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Research Article

ASSEMBLAGES OF WOOD-INHABITING MACROFUNGI SPECIES IN BEECH FORESTS FROM EASTERN ROMANIA

Ciprian Constantin BÎRSAN[1*], Constantin MARDARI[1], Cătălin TĂNASE[2]

[1] "Anastasie Fătu" Botanical Garden, "Alexandru Ioan Cuza" University of Iași, Dumbrava Roșie Street no. 7-9, 700487 Iași – Romania.
[2] Faculty of Biology, "Alexandru Ioan Cuza" University of Iași, Carol I 20A, 700505 Iaşi – Romania; Romanian Academy, 125 Calea Victoriei, Sector 1, 010071 Bucureşti – Romania.
[*] Corresponding author. E-mail: ciprian.birsan@uaic.ro, ORCID: 0000-0003-3099-634X

Abstract: Species composition and diversity of lignicolous fungi occurring in representative *Fagus* sp. forests have been analyzed along an altitude gradient, in 14 locations from the eastern region of Romania, including mountain forests (Borca, Goşman, Nemţisor, Tarcău), as well as hill forests (Arsura, Bârnova, Dălhăuţi, Dragomirna, Gâdinţi, Homiţa, Humosu, Măgura Ocnei, Runc, Valea Fagilor). The investigations were carried out over three successive years (2020-2022) in a total of 40 study plots with a size of 1000 square meters each. Identification of the main fungal groups was realized using a hierarchical agglomerative clustering procedure, and the differential species were determined based on their indicator value, while the relationship between fungal composition and environmental variables was assessed with detrended correspondence analysis. A total of 89 species of wood-inhabiting fungi has been identified, with approximately 2500 records. These species were grouped in three different clusters: one specific to the mixed broadleaved-coniferous forest in (sub) mountain areas, one specific to hornbeam - beech communities in hilly areas, and one specific to more thermophilic forest communities with *Fagus taurica*. The main ecological factors influencing the fungal composition were altitude, average annual precipitations and average annual temperatures, which also influence the trees species composition of the analyzed forests.

Keywords: assemblages, beech forests, climatic factors, lignicolous fungi, tree species.

Introduction

Beech forests occupy large areas in Europe (approximately 910,000 km^2) [LEUSCHNER & ELLENBERG, 2017], in low altitude areas, from NW and Central Europe to southern Sweden. They have been subjected to great anthropogenic pressure throughout history as they have been used as resources for human populations (e.g. timber, accessory products, etc.) [JAHN, 1991; ELLENBERG & LEUSCHNER, 1996]. Representative areas with natural ancient *Fagus* sp. forests are quite few and strictly protected [PETERKEN, 1996; DIACI, 1999; PARVIAINEN & al. 2014].

In Romania, the beech forests occupy the largest areas of the forestry fund (about 31%), covering about 2 million ha [MILESCU & al. 1967; ŞOFLETEA & CURTU, 2007]. In scientific literature have been described 15 types of beech forest ecosystems, depending on phytosociological associations, forest types, soil types and climatic characteristics [PAUCĂ-COMĂNESCU & al. 1989; DONIŢĂ & al. 1990; DONIŢĂ & al. 2005]. A part of these forest types is also found in the Ukrainian and Polish Carpathians, as well as in the plateaus of the Republic of Moldova reaching the eastern limit of species' distribution in Europe [SZAFER, 1932; STOYKO, 1992; STOYKO, 2005; NEDEALCOV & DONICA, 2019]. *Fagus sylvatica*

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edifies pure or mixed forests in (sub) mountain and hilly areas of the country, and in the case of thermal inversions, it replaces the spruce at higher altitudes. *Fagus taurica*, with Balkan and Crimean areal, is a more thermophilic taxon, sporadically encountered in Romania, that form in Valea Fagilor (Tulcea County) a particular forest habitat [OPREA & al. 2011].

Figure 1. Map presenting the distribution of investigates beech forests in Eastern Romania (adapted from www.geotutorials.ro)

These species present a shade temperament [MILESCU & al. 1967], and have a significant impact on the modification of forest ground light regime, on soil humidity and on buffering temperature and humidity changes. These properties favorably affect biological activity, and contribute at maintaining an increased biodiversity [SOLON, 2002]. Adult specimens are resistant to diseases and pests. Still, they are vulnerable to the attack of wood-inhabiting fungi, through mechanical wounds mainly generated by forestry operations, which are successful entry points for infections [IGMÁNDY, 1964]. A great asset of the forest ecosystems in Romania is their naturalness, depending, among other, on diversity and structure of trees layer, ground vegetation, the quantity of dead wood and management type. There have been preoccupations about habitat restoration by increasing the volume of dead wood, thus leading to increased biodiversity throughout the ecological chain. In protected areas and ancient forests, dead wood exists in different forms, from dead trees on the ground, to large pieces (fallen logs) and small wood fragments [CHRISTENSEN & al. 2005].

The different types of dead wood (roots, branches and logs) in different stages of decomposition offer a wide range of ecological niches and favor a high diversity of wood-inhabiting fungi. It is estimated that approximately half of the macrofungi species in forests are wood decomposers, lignicolous fungi playing an important role in the functioning of forest ecosystems [KÜFFER & SENN-IRLET, 2005; MÜLLER & al. 2007; STOKLAND & al. 2021].

Although fungi are components of natural communities, they were rarely used to describe them. Because of their specificity to particular microhabitats and environmental conditions, some fungal taxa could be better suited as indicator species than plants. Despite of the ecological importance of lignicolous macrofungi species in forest ecosystems, this group of organisms was generally neglected in the biodiversity studies carried out in Romania. Although some studies tried to document their diversity at the species level, there are very rare cases when plot-based sampling in combination with multivariate analyses were used [BÎRSAN & al. 2014; COPOŢ & al. 2020] in order to distinguish some macro-fungal communities.

The current study was focused on identifying the main assemblages of wood-inhabiting fungi specific to the *Fagus* forests from the eastern part of Romania, on identification of differential species for these assemblages and on the main abiotic drivers shaping their species composition.

Material and methods

The present study was carried out in 14 forests (13 edified by *Fagus sylvatica* and one by *Fagus taurica*) located in the eastern part of Romania (Figure 1). The sampling plots were established along an altitude gradient, from low elevation areas, of approximately 150 meters altitude (Valea Fagilor), to mid-altitudes ranging from 361 meters to 540 meters (Arsura, Bârnova, Dălhăuţi, Dragomirna, Gâdinţi, Homiţa, Humosu, Măgura Ocnei, Runc), up to relative high altitudes of about 900 m in the mountain area (Borca). The silvicultural history differs between the study areas. Some forests were completely logged in the past and now have a uniform age structure with dominant tree ages ranging from 100 to 250 years. The selected sample areas cover the main types of beech forests and are characterized by various degrees of human interventions.

The mycological investigations were carried out in 40 plots, each with a size of 1000 square meters, over 3 successive years (2020-2022), starting from April to November. The forests massifs are subject to different types of management, a part of them characterized by various degrees of intervention (e.g. Bârnova, Gâdinţi, Homiţa, Măgura Ocnei, Borca, Tarcău), while other sites were declared natural reserves (e.g. Arsura, Dragomirna, Dălhăuţi, Goşman, Nemţisor, Humosu, Runc, Valea Fagilor). In all the investigated sample areas, beech is the dominant species, but other tree species that form the respective forest stands also have a major importance, as host species for various lignicolous fungi. For each sample, the geographical coordinates, altitude, slope and aspect using a geographic positioning device (GPS II Plus Garmin Ltd.) were recorded (Table 1). The species of lignicolous fungi collected were identified at the species level based on macroscopic and microscopic characters, according to identification keys and reference guides [SĂLĂGEANU & SĂLĂGEANU, 1985; BREITENBACH & KRÄNZLIN, 1986; GERHARDT, 1999; BERNICCHIA, 2005; TĂNASE & al. 2009; COURTECUISSE & DUHEM, 2013]. Nomenclature follows the Index Fungorum database [http://www.indexfungorum.org/Names/Names.asp].

Table 1. Geographical features of sampling plots investigated

Acr.	Sampling plots	Localities / County	Latitude	Longitude	Altitude (m a.s.l.)	Aspect	Slope
A 1	Arsura 1	Gârleni BC	46˚38'20.27"	26˚47'17.68"	361	NNV	small
A 2	Arsura 2	Gârleni BC	46˚37'48.37"	26˚48'40.34"	364	NV	medium
A 3	Arsura 3	Gârleni BC	46˚37'44.88"	26˚48'34.72"	343	NE	small
B 1	Bârnova 1	Bârnova IS	47˚00'28.75"	27˚35'10.10"	404	ENE	small
B 2	Bârnova 2	Bârnova IS	47˚00'34.54"	27˚35'12.58"	394	E	medium
Bo 1	Borca 1	Borca NT	47˚14'44.36"	25˚52'30.66"	868	VNV	high
Bo 2	Borca 2	Borca NT	47˚14'33.93"	25˚52'26.19"	877	V	small
Bo 3	Borca 3	Borca NT	47˚14'31.40"	25˚52'30.07"	895	V	high
D 1	Dălhăuți 1	Dălhăuţi VN	45˚41'47.21"	27˚00'50.46"	413	E	medium
D 2	Dălhăuți 2	Dălhăuţi VN	45˚41'34.91"	27˚00'41.43"	470	NE	small
D 3	Dălhăuți 3	Dălhăuţi VN	45˚41'52.22"	27˚00'51.92"	401	N	medium
Dr 1	Dragomirna 1	Dragomirna SV	47˚46'43.50"	26˚13'09.89"	460	ESE	small
Dr 2	Dragomirna 2	Dragomirna SV	47˚46'42.89"	26˚13'05.17"	460	V	medium
Dr 3	Dragomirna 3	Dragomirna SV	47˚46'45.55"	26˚13'02.85"	469	V	small
Dr 4	Dragomirna 4	Dragomirna SV	47˚46'42.48"	26˚13'00.11"	466	ESE	high
G 1	Gâdinți 1	Roman NT	46˚55'15.56"	27˚04'11.41"	418	NE	medium
G 2	Gâdinți 2	Roman NT	46˚55'05.53"	27˚04'13.76"	394	NE	medium
G 3	Gâdinți 3	Roman NT	46˚55'21.06"	27˚04'06.16"	382	NNV	high
Go 1	Goşman 1	Brateş NT	46˚42'50.79"	26˚12'12.08"	823	NV	high
Go 2	Goşman 2	Brateş NT	46˚42'49.62"	26˚12'08,31"	803	ENE	medium
H 1	Homița 1	Cristeşti IS	47˚16'39.37"	26˚36'21.20"	412	NNE	medium
H 2	Homița 2	Cristeşti IS	47˚16'37.48"	26˚36'31.24"	413	NNE	medium

H 3	Homița 3	Cristeşti IS	47°18'16.71"	26°36'19.90"	401	ESE	small
Hu 1	Humosu 1	Pârcovaci IS	47°29'43.64"	26°43'29.23"	439	ESE	small
Hu 2	Humosu 2	Pârcovaci IS	47°29'49.50"	26°43'24.97"	458	E	small
Hu 3	Humosu 3	Pârcovaci IS	47°29'55.70"	26°43'17.23"	470	ENE	small
Hu 4	Humosu 4	Pârcovaci IS	47°30'05.19"	26°43'15.15"	475	ENE	small
MO 1	Măgura Ocnei 1	Târgu Ocna BC	46°15'56.62"	26°35'34.47"	540	ESE	medium
MO 2	Măgura Ocnei 2	Târgu Ocna BC	46°16'13.61"	26°35'18.77"	500	E	medium
N 1	Vânători Neamţ 1	Mrea Neamţ NT	47°16'27.84"	26°07'47.88"	656	V	high
N 2	Vânători Neamţ 2	Mrea Neamţ NT	47°16'28.60"	26°07'38.20"	652	ESE	medium
N 3	Vânători Neamţ 3	Mrea Neamţ NT	47°16'45.00"	26°06'10.16"	692	S	high
R 1	Runc 1	Buhuşi BC	46°45'11.74"	26°44'48.34"	461	S	small
R 2	Runc 2	Buhuşi BC	46°45'18.67"	26°45'23.37"	454	VNV	small
R 3	Runc 3	Buhuşi BC	46°45'22.51"	26°45'29.81"	480	VNV	small
T 1	Tarcău 1	Tarcău NT	46°53'09.53"	26°07'30.79"	530	NV	high
T 2	Tarcău 2	Tarcău NT	46°53'08.00"	26°07'35.39"	525	SE	high
VF 1	Valea Fagilor 1	Luncaviţa TL	45°12'58.33"	28°18'24.98"	130	NE	medium
VF 2	Valea Fagilor 2	Luncaviţa TL	45°12'55.15"	28°18'25.44"	153	ENE	medium
VF 3	Valea Fagilor 3	Luncaviţa TL	45°12'52.61"	28°18'32.82"	163	E	high

Identification of the main groups of wood inhabiting fungi was realized in a hierarchical agglomerative clustering method, using the Sorensen index (on presence-absence data) and the UPGMA algorithm. The main gradients in fungal composition and the environment (altitudes, mean annual temperatures, mean annual precipitations, etc.) influence on fungal assemblages were assessed using detrended correspondence analysis (DCA). Climatic variables were extracted from WorldClim database [HIJMANS & al. 2005]. Differential species were assessed using the indicator value index [DUFRÊNE & LEGENDRE, 1997] and a permutation test allowed the selection of those species statistically significant associated to the clusters [DE CÁCERES & LEGENDRE, 2009]. The clustering procedure has been realized using the GINKGO software [DE CÁCERES & al. 2003; BOUXIN, 2005]. DCA have been realized in CANOCO 5 programme [TER BRAAK & ŠMILAUER, 2012].

Results and discussions

Field mycological investigations had as result the identification of 89 lignicolous fungi species, with approximately 2500 records. The most species rich plot included 47 species, while the poorest species plot had only 10 species. Among the most frequent species, inventoried in more than 40% of all records, were: *Xylaria polymorpha*, *Trametes versicolor*, *Stereum hirsutum*, *Schizophyllum commune*, *Fomes fomentarius*, *Apioperdon pyriforme*. In addition, in the investigated sample areas were found more species less frequent such as *Amylostereum areolatum*, *Coniophora puteana*, *Dasyscyphella nivea*, *Gloephyllum odoratum*, *Gymnopus erythropus*, *Mycena aetites*, *Mycena haematopus*, *Pluteus granulatus*, *Sarcoscypha austriaca*, *Trametes cinnabarina*.

The areas with the greatest fungal diversity were those from Humosu, Valea Fagilor and Dragomirna, some of the most important natural reserves for conservation of *Fagus sylvatica* in our country. In contrast with areas where the forest management is strongly focused on conservation, in the areas with intensive silvicultural management, focused on economic activities, and do not benefit of protection status, lignicolous fungal diversity is lowest, for example Tarcău, Măgura Ocnei and Gâdinţi with 13, 10 and 11 species (Figure 2).

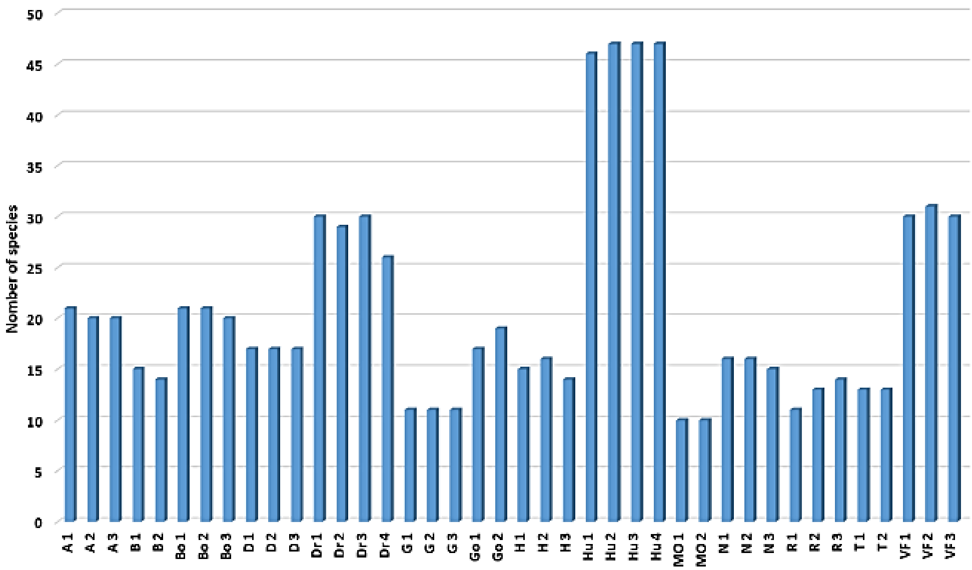

Figure 2. Species richness of wood inhabiting fungi in the investigated plots.

This aspect is related to the quantity and the different types of dead wood, with different decomposition stages, offering a wide range of ecological niches for wood-inhabiting fungi. In all investigated beech natural reserves were identified dead trees which represent true treasures for biodiversity. As the amount of dead wood decreased, a decline in the number of fungi species was observed due to the lack of support for their development. From the point of view of forest management and administration, dead wood has been perceived negatively, supposedly indicating lack of management, negligence and waste. Articles have also been published on the functions of dead wood, thus improving people's perception functions of this type of substrate [MERGANIČOVA & al. 2012]. Nowadays, the amount of dead wood is increasing not only due to ecologists but also due to foresters and scientific research. The amount of dead wood is increasing after its component and importance in the functioning of forest ecosystems have been demonstrated [BÄSSLER & al. 2011; GAO & al. 2015]. Dead wood has become an indicator of sustainable forest management, being of major importance for biodiversity. Fungi are the main saproxylic taxa, first colonizing wood and starting its decomposition. The presence of large amounts of dead wood in forest ecosystems is often connected to low anthropogenic influences, and in regions where human activities are reduced, the degree of naturalness is expected to increase over time and space [LOMBARDI & al. 2008]. In conclusion, the greater the volume of dead wood, the greater the number of lignicolous species were encountered, some of which were rare.

In the dendrogram generated by hierarchical clustering (Figure 3), a first separation was highlighted between fungal assemblages in mountain mixed deciduous - coniferous forests (cluster 1) and fungal assemblages in pure deciduous forest stands. Further, the assemblages in deciduous forests differentiated into communities from more mesothermic and mesophilic forests typical to hilly areas (cluster 3) and more thermophilic and more xeric forests in lower areas (cluster 2). This separation highlights besides the importance of climate in fungal species distribution, the fundamental role of the host tree species.

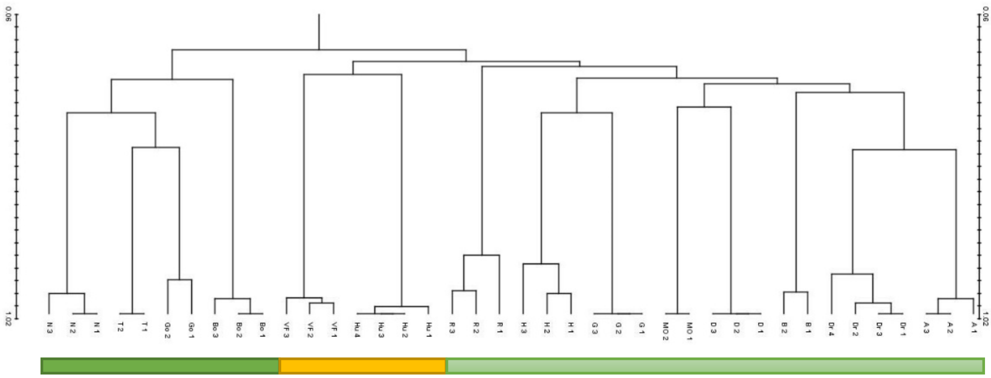

Figure 3. Dendrogram highlighting the three clusters resulted from hierarchical clustering of the 40 sampled plots (cluster 1 – dark green, cluster 2 – yellow, cluster 3 – light green)

Cluster 1 – groups the plots from the mountain and submountain areas, in Borca, Goşman, Tarcău, and Nemţisor areas. The forests stands are edified by *Fagus sylvatica* mixed with *Abies alba*, *Picea abies*, *Betula pendula*, etc., and are characterized by low means of annual temperatures, larger temperature amplitudes, short vegetation seasons, and high values of precipitations. Most of the diagnostic species were identified on logs and woody debris from conifers, in various degrees of decomposition: *Hypholoma capnoides*, *Pseudohydnum gelatinosum*, *Plicaturopsis crispa*, *Flammulina velutipes*, *Pholiota squarrosa*, *Meripilus giganteus*, *Amylostereum areolatum*, *Gloeophyllum odoratum*, *Mycena polygramma*, *Trametes pubescens*.

Cluster 2 – included the plots in more thermophilic forest stands from Valea Fagilor as well as plots investigated in Humosu forest reserve. These areas share a great number of common fungal species (among them) and are characterized by a continental, dry climate, a suboptimal precipitation level. The forests are edified by *Fagus taurica* (in Dobrogea) and *Fagus sylvatica* (in Moldova) with *Carpinus betulus*, *Tilia tomentosa*, *Tilia platyphyllos*, *Fraxinus excelsior*, *Acer campestre*, *Acer platanoides*, *Ulmus glabra*, etc. in the tree layer. The fungal species which differentiate from the other clusters are: *Chondrostereum purpureum*, *Ganoderma lucidum*, *Auricularia auricula-judae*, *Cerioporus varius*, *Hericium coralloides*, *Trametes gibbosa*, *Armillaria cepistipes*, *Mucidula mucida*, *Phlebia tremellosa*, *Pluteus cervinus*, *Auricularia mesenterica*, *Bulgaria inquinans*, *Cerioporus squamosus*, *Collybiopsis* *ramealis*, *Coprinellus micaceus*, *Daldinia concentrica*, *Gymnopus fusipes*, *Ischnoderma resinosum*, *Phlebia rufa*, *Mycena galericulata*, *Lycogala epidendrum*, *Calycina citrina*, *Daedaleopsis confragosa*, *Xerula pudens*, *Schizophyllum commune*, *Hymenochaete rubiginosa*, *Pleurotus ostreatus*, *Volvariella bombycina*, *Coniophora puteana*, *Dasyscyphella nivea*, *Pluteus ephebeus*, *Pluteus granulatus*, *Pluteus leoninus*, *Sarcoscypha austriaca*, *Trametes cinnabarina*, *Panellus stipticus*, *Ganoderma applanatum*, *Daedaleopsis tricolor*, *Hypoxylon fragiforme*, *Sarcoscypha coccinea*.

Cluster 3 – included the plots analyzed in mesophilic *Fagus sylvatica* forests from the nemoral zone (e.g. Bârnova, Dragomirna, Gâdinţi, Homiţa, etc.). The tree layer includes other species, such as *Carpinus betulus*, *Cerasus avium*, *Acer platanoides*, *Tilia cordata*, and were subjected to a high degree of human intervention. As a consequence, these are species poor stands, and are characterized by only one diagnostic species, namely *Hymenopellis radicata*.

The influence of host tree species in determining fungal assemblages is well known in the literature [BERNICCHIA & al. 2007; KEBLI & al. 2011]. It was found that areas dominated by deciduous species present different communities of lignicolous fungi compared to those dominated by conifers [BLASER & al. 2013]. The ecological profile of the component species in the sampled areas indicates a variety of diagnostic species in the assembly of wood-inhabiting fungal communities, closely related to the composition of host tree species, achieving a clear distinction between conifers and deciduous trees, which was also demonstrated in other studies [KÜFFER & al. 2008; O'HANLON & HARRINGTON, 2012]. Although in the current study the most common deciduous species was *Fagus sylvatica*, the presence of other tree species in the investigated forest stands determined the agglomeration of fungal species in different types of assemblages.

In DCA, the most important factors influencing the distribution of lignicolous macrofungi species, as well as the composition of the groups of wood inhabiting fungi, in the studied forests, were the average annual precipitations and mean annual temperatures, which depend, at larger scales, of terrain elevation (Figure 4). Thus, the mountainous and sub-montane regions are characterized by a higher amount of annual precipitation and lower temperatures that maintain moisture for longer periods of time. As demonstrated in other studies [BODDY, 1993; HEILMANN-CLAUSEN & CHRISTENSEN, 2003; KARADELEV & al. 2008], water (along with temperature) is the main limiting factor in the appearance of sporocarps and for the distribution of lignicolous species. In addition, the floristic composition of trees and shrub layers separate fungal assemblages from higher altitudes from those in medium and low hilly areas. The slope aspect of the sampled areas was also important for the distribution of lignified species, because those that are exposed to high brightness (especially southern aspect) lose moisture faster than those that have shade for longer periods of time. The edges of the massif, with greater fluctuations in humidity and solar radiation are poorer in species than those located under the canopy of secular beech forests, with a high degree of shading at ground level [BÄSSLER & al. 2010; ABREGO & SALCEDO, 2014].

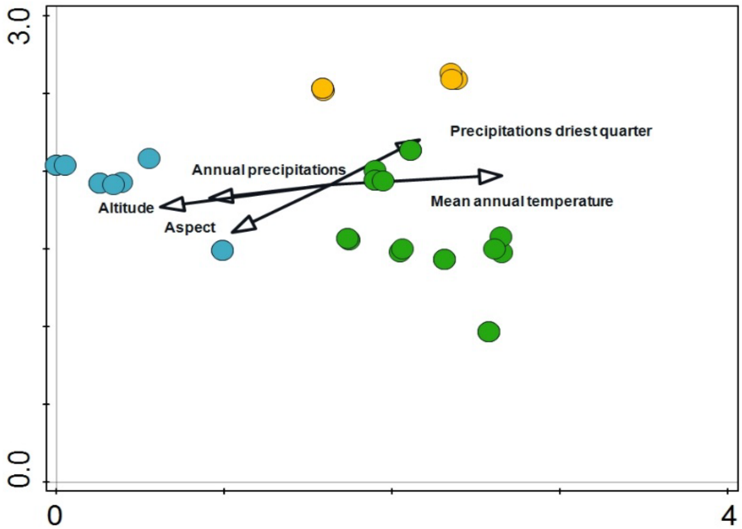

Figure 4. DCA ordination diagram of the 40 investigated mycological plots (only first two axes are presented). Explained variation: 44.1%. Eigenvalues: Axis 1 – 0.412, Axis 2 – 0.223. Fungal assemblages resulted from hierarchical clustering: cluster 1 – blue circles, cluster 2 – yellow circles, cluster 3 – green circles.

Conclusions

The communities of wood-inhabiting fungi were separated into three main groups: one of mixed broad-leaved - coniferous forests in higher areas, a group typical to mesothermic and mesophilous forests from hilly to sub-montane areas and another group specific to more thermophilic *Fagus* sp. forests in lower areas. Average annual precipitations and mean annual temperatures (an underlying effect of altitude) were the main factors influencing their composition. These climatic characteristics induce regional differences between sample plots having as a result different tree composition of forests and consequently, different fungal assemblages. Different tree species in the forest stands provide various quantities of dead wood, with different physical and chemical properties, which are colonized by different fungal species. A greater fungal diversity was recorded in protected areas (forest reserves) than in those with strong anthropogenic influences, mainly due to the amount of the available dead wood.

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