University of South Bohemia in České Budějovice

Faculty of Science

Diversity of mycorrhizal communities in roots of selected grassland species

Bachelor´s thesis

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ANNOTATION

Communities of arbuscular mycorrhizal fungi (AMF) in roots of 14 grassland plant species were examined in the quantitative as well as qualitative way. For quantification, the AMF colonisation was estimated in stained plant roots using the light microscope. For qualitative evaluation of samples, NGS method followed by biostatistical analysis were used. Basic statistical methods were used to compare root colonisation of plant species and plant functional groups, as well as to compare colonisation estimates of two researchers.

DECLARATION

I declare that I am the author of this qualification thesis and that in writing it I have used the sources and literature displayed in the list of given sources only.

České Budějovice, 12. 05. 2021

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Anna Marie Mikulecká

List of abbreviations

AM, AMF – Arbuscular Mycorrhizal Fungi

PCR – Polymerase chain reaction

NGS – Next generation sequencing

VTX – Virtual taxon

G – grass

F – forb

ACKNOWLEDGEMENTS

I need to thank a lot to my supervisor dr. Marie Šmilauerová for the management in the field, in the lab, and supporting me in the theoretical part. She also gave me an opportunity to try the sorting of grassland biomass and field work. It was a nice experience and a part-time job, also I can not forget to thank her for the nice time and useful advice.

I need also to thank a lot for the molecular leading, processing of data (their explanation and going across to them), data corrections, good advice, and of course for the big support from dr. Jiří Košnar.

I thank dr. Petr Šmilauer for the nice management during biostatistical processing and for the patience with me and supporting me.

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CONTENTS

1 INTRODUCTION

1.1 Mycorrhizal symbiosis

Mycorrhizal symbiosis is one of the most widespread symbiotic relationships on Earth. It is generally classified as a mutualistic relationship among fungi and host plants based on reciprocal benefits in nutrition, growth, and the protection against pathogens (Hodge et al. 2010, Cosme et al. 2018). Mycorrhizal fungi colonise roots of a broad spectrum of plant species. Hyphae of mycorrhizal fungi interconnects soil with the inner space of the root and allows the exchange of nutrients (ions) from fungi to plant and carbohydrates from plant to the fungi. Fungi colonise rhizodermis and primary root cortex (Fig. 1).

Mycorrhizal symbiosis is classified into several types according to the character of the fungal colonisation of roots (Fig. 1). This thesis is focused on the arbuscular mycorrhiza (Gryndler et al. 2004).

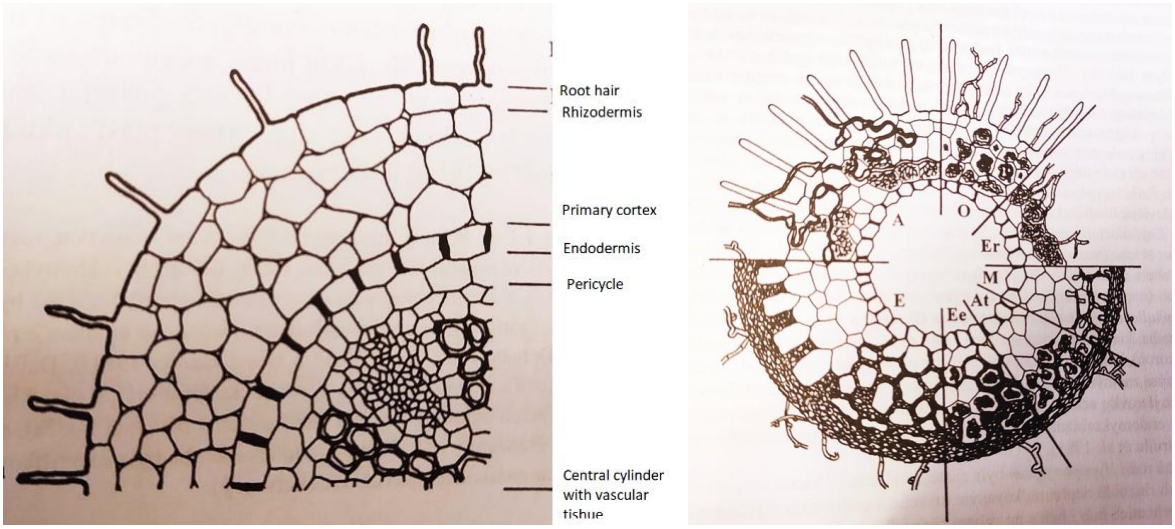

Figure 1: Scheme of a cross-section of a root (on the left). Schematic illustration of different types of mycorrhiza in the cross-section of a root (on the right): A – arbuscular mycorrhiza, E – ectomycorrhiza, Ee – ectendomycorrhiza, At – arbutoid mycorrhiza, M – monotropoid mycorrhiza, Er – ericoid mycorrhiza, O – orchideoid mycorrhiza. (Gryndler et al. 2004)

1.2 Arbuscular mycorrhizal fungi (AMF)

Arbuscular mycorrhizal symbiosis involves approximately 80% of land plants and around 250 morphologically defined or, alternatively, 350 to 1000 molecularly defined AMF species (Davison et al. 2015).

AMF belongs to *Glomeromycotina* subphylum of the *Mucoromycota* phylum, a basal fungal taxon, and they are in the present day considered phylogenetically related to *Mucoromycotina*, based on the genome sequence data from *Rhizophagus irregularis* (Genre et al. 2017).

AM fungi penetrating into the host plant roots create characteristic structures in the primary cortex: intraradical mycelium, arbuscules, and vesicles. Intraradical mycelium consists of thick hyphae, which creates densely branched structures, called arbuscules, in cortical cells (Gryndler et al. 2004). Arbuscules facilitate the transfer of nutrients to the root cells (Hao et al. 2019).

Vesicles (from the Latin vesicula, bladder) of spherical or irregular shapes arise from hyphae of the root intraradical mycelium. Their function is not exactly known, but they are assumed to be fungal storage organs.

It is important that AMF do not penetrate the cytoplasmatic membrane of host‘s cells. Hyphae getting through the cortical cell wall consequently push the cytoplasmatic membrane of the plant cell inwards and the final appearance can remind the penetration (Gryndler et al. 2004, Fig. 2).

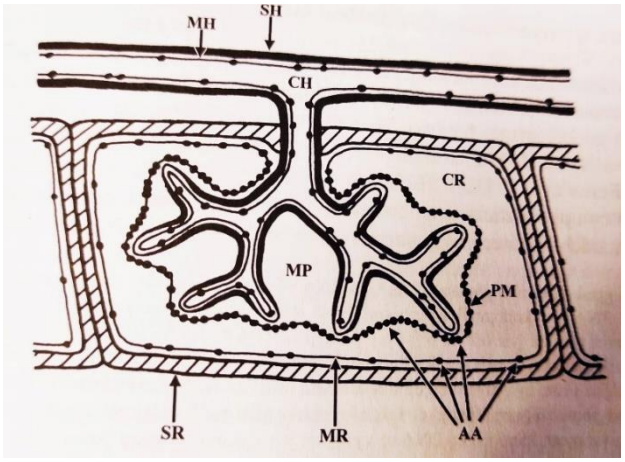

Figure 2: Scheme of the arbuscule formation: SR – cell wall of the host plant cell, MR – the cytoplasmatic membrane of the plant cell, CR – cytoplasm of the plant cell, CH – fungal cytoplasm, MH – the cytoplasmatic membrane of the fungal cell, SH – cell wall of the fungal cell, PM – periarbuscular membrane (expansion of the cytoplasmatic membrane of plant cell), MP – periarbuscular space, AA – localization of ATPase activity. (Gryndler et al. 2004)

1.3 Host species for AMF

AM symbiosis is widespread, but it is not present in all vascular plants. Approximately 20–29% of all vascular plants (including some important crops) do not host AMF. But also non-mycorrhizal vascular plants can under certain conditions contain some AMF (Cosme et al. 2018). A review of the mycorrhizal status of plant species in the British flora (relevant for most of the species of Central Europe) was published by Harley and Harley (1987), and further expanded to a global scale by Soudzilovskaia (Soudzilovskaia et al. 2020).

Vascular host plants exhibit a higher small-scale richness of AMF communities in grasslands, but the total richness is higher in forests (Davison et al. 2015).

1.4 AMF communities

AMF communities show temporal (Dumbrell et al. 2011) as well as spatial variability (Carvalho et al. 2003, Mummey and Rillig 2008, Moll et al. 2016, Avio et al. 2020). Hart et al. (2015) demonstrated changes in AMF community composition even with the soil depth.

For AMF communities composition, the identity of host plant as well as of neighboring plants, availability of AMF taxa on locality, the composition of coexisting fungal microbial communities, distribution and amount of available nutrients, and other soil properties (as soil moisture or pH) are important. The phylogenetical structure of associated AMF communities

can change along a gradient from more ruderal host plants onto plants with other functional traits, and that can be a reason for changes in the dominance of AMF taxa. The trait differences among host plants are often summarized by their classification into functional groups (Dassen et al. 2017; Gui et al. 2018). It is expected that different AMF communities are affiliated with different plant functional groups (Chagnon et al. 2013; López-García et al. 2017). Some studies show differences across the AMF communities in the grassland ecosystems, namely between grasses and forbs (dicotyledonous plant species) (Albarracín et al. 2016; Gui et al. 2018).

To study the effects of individual factors on the AMF community composition, it was essential to implement molecular methods enabling the determination of AMF taxa. Even early studies using analysis of SSU (nuclear ribosomal small sub-unit) sequences from mycorrhizal roots revealed considerable phylogenetic diversity of AMF fungi (Vandenkoornhuyse et al. 2002). The taxonomy of AMF at species level was further evaluated by Öpik et al. (2010), who compiled MaarjAM database of known virtual AMF taxa (VTX) based on phylogenetic analysis of available DNA sequences. The next-generation sequencing technique supports the identification of taxa from hundreds of samples simultaneously (Tedersoo et Nilsson 2017; Glenn 2011; Lindahl et al. 2013). This method is often used as a semi-quantitative technique, providing information about the relative abundance of individual taxa present in AMF communities (Šmilauer et al. 2020).

Unfortunately, it is impossible to determine the intensity of total AMF colonisation in the host plant roots with molecular methods. For this purpose, the staining of AMF structures in roots followed by microscopic evaluation is necessary. In this way, it is possible to determine the intensity of total mycorrhizal colonisation as well as the presence and relative proportions of typical structures (hyphae, vesicules, arbuscules) in roots.

This thesis addresses the following research questions:

1) Are the selected coexisting grassland plant species and plant functional groups (grasses and forbs) different in the degree of AMF root colonisation? Is such a difference detectable in the total AMF colonisation, arbuscular or vesicular colonisation levels?
2) Do estimates of mycorrhizal colonisation differ between researchers?
3) Does the species richness of AMF communities differ among the host species or plant functional groups?

1.5 Goals of the thesis

This work follows the research of my supervisor dr. Šmilauerová and her husband, dr. Šmilauer. Practical goals of this thesis were:

1) Introduction to methods of collecting and processing of host plant roots for the subsequent analysis of AMF community composition and for the estimation of mycorrhizal colonisation rates.
2) Quantification of mycorrhizal colonisation rate in roots, using a light microscope.
3) Comparison of my own and other researcher's estimates of the AMF colonisation rates using the same sample collection.
4) Introduction to the current use of the NGS method in research of mycorrhizal fungal communities, with the help of literature.
5) Work in molecular laboratory (isolation of AMF DNA from host plant roots, and subsequent processing up to sending the samples for the NGS analysis by Illumina MiSeq device).

2 MATERIALS AND METHODS

2.1 Locality characterization

Plants were collected from a meadow near the village Zvíkov in the Czech Republic (48°59‘20“N, 14°36‘28“E), c. 500 m above sea level. This traditionally managed meadow is situated in a shallow valley slope with oligotrophic vegetation (without fertilization in the past 30 years, mown once a year). Cambisol is the dominant soil in the field with a low ability of macronutrients (Šmilauer et al. 2020).

Sampled meadow is a species-rich plant community (about 85 species), with the most abundant species at hay-cut time *Alopecurus pratensis*, *Anthoxanthum odoratum*, *Holcus lanatus*, *Plantago lanceolata,* and *Poa angustifolia*. The nomenclature of the plant species follows Kubát et al. 2002.

2.2 Plant species specification and plant sampling

Fourteen selected species (see Table 1) represent substantial fraction of the plant cover (63%) of the grassland community at the locality. They represent two major functional groups of this plant community: forbs (dicotyledons) and grasses.

Table 1: List of species with the date of plant collection, their membership in functional group (G–grasses) or F–forbs), and final number of individuals used for microscopic evaluation of AMF colonisation.

Abbreviations	Host Species	Functional group	Sampling date	Final number of quantified samples
AM	*Achillea millefolium*	F	24-June 3-July	5
BO	*Betonica officinalis*	F	3-July	5
CJ	*Centaurea jacea*	F	3-July	4
KA	*Knautia arvensis*	F	21-June 24-June	5
PL	*Plantago lanceolata*	F	3-July	5
RA	*Ranunculus acris*	F	9-May	5
VC	*Veronica chamaedrys*	F	7-May 9-May	5
AT	*Agrostis tenuis*	G	12-July	4
AP	*Alopecurus pratensis*	G	12-July	5
AO	*Anthoxanthum odoratum*	G	2-May	5
FP	*Festuca pratensis*	G	21-June	4
FR	*Festuca rubra*	G	21-June	4
HL	*Holcus lanatus*	G	21-June	5
PA	*Poa angustifolia*	G	9-May	5

Plants were sampled from 5 different places of the experimental meadow within the period May–July 2019. In each plant species a flowering individual was collected from each of the sampling places, so totally five plants per all species but one (*Festuca rubra* with four individuals) were collected. Plants were sampled together with the soil surrounding their roots and transported to the laboratory for further processing.

2.3 Sample processing

2.3.1 Root sample preparation

Roots of sampled plants were separated from aboveground parts, cleaned from the soil and other plant's roots, and washed by tap water. Cleaned roots were split into two groups: one for the molecular analysis and the other one for staining and AMF colonisation evaluation. Samples for molecular analysis were dried and stored in a freezer.

2.3.2 Root staining

Samples for estimating mycorrhizal colonisation were cleaned in 10% KOH solution at room temperature overnight (12 h), or for a shorter time (8 h) in grasses. KOH cleaning was followed by tap water washing and 2 min. neutralization in 3.5% HCl solution, and staining in 0.03% w/v solution of Chlorazol Black E in lactoglycerol (14:1:1 lactic acid, glycerol, deionized water) at 90 °C for 60 min (Šmilauer et al. 2020). Stained roots were spread in a drop of lactoglycerol on microscopic slides, covered by coverslip, and sealed with transparent varnish.

2.4 Quantification of AMF colonisation of roots

The colonisation was quantified under a light microscope (Olympus BX50). Magnification 200x was used. AMF colonisation was distinguished into three types: total, arbuscular, and vesicular colonisation. Other fungal colonisation and abnormalities (spores, hyphae of fine endophyte morphotype or non-mycorrhizal fungi, extraradical hyphae) were also recorded.

Estimates of the three categories of fungal colonisation (total, arbuscular and vesicular) were based on estimating the percentage of root length with the AMF structures in each examined field. The estimates were combined across all recorded fields of the individual sample.

As an arbuscule was classified a branched hyphal structure inside a root cell, sometimes rolled into a ball. An example of densely packed arbuscules of AMF in roots are shown in Figs. 3–4.

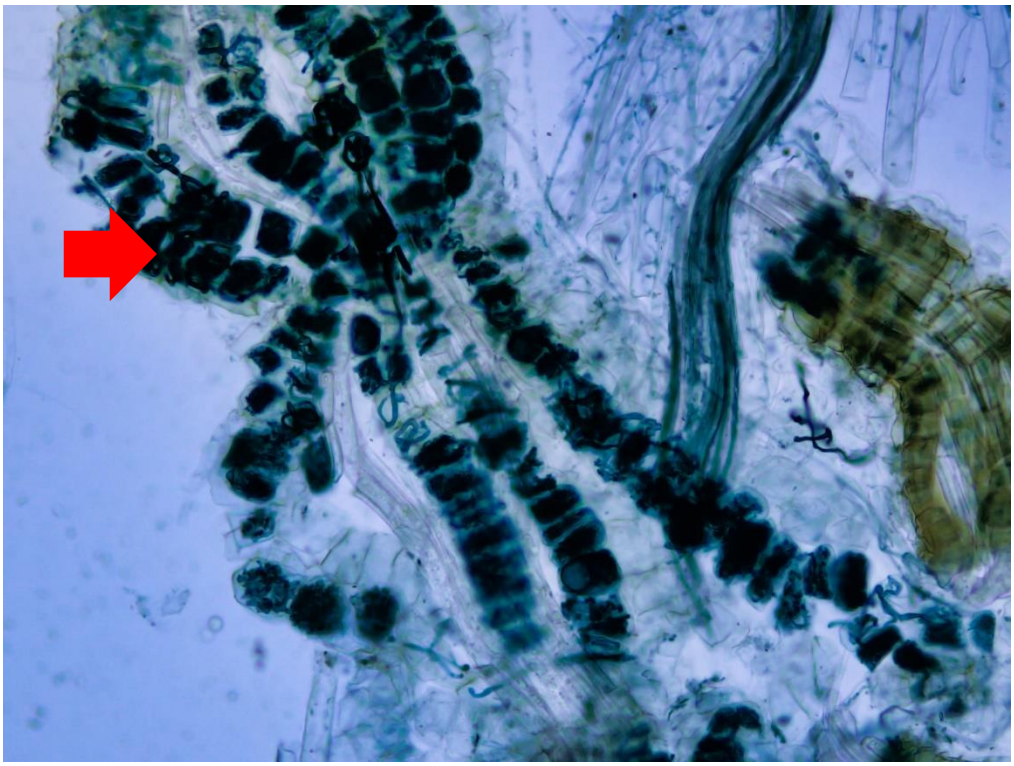

Figure 3: Arbuscules (red arrow) in *Alopecurus pratensis* roots.

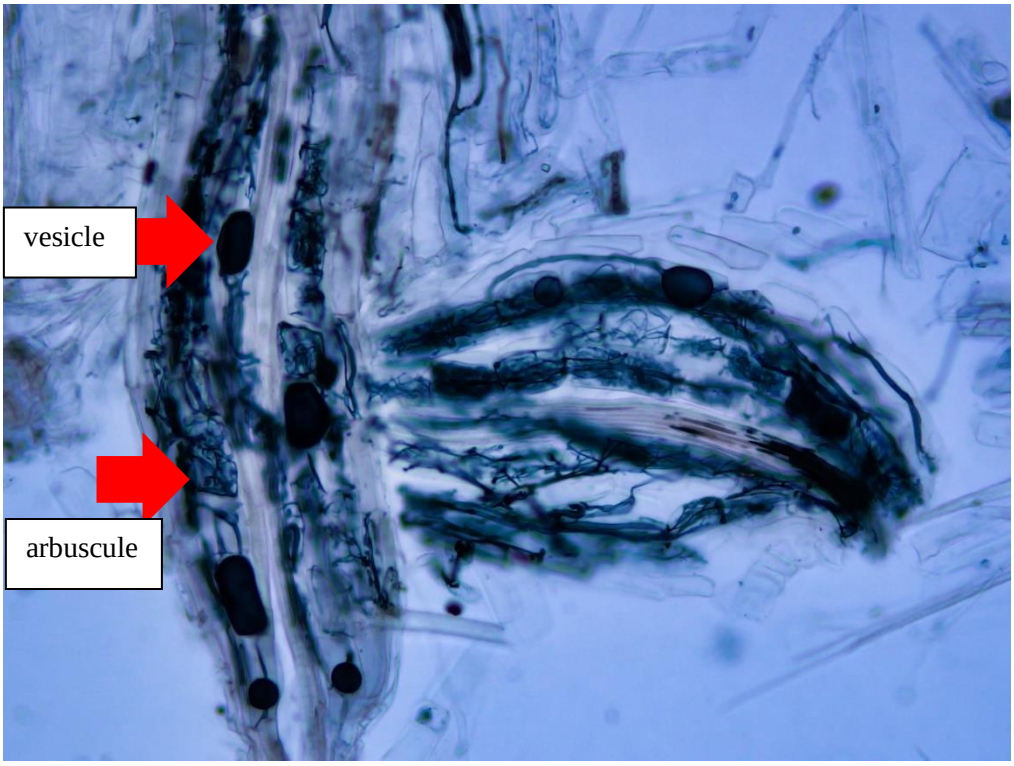

Figure 4: Arbuscules and vesicules in *Festuca pratensis* roots.

As a vesicule was classified a structure of oval shape connected to AMF hyphae (Figs. 4–7).

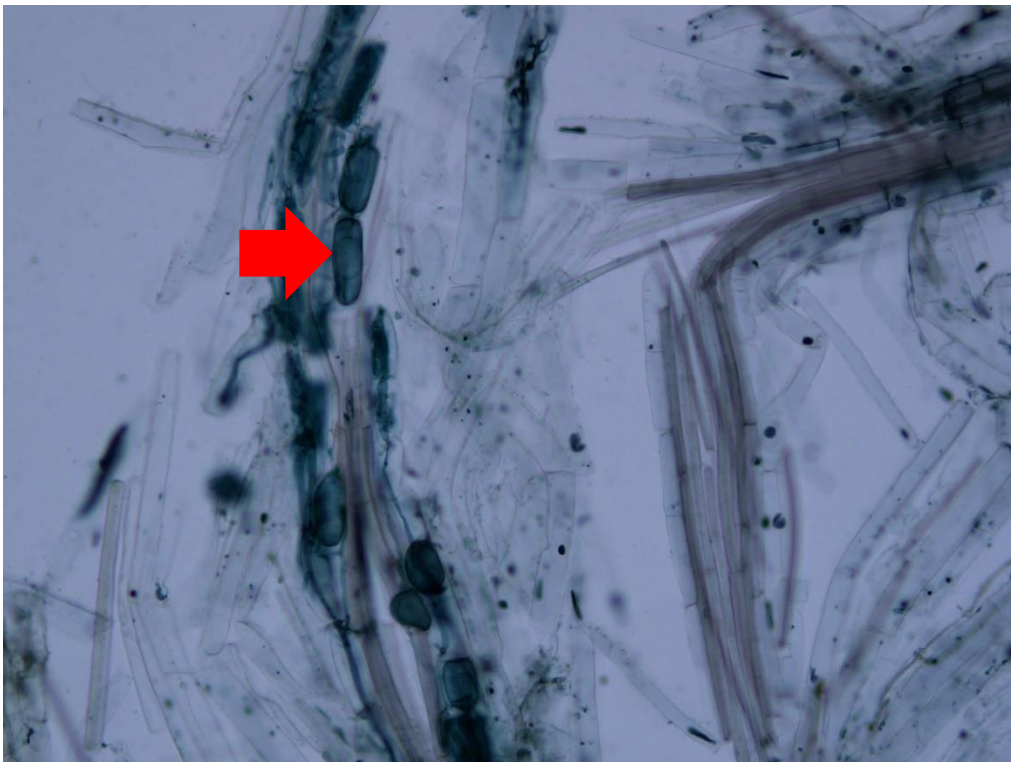

Figure 5: Vesicules (red arrow) in the *Alopecurus pratensis* roots.

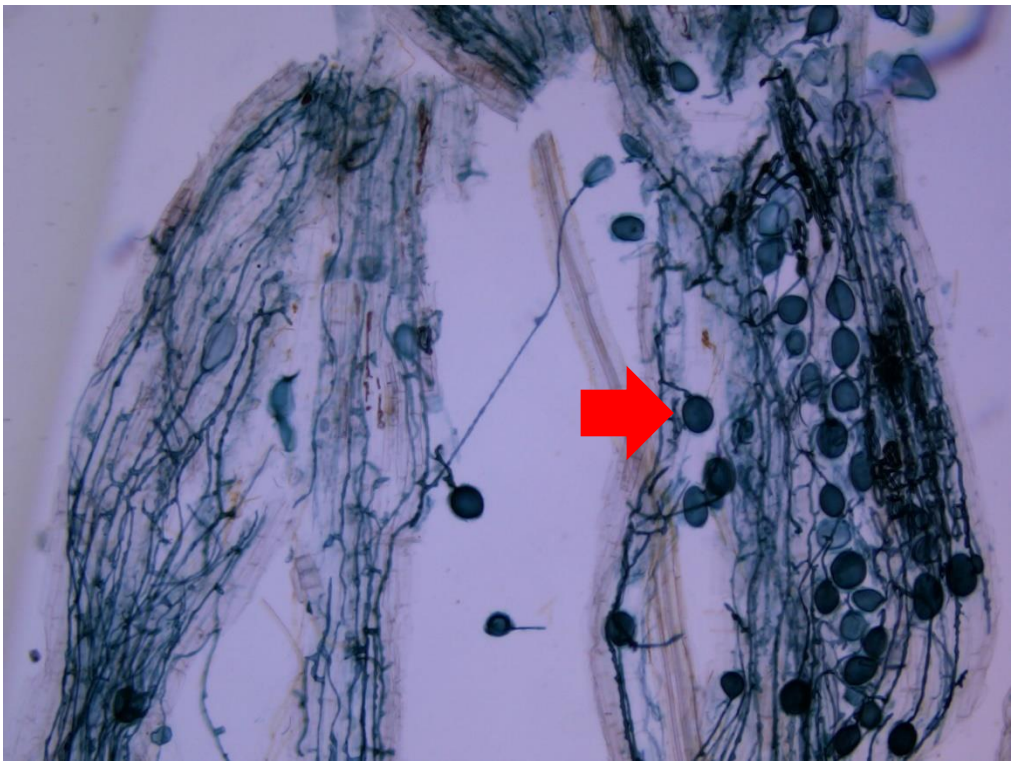

Figure 6: Vesicules (red arrow) in the *Centaurea jacea* roots.

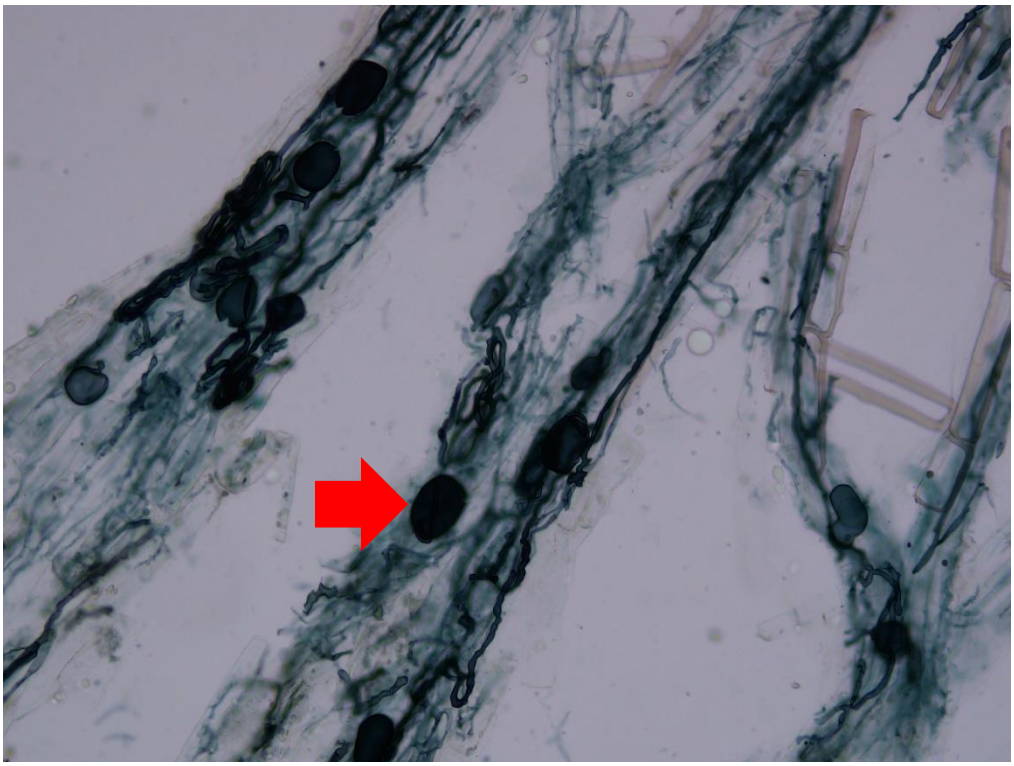

Figure 7: Vesicules (red arrow) in the *Plantago lanceolata* roots.

2.5 Molecular analysis

2.5.1 Isolation of DNA from plant material

Root samples were homogenised by vortexing with eight mirrables from the stainless steel in the mixer mill Retsch 400MM for 20 min at 25 Hz. The isolation itself was done according to the CTAB method (Doyle and Doyle 1987). CTAB (cetyl trimethyl ammonium bromide) works as a detergent, which releases DNA from the membranes and proteins. Hydrophobic contaminants and proteins are removed using chlorophorm extraction, and the water phase containing DNA is subsequently purified using alcohol precipitation. Following minor modifications of the protocol were used:

step 3: to the crushed material was added 800 µL of CTAB solution and 11.4 µL of 2-mercaptoethanol

step 10: addition of 600 µL cold isopropanol (from the freezer)

step 19: centrifugation 5 min at 10,000 rpm

2.5.2 Sample purification

The DNA isolates obtained by CTAB method were often coloured and contained inhibitors blocking PCR amplification. Therefore, DNA samples were further purified by DNeasy PowerClean Cleanup Kit (Qiagen, Venlo, the Netherlands) according to the manufacturer's protocol, except that centrifugation speed was set to 10,500 x g for 2 min, and the amount of solution EB in step 13 was decreased to 40 μL.

2.5.3 PCR

Polymerase chain reaction (PCR) allows amplification of certain parts of DNA in vitro. The amplified region is defined with primers – oligonucleotides (short single-chain DNA) about the length of 18 to 25 bases pairing with a complementary sequence in DNA template and working as a starting point for replication of DNA. The basic arrangement of PCR involves mixing of all agencies (buffer, DNA polymerase, $MgCl_2$, nucleotides, primers, template DNA) and subsequent cyclic temperature changes (in the basic steps: denaturation, annealing of primers, elongation). The PCR amplifications results in exponential increase of amplified DNA.

In my samples approximately 550 bp long fragment of fungal SSU (nuclear ribosomal DNA) was amplified according to the Šmilauer et al. 2020. This genomic region is suitable for easy identification of virtual AMF taxa from MaarjAM database (Öpik et al. 2010).

In the molecular process was used semi-nested PCR design. For first PCR were used NS31 (forward) and AML2 (reverse, Lee et al. 2008) specific primers. The second PCR involved Wanda primer combined with sample-specific barcode sequence, allowing identification of individual samples in NGS output, and AML2 primer.

In the first PCR, all reagents were mixed according to Table 2 and centrifugated. Afterward, 12.9 μL of the mixture was pipetted to the 8-well PCR strip (sufficient for 8 samples) and to a single tube for a negative control. In the following step, 2.1 μL of DNA was added (except to the negative control). Then all samples were mixed, centrifugated and 5 μL of each sample was transferred to another two strips to obtain three independent PCR replicates.

The first PCR was performed under following conditions: an initial denaturation at 98 °C for 30 s, followed by 35 cycles of 10 s at 98 °C, 30 s at 68 °C, and 15 s at 72 °C; and a final extension at 72 °C for 5 min (Šmilauer et al. 2020).

Table 2: Reaction mixture for the 1st PCR (calculated for processing eight samples)

	8x / µL
H_2O	63.45
5x HF buffer	27
10mM sNTPs	2.7
Primer NS31/ 5 pmol μL^{-1}	10.8
Primer AML2 / 5 pmol μL^{-1}	10.8
Phusion polymerase / 2U μL^{-1}	1.35

The results of PCR were verified on 1.5% (w/v) agarose gel in 1x TBE buffer, yielding visible DNA products in approximately 2/3 of the samples (see Fig. 8). If negative control was without amplification, it was continued with 2nd PCR.

Negative control from 1st PCR was used as a template for the 2nd PCR described below and the product was verified on agarose gel. As no amplification was observed in this second amplification of negative control, amplifications of individual samples from 1st PCR were used as a template for 2nd PCR.

For second PCR, all reagents were mixed according to Table 3 and centrifugated. Afterward, 30.45 µL of the mixture was pipetted to the 8-well PCR strip (sufficient for eight samples), and 2.8 µL of a single uniquely barcoded primer WANDA (5 pmol μL^{-1}) was added to each sample tube. Everything was mixed and centrifugated. 4.75 µL of the mixture was taken from each sample tube to the new strip as a negative control. 1.5 µL of the products of 1st PCR was used as a template and added to the sample tubes. Samples were again mixed and centrifugated and 10 µL of the mixture was pipetted to another 2 strips to obtain three independent PCR replicates.

Table 3: Reaction mixture for the 2nd PCR (calculated for processing 8 samples)

	8x / µL
H_2O	166.6
5x HF buffer	59.5
10 mM dNTPs	5.95
Primer AML2 / 5 pmol μL^{-1}	23.8
Phusion polymerase / U μL^{-1}	2.975

PCR setup was used as in the first PCR reaction, except that the annealing temperature was set up to 60 °C and only 10 cycles were used (Šmilauer et al. 2020).

The results of PCR were verified on 1.5% (w/v) agarose gel in 1x TBE buffer (see Fig. 8) and if negative control was without amplification, successfully amplified samples were further processed.

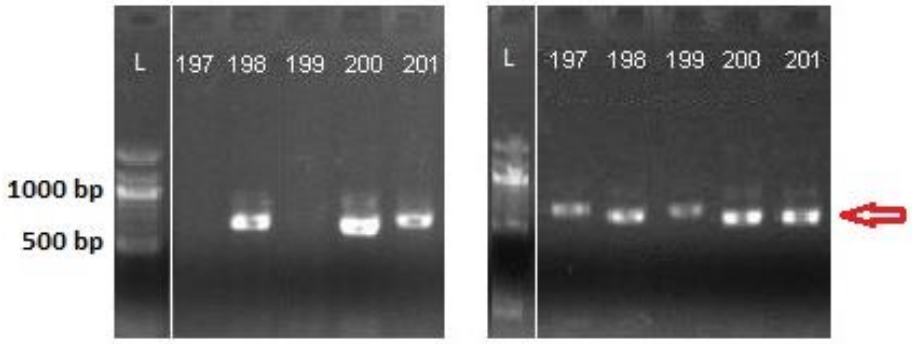

Figure 8: Electrophoretic visualization of 1st PCR products (left) and 2nd PCR products (right) for five selected samples. Red arrows points at target PCR product. L - DNA marker. Showed species: 197 – *Agrostis tenuis*, 198 – *Betonica officinalis*, 199 – *Centaurea jacea*, 200 – *Holcus lanatus*. 201 – *Knautia arvensis*.

2.5.4 PCR purification from agarose gels

Agarose gel after 2nd PCR revealed occasional non-specific amplification in some of the samples (Fig. 8). These PCR products were removed by cutting target PCR product from 1.5% (w/v) agarose gel in 1x TBE buffer.

The subsequent purification of agarose slices containing target DNA was done using PCR clean-up Gel extraction kit (Macherey-Nagel, NucleoSpin®Gel and PCR Clean-up, 2017) with these changes:

In step 3, the volume of Buffer NT3 was reduced to 650 µL and the recommended second washing step was not used. Then in step 5, 30 µL of buffer NE was added and it was centrifugated for 1 min at 10,000 rpm.

2.5.5 Preparation for Illumina sequencing

DNA concentration of purified barcoded samples was measured using Quibit 2.0 fluorometer (dsDNA BR Assay Kit). Samples were equimolarly pooled in a single tube and sent for Illumina MiSeq sequencing performed at SEQme Company (Dobříš, Czech Republic).

Illumina sequencing platform involves parallel sequencing of a large amount of DNA molecules (each molecule is sequenced separately, Egan et al. 2018).

MiSeq 300 bp paired-end sequencing was used, covering the first and last 300 bp of each particular molecule. Assembly of both sequencing directions yielded complete sequence of the target molecule as its length was about 550 bp.

2.6 Analysis of NGS data

Data obtained from the SEQme company were processed (Fig. 9) using the software tools realized in SEED v.2.0 (Větrovský et al. 2018), Mothur v.1.29.5 (Schloss et.al. 2009), Usearch (Edgar et al. 2014) and PipeCraft v.1.0 (Anslan et al. 2017). Paired-end reads were assembled into complete continuous sequences of the sequenced DNA molecules using FLASH v1.2.11 (Magoč et Salzeberg 2011, performed in PipeCraft v.1.0) with the following settings: minOverlap = 10 bp, mismatch ratio = 0.3, average read length = 550, SD = 35. In order to remove low-quality sequences, quality trimming was performed using vsearch v1.11.1 (Rognes et al. 2016, performed in PipeCraft) with the following settings: trucqual = 6, maxee = off, maxee_rate = 0.01, minlen = 450, maxns = 0. The resulting sequences were demultiplexed using SEED v.2.0 with no barcode mismatch allowed and primer mismatch set to one, yielding 844 924 sequences spanning the complete amplicon length. Putative PCR chimeras resulting from premature termination of DNA elongation and subsequent reannealing of the incomplete strand of different molecule was removed using Usearch Uchime algorithm (Edgar et al. 2014) in reference database mode with default settings and MaarjAM database (Öpik et al. 2010; accessed November 2018) of SSU ribosomal DNA type sequences of known virtual AMF taxa (VTX, Bruns et al. 2018). For each sample was chosen up to 1000 sequences using SEED.

SSU pipeline (Vasar et al. 2017) was used to identify Illumina sequences against so-called reference database. The pipeline performed BLAST search against MaarjAM database containing sequences of known AM taxa (2 760 sequences distributed in 352 virtual taxa).

BLAST search found the most similar database hit for each analyzed sequence, and the following criteria were subsequently required to count the most similar sequence as a true match: sequence similarity <= 97%; alignment length not differing from the length of the shorter of the query and subject sequences by > 5%; and an e-value in Blast < 1e-50. As the comparison with the database of known AMF virtual taxa alone is not sufficient to detect new unknown taxa, as the putative novel AMF taxa were considered sequences with similarity to closest available AMF sequence exceeding 90% but below 97% (12 670 sequences), and were clustered using Usearch with a 97% similarity threshold. The resulting clusters of sequences were further carefully evaluated to minimize the risk of elevating sequencing artifacts as a false novel species. Only clusters containing more than 100 sequences were preserved. Random selection of twenty sequences of each cluster was made and aligned with MaarjAM database type of sequence using MAFFT v.7 (Katoh et al. 2017) with default settings and cropped according to the length of Illumina sequences. With the use of TOPALi v.2.5 (Milene et al. 2009) NJ tree was constructed with default settings and 500 bootstrap replicates. Accepted were only clusters that formed well-supported (bootstrap values >= 75) monophyletic clades. With the use of these criteria were not detected any novel AMF taxa. Therefore only AMF sequences with similarity >= 97% to the taxa from the extended versions of the MaarjAM database were obtained from the dataset. For each sample were randomly picked up to 400 of such sequences, yelding a total of 32 180 sequences, which were summarized using SSU pipeline to obtain final table of VTX present in individual samples. From statistical analysis were removed samples with less than 90 sequences.

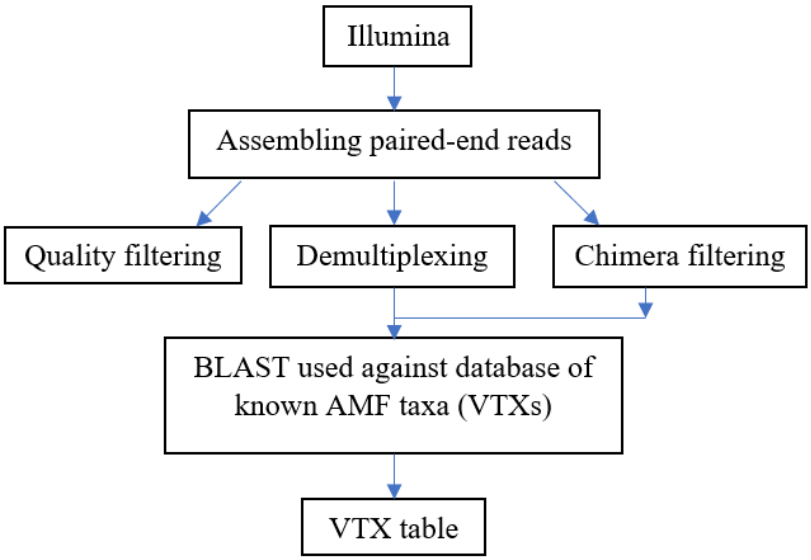

Figure 9: Workflow of analyzing NGS data. Picture inspired by Anslan et al. 2017.

2.7 Statistical analysis of data

One-way ANOVA was used to compare colonisation between species or functional groups. The tests were complemented by plotting by the „whiskers“ boxplots graphs. Tukey *post-hoc* test was used to identity singificantly different host plant species. To compare colonisation estimates by the author and by dr. Šmilauer, paired t-tests were used.

Statistical analysis of the data was done according to Lepš et Šmilauer (2016 and 2020).

In the „box and whisker“ plots presented in Results, thick black lines represent median values, upper and lower edges of boxes represent upper and lower quartiles, whiskers represent minimum and maximum (except outlier observations), and circles represent outliers.

3 RESULTS

3.1 AMF root colonisation rate

In general, total AMF colonisation in host plant species was high (Fig. 10), with the exception of *Alopecurus pratensis* and *Veronica chamaedrys*. Significant differences (p-values < 0.05) were found in all three characteristics of AMF colonisation (Table 4; Figs. 10–12) among individual host species. On the contrary, the two functional groups differed in vesicular colonisation only, which was lower in grasses (see Table 4, Fig. 13).

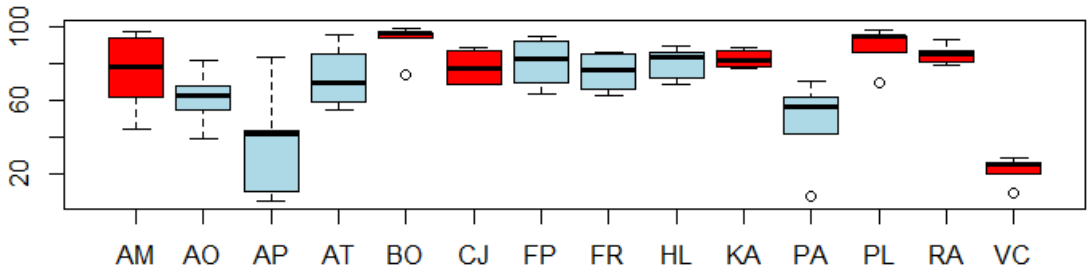

Figure 10: Total AMF colonisation of 14 plant species. Results are presented as box and whisker plots. Forbs are presented by red boxes, grasses by blue boxes. For species abbreviation see Table 1 in Methods.

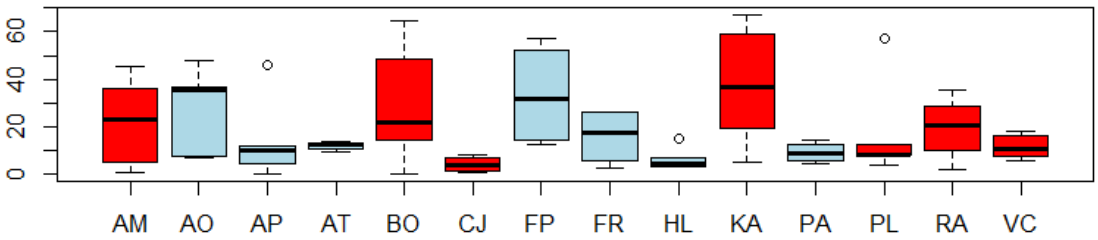

Figure 11: Arbuscular colonisation of 14 plant species. Results are presented as box and whisker plots. Forbs are represented by red boxes, grasses by blue boxes. For species abbreviations see Table 1 in Methods.

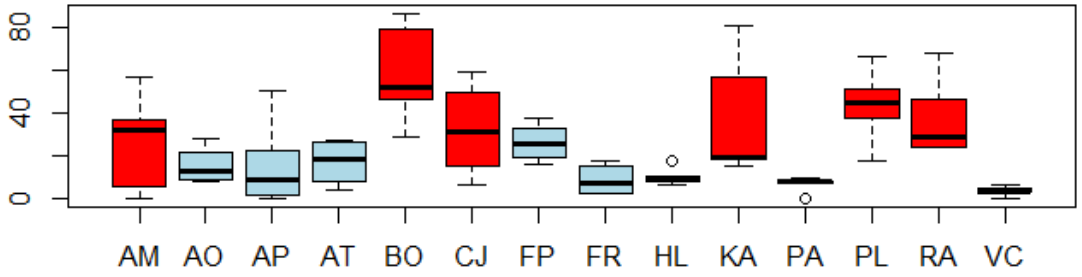

Figure 12: Vesicular colonisation of 14 plant species. Results are presented as box and whisker plots. Forbs are represented by red boxes, grasses by blue boxes. For species abbreviations see Table 1 in Methods.

Table 4: Tests of difference in three characteristics of AMF colonisation among species and between functional groups. F statistic with degrees of freedom and corresponding Type I error probability estimates are shown.

	species		functional groups	
	$F_{13, 52}$	p	$F_{1, 12}$	p
Total AMF colonisation	8.24	< 0.001	0.78	0.395
Arbuscular colonisation	4.81	< 0.001	0.40	0.540
Vesicular colonisation	4.34	< 0.001	6.68	0.024

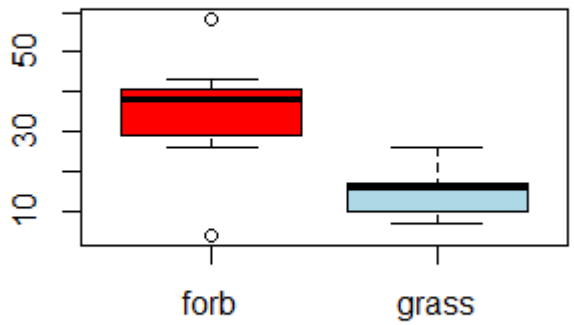

Figure 13: Vesicular colonisation in forbs and grasses.

Tables 5 and 6 present results of the Tukey-test for multiple comparisons of the total AMF colonisation and the vesicular colonisation, within pairs of host species. Both tables show only pairs of significantly different plant species, with adjusted p-values < 0.05. The species given first in the pairs at the start of the table (or those given as second in the pairs at the end of the table) are those with the highest AMF colonisation. *Veronica chamaedrys* is a species with the lowest total AMF colonisation differing from most of the other species (11 out of 14), whereas *Betonica officinalis* is the species with the highest vesicular colonisation differing significantly from 7 other species (6 of them are grass species). For arbuscular colonisation, Tukey-test for multiple comparisons did not find any pair of host species differing significantly with the adjusted p-value < 0.05.

Table 5: Tukey-test comparings the total AMF colonisation between individual host plant species. Out of the 105 possible comparisons, only the significant ones ($p < 0.05$) are shown. The adjusted p-value represents the significance of the pair difference. Difference values were sorted from the highest positive value to the lowest negative value. For species abbreviations see Table 1 in Methods.

Compared species	Difference	Adjusted p-value
BO-AP	55.4	< 0.001
PL-AP	52.0	< 0.001
RA-AP	48.3	0.001
KA-AP	45.8	0.003
FP-AP	44.2	0.010
HL-AP	43.3	0.006
CJ-AP	41.6	0.020
PL-PA	41.1	0.012
FR-AP	38.9	0.040
RA-PA	37.5	0.032
AP-AM	-38.6	0.024
VC-AO	-39.6	0.018
PA-BO	-44.5	0.004
VC-AT	-50.8	0.001
VC-AM	-53.7	0.000
VC-FR	-54.0	0.001
VC-CJ	-56.7	< 0.001
VC-FP	-59.3	< 0.001
VC-HL	-58.4	< 0.001
VC-KA	-61.0	< 0.001
VC-RA	-63.5	< 0.001
VC-PL	-67.1	< 0.001
VC-BO	-70.5	< 0.001

Table 6: Tukey-test comparing the abundance of vesicules in the AMF colonisation between individual host plant species. Out of the 105 possible comparisons only the significant ones ($p < 0.05$) are shown. The adjusted p-value represent the significance of the pair difference. Difference values were sorted from the highest positive value to the lowest negative value. For species abbreviations see Table 1 in Methods.

Compared species	Difference	Adjusted p-value
BO-AO	42.6	0.015
BO-AP	42.0	0.017
BO-AT	41.4	0.037
VC-PL	-39.7	0.031
HL-BO	-48.2	0.003
FR-BO	-49.6	0.004
PA-BO	-51.7	0.001
VC-BO	-54.9	< 0.001

3.2 Differences between researchers in mycorrhizal colonisation estimates

The estimates of AMF colonisation differ sigificantly between both researchers, in all estimated colonisation types (total AMF colonisation, vesicular and arbuscular colonisation), see Table 7.

Table 7: Tests of differences in estimates of three characteristics of AMF colonisation between two researchers. t-statistics and corresponding significance estimates are shown.

	species	
	t_{65}	p
Total AMF colonisation	7.095	< 0.001
Arbuscular colonisation	-9.7084	< 0.001
Vesicular colonisation	7.0013	< 0.001

The average AMF colonisation rates estimated by myself were 69.6% for total AMF colonistion, 18.8% for arbuscular colonisation, and 24.6% for vesicular colonisation. The estimates made by dr. Šmilauer had average 57.5% of total AMF colonisation, 42.2% of arbuscular colonisation, and 11.6% of vesicular colonisation. Mean difference between my and dr. Šmilauer's estimation of the total AMF colonisation, the arbuscular and the vesicular colonisation are, respectively, 12.1, -23.4, and 13.0%.

Comparison of Figures 10 and 14 shows higher estimates of arbuscular colonisation made by dr. Šmilauer for most of species, with the exception of grasses *Alopecurus pratensis* and *Poa angustifolia*, where the estimates are similar.

For vesicular colonisation, species medians from dr. Šmilauer estimates for all but one (*Betonica officinalis*) species were below 20% while my estimates for all forb species were above 20% (Fig. 11 and 15).

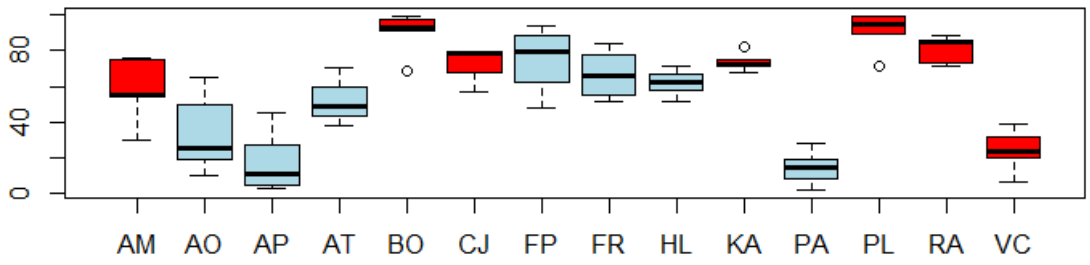

Figure 14: Total AMF colonisation of 14 plant species as estimated by dr. Šmilauer. Results are presented as box and whisker plots. For species abbreviations see Table 1 in Methods.

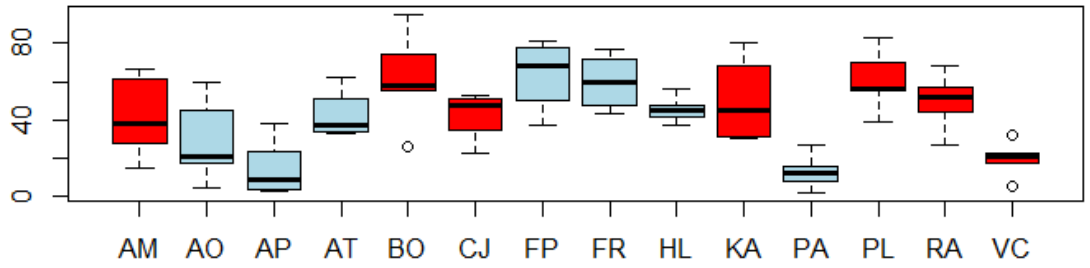

Figure 15: Arbuscular colonisation of 14 species as estimated by dr. Šmilauer. Results are presented as box and whisker plots. For species abbreviations see Table 1 in Methods.

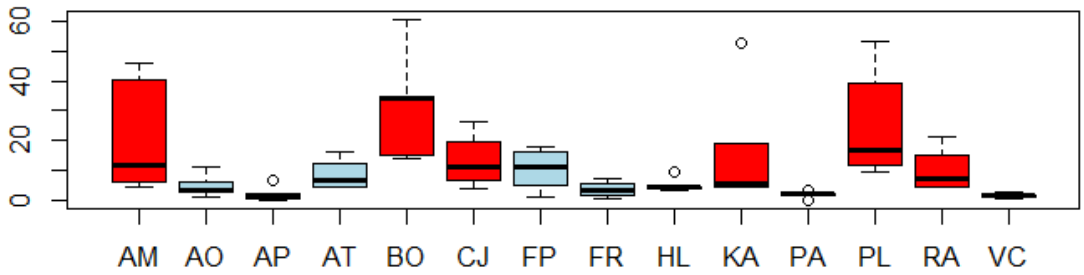

Figure 16: Vesicular colonisation of 14 plant species as estimated by dr. Šmilauer. Results are presented as box and whisker. For species abbreviations see Table 1 in Methods.

Tables 9 to 11 present the results of multiple comparisons of AMF colonisation characteristics (the total AMF colonisation, arbuscular and vesicular colonisation) for pairs of host species evaluated by dr. Šmilauer. Tables show only the pairs of species with adjusted p-values < 0.05.

Consequently, the species given first in the pairs at the start of the table (or those given as second in the pairs at the end of the table) are those with the highest AMF colonisation.

Table 8: Tests of difference among species in three characteristics of AMF ation as estimated by dr. Šmilauer. F statistics with degrees of freedom and corresponding significance estimates are shown.

	Species	
	$F_{13, 52}$	p
Total AMF colonisation	8.24	< 0.001
Arbuscular colonisation	4.81	< 0.001
Vesicular colonisation	3.37	0.009

Table 9: Tukey-test comparing the total AMF colonisation between individual host plant species, as estimated by dr. Šmilauer. Only the adjusted p-value is representating the signifikance of the pair difference. For species abbreviations see Table 1 in Methods.

Compared species	Difference	Adjusted p-value
PL-PA	76.2	< 0.001
PL-AP	72.5	< 0.001
BO-AP	71.5	< 0.001
RA-PA	66.0	< 0.001
RA-AP	62.3	< 0.001
PL-AO	56.9	< 0.001
FP-AP	56.7	< 0.001
BO-AO	56.0	< 0.001
KA-AP	55.3	< 0.001
CJ-AP	55.3	0.001
FR-AP	48.3	0.001
RA-AO	46.7	0.001
HL-AP	43.7	0.001
FP-AO	41.1	0.004
CJ-AO	39.7	0.006
KA-AO	39.7	0.003
PL-AT	39.4	0.007
BO-AT	38.4	0.009
AT-AP	33.1	0.046
PL-AM	32.8	0.028
BO-AM	31.9	0.038
VC-AM	-33.7	0.021
PA-AT	-36.8	0.015
VC-HL	-37.6	0.006
AP-AM	-39.7	0.003
VC-FR	-42.2	0.003
PA-AM	-43.4	0.001
PA-HL	-47.4	< 0.001
VC-KA	-49.2	< 0.001
VC-CJ	-49.3	< 0.001
VC-FP	-50.7	< 0.001
PA-FR	-52.0	< 0.001
VC-RA	-56.3	< 0.001
PA-KA	-59.0	< 0.001
PA-CJ	-59.0	< 0.001
PA-FP	-60.4	< 0.001
VC-BO	-65.5	< 0.001
VC-PL	-66.4	< 0.001
PA-BO	-75.3	< 0.001

Table 10: Tukey-test comparing the arbuscular colonisation between individual host plant species, as estimated by dr. Šmilauer. The adjusted p-value representings the significance of the pair difference. For species abbreviations see Table 1 in Methods.

Compared species	Difference	Adjusted p-value
FP-AP	48.3	0.008
PL-PA	47.7	0.004
BO-AP	46.3	0.006
PL-AP	45.3	0.008
FR-AP	44.4	0.021
VC-PL	-40.7	0.028
VC-BO	-41.7	0.021
VC-FP	-43.7	0.024
PA-FR	-46.8	0.011
PA-BO	-48.7	0.003
PA-FP	-50.7	0.004

Table 11: Tukey-test comparing the vesicular colonisation between individual host plant species, as estimated by dr. Šmilauer. The adjusted p-value represents the significance of the pair difference. For species abbreviations see Table 1 in Methods.

Compared species	Difference	Adjusted p-value
BO-AP	29.6	0.012
BO-AO	26.8	0.035
HL-BO	-26.6	0.038
FR-BO	-28.1	0.039
PA-BO	-29.9	0.011
VC-BO	-30.3	0.009

Poa angustifolia and *Veronica chamaedrys* are species differing in total AMF colonisation from most of the other species (10 resp. 9 out of 14), *Betonica officinalis* is the species with the highest vesicular colonisation differing significantly from six other species (five of them are grass species).

3.3 Differences in species richness of AMF communities in coexisting grassland plant species

The richness of AMF molecular VTX (Table 12, Fig. 17) differs significantly between host plant species ($F_{13,58} = 2.6$; $p = 0.006$) in ANOVA data evaluation. The richest plant species in AMF taxa are *Poa angustifolia*, *Betonica officinalis*, and *Ranuncus acris*. Differences between the two plant functional groups (forbs and grasses) are nearly significant ($F_{1,12} = 3.8$; $p = 0.07$), with higher richness in forbs (Fig. 18).

Table 12: Average counts of AMF VTX for individual host plant species

Species	Group	Avg. VTX count.
(AM) *Achillea millefolium*	F	15.0
(BO) *Betonica officinalis*	F	22.2
(CJ) *Centaurea jacea*	F	20.5
(KA) *Knautia arvensis*	F	19.2
(PL) *Plantago lanceolata*	F	20.3
(RA) *Ranunculus acris*	F	21.2
(VC) *Veronica chamaedris*	F	18.2
(AO) *Anthoxanthum odoratum*	G	17.0
(AP) *Alopecurus pratensis*	G	14.6
(AT) *Agrostis tenuis*	G	17.0
(FP) *Festuca pratensis*	G	16.0
(FR) *Festuca rubra*	G	16.3
(HL) *Holcus lanatus*	G	14.4
(PA) *Poa angustifolia*	G	22.6

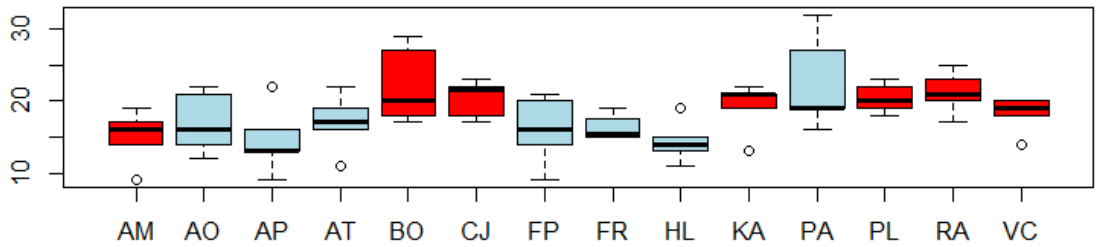

Figure 17: Counts of AMF VTX in the roots of sampled plant species. Results are presented as box and whisker plots. Forbs are representated by red boxes, grasses by blue boxes. For species abbreviations see Table 1 in Methods.

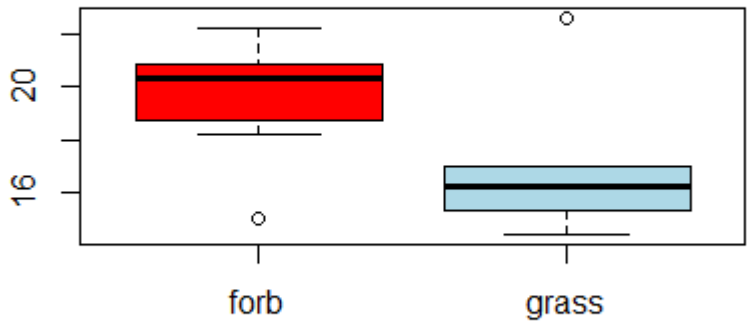

Figure 18: The AMF richness defined as VTX counts in forbs and grasses.

4 DISCUSSION

The examined grassland plant species showed differences in AMF colonisation between the species, and for vesicular colonisation also among functional groups. *Veronica chamaedrys* was a species with the lowest total mycorrhizal infection (average colonisation 21.85%), but with a high proportion of arbuscules within the colonised segments of roots (about 50% of colonised root length). Similar proportion of arbuscules within the colonised root segments had the forb *Knautia arvensis* and grasses *Alopecurus pratensis* and *Agrostis tenuis*. Within such segments, nutrient exchange can be quite intensive and profits for the host plant can exceed that in species with high total colonisation but with a low arbuscular proportion (i.e. *Holcus lanatus* or *Centaurea jacea*).

Examined grasses and forbs considerably differ in the state of the samples. Forb roots were well preserved and not damaged, while root samples of grasses were damaged during the dying process and in some places highly disorganized. This difference can be due to specific structure and properties of the roots of monocotyledonous (grasses) and dicotyledonous plants (forbs).

Significant difference in estimation of AMF colonisation among two researchers could be caused by the experience of the senior examinator, or difference in the time of the

examination, because my examination was done later than dr. Šmilauer's and there could be some effect on the quality (brightness level) of samples.

The difference of arbuscular AMF colonisation among species, as evaluated by dr. Šmilauer was significant according to ANOVA (Table 4), and the *post-hoc* comparisons revealed differences between species pairs. My own results were also conclusive for among-species differences, but post hoc comparison were not. This result could be caused by the fact that Tukey-test used for pair comparison has lower power to identify differences than the overall one-way ANOVA.

Significant differences in species richness of AMF communities were found among the species Interesting is the fact that *Poa angustifolia*, the species with the lowest total AMF colonisation, harbours the richest AMF community. Differences between the two functional goups were also almost significant – lower significance can be caused by smaller number of replicates (7 grasses and 7 forbs).

Comparison was made also with the work of Šmilauer et al. (2020), which was carried out at the same locality and studied fourteen plant species (seven grasses and seven forbs). They found differences in all three examinated types of AMF colonisation not only among species, but also between forbs and grasses. Total, arbuscular and vesicular colonisation of AMF in their study was twice as large in forbs than in grasses.

Contrary to my results, they found no difference in AMF richness among individual plant species.

For their experiment, Šmilauer et al. (2020) used seedlings, but in my thesis I worked with data from adult plants. It is possible that during the process of ontogenesis changes in AMF colonisation in roots take place together with changes in the AMF community within plant roots. Another possible explanation of the differences could be the fact that Šmilauer et al. (2020) examined only 8 plant species, while I used data form 14 plant species. From this point of view, mine could be closer to the reality.

According to Gui et al. (2018), plant functional groups differ in the AMF colonisation (higher colonisation in forbs than in grasses). In my work, the biggest differences were between plant species, which exceeded differences between the two functional groups. The difference of my study from Gui et al. (2018) could be due to different studied type of grassland vegetation (steppe in China).

5 CONCLUSIONS

This study examinated mycorrhizal communities in roots of 14 selected grassland plant species. AMF colonisation was quantified by microscope-based evaluation of the AMF structures (vesicules, arbuscules). AMF community composition was analysed by molecular methods, (NGS Illumina sequencing), with subsequent bioinformatics and statistical processing.

Microscopy showed significant differences in all three characteristics of AMF colonisation (total, arbuscular, vesicular) among studied plant species. Comparison of plant species from two functional groups (forbs vs. grasses) revealed significant differences in vesicular colonisation only. Comparison of microscopy results with the estimates of another researcher dr. Šmilauer revealed significant differences in all three characteristics of AMF colonisation. Molecular analysis revealed significant differences in AMF richness among studied plant species, and considerable differences between the two plant functional groups.

6 RESOURCES

Albarracín Orio A. G., Bruecher E., Ducasse D. A. (2016): Switching between monocot and dicot crops in rotation schemes of Argentinean productive fields results in an inkrement of arbuscular mycorrhizal fungi diversity. *Applied Soil Ecology*, **98**: 121–131.

Anslan S., Bahram M., Hiiesalu I., Tedersoo L. (2017): PipeCraft: flexible open-source toolkit for bioinformatics analysis of custom high-throughput amplicon sequencing data. *Molecular Ecology Resources*, **17**: e234–e240.

Avio L., Njeru E. M., Oehl F., Turrini A., Bocci G., Bàrberi P., Giovannetti M., Sbrana C. (2020): Small-scale soil heterogeneity affects the distribution of arbuscular mycorrhizal fungal species in a hot-spot field in a Mediterranean site. *Applied Soil Ecology*, e103631.

Bruns T. D., N. Corradi, Redecker D., Taylor J. W., Öpik M. (2018): Glomeromycotina: what is a species and why should we care? *New Phytologist*, **220**: 963–967.

Carvalho L.M., Correia P. M., Ryel R. J., Martins-Loução M. A. (2003): Spatial variability of arbuscular mycorrhizal fungal spores in two natural plant communities. *Plant and Soil*, **251**: 227–236.

Chagnon P. L., Bradley R. L., Maherali K., Klironomos J. N. (2013): A trait-based framework to understand life history of mycorrhizal fungi. *Trends in Plant Science*, **18**: 484–491.

Cosme M., Fernández I., Van der Heijden M. G. A., Pieterse C. M. J. (2018): Non-Mycorrhizal Plants: The Exceptions that Prove the Rule. *A Cell Press journal* [online]. May 09, 2018, **23**(7): P577–587 [cit. 2021-04-01]. Available from: https://www.cell.com/trends/plant-science/pdf/S1360-1385(18)30088-8.pdf doi:https://doi.org/10.1016/j.tplants.2018.04.004.

Dassen S., Cortois R., Martens H., de Hollander M., Kowalchuk G. A., van der Putten W. H., De Deyn G. B. (2017): Differential responses of soil bacteria, fungi, archea and protists to plant species richness and plant functional group identity. *Molecular Ecology*, **26**: 4085–4098.

Davison J., Moora M., Öpik M., Adholeya A., Ainsaar L., Bâ A., Burla S., Diedhiou A. G., Hiiesalu I., Jairus T., Johnson N. C., Kane A., Koorem K., Kochar M., Ndiaye C., Pärtel M., Saks Ü., Singh R., Vasar M., Zobel M. (2015): Global assessment of arbuscular mycorrhizal fungus diversity reveals very low endemism. *Science*, **349**, Issue 6251.

Doyle J.J., Doyle J.L. (1987): A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*, **19**: 11–15.

Dumbrell A. J., Ashton P. D., Aziz N., Feng G., Nelson M., Dytham C., Fitter A. H., Helgason T. (2011): Distinct seasonal assemblages of arbuscular mycorrhizal fungi revealed by massively parallel pyrosequencing. *New Phytologist*, **190**: 794–804.

Edgar R. C., Haas B. J., Clemente J. C., Quince C., Knight R. (2014): UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics*, **27**: 2194–2200.

Egan C. P., Rummel A., Kokkoris V., Klironomos J., Lekberg Y., Hart M. (2018): Using mock communities of arbusculat mycorrhizal fungi to evaluate fidelity associated with Illumina sequencing. *Fungal Ecology*, **33**: 52–64.

Genre A., Bonfante P. (2017): The structure of arbuscular mycorrhizas: A cell biologist's view. Molecular Mycorrhizal Symbiosis. *John Wiley & Sons, Inc*. **First edition**.

Glenn T. C. (2011): Field quide to next-generation DNA sequences. *Molecular Ecology Resources*, **11**: 759–769.

Gui W., Ren H., Liu N., Zhang Y., Cobb A. B., Wilson G. W. T., Sun X., Hu J., Xiao Y., Zhang F. et al. (2018): Plant functional group influences arbuscular mycorrhizal fungal abundance and hyphal contribution to soil CO2 efflux in temperate grasslands. *Plant and Soil*, **432**: 157–170.

Gryndler M., Baláž M., Hršelová H., Jansa J., Vosátka M. (2004): Mykorhizní symbióza – O soužití hub s kořeny rostlin. *Academia*.

Hart M. M., Aleklett K., Chagnon P. L., Egan C., Ghignone S., Helgason T., Lekberg Y., Öpik M., Pickles B. J., Waller L. (2015): Navigating the labyrinth: a guide to sequence-based, community ecology of arbuscular mycorrhizal fungi. *New Phytologist*, **207**: 235–247.

Hao Z., Xie W., Chen B. (2019): Arbuscular Mycorrhizal Symbiosis Affects Plant Immunity to Viral Infection and Accumulation. *Viruses* [online]. 2019 Jun 8, **11**(6): 534 [cit. 2021-04-01]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6630321/ doi:10.3390/v11060534.

Hodge A., Helgason T., Fitter A. H. (2010): Nutritional ecology of arbuscular mycorrhizal fungi. *Fungal Ecology*, **3**: 267–273.

Harley J. L., Harley E. L. (1987): A check-list of mycorrhiza in the British flora. *New Phytologist*, **105**: 1–102.

Katoh K., Rozewicki J., Yamada K. D. (2017): MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Brief. Bioinform.*, Sep 6 2017. doi: 10.1093/bib/bbx108.

Kubát K., Kaplan Z., Danihelka J., Chrtek J., Kirschner J., Stech M., Stepanek J. (2002): Klíč ke květeně České republiky. *Academia*, 928.

Lee J., Lee S., Young J.P.W (2008): Improved PCR primers for the detection and identification of arbuscular mycorrhizal fungi. *FEMS Microbiology Ecology*, **65**: 339–349.

Lepš J., Šmilauer P. (2016): Biostatistika. Episteme. **First edition**.

Lepš J., Šmilauer P. (2020): Biostatistic with R. An Introductory Guide for Field Biologist. *Cambridge University Press*. **First edition**, doi: 10.1017/9781108616041.

Lindahl B. D., Nilsson R. H., Tedersoo L., Abarenkov K., Carsen T., Kjøller R., Koljalg U., Pennanen T., Rosendahl S., Stenlid J., Kauserud H. (2013): Fungal community analysis by

high-throughput sequencing of amplified markers – a user's guide. *New Phytologist*, **199**: 288–299.

López-García A., Varela-Cervero S., Vasar M., Öpik M., Barea J. M., Azcón-Aguilar C. (2017): Plant traits determine the phylogenetic structure of arbuscular mycorrhizal fungal communities. *Molecular Ecology*, **26**: 6948–6959.

Magoč T., Salzberg S. L. (2011): FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics*, **27**: 2957–2963.

Milne, Lindner D., Bayer M., Husmeier D., McGuire G., Marshall D. F., Wright F. (2009): TOPALi v2: a rich graphical interface for evolutionary analyses of multiple alignments on HPC clusters and multi-core desktops. *Bioinformatics*, **25**: 126–127.

Moll J., Hoppe B., König S., Wubet T., Buscot F., Krüger D. (2016): Spatial Distribution of Fungal Communities in an Arable Soil. *Plos One*, **11**: e0148130.

Mummey D. L., Rillig M. C. (2008): Spatial characterization of arbuscular mycorrhizal fungal molecular diversity at the submetre scale in a temperate grassland. *FEMS Microbiology Ecology*, **64**: 260–270.

Öpik M., Vanatoa A., Vanatoa E., Moora M., Davison J., Kalwij J. M., Reier U., Zobel M. (2010): The online database MaarjAM reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). *New Phytologist*, **188**: 223–241.

Rognes T., Flouri T., Nichols B., Quince Ch., Mahé F. (2016): VSEARCH: a versatile open source tool for metagenomics. *Peer Journal*, **4**: e2584.

Schloss P.D., Westcott S. L., Ryabin T., Hall J. R., Hartmann M., Hollister E. B., Lesniewski R. A., Oakley B.B., Parks D.H., Robinson C.J., Sahl J.W., Stress B., Thallinger G.G., Van Horn D.J., Weber C.F. (2009): Introducing Mothur: open-source, platform-independent, communitysupported software for describing and comparing microbial communities. *Appl. Environm. Microbiol*., **75**: 7537–7541.

Soudzilovskaia N. A., Vaessen S., Barcelo M., He J., Rahimlou S., Abarenkov K., Brundrett M. C., Gomes S. I. F., Merckx V., Tedersoo L. (2020): FungalRoot: global online database of plant mycorrhizal associations. *New Phytologist*, **227**: 955–966.

Šmilauer P., Košnar J., Kotilínek M., Šmilauerová M. (2020): Contrasting effects of host identity, plant community, and local species pool on the composition and colonisation levels

of arbuscular mycorrhizal fungal community in a temperate grassland. *New Phytologist*, doi: 10.1111/nph.16112.

Tedersoo L., Nilsson R.H. (2017): Molecular identification of fungi. *Molecular Mycorrhizal Symbiosis*. John Wiley & Sons, 2017, **First Edition**, 301–322.

Vasar M., Andreson R., Davison J., Jairus T., Moora M., Remm M., Young J.P.W., Zobel M., Öpik M. (2017): Increased sequencing depth does not increase captured diversity of arbuscular mycorrhizal fungi. *Mycorrhiza*, **27**: 761–773.

Větrovský T., Baldrian P., Morais D. (2018): SEED 2: a user-friendly platform for amplicon high-throughput sequencing data analyses. *Bioinformatics*, **34**: 2292–2294.

Vandenkoornhuyse P., Baldauf S. L., Leyval C., Straczek J., Young J. P. W. (2002): Extensive fungal diversity in plant roots. *Science* **295**: 2051.