

## Diversity and sporocarp development of lignicolous myxomycetes in young timber forests of western Germany

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**Abstract:** We investigated the biodiversity and timing of fruiting-body development of lignicolous myxomycetes in three humid young timber forests dominated by *Fagus sylvatica*, *Quercus robur* and *Pinus sylvestris* located in North Rhine-Westphalia (Germany) for a complete growing season. A total of 571 specimens assigned to 58 species were collected, and the geographical distributions of specimens are documented herein. Fructifications appearing within a defined area were examined for a complete vegetation period. Relationships between habitat characteristics and occurrence of myxomycetes were investigated. Myxomycete abundance was determined mainly by the characteristics of the forest. Most myxomycetes fruited in July and September, and these events were correlated with heavy rains one or two days before the appearance of the sporocarps.

**Zusammenfassung:** Wir haben die Artenvielfalt und Zeit der Fruchtkörperbildung von Myxomyceten untersucht. Die drei untersuchten Gebiete sind Niederwälder mit vorwiegend *Fagus sylvatica*, *Quercus robur* und *Pinus sylvestris* in Nordrhein-Westfalen (Deutschland). Es wurden Exkursionen von Mai bis Oktober in zweitägigen Abständen durchgeführt und 571 Exsikkate aufgesammelt. Diese konnten 58 Arten zugeordnet werden. Die geografische Position wurde für jeden Fruchtkörper bestimmt. Habitateigenschaften und räumliche Verteilung der Fruchtkörper wurden in Beziehung gestellt mit den Charakteristika des Waldes.

Myxomycetes (*Myxogastria*) or slime molds are common fungi-like protists associated with, e.g. soil, litter and decaying coarse woody debris. The life cycle of myxomycetes is characterized by two distinct stages – a haploid stage represented by a spore, myxoflagellate or myxamoeba and a diploid stage represented by a multinuclear life form, the plasmodium (GRAY & ALEXOPOULOS 1968, HOPPE & KUTSCHERA 2010). Within the sporocarps, spores develop in response to environmental signals (MARWAN 2003). The sporocarps are often studied in the field, but only a few publications deal with a longer time span of a defined area (HEILMANN-CLAUSEN 2001; TAKAHASHI & HADA

2009, 2010; NAJAGAKI & GUY 2007; NAKAGAKI & al. 2007). To investigate microhabitat conditions for their trophic stages, it is necessary to use various cultivation methods (FEEST & MADELIN 1985, 1987; MITCHEL 1978) or molecular methods (KAMONO & al. 2013). In spite of a mass of data on their taxonomic diversity in various regions and ecosystems throughout the world, the functional role of this group of organisms in the ecosystem is still poorly understood (FEEST & MADELIN 1987). In 2009, TAKAHASCHI & HADA investigated the biogeographical distribution of myxomycetes associated with *Pinus densiflora* in Japan. Similarities were noted between the stage of decomposition of the coarse woody debris and species richness of myxomycetes. Nevertheless, they did not determine the exact time of fruiting-body formation and also assumed that despite their mobility the location of amoebae and plasmodia correlated with the position of the sporocarps.

Herein we present data for myxomycete diversity in three temperate forests for a complete season with regular surveys in 2012. The objective of this study was to collect data of the time of fructification and environmental condition (e.g. climatic events) for myxomycetes in western Germany, following the methods of LADO & al. (2007) and NOVOZHILOV & al. (2006 a, b).

Not all myxomycetes fructificate at the same time. The spatial disposition of the fruiting bodies depends on the habitat (microhabitat) of the amoebas and plasmodia. These stages are able to move in a defined area. Environmental events influence the fruiting-body development. Only the comparison of precise distribution allows characterization of several species that can vary between different areas. We give here an overview of the temporal distribution of the fruiting bodies for an entire vegetation period.

## Materials and methods

The forests are located near the city of Siegen (50° 52' N, 08° 01' E), 200–440 m s. m. The soil is formed from slate and sediments. The climate is temperate and humid (precipitation: 1160 mm). During the 2012 sampling period, three localities were chosen and investigated in a second or third day interval. The three forests are not uniform in age structure and the amount of decaying wood. Three categories of woodland were specified: category 1 is characterized by a large amount of old, decaying wood, mostly tree stumps, category 2 is characterized by a moderate amount of decaying wood, mostly smaller branches (< 2 cm in diameter) and leaf litter, category 3 is characterized by the low quantity of decaying wood and a higher light exposure. All sporocarps of myxomycetes were geo-referenced with a portable GPS receiver (eTrex 20, Garmin, WGS84). The whole of each sporocarp discovered was transferred into a plastic box, fixed with glue, labeled with the GPS data and air dried. Slides for light-microscopy were prepared with HOYER's medium (NEUBERT & al. 1993). Microscopic investigations were carried out by light microscopy (Primo Star Zeiss, Germany) and a scanning electron microscopy (S-4000, Hitachi, Japan). The species were determined by current literature (LADO 2001, NEUBERT & al. 1993, 1995, 2000; POLAIN & al. 2011). The sporocarps were deposited in the herbarium of the first author (MYX542–MYX1397).

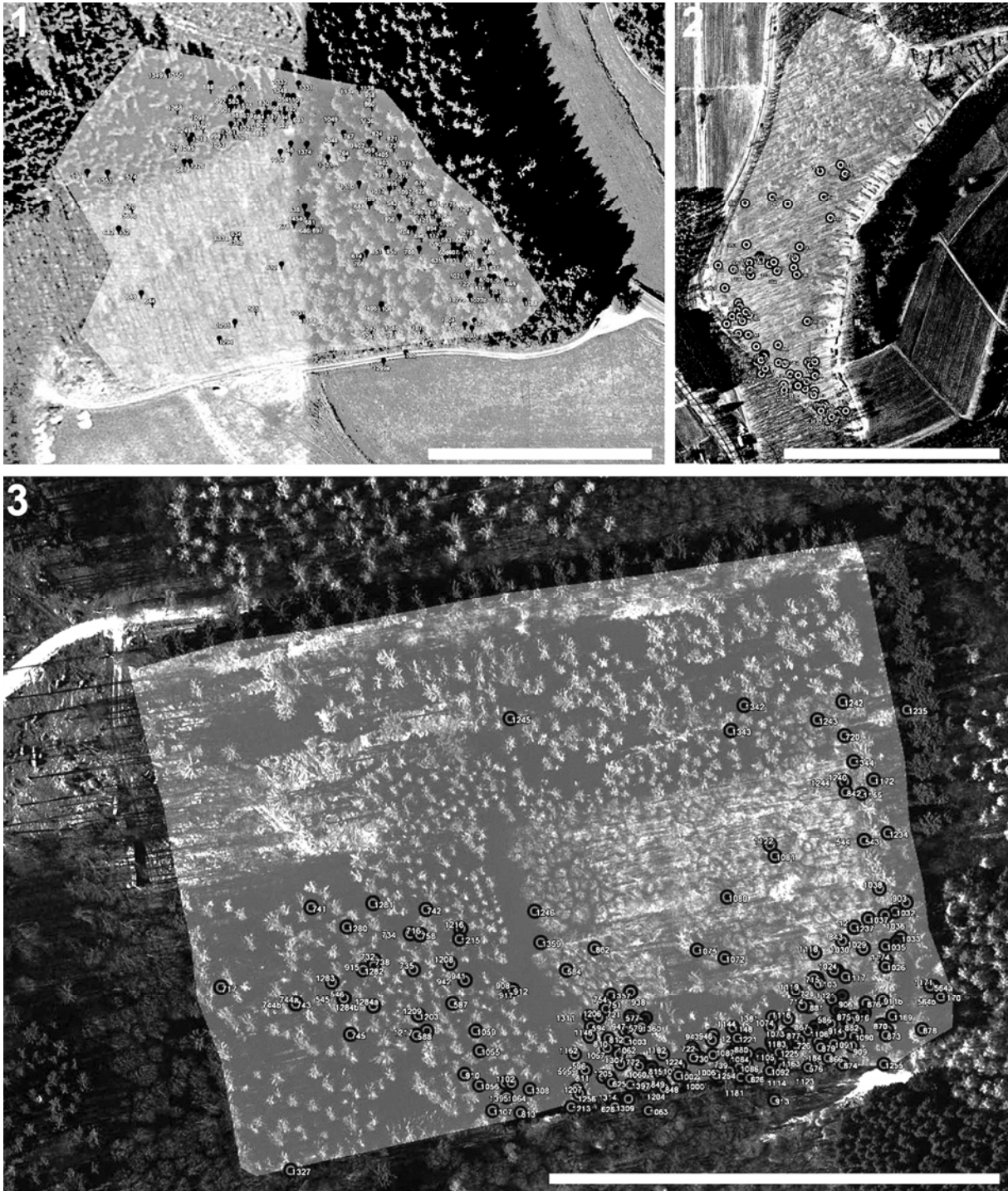
We used SPADE (CHAO & al. 2008) for calculation of the similarity matrix between the three different study areas and the different categories of woodland.

## Site description

The research localities (Figs. 1–3) were nearly in the same distances from each other. The forest of Faule Birke (FB, 50°50' 23.61" N, 08° 02' 10.62" E, 430 m s. m.) is 48700 m<sup>2</sup>. The vegetation was a mixed forest dominated by oak (*Quercus robur*)

and beech (*Fagus sylvatica*). *Acer pseudoplatanus* and *Picea abies* are occasionally present. Recently, other trees have been planted for better growth under changing climatic conditions.

The forest of Oberholzklau (OHK, 50° 55' 12.35" N, 07° 56' 23.92" E, 336 m s. m.) is 22000 m<sup>2</sup> and was surrounded by managed fields. The forest slopes (approx 24%) to a plateau. *Fagus sylvatica* showed a relative cover of 97% in the deeper areas. This forest is approx. 5 to 30 years old and clearly separated from those forests consisting of the younger oaks (relative cover 2.6%). Following initial planting, no



Figs. 1–3. Investigated localities: 1. Oberholzklau. 2. Obersetzen. 3. Faule Birke. Dots show the GPS-located position of the specimens. Bar: 100 m.

Table 1. Occurrence of myxomycetes in the study areas. A – abundant, C – common, O – occasional and R – rare, R: <3 records; O: >3<10 records; C: >10<20 records; A: >20 records; FB – forest of Faule Birke; OHK – forest of Oberholzklau; OS – forest of Obersetzen.

Species		Whole area	Estim_ FB	FB	Estim_ OHS	OHK	Estim_ OS	OS
<b>Records</b>		<b>571</b>		<b>251</b>		<b>212</b>		<b>108</b>
<b>Species total **</b>		<b>79</b>		<b>42</b>		<b>47</b>		<b>48</b>
<i>Arcyodes incarnata</i>	R	1	R	1				
<i>Arcyria cinerea</i>	A	45	A	21	C	17	O	7
<i>Arcyria denudata</i>	O	6	R	1	O	4	R	1
<i>Arcyria ferruginea</i>	R	2			O	2		
<i>Arcyria globosa</i>	R	1	R	1				
<i>Arcyria incarnata</i>	O	3	R	2			R	1
<i>Arcyria minuta</i>	R	1					R	1
<i>Arcyria obvelata</i>	R	2	R	1			R	1
<i>Arcyria pomiformis</i>	C	16	R	1	O	6	O	9
<i>Arcyria sp.</i>	R	1			R	1		
<i>Ceratiomyxa fruticolosa</i>	C	9	O	4	O	4	R	1
<i>Clastroderma debaryanum</i>	O	4	O	3			R	1
<i>Comatruchia elegans</i>	R	1					R	1
<i>Comatruchia hyperopta</i>	R	1	R	1				
<i>Comatruchia laxa</i>	O	8	R	1			O	7
<i>Comatruchia nigra</i>	R	2			R	1	R	1
<i>Comatruchia</i> or <i>Paradiacheopsis</i>	O	3			R	1	R	2
<i>Comatruchia</i> or <i>Stemonitopsis</i>	R	2			R	1	R	1
<i>Comatruchia pulchella</i>	O	3					O	3
<i>Comatruchia sp.</i>	O	7	R	1	R	1	O	5
<i>Comatruchia sp.</i> (cf. <i>alta</i> )	R	1			R	1		
<i>Cribraria argillacea</i>	C	16	C	10	O	5	R	1
<i>Cribraria cancellata</i>	A	81	A	68	C	11	R	2
<i>Cribraria persoonii</i>	R	1					R	1
<i>Cribraria rufa</i>	C	18	C	8	O	9	R	1
<i>Cribraria sp.</i>	C	12	O	4	C	5	O	3
<i>Cribraria sp.</i> (cf. <i>rufa</i> )	R	1	R	1				
<i>Cribraria tenella</i>	O	4	O	3			O	1
<i>Diachea leucopodia</i>	O	5					O	5
<i>Diderma cinereum</i>	R	1					R	1
<i>Diderma testaceum</i>	R	2					R	2
<i>Didymium melanospermum</i>	R	1					R	1
<i>Didymium nigripes</i>	R	1			R	1		
<i>Echinostelium corynophorum</i>	R	2			R	1	R	1
<i>Echinostelium minutum</i>	R	1					R	1
<i>Enerthenema papillatum</i>	O	6	R	2	O	3	R	1
<i>Enteridium lycoperdon</i>	R	2		1	R	2		
<i>Fuligo septica</i> var. <i>candida</i>	A	35	A	22	O	9	O	4
<i>Fuligo septica</i> var. <i>flava</i>	A	53	A	30	A	21	O	3

Table 1 continued.

Species		Whole area	E- stim_FB	FB	E- stim_O HS	OHK	E- stim_OS	OS
<i>Hemitrichia calyculata</i>	R	2			R	2		
<i>Licea parasitica</i>	R	1					R	1
<i>Licea operculata</i>	O	4	R	2	R	2		
<i>Licea testudinacea</i>	R	1			R	1		
<i>Licea variabilis</i>	O	4	R	1	R	1	R	2
<i>Lycogala epidendrum</i>	A	35	C	19	C	12	R	2
<i>Macbrideola cornea</i>	R	2			R	2		
<i>Paradiacheopsis fimbriata</i>	R	1			R	1		
<i>Paradiacheopsis</i> or <i>Stemonitopsis</i>	R	1	R	1				
<i>Paradiacheopsis solitaria</i>	A	20	R	2	C	8	C	10
<i>Paradiacheopsis</i> sp.	C	11	O	4	C	5	R	2
<i>Physarum album</i>	A	39	C	5	A	27	O	7
<i>Physarum citrinum</i>	R	1	R	1				
<i>Physarum gyrosum</i>	R	1					R	1
<i>Physarum leucophaeum</i>	O	3			O	3		
<i>Physarum robustum</i>	R	2			R	1	R	1
<i>Physarum</i> sp.	O	3			O	3		
<i>Physarum</i> sp. (cf. <i>leucophaeum</i> )	R	1	R	1				
<i>Physarum viride</i>	O	3		1	O	5		
<i>Stemonaria irregularis</i>	R	1			R	1		
<i>Stemonaria</i> or <i>Stemonitis</i>	R	1	R	1				
<i>Stemonaria</i> sp.	O	3	R	1	R	1		
<i>Stemonitis axifera</i>	O	4			O	3	R	1
<i>Stemonitis flavogenita</i>	R	1			R	1		
<i>Stemonitis foliicola</i>	R	1			R	1		
<i>Stemonitis fusca</i>	C	19	C	5	C	10	O	4
<i>Stemonitis</i> or <i>Comatrachia</i>	R	1					R	1
<i>Stemonitis</i> or <i>Stemonaria</i>	O	4	R	3				
<i>Stemonitis pallida</i>	R	1	R	1				
<i>Stemonitis smithii</i>	O	8	O	4	O	4		
<i>Stemonitis</i> sp.	C	9	O	4	O	5		
<i>Stemonitis</i> sp. (cf. <i>axifera</i> )	R	1					R	1
<i>Stemonitis</i> sp. (cf. <i>flavogenita</i> )	R	1	R	1				
<i>Stemonitis splendens</i>	R	1			R	1		
<i>Stemonitopsis typhina</i>	O	3	R	2	R	1		
<i>Trichia contorta</i>	R	1					R	1
<i>Trichia decipiens</i>	O	4			O	3	R	1
<i>Trichia</i> sp.	R	1	R	1				
<i>Trichia varia</i>	O	7	O	3	R	2	R	2
<i>Tubulifera arachnoidea</i>	R	2	R	1			R	1

thinning has taken place. The beeches are about 1 m apart and 5 cm in diameter. The Light levels are very low. In the edge area, there are mostly *Q. robur* (relative cover

58%), *F. sylvatica* (relative cover 25%) and *Larix decidua* MILL. (relative cover 15%) approx. 60 years old and a distance of 6–10 m to each other. On the plateau are *P. abies* and *P. menziesii* (approx. age 60 years) and younger trees of *F. sylvatica* (7–9 years). At the edge, a lot of dead wood is found, but this is not the case in core area. The trees are older and have not been managed for some time (approx. 25 years).

The forest of Obersetzen (OS, 50° 56' 13.60" N, 08° 2' 20.54" E, 320–370 m s. m.), called Hauberg (HOPPE 2013), represents a typical low timber forest and is 48250 m<sup>2</sup>. The dominant tree species is *Q. robur*. Hillside slopes are from up to 42% (east to west alignment) and 25% (north to south alignment). The steep slopes mean that the area is dry except for an area around a spring. At the edge, there is an area of approx. 30 m (in diameter) of a clearcut forest where there are only isolated old trees of *Q. robur* (distance more than 20 m). The remainder is mainly unmanaged, resulting in a lot of decaying wood being present.

Table 2. Similarity matrix of: a. the three categories of decaying wood (for calculation 58 distinct species were used, 38 species for OS, 35 species for OHK and 31 species for FB). b. the three investigated areas. Only exactly determined species were used for calculation.

a)	Category 1	Category 2	Category 3	b)	OS	OHK	FB
Category 1	1	0.665	0.78	OS	1	0.757	0.379
Category 2		1	0.92	OHK		1	0.661
Category 3			1	FB			1

## Results

Alltogether 571 specimens of 58 morphological species were collected at 180 field trips (Table 1): 38 species (108 specimens) for OS, 35 species (212 specimens) for OHK and 31 species (253 specimens) for FB. Species with small sporocarps, like *Echinostelium*, *Licea*, *Macbrideola* and *Paradiacheopsis* have been collected indirectly. The fruiting bodies were collected together with other species. Because of their small size, species of these genera are difficult to find directly in the environment. Hence, the counts given here are the appearance of the species only, and not the number of sporocarps in the field. In May and June there were numerous fructifications of *Lycogala epidendrum* FR. and *Ceratiomyxa fruticulosa* T. MACBR. Overall the most common species were *Arcyria cinerea* PERS. (44 specimens), and *Cribraria cancellata* NANN.-BREMEK. (85 specimens), *Fuligo septica* F. H. WIGGERS (86 specimens) and *Physarum album* RAEUSCH. (40 specimens).

We calculated the similarity indices for the three categories of the forests (Table 2 a). Category 2 to 3 were similar. The similarity of the three localities showed the similarity values highest between Obersetzen and Oberholzklau and the lowest between Obersetzen and Faule Birke (Table 2 b).

In 2012, weather conditions were moderate, with average temperatures for May to June: 18.8 °C, for July to September: 22.8 °C and October to November: 11.5 °C. In addition, most temperatures exceeded 5 °C and showed strong variations every 14 days. From the middle of October onwards, the temperature dropped below 10 °C. Weekly humidity levels fluctuated between 60 and 90%, with a rise at the end of the investigation period. It was rainy from May to July, and from August to September it was comparatively very dry (WETTERONLINE 2014).

For Oberholzklau, 86 specimens were collected in the core area and 125 specimens in the edge area. The specimens were found mostly at the edge, on ca. 60 year-old *L. decidua* and beeches.

In Obersetzen, decaying wood is extensively distributed throughout the area and specimens could be found in the whole area (particularly in the area downhill). The distribution was biased to the more humid zone around the spring.

If we compared the number of specimens between OHK and OS, there were marked disparities in the number of specimens (212 vs 102). In both forests, species richness numbers are comparable (46 vs 31). The development of the fruiting bodies of most myxomycetes was in July, but there was a second fruiting-body forming event in

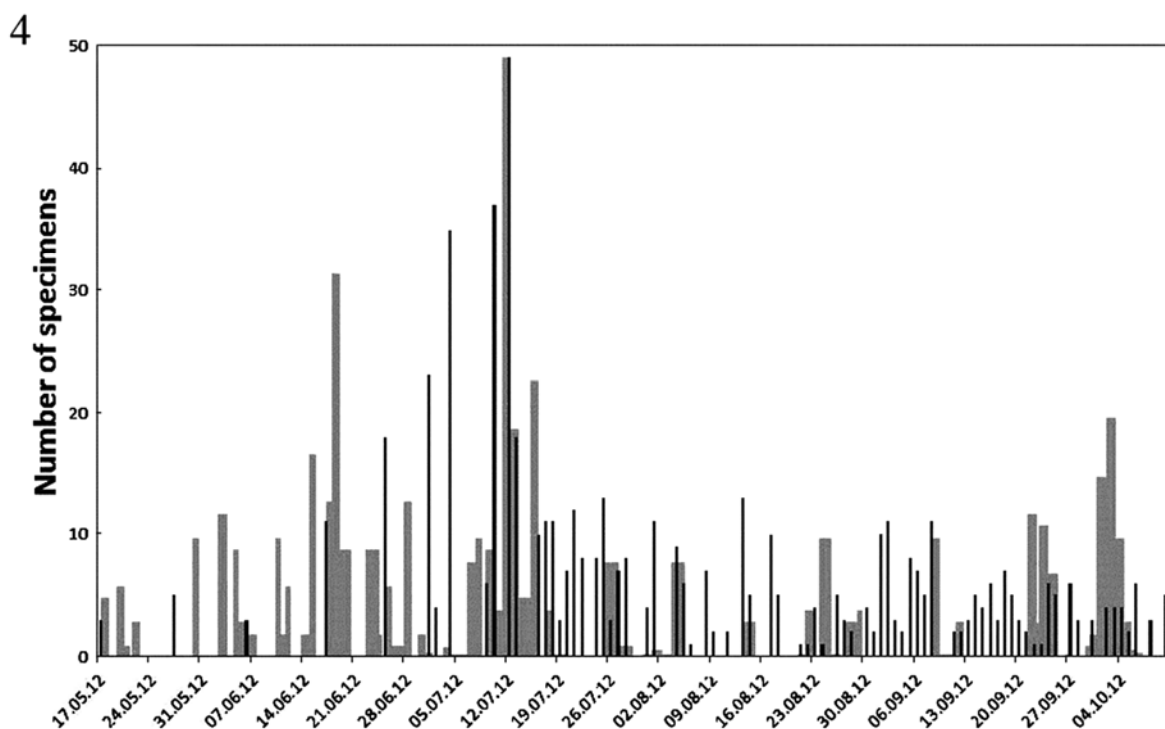
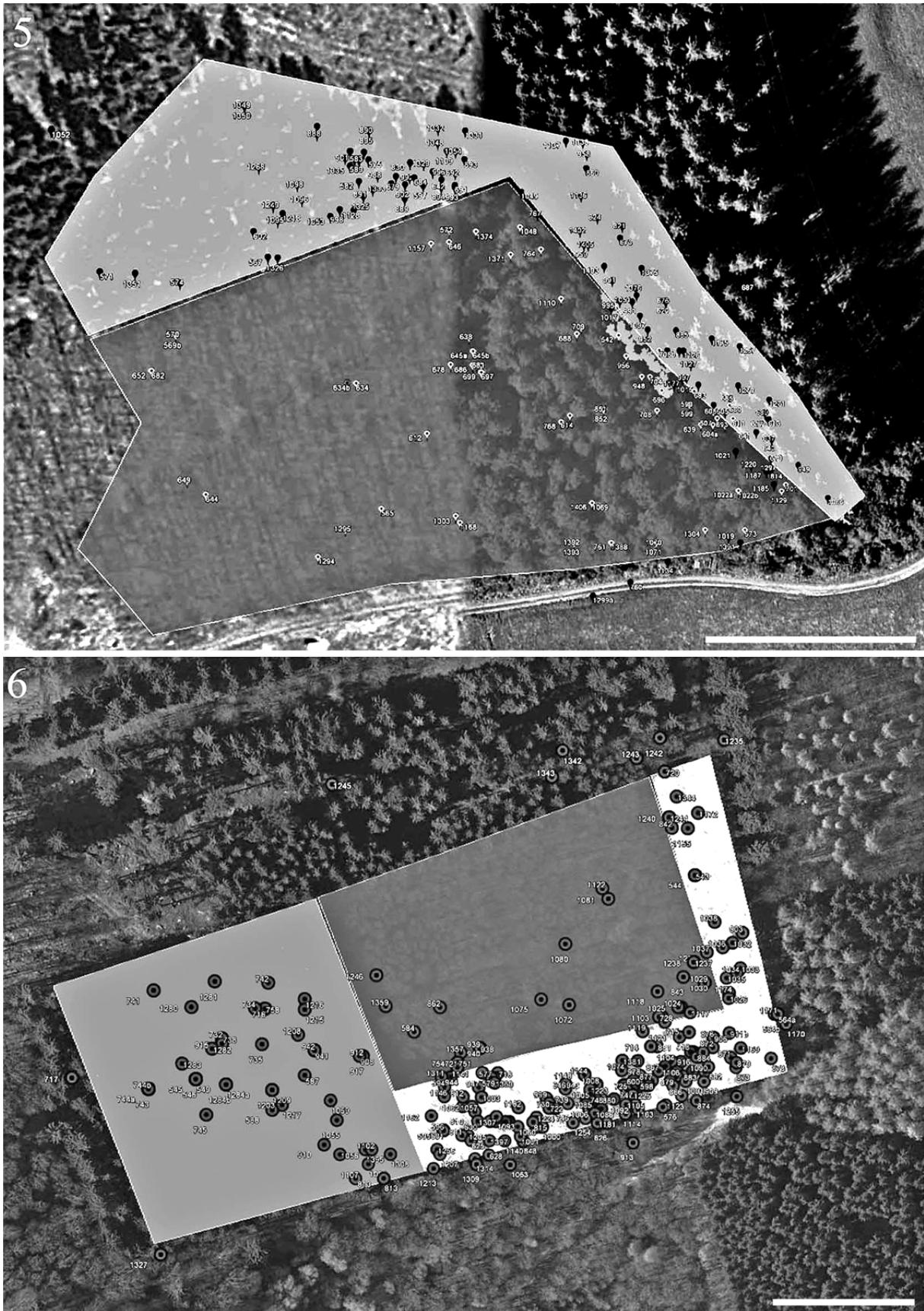


Fig. 4. Sampled specimens and the data of precipitation within the sampling period: grey bar: sampled sporocarps, black bar: rain in mm.

September, especially of species belonging to the orders *Physarales* and *Stemonitales*. The comparison of rain events and the collecting data showed an increase of the sporocarps one or two days after (Fig. 4) rain.

## Discussion

The myxomycete community differs conspicuously between the different localities and categories (Tables 1, 2). For example the species of the genus *Arcyria* are often found in the shadowed and humid habitat (category 2). In contrast, the genus *Cribraria* was found mostly in the category 3 biotope. The distribution of species of other genera was nearly the same (Table 2). The spatial distribution of the fruiting bodies was markedly associated with the edge area (category 1) and the west facing sides (category 3) (Figs 5-6). In the more harvested areas remarkably few sporocarps were found. However, correlations can be made between rain events and subsequently dis-



Figs. 5–6. The position of the sampled fruiting bodies and the degree of the coverage (white – Category 1, black – Category 2, grey – Category 3) of the trees are shown in Fig. 5 for OHK and in Fig. 6 for FB. The GPS-position of the fruiting bodies in relation to the decaying wood is shown. Bar: 100 m.



covered fruiting bodies (Fig. 4). The influence of other outer and inner factors cannot be excluded.

The ecology of myxomycetes in various habitats has been studied during the past few decades in North and Central America (KELLER 2004; STEPHENSON 1988, 1989, 2004; STEPHENSON & al. 2004; NOVOZILOV & al. 2000), Brazil (CAVALCANTI & al. 2006) and Europe and Asia (SCHNITTLER & NOVOZILOV 2002, HÄRKÖNEN & al. 2004). Hence, this is a good overview about the time of fruiting of myxomycetes. There is a lack of knowledge of the exact time of fructification of particular species (TAKAHASCHI & HADA 2009). Lignicolous species such as *C. fruticulosa*, *L. epidendrum*, *A. cinerea* and *C. cancellata* were sampled extensively in these forests. In contrast, smaller species such as members of the genera *Licea* or *Paradiacheopsis* are under-represented. These smaller myxomycetes are difficult to collect in the field. The moist chamber method is suited to their study. This shows the difficulty of examining myxomycetes in the field. Time-consuming trips must be made and combinations of different methods be carried out. Here we show the fructification period for different species for one year. We think, that the low number of sporocarps results from the intensive forest management in the past decades. The importance of decaying wood for the microbiota has been discussed by SAMUELSSON & al. (1994) and CHRISTENSEN & EMBORG (1996). In the forests we studied decaying wood is reduced to a minimum. Most of the myxoamoebae need bacteria (GUTTES & GUTTES 1960, KERR 1963, HOPPE 2009). The absence of some species could be a sign of a discontinuous food chain. The myxomycetes community of this localities was mostly non-specialized, with most of them are not dependent on a certain time for fructification. The fast recolonization of forests is possible by means of spore spreading and the short generation-times. The quick colonization is advantageous for these species to cope with varying conditions of managed forests. Climatic factors also influence the myxomycetes community. In future other long-term studies must be made considering the influencing factors tree or plant species, stage of decaying wood, micro-climatic conditions and light quality.

The studies should be small-scale to detect changes within a niche and combine field collecting with laboratory incubation for small species. The problem of the overlapping of the niches was discussed before by STEPHENSON (1988) and it is difficult to identify these niches, but this knowledge is an important step for a better understanding these microorganisms.

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