots with beech forest soil had higher bryophyte cover than pots with riparian forest soil. The bryophyte species richness was significantly higher in the *A. ursinum* and *A. nemorosa* planting treatments than in the *A. podagraria* planting treatment (Table 1, Fig. 2e) and pots with riparian forest soil showed significantly higher bryophyte species richness.

AMF root colonization degree

The highest mean values of relative mycorrhizal root length (M_{AMF}) and relative arbuscular richness (A_{AMF}) were observed in *A. ursinum* in both soils and the lowest in the *A. nemorosa*, *A. podagraria*, and the mixture of *Allium-Aegopodium* planting treatments from beech forest soil (Table 1, Fig. 3).

AMF abundance in soil

The PLFA 16:1 ω 5 and NLFA 16:1 ω 5 concentrations were influenced by both planting treatment and soil origin and their interaction (Table 1). They were highest in the *Allium-Aegopodium* mixture and the mixture of four species in the riparian forest soil (Fig. 4a, b). The highest AMF spore density was recorded for *A. podagraria* grown in beech forest soil, whereas the lowest in the *A. nemorosa* and the mixture of *Allium-Aegopodium* from beech forest soil and all planting treatments from riparian forest soil (Table 1, Fig. 4c). As regards AMF morphospecies richness, bare soil from beech forest soil was characterized by the highest values of this parameter (Fig. 4d).

Correlations between plant and AMF parameters

As a rule, vascular plant cover and vascular plant shoot mass and belowground mass positively

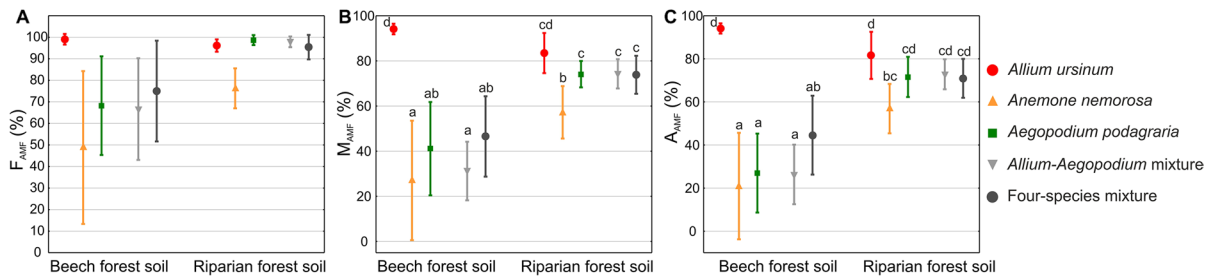


Fig. 3 Root colonization parameters of arbuscular mycorrhizal fungi (AMF) (mean $\pm$ SD): (A) mycorrhizal frequency ($F_{AMF}\%$), (B) relative mycorrhizal root length ($M_{AMF}\%$), and (C) relative arbuscular richness ($A_{AMF}\%$). Lowercase letters above the whiskers indicate a statistically significant interaction

between the planting treatment and soil origin effects ($p < 0.05$). See Table 1 for the results of two-way ANOVAs. No statistical analysis was done for F_{AMF} due to lack of variance homogeneity even after transformations

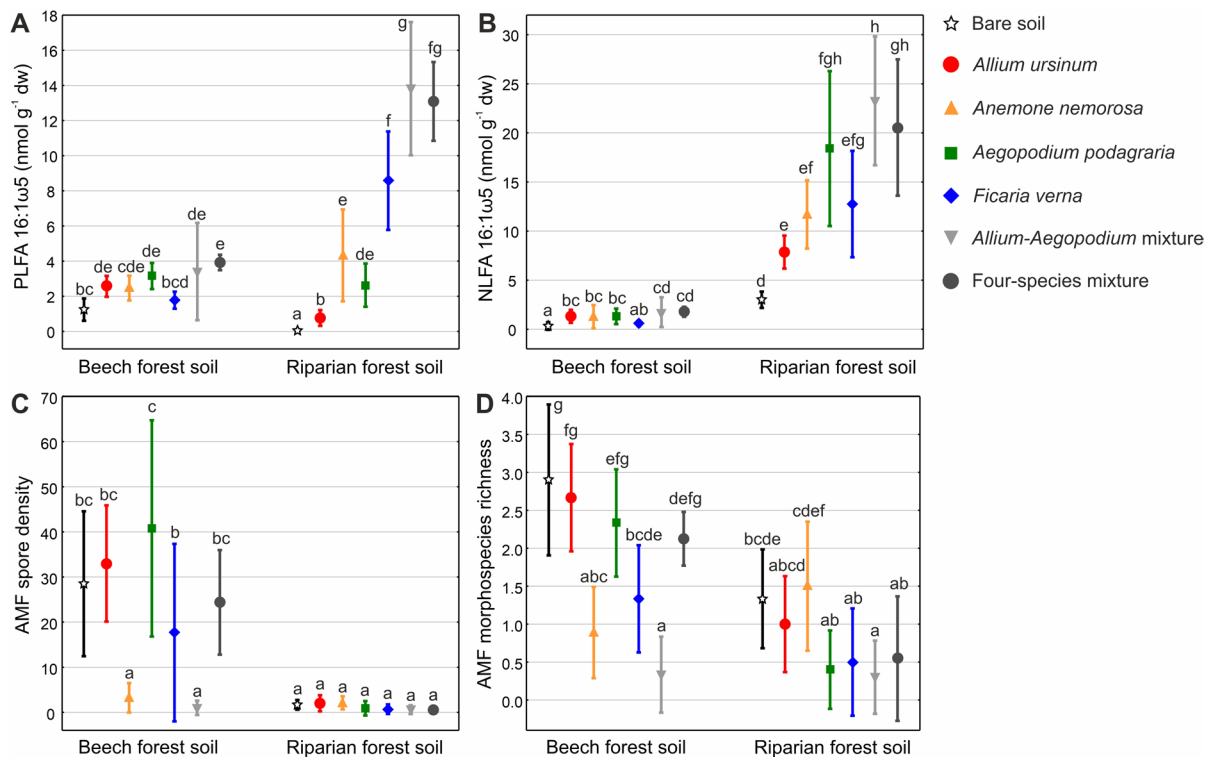


Fig. 4 The abundance and species richness of arbuscular mycorrhizal fungi (AMF) in the soils of the experimental treatments (mean $\pm$ SD): (A) the concentration of PLFA 16:1 ω 5, (B) the concentration of NLFA 16:1 ω 5, (C) AMF spore density in 50 g of fresh soil, and (D) AMF morphospecies richness

in 50 g of fresh soil. Lowercase letters above the whiskers indicate a statistically significant interaction between the planting treatment and soil origin effects ($p < 0.05$). See Table 1 for the results of two-way ANOVAs

correlated with PLFA 16:1 ω 5 and NLFA 16:1 ω 5 concentrations, AMF root colonization parameters, but negatively with AMF morphospecies richness in soil (Fig. 5, Fig. S1) Such dependencies apply both to

all data and to both soils analyzed separately. In the case of the soil from the riparian forest, the content of $P-PO_4$, $N-NO_3$, K_{EX} , and Ca_{EX} negatively correlated with vascular plant cover and vascular plant shoot

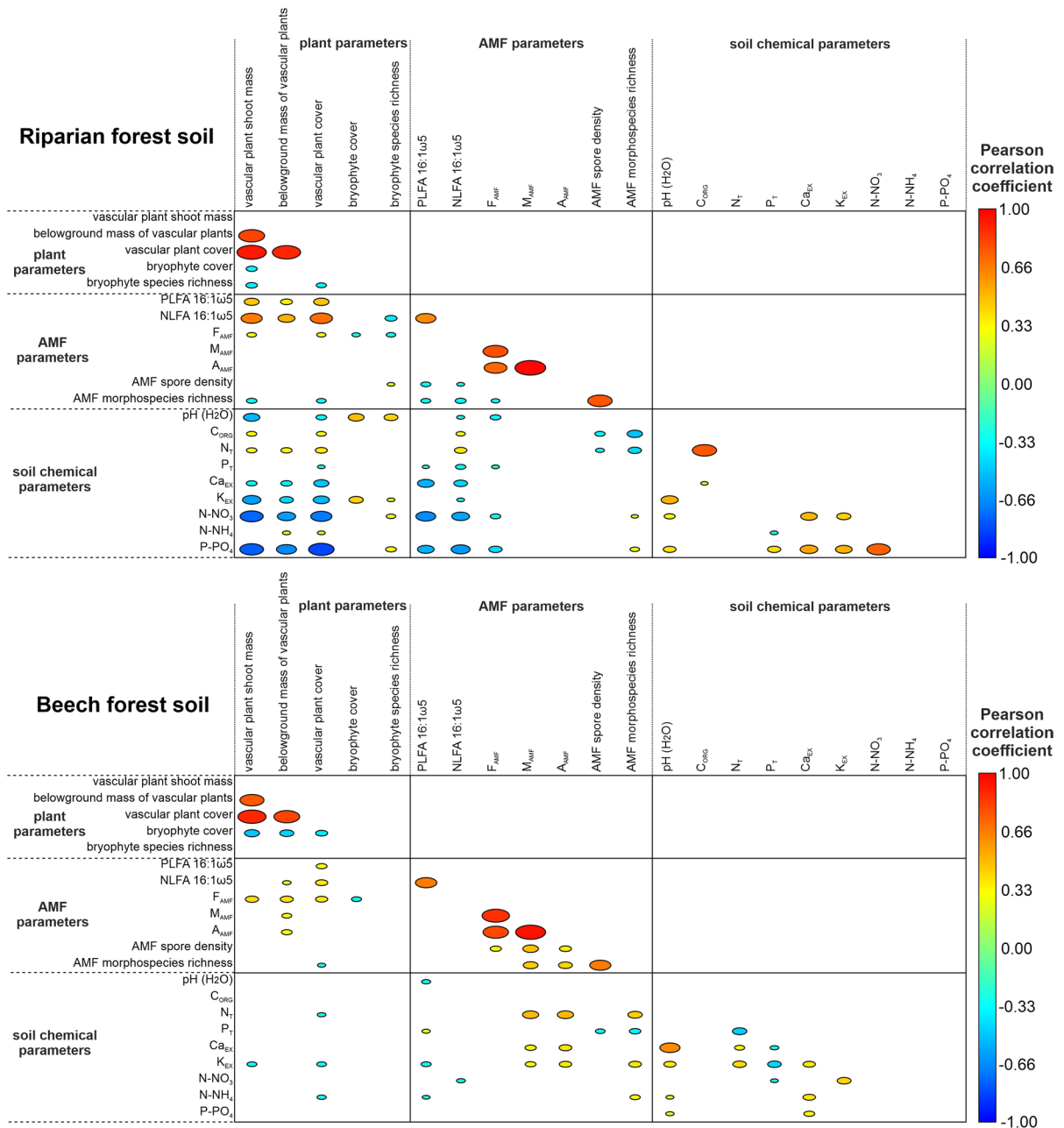


Fig. 5 Exploratory heatmaps showing Pearson's correlation coefficients for plant, arbuscular mycorrhizal fungi (AMF), and soil chemical parameters for two forest soils. Only statistically significant ($p < 0.05$) correlations are shown

mass and belowground mass. The regression analysis showed a significant dependence of PLFA 16:1ω5 and NLFA 16:1ω5 on plant parameters (Fig. 6). However, the greater percentage of variability in NLFA 16:1ω5 is explained by predictors related to plant parameters compared to PLFA 16:1ω5. With regard

to the relationships between soil chemical parameters and AMF, in riparian forest soil $P-PO_4$, $N-NO_3$, K_{EX} , and Ca_{EX} were negatively related to PLFA 16:1ω5, NLFA 16:1ω5 and F_{AMF} , whereas in beech forest soil only pH, $N-NH_4$, and K_{EX} negatively influenced PLFA 16:1ω5 concentrations (Fig. 5).

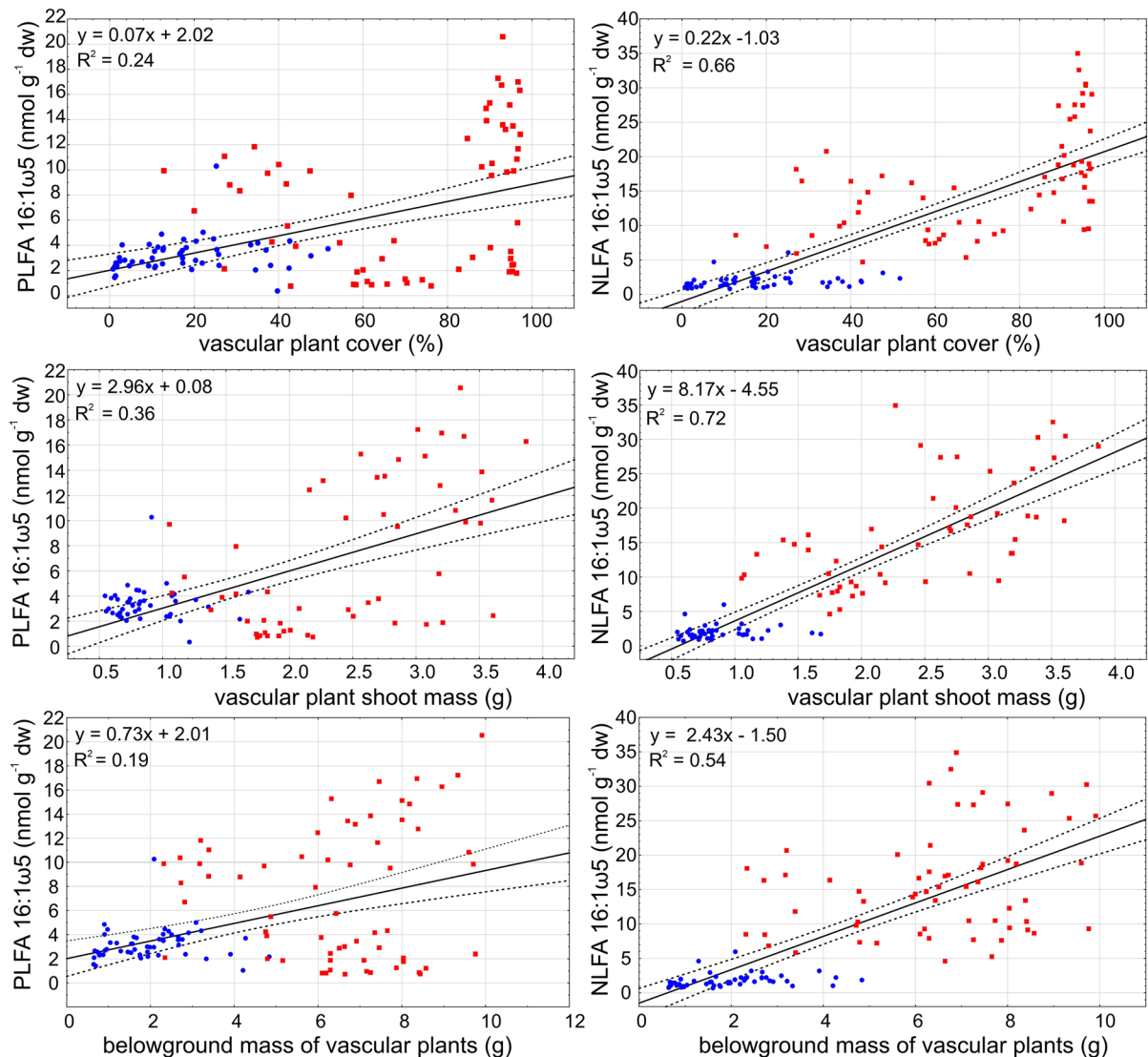


Fig. 6 Simple linear regression analysis of vascular plant cover, vascular plant shoot mass, belowground mass of vascular plants and NLFA 16:1ω5 as well as PLFA 16:1ω5. The blue circles represent the beech forest soil, and the red squares

represent the riparian forest soil. Coefficients of determination (R^2) are provided on the figure. The dashed line indicates the 95% confidence interval

AMF and soil chemical factors differentiating experimental treatments

The factors differentiating planting treatments varied depending on soil origin. Seven factors, including two AMF and five soil chemical parameters, contributed most to the discrimination of particular planting treatments with beech forest soil, whereas the remaining parameters did not exert significant effects (Table 2). P_T , N_T , and K_{EX} contributed

the most to separation of planting treatments along the first canonical discriminant axis, along which the bare soil and *A. ursinum* planting treatments were the most separated ones from the mixture of *Allium-Aegopodium* and the mixture of four species due to higher N_T and K_{EX} concentrations and lower P_T concentrations. The *A. nemorosa* planting treatment was separated along the second canonical discriminant axis from the mixture of four species due to its higher soil pH (Fig. 7a).

Table 2 Results of forward stepwise discriminant analysis. Wilks' lambda, partial Wilks' lambda, F to remove and p values for arbuscular mycorrhizal fungal (AMF) attributes and chemical parameters of a pot experiment with two forest soils are shown. The variables are arranged according to the size of Wilks' lambda statistics; only significant variables are shown

Variable	Wilks' lambda	partial Wilks' lambda	F to remove value	p
Beech forest soil (n = 64)				
P _T	0.020	0.491	7.94	<0.001
K _{EX}	0.017	0.603	5.04	<0.001
pH (H ₂ O)	0.015	0.684	3.54	0.006
N _T	0.014	0.692	3.42	0.007
AMF morphospecies richness	0.014	0.736	2.75	0.023
PLFA 16:1ω5	0.013	0.749	2.57	0.031
P-PO ₄	0.013	0.762	2.39	0.043
Riparian forest soil (n = 74)				
PLFA 16:1ω5	0.003	0.335	18.85	<0.001
K _{EX}	0.002	0.435	12.36	<0.001
P-PO ₄	0.002	0.625	5.69	<0.001
N _T	0.002	0.627	5.66	<0.001
pH (H ₂ O)	0.002	0.671	4.66	0.001
Ca _{EX}	0.002	0.684	4.39	0.001
N-NO ₃	0.001	0.707	3.95	0.002
NLFA 16:1ω5	0.001	0.777	2.73	0.021
P _T	0.001	0.787	2.58	0.028

The planting treatments with riparian forest soil were more clearly separated from each other than those with beech forest soil (Fig. 7b) by nine influential factors having significant discriminating power (Table 2). The *Allium-Aegopodium* mixture and the mixture of four species were clearly separated from the remaining planting treatments along the first canonical discriminant axis defined by lower N-NO₃ and P-PO₄ concentrations, and higher PLFA 16:1ω5 concentrations in these treatments. The *F. verna* planting treatment was most clearly separated along the second canonical discriminant axis defined the most by K_{EX} concentration in soil (Fig. 7b).

AMF community composition

In total, spores of 16 AMF morphospecies were isolated from all samples. Two-way PERMANOVA indicated a significant effect of both planting treatment and soil origin factors as well as their interactions on AMF community composition (Table S2). Pairwise comparisons among levels of planting treatment factor within each level of soil origin factor revealed greatest differences among planting treatments for beech forest soil (Table 3). For example, AMF

community composition of soil under *A. nemorosa* differed significantly from that of soils under all other planting treatments. For riparian forest soil, the only significant differences were found between the AMF communities of the mixture of *Allium-Aegopodium* and bare soil and soil under *A. ursinum*. All planting treatments differed significantly between beech and riparian forest soils (Table 3).

The seriation of AMF morphospecies occurrence across particular planting treatments is presented in Fig. 8. Arbuscular mycorrhizal fungi species occurrence was the most distinct between the two soil origins. As many as eight AMF morphospecies were exclusively found in beech forest soil samples while only four were exclusively found in riparian forest soils at the end of the experiment. Only four morphospecies were found in both soils (Fig. 8). Particular planting treatments are not so clearly separated, except for a few samples of *A. nemorosa* from beech forest soil that were very similar to samples representing riparian forest soil. This was also confirmed by DCA analysis based on the frequency of AMF morphospecies occurrence in particular planting treatments. The planting treatments with *A. nemorosa* from beech forest soil was grouped together with

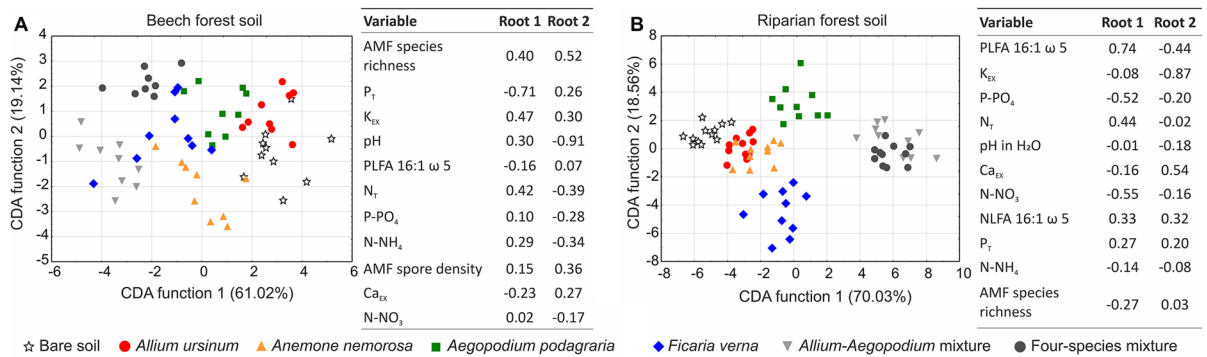


Fig. 7 The scatterplots presenting the results of canonical discriminant analysis of experimental treatments representing different planting treatments for two soils separately onto the first

and second discriminant functions (canonical roots). Standardized coefficients for canonical variables are also provided

planting treatments representing the riparian forest soil (Fig. 9). DCA analysis performed separately on both types of soil also confirmed that *A. nemorosa* planting treatment is the most different in terms of AMF community composition (Fig. S2).

Discussion

We used for the first time an experimental approach to assess the influence of temperate forest herbaceous species with varying traits on AMF abundance and diversity in soil. Our results show that the four plants can form niches in the forest soil differing in AMF abundance and diversity, thereby contributing to the spatial heterogeneity of forest micro-sites.

In our experiment, the soil from the riparian forest promoted vascular plant growth 3–4-fold, compared to the soil from the beech forest. Probably, as a consequence of higher vascular plant mass, higher mycorrhizal colonization of roots and the concentration of AMF biochemical markers in soils were found, in general, in the riparian forest soil. Moreover, there was also a strong effect of soil origin on the composition of AMF morphospecies. The aforementioned effects may result from differences in soil chemical properties, since almost all parameters were higher in the riparian forest soil than in the beech forest soil. The differences were especially pronounced in case of pH and the concentrations of available forms of Ca, K, N, and P. However, variations in soil biota abundance and composition between the soils may also

play a role in determining growth of plants and their impact on AMF community (Ardestani et al. 2022).

The highest mean values of mycorrhizal parameters were found in *A. ursinum* on both soils. Similarly, high mycorrhizal colonization rates of this plant species in comparison to other herbaceous plants were found in temperate forests (Grünfeld et al. 2019; Zubek et al. 2021). Other species of the genus *Allium*, both wild and cultivated, were also reported to form AM (Wang and Qiu 2006), with a high degree of root colonization (Hewins et al. 2015; Nobis et al. 2015; Piszczek et al. 2019). Seasonal changes in root colonization were shown in *A. tricoccum*, but AMF root communities appeared unaffected by plant phenology (Hewins et al. 2015).

The PLFA 16:1 ω 5 and NLFA 16:1 ω 5 concentrations tended to be higher in the mixture of *Allium-Aegopodium* and the mixture of four species. These biochemicals are attributed to AMF hyphal biomass and AMF storage lipids markers, respectively (Olsson et al. 1997; Olsson 1999; Sharma and Buyer 2015). As a highly mycorrhizal species (Zubek et al. 2021), *A. ursinum* seems to support AMF growth in soils. Similarly, *A. podagraria* was also reported to form AM (Zubek et al. 2021), and may thus contribute to the increased PLFA 16:1 ω 5 and NLFA 16:1 ω 5 concentrations. This suggests that both AM plants have allocated a part of their photosynthates to AMF extraradical hyphae, and that the fungi have re-allocated them to storage lipids (van Aarle et al. 2003). The short period of photosynthetic activity of *A. ursinum* due to fast leaf senescence seems not to negatively impact AMF communities associated with this

Table 3 PERMANOVA pairwise comparisons of arbuscular mycorrhizal fungi community composition among levels of planting treatments within soil origins and between soil origins within planting treatments, calculated as multivariate pseudo-t

Factor	Bare soil	<i>Allium ursinum</i>	<i>Anemone nemorosa</i>	<i>Aegopodium podagraria</i>	<i>Ficaria verna</i>	<i>Allium - Aegopodium mixture</i>	Four-species mixture
Soil	Bare soil		1.21	3.62	1.50	2.34	2.36
	<i>Allium ursinum</i>	0.192		4.71	0.23	2.46	3.55
	<i>Anemone nemorosa</i>	0.001	0.001		5.43	4.82	3.72
	<i>Aegopodium podagraria</i>	0.088	0.934	0.002		2.52	4.63
	<i>Ficaria verna</i>	0.001	0.005	0.001	0.023		1.57
	<i>Allium - Aegopodium mixture</i>	0.006	0.004	0.009	0.001	0.238	
	Four species mixture	0.013	0.186	0.001	0.342	0.049	0.006
	Bare soil		0.89	1.44	0.96	1.36	2.13
	<i>Allium ursinum</i>	0.572		0.86	0.63	1.08	2.18
	<i>Anemone nemorosa</i>	0.074	0.524		0.30	0.68	2.12
Riparian forest soil	<i>Aegopodium podagraria</i>	0.468	0.773	0.921		0.33	1.46
	<i>Ficaria verna</i>	0.110	0.280	0.663	0.731		1.32
	<i>Allium - Aegopodium mixture</i>	0.002	0.016	0.076	0.429	0.438	
	Four species mixture	0.227	0.344	0.715	0.833	0.837	0.247
	Bare soil		0.89	1.44	0.96	1.36	2.13
	<i>Allium ursinum</i>	0.572		0.86	0.63	1.08	2.18
	<i>Anemone nemorosa</i>	0.074	0.524		0.30	0.68	2.12
Plant	<i>Aegopodium podagraria</i>	0.468	0.773	0.921		0.33	1.46
	<i>Ficaria verna</i>	0.110	0.280	0.663	0.731		1.32
	<i>Allium - Aegopodium mixture</i>	0.002	0.016	0.076	0.429	0.438	
	Four species mixture	0.227	0.344	0.715	0.833	0.837	0.247
	Bare soil		0.89	1.44	0.96	1.36	2.13
	<i>Allium ursinum</i>	0.572		0.86	0.63	1.08	2.18
	<i>Anemone nemorosa</i>	0.074	0.524		0.30	0.68	2.12
Beech forest soil vs. Riparian forest soil		t = 2.68 p = 0.001	t = 4.12 p = 0.001	t = 2.27 p = 0.015	t = 4.12 p = 0.001	t = 4.15 p = 0.004	t = 4.14 p = 0.004
							t = 4.41 p = 0.002

statistics and p values obtained using permutation procedure. Lower diagonal – p values by permutation, upper diagonal – pseudo-t statistics; values in bold are significant ($p < 0.05$)

species. Hewins et al. (2015) observed no clear seasonal changes in AMF root community structure of *A. tricoccum* and suggested that this was due to the constant presence of roots throughout the growing season. Hewins et al. (2015) also showed an increase in *A. tricoccum* nutrient content in bulbs during the time when it lacks leaves. The authors suggested that during this time, the roots and/or associated AMF are active, acquiring nutrients, despite the absence of photosynthesis and thus C flows to AMF. We may find a similar case in *A. ursinum*, namely, there may be little seasonal variability in C resource availability within roots, and thus no negative effects on AMF abundance in soil are observed. Alternatively, the higher concentrations of PLFA 16:1 ω 5 and NLFA 16:1 ω 5 may also result from the fact that in both planting treatments, vascular plant cover was high and that these planting treatments were not monocultures. It was shown that higher vascular plant cover increases AMF abundance in temperate forest soils (Bahnmann et al. 2018; Zubek et al. 2021). Literature data also indicate that higher plant diversity has a positive influence on AMF diversity (Helgason et al. 1998; Burrows and Pfleger 2002; Johnson et al. 2004).

Although in most AMF a large proportion of total fatty acids is found as PLFA 16:1 ω 5, this compound occurs also in some bacteria (Olsson 1999). Thus,

our result concerning PLFA 16:1 ω 5 concentrations in the soils may be affected by the presence of bacteria. Nevertheless, the tendencies in PLFA 16:1 ω 5 concentrations were supported by the trends in NLFA 16:1 ω 5 concentrations.

Allium ursinum disperses by seeds in natural conditions, forming dense, in many cases also mono-specific, patches. *Aegopodium podagraria*, which spreads mainly by its rhizomes, is similarly effective in colonizing forest floor (Witkowska-Żuk 2008). These traits of both mycorrhizal plants result in their support of AMF abundance in temperate forests. *Aegopodium podagraria* has the status of an invasive species in the North American forests (D'Hertefeldt et al. 2014; USDA Forest Service 2022). To the best of our knowledge, no studies have focused so far on *A. podagraria* effects on soil microbial communities in the area of its invasion. As knowledge on the impact of invaders on the environment is crucial for both ecological and practical reasons, our results, which reveal the ability of this plant to impact AMF communities in the native range, contribute to a better understanding of its ecology. Furthermore, *A. podagraria* occurs also in anthropogenic habitats (Witkowska-Żuk 2008), and is considered as a weed, especially in shaded gardens (Afonin et al. 2008). Knowledge of its ecology in interactions with AMF may be essential in the management of arable soils.

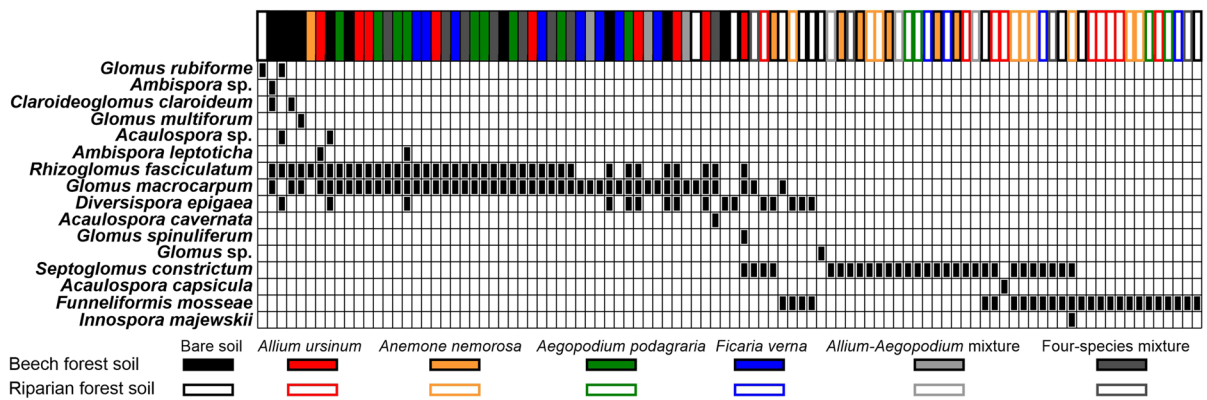


Fig. 8 The seriation diagram of arbuscular mycorrhizal fungi morphospecies occurrence (absence/presence) in soil samples representing particular experimental treatments on an unconstrained algorithm

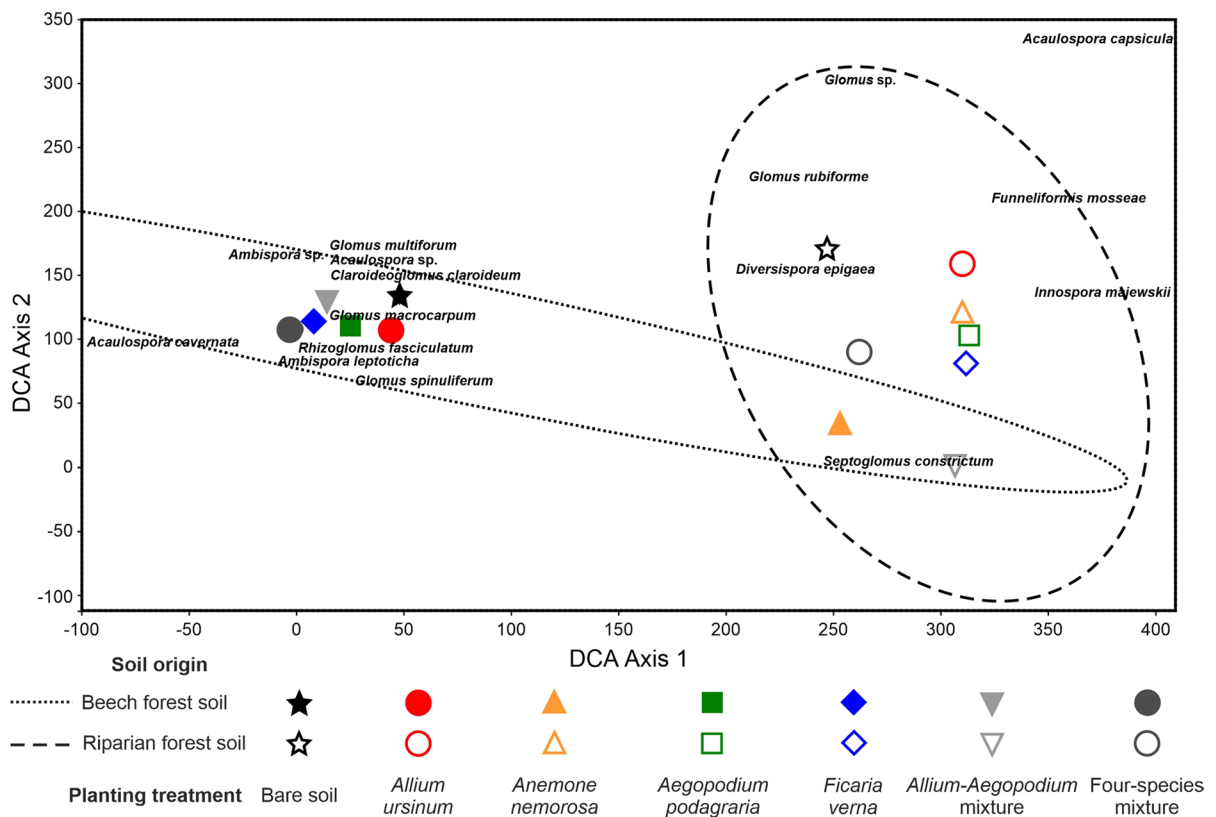


Fig. 9 Detrended correspondence analysis (DCA) scatter plot of analyzed experimental treatments and particular arbuscular mycorrhizal fungi morphospecies along the first two axes. 95% confidence ellipses are included

The highest AMF spore density was recorded in the *A. podagraria* planting treatment from beech forest soil, whereas the lowest in the *A. nemorosa* and

the mixture of *Allium-Aegopodium* treatments with beech forest soil and all planting treatments with riparian forest soil. Although high NLFA 16:1ω5

concentrations were found in the mixture of *Allium-Aegopodium*, surprisingly, spore density was the lowest. This could have resulted from the fact that storage lipids may not only be present in spores but also accumulate in hyphae (Smith and Read 2008).

In general, low AMF morphospecies richness was recorded in both soils, which is in line with the observations of Rožek et al. (2019, 2020) and Zubek et al. (2021), who found low AMF morphospecies richness in temperate forests. The highest morphospecies richness was observed in the *A. ursinum*, *A. podagraria*, and the mixture of four species planting treatments with beech soil. This may be related to the aforementioned character of mycorrhizal associations of both plants. Both species stimulated the growth and sporulation of AMF species. Surprisingly, also the bare soil planting treatment with beech forest soil was characterized by a similar AMF morphospecies richness. In this treatment, high bryophyte cover was observed. Wang and Qiu (2006) reported that 46% and 71% of the bryophyte, namely liverworts and hornworts, species and families have fungal associations, and some of them were reported as ‘AM-like’. In the case of liverworts and hornworts, the structures involved in fungal associations are rhizoids and thalli of gametophytes (Smith and Read 2008). As was shown in a liverwort, *Marchantia paleacea*, and also in other species from this genus, AMF form coils in some cells, many of which with arbuscules. AMF improved growth and fitness of *M. paleacea* through enhancing nutrient uptake, especially P, and the liverwort enhanced AMF biomass in soil (Humphreys et al. 2010). Therefore, there is a possibility that the presence of AMF species in the bare soil treatment was due to AMF associations with liverworts.

In the present study, AMF community composition depended on soil origin and was at the same time affected by planting treatment. This is in line with our field observation, which showed that AMF community composition differed between beech and riparian forests (Zubek et al. 2021). The results of the present experimental study suggest that the herbaceous plant species colonizing forest sites may form soil niches differing in AMF diversity.

Although *A. nemorosa* was reported to form AM (Rožek et al. 2019), the mycorrhizal colonization rates of this species were low and its effect on AMF abundance in soil was rather weak in the present study. Nevertheless, AMF community composition

related to this plant differed the most from other planting treatments. However, as we used the classical method of AMF species identification, AMF diversity may have been underestimated. We may have overlooked AMF species that rarely produce spores (Clapp et al. 1995; Hempel et al. 2007; Njeru et al. 2015; Varela-Cervero et al. 2015).

Similarly to *A. nemorosa*, the effects of *F. verna*, another small spring ephemeral, on soil AMF communities were not pronounced. However, due to poor growth of *F. verna* in some treatments of our experiment, and thus the lack of shoot biomass data, as well as the scarce presence of roots and consequent lack of AMF colonization degree analysis, we are not able to be conclusive about the *F. verna* effects on AMF communities. Although AMF root colonization degree of *F. verna*, as well as spore and morphospecies numbers in soils related to this species were low, the values tended to be the highest of all studied plant species and their combinations in a riparian forest (Zubek et al. 2021). *Ficaria verna* has become a highly invasive species in temperate deciduous forests in North America (Invasive Species Specialist Group 2022). Paolucci et al. (2021) reported low AMF colonization rates of *F. verna* roots in its introduced range. The authors suggested that although *F. verna* may form AM, its AMF root colonization is not extensive and thus *F. verna* does not have strong relationships with AMF.

Anemone nemorosa and *F. verna* belong to the Ranunculaceae, whose representatives are AM-forming species (Wang and Qiu 2006). Although colonized by AMF, the responsiveness of plants of this family to AMF may vary (Kytöviita and Ruotsalainen 2007; Zubek et al. 2009; Borriello et al. 2017). As shown in the experiment conducted by Rožek et al. (2021), some temperate forest herbaceous species, even those belonging to families whose representatives are usually highly colonized by and responsive to AMF, may be less dependent on AMF. In turn, they may have little impact on AMF soil community, as observed for *A. nemorosa* and *F. verna* in the present study. This assumption, however, needs further experiments concerning the impact of AMF on the performance of both plant species.

In the present study, in general, vascular plant cover and vascular plant shoot and belowground mass positively correlated with PLFA 16:1 ω 5 and

NLFA 16:1ω5 concentrations, AMF root colonization parameters, but negatively affected AMF spore density and morphospecies richness in soil. These results confirm observations from the field that in forests, AMF abundance in soil and AMF root colonization may be increased by a greater plant coverage (Zubek et al. 2021). The negative effect of vascular plant cover and biomass on AMF spore density and AMF morphospecies richness in soil found in the present experiment is in contrast to our field observations (Zubek et al. 2021) and difficult to explain. We also observed that the cover of bryophytes negatively correlated with NLFA 16:1ω5 concentration in soil, AMF root colonization parameters as well as all soil parameters. This effect may be related to AMF associations with bryophytes, namely weak support of AMF by C transfer. However, the negative effect of bryophytes on AMF parameters could also be a result of the lower vascular plant cover in the treatments rather than the high bryophyte cover itself.

In the present study, AMF abundance and diversity in soil was also influenced by soil chemical properties which was especially noticeable in the soil from the riparian forest. Concentrations of N, P, K, Ca or pH were responsible for these effects. This is in line with numerous observations that macronutrient contents and soil pH affect AMF communities in temperate forests (Burke et al. 2011; Koorem et al. 2014; Rożek et al. 2021; Zubek et al. 2021). For example, Burke et al. (2011) found that AMF community diversity is positively correlated with soil inorganic N and P content, whereas Koorem et al. (2014) showed that the abundance of AMF was negatively related to soil phosphorus and positively influenced by soil nitrogen content. It was also found that AMF abundance in soils and roots increased along with increasing soil alkalinity and macronutrient levels (Rożek et al. 2019).

The majority of AMF species found in the soils used in our experiment are widely distributed in the world and present in numerous natural and anthropogenic habitats (Błaszowski 2012). The species have already been found in temperate forest soils (Błaszowski 2012; Rożek et al. 2019, 2020; Stanek et al. 2021; Zubek et al. 2021). Although *Acaulospora cavernata* is acknowledged as a rare species (Błaszowski 1994; Gai et al. 2006), its spores were found recently in temperate forest soils (Rożek et al. 2019, 2020). Similarly, *Glomus spinuliferum* is known, so far, from only a few locations in Europe,

mainly grasslands and vineyards (Błaszowski 2012); however, it was also recently found in a temperate forest (Zubek et al. 2021). This is the first report of *Glomus multiforum* occurrence in forest soil, as this species is known so far only from grasslands and dunes (Błaszowski 2012). Although literature data show that *Ambispora leptoticha* spores are rather rarely found in the world, results of molecular environmental analyses (Bills and Morton 2015; Marinho et al. 2018) suggest that the species also has a wide distribution and occurs in different habitats of the world.

In conclusion, we demonstrated that herbaceous plants under study may form niches in the forest soil differing in AMF abundance and diversity. Highly colonized plant species that are able to form large patches on the forest floor, namely *A. ursinum* and *A. podagraria*, were found to be the most successful in supporting AMF abundance. Although *F. verna* and *A. nemorosa* are AM-forming plants, their impact on AMF communities in soil were found to be rather weak. We also showed that the effect of plant species identity on AMF abundance and diversity is dependent on soil chemical properties. Finally, our findings support earlier observations that temperate forest soils are characterized by low AMF species numbers and that herbaceous species of the forest floor show highly variable root colonization levels. This study provided an insight into the rarely-studied interactions of forest herbaceous plants and Glomeromycota. A greater understanding of the biology and ecology of forest herbaceous plants, i.e. how they interact with microorganisms that contribute to their persistence in forests, may be essential for long-term management of these plant species and communities (Hewins et al. 2015). Further studies should elucidate the character of AMF associations with the plant species under study, namely the impact of AMF on the performance of these species.

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Declarations

Conflict of interest The authors have no conflict of interest.

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