RECOMMENDED PROTOCOLS FOR SAMPLING MACROFUNGI

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Many factors influence the diversity of macrofungi at a particular site (see "Factors Influencing Species Richness," earlier in this chapter). Those factors include the nature of the habitat (e.g., grassland or forest, natural or planted, early successional or mature), the diversity of plant species (particularly host plants), the diversity of substrata, geographic location (latitude and elevation), soils types, and climate. Site factors are compounded by the amount of natural and manmade disturbance, site-management practices, and exposure to pollution. The protocols used in a study also have an impact on the observed diversity of macrofungi.

Schmit and associates (in review) undertook a meta–data analysis of plot-based studies that measured the diversity of both macrofungi and trees. They performed two analyses on 25 studies involving a total of 184 plots located in North America, Europe, China, and Costa Rica. The first analysis determined that, although plots contained more macrofungi than trees, the macrofungi were neither more nor less widely distributed than tree species. The second analysis looked at the effects of sampling effort (plot size, number of years sampled, number of visits), tree diversity (number of tree species present), number of ectomycorrhizal tree species present, and the type of tree present (conifers versus hardwoods), and latitude on the number of macrofungal species present in the plot. Although sampling effort had a major impact on the diversity of macrofungi discovered at a particular site (in general, more taxa are found with an increased effort), habitat type and tree diversity played a larger role in explaining differences between studies than did sampling effort. However, differences in the sampling protocols used in the 19 studies interfered with direct comparisons of their results.

Fortunately, protocols used to sample terrestrial macrofungal diversity have begun to converge in recent years (e.g., Richardson 1970; Jansen 1984; Tyler 1985, 1989; Bills et al. 1986; Villeneuve et al. 1989; Gulden et al. 1992; Salo 1993; Leacock 1997; Rossman et al. 1998; O'Dell et al. 1999; Schmit et al. 1999; Straatsma et al. 2001; P. R. Leacock and D. J. McLaughlin, unpublished manuscript). Many of the investigators used variations of a common sampling theme–that is, sampling all of the macrofungi occurring in relatively small circular plots set out evenly along transects of defined lengths, with a total area of 0.1 hectares sampled per site. The most commonly used protocols differed in the sizes of the circular subplots (4 m^2 versus 5 m^2) and, consequently, the numbers of subplots and the lengths of the transects. Even if the total area surveyed is the same, using subplots of different sizes could influence frequency data because species frequency is calculated as the proportion of all subplots in which it is present. A 0.1-hectare area contains 250 4-m^2 subplots but only 200 5-m^2 subplots. We recommend the use of 5-m^2 circular subplots for two reasons: (1) the remaining forest fragments in many of the areas where we are working (e.g., Central America, China, Midwestern North America) are small, making it difficult to establish long transects; and (2) we can make use of grid markers (usually based on 10-m × 10-m grids) established as part of plant inventory projects as the same study sites. Using established grids facilitates setting up transects and, more importantly, facilitates comparison of the plant and macrofungal diversity data. Setting up the 200 5-m^2 subplots is straightforward, and sampling them is efficient; in addition, our collecting team of students, interns, volunteers, and parataxonomists has expressed satisfaction with the layout. Sampling frequency and number of plots per site given here (see "Recommended Protocols") are minimal values. If resources are available, we suggest increasing the number of plots per site and/or sampling intensity.

Protocols for sampling macrofungi occurring on large woody substrata also are becoming more similar (Lindblad 1998,2000,2001; D. L. Czederpiltz, unpublished data). The primary difference between our recommended protocols and some other currently used protocols (eg., protocol used by Czederpiltz et al. 1999; D. L. Czederpiltz, unpublished data) is that the latter protocols require destructive sampling (e.g., rolling logs, removing bark), whereas our protocols minimize disturbance to the site. Destructive sampling enables one to record all of the fungi fruiting on a substratum at the time of sampling but precludes resampling of that substratum. Our protocol allows for resampling, thereby allowing one to record macrofungi fruiting on the surface of the same logs over time. Because of temporal changes, in the species fruiting, we prefer to sample over time, although we may miss fungi that potentially are fruiting on the underside of logs or under their bark.

We recommend using an integrated set of sampling protocols for macrofungi, including opportunistic sampling, sampling of fixed-size plots, and sampling of a fixed number of downed logs. That practice will optimize the number ofmacrofungal species recorded at a site by including all the conspicuous fungi while also providing quantitative data that are comparable with data from other sites. Repeated sampling of relatively small fixed-size plots and large trunks ensures that inconspicuous fungi and fungi on relatively scarce substrata are included in the study. These protocols are being used in the Costa Rican National Fungal Inventory (Mueller and Mata 2000).

RECOMMENDED PROTOCOLS

OPPORTUNISTIC SAMPLING OF MACROFUNGI

By opportunistic sampling we mean carefully walking through a study site and collecting conspicuous sporocarps. Collectors should sample as many habitats in the site as possible. This technique does not yield quantitative data. Nevertheless, it is an important adjunct to the plot-based quantitative methods discussed next because additional species of macrofungi will be seen "off plot" as a result of the patchy distribution of sporocarps. Thus, a combination of opportunistic and plot-based sampling is necessary to maximize the macrofungal diversity documented at a site.

SAMPLING CONSPICUOUS MACROFUNGI USING FIXED-SIZE PLOTS

The protocol for sampling conspicuous macrofungi using fixed-size plots includes the following steps:

1. The investigator selects an area within the site for the permanent plot. The area should be representative of an important forest or grassland type at the site and should be chosen to optimize the diversity of habitat types sampled by the entire study (i.e., if the study covers grasslands, open woodlands, and dense forest, at least one plot should be set up in each habitat type). The plot should be established in as homogeneous an area as possible and as easily accessible from the road or trail as possible, without being susceptible to "edge effects" because it will be visited repeatedly.
2. Each plot consists of 10 transects that are 100 m long. Typically, transects are laid out parallel to one another at 10-m intervals. If the shape of the area to be sampled does not allow that, however, some transects can be laid out end to end. Transects are marked every 5 m with a flag or stake. Each transect is assigned a unique letter, and each flag should be numbered sequentially within a transect (i.e., A1–A20; B1–B20; . . . ; J1–J20).
3. Each person sampling carries a plastic pipe or wooden pole and a rope that is 1.262 m long; the rope is used to circumscribe 5-m^2 circular subplots around each flag in a transect, giving rise to 20 5-m^2 subplots per transect, for a total of 200 subplots, or a sampling area of 1000 m^2 (0.1 ha) per plot. Care should be used to not walk in or unnecessarily disturb the subplots.
4. All macrofungi occurring in a subplot are collected, labeled with the transect letter and subplot number, and placed in an appropriate bag or container. At the end of the collecting day, specimens are transported back to the field station for sorting, describing, photographing, and drying. The substratum (soil, leaf litter, wood) is noted for each specimen.

Ideally, subplots should be sampled every 2 weeks during the fruiting season. Often, however, sampling intensity must be reduced because of limited resources. Generally, it is possible to accomplish that without compromising the quality of the diversity information being collected. G. M. Mueller and his colleagues (unpublished data) intensively sampled plots in the Chicago area for 1 year. Based on the data from that year and on herbarium records, they determined peak fruiting times for the region and adjusted their sampling schedule accordingly so as to optimize their sampling effort.

SAMPLING SMALL ASCOMYCETES USING MICROPLOTS

The goal when sampling small ascomycetes is not to cover a lot of area but to obtain a quantitative sample of the microfungi growing on small substrata, especially those species that are infrequently or rarely collected. The sampling protocol for microfungi is similar to that for macrofungi and involves the following steps:

1. Investigators establish a microplot for sampling microfungi adjacent to a macrofungal subplot along the transects. Because substrata in the plots are collected, different plots are laid out each time a sample is taken.
2. Each person sampling carries a 0.56-m-long (or 1.128-m-long) plastic pipe or wood pole, which is used to circumscribe 1-m^2 circular subplots. The number of subplots per site to be sampled depends on the team's resources. Sampling the plots is time consuming and labor intensive, involving careful examination of all small substrata present. The time necessary to complete a sample varies with the diversity of substrata in the plot and the number of people sampling them. In Costa Rica, team members were instructed to sample as many plots as they could within 1 week. Most individuals sampled only two or three 1-m^2 subplots in a week because of the time involved in scanning the substrata to observe the microfungi.

3. All microfungi on twigs, branches, and leaves occurring within a plot are collected, labeled with the transect letter and subplot number, and placed in appropriate bags for transport back to the field station at the end of the collecting day. The samples then are sorted, examined under a stereomicroscope to determine if they are fertile, described, divided, and dried. The substratum (branch, twig, leaf) is noted for each specimen.
4. At a minimum, microfungal plots should be sampled twice a year—in the middle and at the end of the fruiting season.

SAMPLING A FIXED NUMBER OF DOWNED LOGS

Restricting quantitative sampling to the 0.1 -ha plots and the microplots would exclude most of the fungi found on large pieces of wood because the frequency of those larger substrata within small subplots is generally very low. Therefore, fungi occurring on larger pieces of wood must be sampled separately. The following protocols should capture a good percentage of the fungal diversity on those substrata as well as provide quantitative data on abundance and host and size-class specificity that can be compared with such data from other areas and studies.

1. Logs to be sampled should measure more than 20 cm in diameter and more than 2 m in length and should be lying on the ground.
2. At each site, the investigator selects 30 logs in each of the following decay classes:
   Class 1: Relatively newly fallen, usually retaining its bark
   Class 2: Medium rotten; bark fallen off; knife can penetrate–2cm into the wood without undue pressure
   Class 3: Thoroughly rotten; knife can penetrate into the wood without much pressure; the wood can be partly destroyed with the fingers.
3. Each log is marked with a colored plastic band and given a number, and its position is mapped. If possible, the tree is identified to genus or species. The length and diameter of each log is measured and recorded. If the diameter of the log varies greatly from one end to the other, the investigator measures the diameter near both ends and at the middle. If the log has major branches, each of those also is measured.
4. Generally, only two to three sporocarps of each common species are collected from each log; multiple sporocarps of species not or only infrequently encountered previously are collected. If many sporocarps of a particular species are present, a pin or other marker can be used as a reminder not to collect the same species again on the next trip.
5. Small specimens, such as those of corticoid fungi and ascomycetes, are collected whole, whereas large polypores are sectioned radially at a width of approximately 1.0-1.5 cm to enhance drying and discourage mold. Specimens are labeled with the log number and placed in appropriate containers. At the end of the collecting day, specimens are transported back to the field station for sorting, describing, photographing, and drying. When possible, the genus or species of the host tree is noted.

As mentioned earlier (see "Determining Adequate Sampling'' and "Sampling Conspicuous Macrofungi Using Fixed-Size Plots"), sampling should be carried out every 2 weeks to ensure that all fleshy agaric species are collected. If that sampling intensity is not possible because of limited human and/or financial resources, good diversity information on lignicolous macrofungi still can be obtained with less frequent sampling. Sampling logs four times per year (in the dry season and at the beginning, middle, and end of the rainy season), for example, has worked well for the Costa Rican National Fungal Inventory.

SUMMARY

The material in this chapter is intended to provide investigators with some direction in planning and conducting inventories of macrofungi. We hope that the recent growth of such studies will continue and be nurtured by the information that we have provided. In particular, we emphasize the need for well-planned research with clearly stated goals. Monitoring of fungi is a more recent undertaking that may be useful in detecting anthropogenic disturbances, such as air pollution, and quantifying their impacts.

Project planning should include background literature research on the vegetation and geology of the study area, as well as on the taxa of fungi likely to be encountered. Pilot studies or preliminary sampling of the fungi are useful in determining the intensity of sampling required to achieve the goals of the survey. Such studies also will provide insight into the numbers of specimens likely to be acquired and the taxonomic difficulty of the project.

Executing the project involves careful collection of specimens; documentation of the resulting specimens

with written descriptions, photographs, spore prints, cultures, and macrochemical tests; preservation of specimens; and archiving of voucher specimens and data in recognized herbaria. The processing of large numbers of specimens involves some prioritization because not all specimens are equally ephemeral or useful. Data analyses can include extrapolation of site richness from samples and complementarity tests to evaluate sampling efficiency. Use of the sampling protocols recommended in this chapter will yield standardized data on the diversity of macrofungi found fruiting on soil, leaf litter, and woody substrata comparable to data from equivalent studies at other times or sites. Nevertheless, research on sampling design remains a priority for improving sampling efficiency at all stages of the study and the quality of the data obtained.

At present, the greatest constraints on studies of macrofungal diversity are the paucity of fungal taxonomists and identification resources. No region of the world as yet has a complete mycota equivalent to a vascular-plant flora, a condition likely to persist for some time. We must not let that obstacle prevent us from carrying out inventories, but it is a limitation that we should strive to overcome.

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Front cover: The slime mold *Physarum roseum* on a decaying leaf. Slime molds are fungal-like organisms traditionally studied by mycologists. Photo by Ray Simons.

Back cover: A species of *Mycena* found in Yunnan, China. Species of the mushroom genus Mycena are commonly encountered throughout the world. Photo by Gregory M. Mueller.