A Mycological Florilegium: Fungal Communities

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FOR ME, ONE of the most appealing aspects of field mycology is the fact that you can walk the same path year after year and always be surprised. When we first see a species after ten years observation of the same spot, we do not know whether it fruits rarely (every 25 years perhaps), whether it is a new arrival destined to stay or an ephemeral transient. We know little about the age and size of fungal individuals and even less about the longevity, survival rate and distribution range of spores.

Assessing the diversity of mushrooms, plants, and animals is more and more important because the environment is changing so rapidly. We have only a vague idea about the numbers of fungi on earth, and only a few small areas have been thoroughly inventoried. This article focuses on approaches to these big and difficult questions. It gives an overview of recent literature which reports on the composition of the mycoflora, makes estimates of which species are common and which are rare, or treats a part of the mycological component at a certain site and a certain time. Many papers fit these categories, varying from articles on methods, to detailed analyses of a small plot in a single forest. Most of the studies only address a part of the mycoflora, especially the ectomycorrhizal fungi, but some studies focus on what changes occur under shifting circumstances. Molecular methods using the non-fruiting parts of the organism are used for identification in many cases, but some studies do not set out to get an idea of the species at all. From all these I present my personal pick of the crop. I have grouped my selections under four headings. Each of these sections begins with a list of papers in the order in which they are discussed, and with only one paper for each team of authors. This should suffice to lead you to the other papers. Abstracts of most of the papers are available online to everyone, free of charge; to find them, try googling the name of the journal.

I hope that you will be stimulated to search for similar papers on research in your own area—or even to consider how you may make your own contribution.

The Fruitbody Approach

Straatsma, G., F. Ayer, & S. Egli. 2001. Species richness, abundance, and phenology of fungal fruit bodies over 21 years in a Swiss forest plot. *Mycological Research* 105: 515–23.

Roberts, C., O. Ceska, P. Kroeger, & B. Kendrick. 2004. Macrofungi from six habitats over five years in Clayoquot Sound, Vancouver Island. *Canadian Journal of Botany* 82: 1518–38.

Norvell, L. L. & R. L. Exeter. 2004. Ectomycorrhizal epigeous basidiomycete diversity in Oregon Coast Range *Pseudotsuga menziesii* forests: Preliminary observations. *Memoirs of The New York Botanical Garden* 89: 159–89.

Walker, J. F. & O. K. Miller, Jr. 2002. Ectomycorrhizal sporophore distributions in a southeastern Appalachian mixed hardwood/conifer forest with thickets of *Rhododendron maximum*. *Mycologia* 94: 221–29.

Unterseher, M., P. Otto, & W. Morawetz. 2005. Species richness and substrate specificity of lignicolous fungi in the canopy of a temperate, mixed deciduous forest. *Mycological Progress* 4: 117–32.

Lindner, D. L., H. H. Burdsall, Jr., & G. R. Stanosz. 2006. Species diversity of polyporoid and corticoid fungi in northern hardwood forests with differing management histories. *Mycologia* 98: 195–217.

Boddy, L. 2001. Fungal community ecology and wood decomposition processes in angiosperms: from standing tree to complete decay of coarse woody debris. *Ecological Bulletins* 49: 43–56 [unfortunately not online].

A Swiss team recorded mushroom fruitbodies in one plot of 1500 m^2 during weekly visits from

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May to November over the course of 21 years and found that only eight out of over 400 species fruited every year. And every year, even in a very dry year and the very last year, species which had never been encountered before were reported. The numbers of species fluctuated from 18 during the driest year of 1989 to 194 in 1992, and the numbers of fruitbodies from 182 during that dry year of 1989 to 8467 in 1993, the wettest year. In total 71,222 fruitbodies were produced. A lower visit frequency was considered, but they found that many species were missed if they only inventoried every other week. This study focused entirely on above-ground terrestrial fruiting bodies, leaving aside all the conks, small leaf-inhabiting discs, and the truffles, to name a few of the other groups. The data collected in this very thorough fashion were used as a comparison with the effects of different treatments (nitrogen addition, picking of fruitbodies, etc.). This study has set a classic example of how to perform fruitbody surveys.

Dunes, bogs, estuaries, forest fringes, second-growth, and old-growth temperate rainforest were the places on the west coast of Vancouver Island where a small group inventoried for five years. No plots, visits in spring and autumn (in most years twice), and a huge effort in identifying the species encountered. The list has over 550 species, and every year it grew by at least 100! So, the real richness of this area is not known at all, and many more years are necessary to measure its extent. The old-growth rainforest with its three-dimensional structure got most attention, because of its intrinsic interest but also because it covers much more ground than the other habitats. It appeared to be the richest in species with 291—more than four times as many as were found in the bogs and estuaries. *Craterellus tubaeformis* occurred in all habitats and was one of 28 species to fruit every year, but most species were only encountered once, in one place. Three hundred ten species were found fruiting during one year only. An impressive number of *Cortinarius* species was discovered (67!), followed by *Mycena* with 38 and *Russula* with 35 species.

An equally long list of species gives some insight into the fungal richness of Douglas-fir forests in Oregon. The effects of stand age, clear-cutting, and thinning were examined, especially for the ectomycorrhizal flora, during four years. *Cortinarius*, *Inocybe* and *Russula* were the most species-rich genera with 95, 62, and 50 species respectively. Heavy thinning decreased the richness of the stands, but moderate thinning, in which half the number of trees was left standing, did not change the richness significantly. The absence of good identification keys for many species-rich genera in this part of the country made work hard, and the authors plead for more taxonomic work to be done!

From the eastern part of the U.S.A. comes a paper on the effect of *Rhododendron* thickets on the mushroom production in mixed woods. It had earlier been shown that *Tsuga canadensis* seedlings in those thickets had fewer ectomycorrhizal fungi on their roots than did seedlings outside the thickets. Yet mushroom production did not seem to be affected at all by the presence or absence of this spreading bush. The same species were found inside and outside the thickets, and also the litter did not have any effects.

Mycologists tend to walk with their heads down—a very good attitude in cities to a) avoid the dog poop and b) find coins—but there is a rich habitat above us! Tree canopies with their dead branches are a haven for bark-inhabiting and wood-rotting fungi. Lots of lofty slime molds have been discovered in the Great Smoky Mountains NP all-species inventory, but fungi have been step-motherly treated in this regard. Fortunately, there is now a nice example of treetop fungi from a German study. A temperate, mixed, deciduous forest is one of the sites for this canopy research, and a big crane was set up to allow people to get up and collect. A three-dimensional world asks for a different sampling approach than one for the two dimensions we are used to on the forest floor. A cube of 3x3x3 m^3 around the cabin hanging from the crane was the plot in which dead branches of different tree species were sampled. The branches were put in a moist chamber to get fruitbodies to grow on them, and the species were identified. Again, there a was wide variety and high number of species; 118 different fungal taxa were identified. Crust-formers were the most numerous, followed by small Ascomycetes. Real mushrooms, especially those with big fruitbodies, such as *Pluteus cervinus*, the deer fungus, were rare. Again, many species were only found once or on one tree, and many were restricted to a single tree species.

The importance of dead wood in forests has

been widely recognized, as it is a habitat for fungi, insects, birds and even for tree seedlings. A steady stream of papers on wood inhabiting mushrooms comes from northern Europe, where boreal forests are species-rich and logging continues to be a threat. American research on the inhabitants of old logs and snags is rarer, so it is nice to see a paper on polypores and crusts of the northern hardwood forests of Wisconsin and the upper peninsula of Michigan. Plots were laid out in different forests (old-growth, uneven-aged, and relatively young even-aged), and wood of various diameter was investigated for the fungi. In total, 255 species of polypores and crusts were identified, with a lot of variation in the number of species on the smaller wood from year to year. This impressive number was the result of sampling for a mere two years! Old-growth forests were characterized by *Cystostereum murrayi* and *Rigidoporus crocatus*. *Oxyporus populinus* on *Acer* was characteristic for the younger even-aged stands. Five species were discovered that had not been reported for the United States previously. A total of 46 species could not be identified at all, and might represent new species.

A fascinating tale on the course of wood decay, and the factors that influence it, is given in an overview article by Lynne Boddy. As soon as wood becomes dysfunctional, fungi take over, starting with colonization from outside or inside—fungi are lying in wait for the moment. Every new arrival changes the (de)composition, and the community is in flux throughout the decomposition process, both in space and in time. The amounts of water and oxygen are important factors affecting the development of the community, and their effects are different in the various types of wood—be it a dead branch in the canopy or a log lying on the forest floor. Mushrooms flag the presence of a species in the wood but do not tell us when the species arrived, how extensive its mycelium is, or what stress factors might have contributed to the formation of the fruitbodies. How fast a piece of wood is broken down depends on which species are involved, though temperature and amount of water play a role as well. *Xylaria hypoxylon* forms a big fence around its territory to maintain a low water content, and keep other species out. But as soon as the plates forming the fence are removed, other fungi take over, and decay happens really fast. In short, decay is not a single-lane process, but a mosaic, and much depends on who was first.

The All-Taxa Molecular Approach

O'Brien, H., J. L. Parrent, J. A. Jackson, J.-M. Moncalvo, & R. Vilgalys. 2005. Fungal community analysis by large-scale sequencing of environtmental samples. *Applied and Environmental Microbiology* 71: 5544–50.

Fierer, N., J. A. Jackson, R. Vilgalys, & R. B. Jackson. 2005. Assessment of soil microbial community structure by use orf taxon-specific quantitavie PCR analysis. *Applied and Environmental Microbiology* 71: 4117–20.

A totally different approach to assessing diversity was chosen by a team from Duke University in Durham (NC); they collected soil and leaf litter from two different forests. DNA was extracted out of a small sample from these collections, two different gene regions were amplified using PCR techniques, cloned and subsequently sequenced. The results were compared with data in public databases and from their own collection of fruitbody samples to find suitable names for the species. This is like using forensic techniques to identify the murderer on account of some skin or blood drops left on the crime scene. The results for the Duke study are quite remarkable. In those small samples, covering only an utterly microscopic fraction of the total forest, more than 400 sequence types were detected, representing chytrids to agarics, with Ascomycetes and Basidiomycetes being the most species rich groups. Even more than in the Swiss fruitbody study reviewed above, the total richness was far from covered, and many more species are supposed to be present in these forests. This analysis gives an idea about the species present in the soil, but not about the form they are in: living hyphae, spores, or digested bits of fruitbodies.

The methods applied here sound almost too good to be true; and in reality there are, just as with the fruitbody-based inventories, many flaws and problems. One of them is the fact that such a big project calls for standard procedures, and that means that species that need something special to detect them fall through the net and are not caught. Furthermore, very abundant species have a much bigger chance to be detected than rare

species, or species for which the method is not a tight fit. The characters (pieces of DNA) used in these analyses might not be the best to delimit species. The differences between species might be very small in one group and big in another. Compare this with using the character "cap color red" in a key: it is an excellent character to recognize the species group of *Russula emetica*, but not the species within this complex. And lastly, getting a fitting name depends on the quality and quantity of data in the databases. Producing those reference data is the task of taxonomists and field mycologists, of writers of keys and floras, who depend on good, well-documented collections.

Ectomycorrhizal Fungi

Taylor, A. F. S. 2002. Fungal diversity in ectomycorrhizal communities: sampling effort and species detection. *Plant and Soil* 244: 19–28.

Izzo, A. D., M. Meyer, J. M. Trappe, M. North, & T. D. Bruns. 2005. Hypogeous ectomycorrhizal fungal species on roots and in small mammal diet in a mixed-conifer forest. *Forest Science* 51: 243–54.

Ashkannejhad, S. & T. R. Horton. 2005. Ectomycorrhizal ecology under primary succession on coastal sand dunes: interactions involving *Pinus contorta*, suilloid fungi and deer. *New Phytologist* 169: 345–54.

Cripps, C. 2004. Ectomycorrhizal fungi above and below ground in a small, isolated aspen stand: A simple system reveals fungal fruiting strategies and an edge effect. *Memoirs of the New York Botanical Garden* 89: 249–65.

Bergemann, S. E. & M. Garbelotto. 2006. High diversity of fungi recovered from the roots of mature tanoak (*Lithocarpus densiflorus*) in northern California. *Canadian Journal of Botany* 84: 1380–94.

Robertson, S. J., L. E. Tackaberry, K. N. Egger, & H. B. Massicotte. 2006. Ectomycorrhizal fungal communities of black spruce differ between wetland and upland forest. *Canadian Journal of Forest Research* 36: 972–85.

Many studies focus only on a part of the mycoflora, in particular the ectomycorrhizal species. They are easier to target as they grow around the tips of their host trees and shrubs, and these mycorrhizal root tips can be found by sifting through soil samples. The fungus on the roots can be visually identified, but more information is gathered from the DNA and its matches with records in databases such as Genbank.

How to sample this underworld is a study on itself, and the numbers of root tips needed to get close to a complete picture are huge. As in all settings, only a few species are very abundant, the vast majority is rarely encountered. For instance, the rarest species in a Swedish study was found on three out of 5,371 tips. The total number of root tips per square meter of forest floor was estimated to be between 70,000 and 720,000.

One interesting facts unearthed by the early ectomycorrhizal studies is that a species can form many big fruitbodies, while it is difficult to find any trace of it on the roots. The picture we get from looking at fruitbodies may be different from what is happening underground. In other words, some species may be very efficient in getting carbon (sugars) from the tree, whereas others invest much less energy in the making of fruitbodies but instead may develop an extensive network of hyphae. Or some species rely heavily on spores for keeping the species alive, others may have long-lived individuals and invest their energy in maintaining their "bodies." We can also wonder whether we look in the right places, both for the fruitbodies and for the root tips.

A nice study by Antonio Izzo and colleagues employed and compared four different sampling methods in making an inventory of the hypogeous fungi in an *Abies* forest in the southern part of the Sierra Nevada in California. They obtained fungal material from root tips (and used DNA for identification); from spores found in the scat of small mammals in the same area, since truffles and the like form a big portion of the diet of these animals (again with DNA for identification); and from fruitbodies of (false) truffles collected over the course of several years in the same general area. Their fourth method was to collect soil, use it to grow pines from seed, then look at the roots of the seedlings and identify the species found there. This is a great method to determine which fungal species persist as viable spores. *Amanita* and *Russula* species fail to show up, but *Rhizopogon* (false truffles) and a few *Suillus* species will often appear. *Thelephora* and *Tomentella* species also have spores that can stay viable for a long time.

However, the hypogeous lifestyle has been invented by many totally different groups of mushrooms, so there is not a simple DNA method to distinguish them from all other mushroom species. In this true fir forest, one fifth of the number of species found on the roots had the hypogeous lifestyle; a total of 29 hypogeous genera was found. Fifteen species were discovered in scat, including one species that is a wood rotting fungus. Two *Rhizopogon* species were detected by all four of the methods, but other species only showed up in the bioassays and were not seen as fruitbodies at all (nor in the scat). Apparently, these are species which rarely fruit and spend years and years as spores in the soil, biding their time.

Research in the coastal dunes in Oregon looked at the establishment of pines in this barren area, and the role of fungi in the colonization of the trees. The question was how do the fungi get dispersed from one tree island to the next? Again different methods were used to detect the fungi, including direct observations of fruitbodies, identification of the fungi on the root tips of trees in the experimental area and on the root tips of seedlings grown in soil from the area, and on sterile soil with deer pellets. It turned out that deer, just like the smaller mammals in the western mountains, eat a lot of mushrooms. They eat both *Suillus* and *Rhizopogon* species, but the spores of the latter stayed viable for at least a year in the animal droppings. Although the forested areas harboured a much wider array of fungal species than these two genera, the species that facilitated the germination of the pines in the open uncolonized areas were especially those Rhizopogons, which got there via the intestinal tracts of the ungulates.

A small aspen stand in Montana was where Cathy Cripps studied ectomycorrhizal fungi, both above and below ground, for four years. This is one of the very few studies in which a whole stand could be studied. Eight species were found in this tree island, and four of them were found fruiting. One species, *Paxillus vernalis,* produced only a single fruitbody during the study although, below ground, it was widespread. The pioneer species *Inocybe lacera* and *Laccaria proxima* formed hundreds of fruitbodies each year, but only at the edge of the stand, though again, their mycelium was encountered throughout the whole stand. The total numbers of mycorrhizal root tips were high: the estimate is between 200,000 and 1.3 million per cubic meter of soil, even higher than in the Swedish studies mentioned above.

A Californian study focused on five tanbark oak stands (*Lithocarpus densiflora*) in the northern coastal part of the state. This tree is suffering under the onslaught of Sudden Oak Death (caused by *Phytophthora ramorum,* a relative of the organism that causes potato blight), and mortality rates are extremely high. Knowing what mycorrhizal species are present may help to defend the tree species. Over 100 species were discovered in the roots from one sampling effort in 15 plots, but the estimate of the total richness is 265. Root endophytes, saprotrophic fungi and especially ectomycorrhizal species were found. This community is rich in species of the Russulaceae and the Thelephorales (such as *Bankera, Hydnellum, Boletopsis,* and *Tomentella* species). Many species remained nameless, as there not enough sequence data for comparison available in Genbank; half of the number of Ascomycetes could not be named.

Communities of black spruce (*Picea mariana*) seedlings were the subject of a study in British Columbia. This tree occurs in a series of habitats ranging from wet to dry and low to uplands, with a changing set of fungi, also depending on the co-occurring mycorrhizal trees (lodgepole pine in the uplands and tamarack in the wetlands). Seedlings were collected once, and fruitbodies and soil were also gathered. Morphologically, there were 33 different types of mycorrhiza recognized on the root tips, but analysis of these showed that there were twice as many molecularly defined types. The richest were the upland stands, where lodgepole pine and black spruce co-exist.

Ectomycorrhizal Fungi and Environmental Changes

Avis, P. G., D. J. McLaughlin, B. C. Dentinger, & P.B. Reich. 2003. Long-term increase in nitrogen supply alters above- and below-ground ectomycorrhizal communities and increases the dominance of *Russula* spp. in a termperate oak savanna. *New Phytologist* 160: 239–53.

Lilleskov, E. A., T. J. Fahey, T. R. Horton, & G. M. Lovett. 2002. Belowground ectomycorrhizal fungal community change over a nitrogen deposition gradient in Alaska. *Ecology* 83: 104–15.

In Europe, there has been massive nitrogen deposition in soils on account of artificial fertilization, industrial pollution, and other human activities. The resultant changes in pH of forest soils have altered the forests and their mycoflora enormously. Grasses have taken over in open pine forests where in the past reindeer lichens of the genera *Cladonia* and *Cladina* were dominant. Many mushroom species have stopped fruiting; *Tricholoma* species, for instance, have become less and less common in the Netherlands during the last century, judging from fruitbody production.

Ectomycorrhizal fungi are essential for the trees in regions where nitrogen is hard to get, as they can access it better than plants. In exchange for the nitrogen the fungus gets sugars (carbon). But when nitrogen is freely available, the trees have a much lower need of the fungi. We know that the fungi do not fruit anymore, but what happens underground, on the roots, and in the soil, is another question. Studies in two totally different settings, one an oak savanna in the midwest, the other a spruce dominated forest in Alaska address these issues.

The effects of long-term application of nitrogen were studied experimentally in an oak savanna in Minnesota over a three-year period. The number of species fruiting in fertilized plots was lower than in unfertilized areas, while the species that were found in the fertilized plots produced more fruitbodies than elsewhere; in the plots with the highest amount of nitrogen, a *Russula* species close to *R. amoenolens* was the dominant fruiter with a total of 875 fruitbodies over a three-year period. Below ground, on the roots, the changes were perhaps a bit less pronounced, but again the total number of species decreased, and *Russula* species replaced *Cortinarius* species, which were dominant in the unfertilized plots.

A nitrogen deposition gradient caused by a fertilizer facility in Alaska also affected the fungi both above and below-ground. The factory had been emitting nitrogen since 1968, but in the mid-'80s control measures were put into place which reduced the output by around 80%. In this study the roots of white spruce (*Picea glauca*) were examined and sampled during a three-month period in 1995; another study by the same authors showed a decrease in fruitbodies in the N-rich plots. The richness both in fruitbodies and in the ectomycorrhizal root tips declined dramatically with an increase in nitrogen. A suite of species (*Amphinema byssoides* and several *Cortinarius*, *Tomentella*, and *Piloderma* species) present in the low-impacted plots was not found in the fertilized plots. They were replaced by ubiquitous species such as *Paxillus involutus*, *Lactarius theiogalus*, *Tylospora fibrillosa,* and *Thelephora terrestris*. The first two of these replacers are specialized for uptake of phosphorus under these high-nitrogen conditions.

Closing Remarks

The strongest impression from all these studies is surely that the richness and abundance of fungi is much greater than previously thought. It has also been demonstrated again and again that many species here in North America are still undescribed.

Accordingly, recording and inventorying should be high on the list of every mushroom club's activities. Mycoblitzes such as the two conducted each year at California's Point Reyes National Seashore since 2005, in which voucher material of all species is preserved for study, are a good start. NAMA has preserved material from their forays since 1997, giving the procedures wide exposure. The species lists and photos of the collections are available online. Mycoblitzes can also serve a broader educational purpose when the general public is invited to participate.

A second strong impression is that we know very little about what the different species actually do and what their life histories are. In this respect, there seems to be an emphasis on the study of ectomycorrhizal species, leading to a woeful neglect of saprotrophic fungi growing on litter and wood.

To find more out about all forest dwellers is the big challenge of the future!