**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, bryo phytes 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).

**MATERIALS AND METHODS**

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**Species richness**

**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).

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