**Abstract**

Keywords:

**INTRODUCTION**

Bryophytes are the second biggest species group within the plant kingdom behind the much larger angiosperms (Crosby et. al. 1999, Frahm & Frey 1992). There are approximately 25,000 species taxonomically divided into hornworths (*Anthocerotopsida*), two classes of the liverworts (*Marchantiopsida, Jungermanniopsida*) and the mosses (*Bryopsida*) (Zechmeister, Grodzinska & Szarek Lukaszewska 2003). The history of bryophyte research began 1718 in Gießen, Germany where J. J. Dillenius first described mosses for botanic research (Drehwald 2013). Recent researches suggest that mosses play an important role as an omnipresent component in plant communities worldwide and strongly influence the water, nutrient and carbon cycle of their habitat (Turetsky et. al. 2012, Gerson 1969, (Gignac 2001). Unlike many other plants bryophytes can reproduce both sexually and vegetative (Frey & Kürschner 2011, Mishler 1985). Their role as the simplest terrestrial plant puts them in the spotlight of research which tries to draw back the lines plant-evolution from aquatic to terrestrial habitats (Cove, Knight & Lamparter 1997). Bryophytes lately interested researchers for many applications: Mosses were successfully used as accumulation indicators for pollutants like trace metals, heavy metals, radionucleides and for toxic organic compounds (Giordano et. Al. 2005, Harmens et. al.2010, Nentwig et. Al. 2009, Zechmeister, Grodzinska & Szarek Lukaszewska 2003). Forest integrity research puts much effort in research because the irreplaceable and vulnerable role of mosses in healthy forest habitats is endangered by actual forest management practices (Fenton 2005, Frego 2007, Mezaka, Brūmelis & Piterāns 2012, Peck 2006). And their vulnerability to abiotic environmental stress makes them a promising indicator species for global change research (During 1979, Gignac 2001, Ogwu 2019).

Because of their small size compared to other plants, bryophytes never truly stood in the focus of nature preservation measures (Drehwald 2013, (Furness & Grime 1982). “The progress in moss taxonomy is years behind that in vascular plants [...] the field is still in the exploratory, floristic stage of development, and many of the commonest species are very poorly understood taxonomically, floristically, and ecologically [...] while a large part of the southern hemisphere still remains undiscovered.” (Anderson 1963). Even in the twenty first century there are huge distribution gaps of common species (based on missing Data) Germany which represents the one of the most studied areas in bryophyte research (Meinunger & Schröder 2007). Mosses were just recently added to the red list of endangered species which hopefully leads to more research measures to enhance the knowledge about their role in diverse ecosystems (Drehwald 2013). Even their antifungal and antifeedant contents find use in the cosmetic industry (Frahm 2004).

The goal of this work is to map the mosses in the Marburg Open Forest near Cölbe (Hesse, Germany) to investigate moss distribution patterns. We hope to find relationships between the occurrence and abundance of moss species in different habitats and growing on different substrates. We investigated if there are species that only occur on certain tree species or on certain substrates (epiphytic, soil, deadwood) and which relations could be derived from these patterns. We chose a nested plot design in which a mainplot contains many subplots. We hope to increase the accuracy of species richness by this plot design (Ilić, Igić, Ćuk & Vukov 2018). Epiphytic mosses were recorded on a variety of tree species and in three levels (one to three meters above the tree-root). Also the moss distribution on dead wood and soil was recorded. We assume that there are similar moss species in the same forest type (e.g. Beech, Spruce, Oak) and tree species. Also we hope to find relationships between the occurrence of moss species and the corresponding substrate it is growing on (e.g. soil, deadwood, epiphytic).

**2. Data and Methods**

**2.1 Study area**

The research was performed from May to Juli 2019 in the Marburg Open Forest (MOF) near the small town Caldern a part of the administrative district of Marburg (Hessen, Germany). Geological the area is dominated by limestone, greywacke, shales and conglomerate stone (Hessisches Landesamt für Umwelt und Geologie 2007). The soil composition in this area are solifluidal sediments and brown earth and is part of the geologic constellation “Rheinisches Schiefergebirge” (Bodenviewer 2020). The highest elevation is the Hungert with 412 meters above sea level (Opentopomap 2020). It represents a typical central European temperate forest and includes departments with mainly *Fagus sylvatica* , *Quercus petrea cf* , *Picea abies, Pseudotsuga menziesii, a* single small department with *Larix decidua* and some clearings, meadows and an abandoned quarry. Additionally there are some tiny creeks which don’t carry water permanently. The *Quercus petrea* departments include a mix of *Quercus petrea* and *Fagus sylvatica* and as typical for economically used forest the departments can include small amounts of other tree species. For our study we focus on the departments of the four main tree species *Fagus sylvatica , Quercus petrea cf , Picea abies* and *Pseudotsuga menziesii* along with the *Larix decidua* department and a clearing (Fig. 1). We didn’t collect data for the quarry or the creeks. The departments north of the primary road are classified as natural reserves where we will not take any samples.

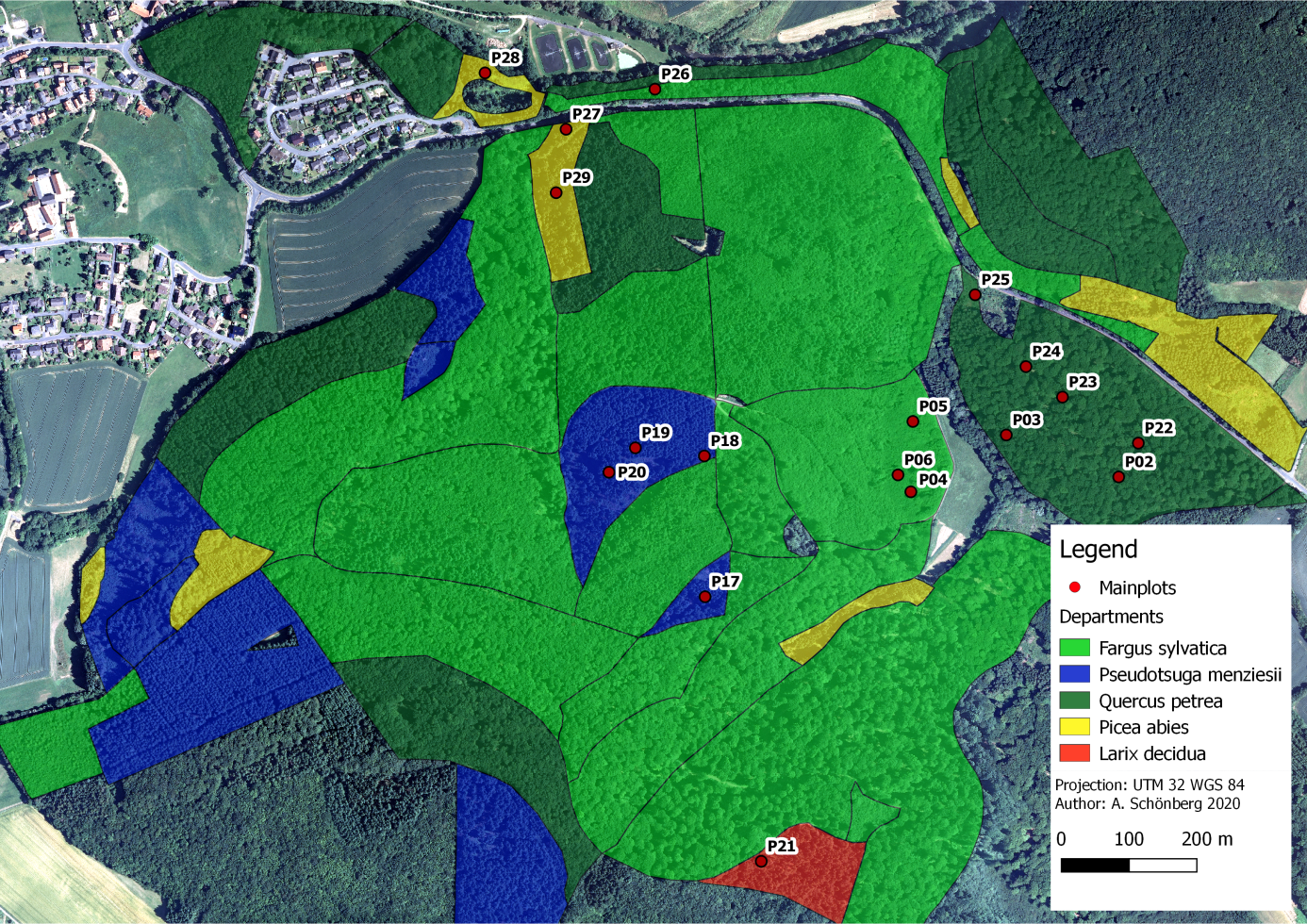


Fig. 1: Study site with vegetation departments

*Entweder hier nur eine karte des Gebietes mit fokus auf den abteilungen und baumalter !ohne plots! Und später die plot karte. Oder hier keine karte und verweis auf data und methoden um keine ähnlichen karten zu doppeln*

**2.2 Field Methods**

“*kurz einleitung”*

For our study and regarding to our hypothesis we will perform a vegetation survey after BRAUN-BLANQUET (1928) and use a nested plot design to collect data for different substrates.

“*entscheidung anzahl mainplots”*

Due to the different sizes of the departments and to collect representative data we calculate the amount of plot per tree species as followed. For the angiosperm species we sample on tree plots in *Fagus sylvatica* departments and five times in *Quercus petrea cf.* because there occure *Fagus sylvatica* too. For the gymnosperm species we sample on one plot for *Larix decidua* because there is only one department in the study area. For *Pseudotsuga menziesii* we sample on four plots along with four plots for P*icea abies* to get equal amounts of plots for the main tree species. Further we sample on a clearing to get data to compare to the forest plots.

“vorgehen für mainplots”

For the planned amount of plots per forest department we searched for most representative position (DIERSCHKE 1994, S.150) within the departments and use a nested plot design to collect data regarding to our hypothesis. Therefore we set up a “main plot” on a representative position and set a 5 meter radius. This area of ca 80 m² is nearly eqaual to a 10x10 meter squared plot and typically used for forest vegetations plots (DIERSCHKE 1994, S.150). First we estimate the coverage for the main plot area for the tree, scrub and herb-layers as well as take the coordinates. Than we set up “subplots” for the different substrates to identify the moos species. This improves the subjectivity of the vegetation survey as well as delivering data about the distribution of moos species within a main plot. For substrates we choose soil, deadwood and epiphyte because we expect to find those substrates on most plots. For every subplot the species are identified and the coverage is estimated based on the Braun Blanquet scale (BRAUN-BLANQUET 1928, S. 22) while the relation is based on the sub plot typ.

**Subplots**

Soil

For the soil subplots we identified all moos species occurring on leave free ground. Typically this substrate contains small elevations which are leave free due to the exposition to the wind. The coverage is estimates in relation to the whole main plots area to prevent high cover values if there is only a small area of soil related to the main plot area. Further this allows comparing high coverage values on leave free areas like clearings or meadows with plots where only small areas are leave free. On the other hand there could be a high amount of low coverage values for plots in the forest.

Deadwood

Like for soil we identified all moos species occurring on deadwood but estimate the coverage in relation to the accumulated area of deadwood in the main plot area instead to the main plot area. Here we expect deadwood occurring relatively common on the main plots and not this high differences than on soil. So we focus our interest on the comparability of the species within a plot instead of the plots among themselves

Epiphyte

For the epiphyte subplots we first identified the tree species within the main plot. For every tree species we set up an epiphyte subplot to later compare the epiphytic moos species by tree species. Therefore we identified the moos species and estimate the coverage by the mean on all trees of the same species with the main area. If there are more than only a few trees we choose representative trees as a sample for the area. Further we divine the plots into elevation levels to later test our hypothesis about the elevation dependent occurrence of moos species by tree class.

|  |  |
| --- | --- |
| Level | elevation |
| 1 | Ground up to 1 meter |
| 2 | 1 meter up to 2 meters |
| 3 | Above 2 meters |

This sampling design can result in single species occurring multiple times on a main plot with different cover values which has to be handled in data preprocessing.

If any type of substrate is missing on a main plot we don’t generate it instead of setting zeros values due to multivariate statistical approaches like ordination cannot handle zeros.

“identifcation of moos species”

In general moos species can be more difficult to identify than other plants. Species which we could not identify in the field are collected and identified later. It is possible that some species where not found in the field but later found within a sample. Than we cannot estimate the coverage.

Further some moos species can be difficult to distinguish for example the *Mezgeria* species or *orthodicranum montanum* and *affine.* Therefore we avoid to artificially generate more different species due to unsure identification. For example if we estimate most *orthodicranum* species to be *montanum* we will avoid to identify a single sample as affine if we are not very sure. Yet it is possible that we found more or less different species due to those identification problems.

**2.3 Data analysis**

First the collected data was digitalized in a single table. Additionally a second table is digitalized containing the information for the main plot like the coordinates and the environmental parameters of tree,- scrub,- and herb-layer coverage.

“datensatz cleanen”

For further analysis the main species table is edited to ensure there are no missing data due to the fieldwork. It is possible that the cover for a species is missing when it was found within a moos sample and not collected on the plot elsewhere. To avoid an information loss this species all get the same value for cover instead of deleting the species. Assuming that with higher coverage’s the species would be collected elsewhere on the plot and to reduce a possible manipulation we assign an “r” to those species. Any moos species with missing name entry will be deleted if we are not able to reproduce the information.

“datensatz bbscale to numeric”

To allow mathematical operations and perform multi-variant statistical methods the Braun-Blanque scale for the coverage is translated into numeric values. We assigned the mean value in percent from the Braun-Blanquet scale as the numeric mean value (see fig.XX).

|  |  |  |
| --- | --- | --- |
| Bran-Blanquet scale | cover | Numeric mean |
| r | Far less than 1 % | 0,1 |
| + | Less than 1% | 0,5 |
| 1 | Less than 5% | 2,5 |
| 2 | 5-25 % | 15 |
| 3 | 25-50 % | 37,5 |
| 4 | 50-75 % | 62,5 |
| 5 | 75-100 % | 87,5 |

“privot und subsets”

Due to our sampling design a moos species can occur multiple times within a main plot on different substrates subplots and on several levels on different tree species. To investigate the differences between the main plots we need to accumulate the coverage for species occurring on multiple subplots. Therefore we used the pivot format table to calculate the mean coverage values to get a new dataset with the species on the main plots independent of the substrate they occur on. To analyze the different substrates by main plot position we further generated datasets for the three substrates (soil, deadwood and epiphyte). To further investigate similarities of the substrate subplots we computed a dataset with all subplots.

**3.2 data analysis**

“Species richness and and total coverage” Berechnung der Werte

First we generate a species table with all moos species occurring in the study area with information about their occurrence on the different plots. Than we calculate the species richness and total coverage every species for the main and subplots to get an overview of the distribution of moss species in the study area. For the total coverage we used the sum of numeric values from every subplot. This grants to compare the dominance of species within a plot but the values should not be compared to other plots. The species richness is the amount of unique species occurring on the plot.

Further to examine the distribution of the species we identified which species occur only on one of the different substrates to see if there are any relationships between the substrates. Further to examine the distribution of species on the substartes we identifie those species only occuring on a single substrate to see if we find typical species for the substrates. If those typical species are missing because they occur on more then one substrate we will further generate two lists with terrestrial species by combining the species occurring on deadwood and soil and test which not occur on epiphyte and epiphytic species by combine epiphyte and deadwood species and check which not occur on soil.

“ordination and cluster analysis”

To investigate our hypothesis that the differences in moos compositions depend on the forest departments we used the multi-variant statistical method of ordination and clusteranalysis.

We used

vegan paket zitieren hierarchic clustering (HC) and K-means clustering (KM). To further visualize the distances of the Objects the function computes a non-metric multi-dimensional scaling (nmds) and detrended correspondence analysis (dca) ordination. We estimate that the three classes are sorted in the three clusters due to similarities of the extracted values. To check the quality of th

“cor tests environmental parameter”

The main plots have different coverage values for the tree, scrub and herb layers. To investigate if there are any correlations with the richness and total coverage of the plots we use “cor.tests”. We tested each total coverage and species richness versus the coverage of tree, scrub and herb layers as well as versus the tree species and the tree class (angiosperm and gymnosperm).

“methods cor test”

At least to test our hypothesis that the elevation distribution of epiphytic moos species depends on the tree species we analyzed the correlation between the maximum level species occurs and the tree species it was collected on. Therefore we use our collected data for the epiphyte subplots with information about the maximum height level any moos species occur for all tree species. Further we assign angiosperm and gymnosperm to the generate the tree-class parameter. Nur mit tree type geht das doch garnicht? 9 klassen gegen 3 höhen testen, was soll da der cor wert auch aussagen, es gibt ja keine skala für treetyp nur für treeclass. Than we used the cor.test with the pearson method to test if there is a correlation between the the treeclass and the maximum height level of epipythes.

Results cor test

In total we found nine tree species on our main plots (with their respective amount of subplots): *Fagus sylvatica* (8), *Quercus petrea cf* (6), *Picea abies* (4), *Carpinus betulus* (2), *Betula pendula* (2), *Larix decidua* (2), *Pseudotsuga menziesii* (4), *Sorbus aucuparia* (1) and *Acer spec* (1). We hypothised that there is a correlation between the tree typ and the maximum height of epiphytes. We hypothised that on angiosperm tree species epipyht moos reaches higher elevation than on angiosperm. Except on one *Larix decidua* gymnosperms only have epiphytes up to one meter while on angiosperms moos species typically reaches up to two meters. The correlation between the tree class and the maximal height of epiphytes delivers a correlation value of -0.6111822

With a p-value of 0.0004283. With this result we can conclude our hypothesis that angiosperm have epiphytes in higher elevations.

“Results cor test environmental parameters”

We could not find any significant correlations between the species richness or total coverage and the tested variables (see fig)

|  |  |  |  |
| --- | --- | --- | --- |
| Variable 1 | Variable 2 | Cor value | p-value |
| Richness | Tree layer coverage | 0.0509392 | 0.8409 |
| Richness | Scrub layer coverage | -0.5039678 | 0.03297 |
| Richness | Herb layer coverage | -0.09983415 | 0.6935 |
| Richness | Tree species | -0.396783 | 0.103 |
| Richness | Tree class | -0.3476427 | 0.1575 |
| Total coverage | Tree layer coverage | -0.1783518 | 0.4789 |
| Total coverage | Scrub layer coverage | 0.03294554 | 0.8967 |
| Total coverage | Herb layer coverage | 0.08304375 | 0.7432 |
| Total coverage | Tree species | 0.1318336 | 0.6021 |
| Total coverage | Tree class | -0.2634893 | 0.2908 |

**Results Basisdaten zu Moosen in Caldern**

For the Caldern Forest we found 32 different Moos-species in total within the soil, deadwood and epiphyte substrates (see fig.x). The soil and epiphyte substrates contain 18 different species each and the deadwood substrate has the highest diversity with 21 species. Most common Species within the main plots is *Hypnum cupressiforme* which is further the most common species by occurrence on plots on deadwood (94%) and epiphyte (100%). On soil the most common species is *Polytrichum formosum* (71%).

In total we found 32 different moos species in the study area based on our sampling design. Most common species overall is *Hypnum cupressiforme* occurring on every main plot (18/18) with the far highest dominance in total coverage (see fig). Further common species are: *Brachyothecium rutabulum* (15/18), *Dicranum scoparium* (12/18), *Politrichum formosum* (11/18) and *Orthotrichum affine* (11/18). For the soil subplots *Politrichum formosum* (10/14), *Brachyothecium rutabulum* (8/14) and *Atrichum undulatum* (8/14) represent the most common species and with their respective total coverage this is the typical composition on soil in the study area. While *Hypnum cupressiforme* occurred very rare on soil compared to the other substrates (3/14) it is the most common species on deadwood (17/18) and epiphyte (30/30) subplots. Typical compositions on deadwood are *Hypnum cupressiforme* (17/18), *Brachyothecium rutabulum* (12/18) *and Dicranum scoparium* (9/18). The epiphyte subplots show a high amount of species occurring only on very few plots compared to the other substrates. Again *Hypnum cupressiforme* (30/30), *Brachyothecium rutabulum* (14/30) represent the typical species along with *Orthotichum affine* (11/30).

Some species only occur on a single substrate others on two or on all tree. Only on soil subplots occurred: *Hylocomium splendens, Plagiomnium affine cf , Plagiomnium undulatum*, *Pleurozium schreberi* and *Rhizomnium punctatum*. On deadwood subplots *Dicranella heteromalla* and *Ditrichum heteromalla cf* and on epiphyte respectively *Bryum capillare, Frullania dilatata, Metzgeria simplex, Scapania nemorosa cf, Ulota crispa* and x. Some typical epiphytes like *Orthotricum affine* and respectively typical terrestrial species like *Polytrichum* are missing in this lists because they occur on deadwood to. With the deadwood substrate combined to soil and epiphyte we receive a list that more likely represent the typical compositions seen in the field. For terrestrial species (not occurring as epiphytes) there are"Atrichum.undulatum" "Eurhynchium.praelongum" "Herzogiella.seligeri"

[4] "Hylocomium.splendens" "Plagiomnium.affine" "Plagiomnium.affine.cf."

[7] "Plagiomnium.undulatum" "Pleurozium.schreberi" "Polytrichum.formosum"

[10] "Rhizomnium.punctatum" "Rhytidiadelphus.squarrosus" "Scleropodium.purum" and for epiphytes (not occurring on soil): [1] "Bryum.capillare" "Ceratodon.purpureus" "Frullania.dilatata" "Metzgeria.furcata"

[5] "Metzgeria.simplex" "Ortho.dicranum.montanum" "Orthotrichum.affine" "Plagiothecium.laetum"

[9] "Scapania.nemorosa.cf." "Thuidium.tamariscinum" "Ulota.crispa" "x"

(hier noch nicht auf deadwood als übergang eingehen?)

**TODO:**

**artenliste von hand verschieben Methode und result**

**MVS Methode und result**

**Literature**

**Ilic 2018:**

**Field sampling methods for investigating forest-floor bryophytes: Microcoenose vs. random sampling**

**Abstract:** Because of the high importance of bryophytes in forest ecosystems, it is necessary to develop standardized field sampling methodologies. The quadrat method is commonly used for bryophyte diversity and distribution pattern surveys. Quadrat size and the position of quadrats within the studied area have a significant influence on different analyses. The aim of the present study was to define the minimum quadrat size appropriate for sampling ground bryophytes in temperate beech forests, to compare two different field sampling methods for research on ground bryophytes, the random and microcoenose methods; and to test the adequacy of the microcoenose sampling method in temperate beech forests. Research was carried out on Fruška Gora mountain (Serbia) at four different sites. All sites contained temperate broadleaf forest vegetation, predominantly Fagus sylvatica, but also included various other tree species. Systematic sampling based on nested quadrats was used to determine the minimum sampling area. Random sampling was performed using 10 or 20 microplots (minimum area quadrat), randomly located within 10x10 m plots. Microcoenose sampling is a systematic sampling method based on the fact that every bryophyte fragment on the forest floor is a separate microcoenose. These methods were compared using the following criteria: species richness; Shannon’s diversity index and evenness measure; coverage of dominant species, and the time needed for sampling. The microcoenose sampling method has proven to be highly applicable in temperate beech forests in terms of species richness and diversity, in contrast to random sampling, which was not suitable for bryophyte flora with a patchy distribution.

Keywords: bryophytes; microplots; sampling; forests; diversity

**INTRODUCTION**

Bryophytes (mosses, liverworts and hornworts) play important roles in forest ecosystems by contributing to species richness [1,2] forest biomass [3,4], water regime and nitrogen budget regulation [5,6], while also providing a microhabitat for other organisms [7]. In general, bryophytes are important components of “forest integrity” [8]. Due to this fact, it is necessary to include bryophytes in all forest ecosystem studies. Vegetation and phytosociological studies in Europe use well-developed and standardized methodology [9-12], but in the majority of these studies, bryophytes have been ignored, despite the fact that these plants have a large influence on ecosystems, phytocoenosis and habitat conditions. A globally accepted method for the quantification of bryophyte abundance in forest communities is still lacking, but there are several approaches that have been standardized. Three commonly used approaches for quantitative bryophyte sampling in forests are: the line intercept method [13-16], floristic habitat sampling [17,18] and the quadrat method [1,19-26]. The main problem with the line intercept method is an increased probability of missing small species [16]. Floristic habitat sampling (FHS) is a method based on the use of microhabitats within the stand as a sampling unit [17]. This method is similar to floristic sampling, and its main advantages are the high possibility of recording rare species and high applicability in bryophyte research over large areas [17]. The main disadvantage of FHS is the fact that this method does not estimate abundance well, so it is not completely appropriate for estimation of statistical inference or good abundance [18]. The quadrat method is commonly used in bryophyte studies of diversity and distribution patterns. There are several disadvantages in using the quadrat method for quantifying bryophytes. The first problem concerns the appropriate quadrat size. Using too small a quadrat can lead to the exclusion of some very important species. The minimum area concept for determination of the minimal appropriate quadrat size (based on species area curves) depends on the scale. According to some authors [27,28], it is hard to fix the minimum area that could properly catch a sufficient proportion of total diversity in any type of habitat. In general, species area curves rarely reach complete saturation [29], and species numbers increase with quadrat enlargement; however, at some point this enlargement slows down. Moravec [30] suggested using the criterion of similarity and confirmation of minimum area by stopping the increase of average similarity through enlarging quadrat size. Although determination of quadrat size by species area-curves is not an “ideal” solution, it is the most efficient and most commonly used. The second problem is how to find an appropriate method for quadrat positioning. Is it better to use a completely random approach, or some form of systematic quadrat positioning? The problem with random positioning is that it mainly excludes the existence of different microhabitats within the plot (phytocoenosis) where the study is performed; this problem can be bypassed by systematic sampling [31]. The third problem with the quadrat method is that different authors use different quadrat sizes (microplots), making results from different studies less comparable [28]. Bryophytes show variations in distribution patterns in different types of ecosystems, and therefore, sampling methods for quantification are highly dependent on the type of ecosystem, environmental factors and the aim of the research [7]. Jiang et al. [32] developed a microcoenose sampling method for ground bryophyte flora in different types of forest vegetation in China, which provides sufficient information in terms of species richness and distribution of bryophytes. However, it is not known if this sampling method is applicable or advantageous in temperate forests in comparison to random sampling methods. The aim of the present study was to address the following issues: (i) what is the minimum quadrat size for the quantification of ground bryophyte flora in temperate forests dominated by Fagus sylvatica, and (ii) which model performs better − completely random sampling or the microcoenose sampling method.

**MATERIALS AND METHODS**

**Study site**

This research was performed during March-April 2016 on Mt. Fruška Gora, located in the north of Serbia in the southern part of the Pannonian plain (Fig. S1), between 45°0’ - 45°15’ N and 16°37’ - 18°01’ E. This mountain is surrounded by the Danube alluvial plain in the north and east, and by two loess plateaus in the south and west. The highest peak is Crveni Čot (539 m a.s.l.). Geologically it is a very diverse area. The largest part is composed of siliceous rocks, and the vegetation probably dates from the Tertiary, because glaciation did not have a significant impact on this mountain [33]. There is a dense hydrological network composed of groundwater, karst springs, mineral and thermal springs, streams (constant and periodical) and some standing water [34]. There are three types of soil on Fruška Gora: chernozem, brown forest soil and brown calcareous soil [35]. This area lies in a mild-continental central European climatic region [36]. The highest precipitation levels are in May-June, September and October [33]. The lowest average temperature is in January and the highest in July [37]. Due to its natural value, Fruška Gora was declared a National Park in 1960. The majority of the protected area is under forest vegetation. For this study, four localities on Mt. Fruška Gora were chosen, all under typical forest vegetation: these were Iriški Venac-Stražilovo (IS), a beech (Fagus sylvatica) forest dominated by bryophytes in the ground layer; Papratski Do (PD), a mixed forest with F. sylvatica as the dominant species and significant participation of Carpinus betulus, Quercus petraea and Tilia platyphyllos; Vrdnik (V), a mountain beech forest with dominant species F. sylvatica and Q. petraea, and Dumbovo waterfall (D), a monodominant beech forest with absolute domination by F. sylvatica.

Sampling scheme

In the present study, bryophytes were considered sensu lato (i.e. including representatives of mosses and liverworts, while hornworts were not found in this area). In addition to species found on the soil, bryophytes that grow on small rocks and roots at elevations less than 5 cm above the soil surface were also considered to be forest floor bryophytes. The main reason for this is that

many species in this area are polyedaphic, and many bryophyte patches are spread across different substrates. At each locality, a sampling area was chosen in the central part of the selected forest sites. Five plots (10x10 m) were then randomly chosen within the boundaries of these sampling areas. On these plots, all bryophyte taxa were listed to obtain the actual species numbers and calculated as the average species number in five 10x10 m plots. To obtain actual coverage values (%), total coverage of all bryophytes, as well as coverage by dominant bryophyte species (species with the highest abundance on the plots) was measured on 10x10 m plots within each site. Actual species numbers and coverage values were used for comparison of the species number and coverage recorded using different sampling methods and calculated as the proportion on 10x10 m plots. Bryophytes were identified in the field or in the laboratory, and deposited in a herbarium. At each study site, a minimum sampling area was determined using a systematic sampling method [32] with some modifications, as follows: five plots (10x10 m) were delineated with nested quadrats with dimensions of 10x10 cm, 20x20 cm, 50x50 cm, 1x1 m and 2x2 m (Fig. S2); the distances between the sampling quadrats was equal; species richness and abundance were measured in all 2x2 m quadrats (125 in total). The minimum sampling area (microplot) was used for testing two different sampling methods. First, a random sampling method was performed using 10 randomly located microplots (Fig. S3A) within each 10x10 m plot (for a total of 50 microplots per study site). Then, the number of microplots was increased to 20 (Fig. S3B) for each plot (for a total number of 100 microplots per study site) in order to test the appropriate number of minimum area quadrats (microplots) for random sampling. Randomness was achieved by placing a wooden frame (50x50 cm, delineated with 1x1 cm quadrats) within the boundaries of the plot (10x10 m). Microplots without bryophytes were also included in the analysis. Second, a microcoenose sampling method [32] was employed. In this case, every bryophyte fragment was considered to be a microcoenose. Plots (10x10 m) were delineated on 25 grids (2x2 m). The microplots were thrown in the center of the largest bryophyte fragment in each of 25 grids (Fig. S3C). Grids without bryophytes were included in the analysis.

**Data analysis**

To determine minimum quadrat size, the following indices were used: species richness (S), the number of species in each analyzed quadrat or plot (10x10 m), and the Sørensen similarity index [38]. A qualitative minimum area curve (species-area curve) was constructed [29] for all studied sites in order to determine the minimum appropriate quadrat size. The turnover point in each species-area curve was determined by the tangent method [39]. A similarity area curve was constructed for confirmation of the species-area curves. The turnover point in each similarity-area curve was based on the point where the similarity values were greater than 80% [40]. For data analysis, the average coverage and species richness of all microplots for each sampling area were used. Four criteria were used for testing the usability of these sampling methods for some quantitative diversity measurements: (i) species richness (S) gained in different types of sampling, (ii) Shannon’s diversity index (H’) and evenness measure (J’) [41], (iii) coverage of dominant species, and (iv) the time needed for sampling, expressed in min. The sampling time was measured only at site D and included species identification, packing of species impossible to identify in the field and measuring of species coverage in all individual microplots. Statistical analyses were performed using the t-test in STATISTICA ® ver. 13.2 software [42]. The diversity index was calculated and compared using PAST ver. 3.15 [43].

**RESULTS**

Actual species number and actual coverage of bryophytesThe total number of species listed by 10x10 m plot size at V, D, IS and PD were 23, 35, 28, 21, respectively. Differences in species richness at the four studied sites were probably related to different ecological conditions in each type of forest. The actual coverage of bryophytes on the 10x10 m plots was similar for V, D and PD (23%, 35% and 21%, respectively), while it was much higher for the locality IS, where it reached 91%.

**Minimum area determination**

Based on the qualitative minimum area curve (speciesarea curve), the turnover point was found to be a quadrat size 50x50 cm (0.25 m 2 ) for all investigated sites (Fig.1). Using a similarity area curve (Fig.2) for each site, the turnover point was found to be a quadrat size 0.25 m 2 , which is based on the similarity between quadrats in which it was higher than 80%. All subsequent quadrats were not significantly different from the 50x50 cm quadrats. Considering the abovementioned characteristics, a quadrat size of 50x50 cm was selected as the minimum quadrat size (microplot) for testing the random and microcoenose sampling methods.

**Species richness**

The random sampling method was tested as an appropriate method for reducing subjectivity in field sampling. The main problem with this method was the difference between the species numbers recorded in all microplots (10 or 20 per plot) and the actual number of species at all studied sites. In the first case, based on 10 randomly located microplots (50x50 cm) at all four localities, a statistically significant difference (p<0.05) was found between the actual species number and the recorded species number for all 50 microplots (Table 1). In the second case, based on 20 randomly located microplots, only one study site (IS) was found without a statistically significant difference (p<0.05) between the actual species number and measured species number (Table 2).