COENOSES 3 (2); 61-68, 1988

SAMPLING MYCOCOENOSES

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Keywords: Mycocoenology, Sampling, Design, Size, Number, Units

Abstract: The literature is reviewed and a sampling design is described which present a reliable basis for the study of the environmental distribution of soil microfungi. An example illustrates the method's application.

Introduction

Although there are currently comprehensive ecological studies of soil microfungi, their sampling designs have not been adequatly described. This is partly due to difficulties inherent in mycocoenological investigations, such as the influence of seasonal trends (Parkinson and Balasooriya 1969, Widden and Parkinson 1973, Mabee and Garner 1974, Gochenaur 1978), vegetation types (Tresner *et al.* 1954, Christensen *et al.* 1962, Saksena *et al.* 1965, Christensen and Whittingham 1965, Gochenaur and Backus 1967, Gochenaur and Whittingham 1967, Christensen 1969, Danielson and Davey 1973, Morrall 1974, Bisset and Parkinson 1979c, Baath 1981), soil depth and other characteristics (Williams 1963, Williams and Parkinson 1964, Parkinson and Balasooriya 1967, Widden and Parkinson 1973, Söderström 1975) relating to changes in microfungal communities. Isolation procedures, taxonomical problems and statistical tests (Christensen 1977, Widden 1979, Bisset and Parkinson 1979a) have been taken into account, but the sampling problem has not yet been solved.

Yet, this is an area of primary interest. Daget and Godron (1982) affirm this and suggest that before the field work begins, it is necessary to carefully consider the study plans. Specifically, biological investigations have to face the problem of the size and number of sampling units, which should be examined before reliable generalizations can be made based on the sample about the population. Jeffers (1972) considers about 50% of English biological investigations carried out before 1971 useless, because of the inappropriate experimental and survey design. While this may be an overstatement, it seems essential that biologists concentrate upon the sampling method more in the future than they have done in the past.

The objective of this study is to assess the geographical and environmental distribution of soil microfungi. Two main points are considered:

1) After 1950, a number of studies have demonstrated, that communities of soil fungi show ecological and geoclimatic specificity in response to environmental factors, rejecting the earlier idea of a cosmopolitan soil fungus flora (Morrall and Vanterpool 1968).

2) Mycocoenosis is the assemblage of fungi within a phytocoenosis, a kind of taxacoenosis dependent, in ecological respects, on communities of vascular plants (Arnolds 1984).

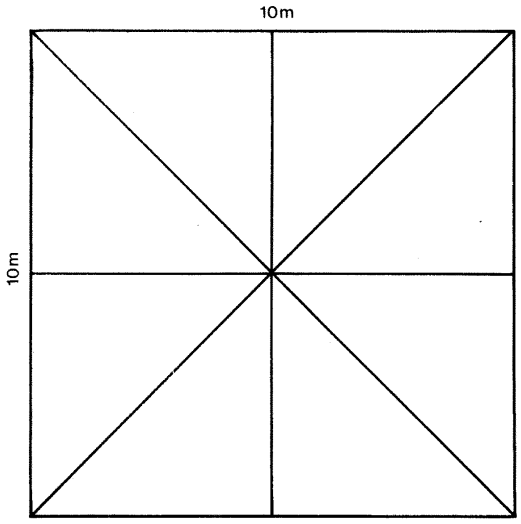
We stress the methodological aspects. Our approach, designed to discover the distribution pattern of soil microfungi indirectly, involves investigating the relationships between mycocoenoses (micromycocoenoses) and their environment in well-known plant communities. Massari (1983) suggested a method of study for micromycocoenoses, which has been tested with encouraging results. In this paper we revisit its merits, and comment on its application.

Sampling considerations and design

If we consider any surface, *e.g.*, a 10×10 quadrat in a plant community, different groups of microfungi might be associated with it, depending on the actual environmental conditions, presented by tree stumps, soil depressions, organic matter, decaying wood on the ground, etc. We have asked: 1) Is a given combination of species related to specific environmental aspects depending on the dominant ecological factors and vegetation types? 2) How, where, and when does such a species combination reoccur? 3) If such a relationship does not exist, what are the consequences upon microfungal distribution?

In order to answer these questions we must first define possible combinations, associated with different environmental conditions. In other words, we need a sampling method covering, as far as possible, most of these environmental conditions, so as to show their differences and similarities. Sampling along the main directions of the study area, as shown in Fig. 1, might satisfy this requirement, if it were not - as it happens with transects - for the subjective choice of sample size (number of sampling units) and the distances between sample points.

The problems of subjective choice have to be solved. It is obvious that a sampling method that excludes the researcher's choice can be regulated only from within, that is by the sampling mechanism itself. The simplest is to monitor the increase of the number of species in



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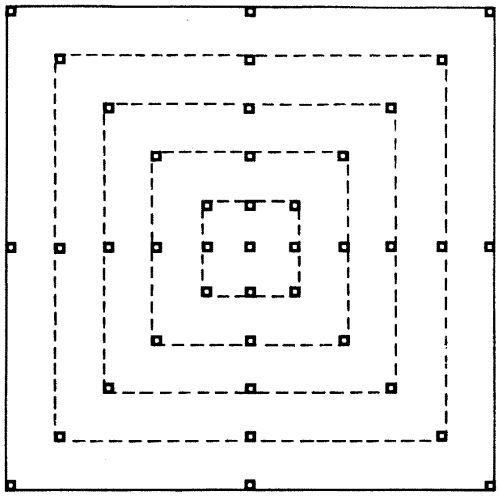

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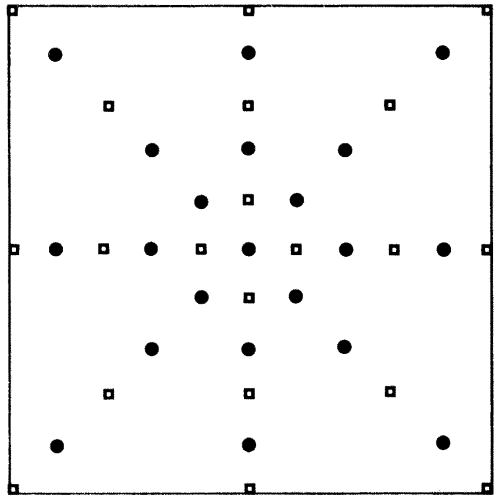

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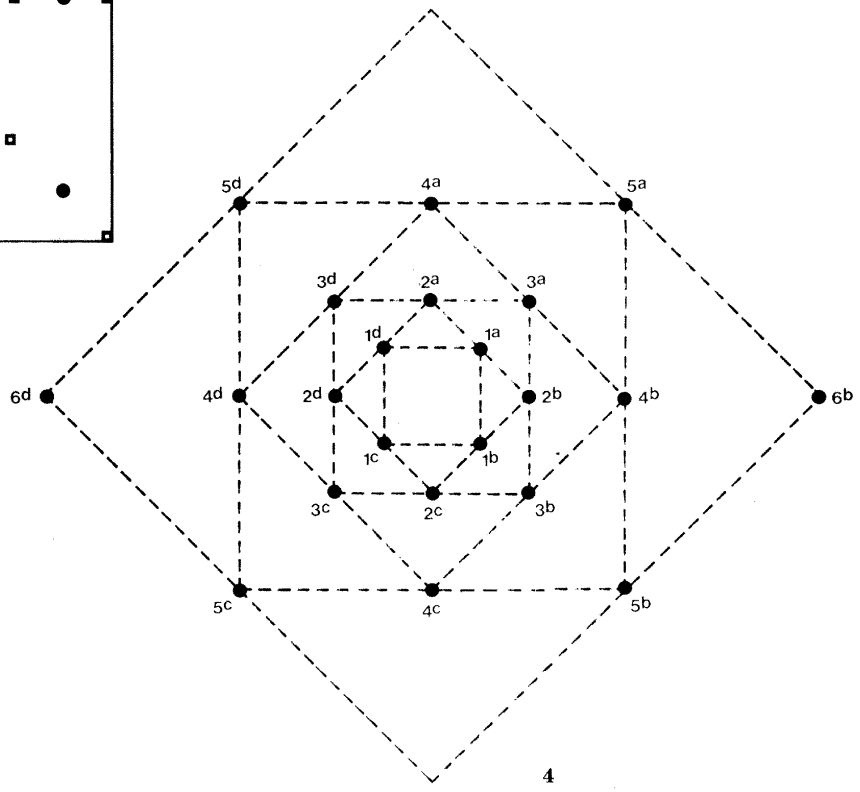

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Figs. 1, 2, 3, 4. Sequence of plans processed to determine a sampling scheme for the study of microfungal communities.

relation to the increase of surface as in the minimal area method. When no more new species are found, or new species become very rare, the sampling can be considered concluded.

We started from the concept of the minimal area in order to apply it to the study of soil microfungi. We are aware that some authors do not agree with the applicability of this method, except in rare cases, since the species-area curve is infinite. However our aim is *not* to define the minimal area, the size of the sampling unit, but the use of the minimal area concept as a potentially useful perspective of sampling. In phytocoenological applications, the analytic minimal area is defined for a stand under study as a representative area. For example, an adequate sample of species of regular occurrence in the stand satisfies this requirement. The size decision may, but will not necessarily, be based on the total number of species in the stand or the usual minimal area curve (Du Rietz 1921, 1930, Vestal 1949, Goodall 1952, 1961, Hopkins 1957, Cain and Castro 1959, Braun-Blanquet 1964, Greig-Smith 1964, van der Maarel 1966, Tüxen 1970).

The minimal area perspective assumes particular importance in the experimental phase of mycocoenological investigations. It should, however, be modified, integrated or, even rejected when indicated by new findings. In order to find a relationships between the number of new species and the number of sampling units, we placed the sampling points along the lines shown in Fig. 1, at arbitrary distances. The advantage of this arrangement is that the units can form wider and wider quadrats (Fig. 2), until they cover the entire area. If this pattern is followed, with the sampling points 1 m apart, the analysis would require 41 soil samples: this would involves preparation of 410 petri dishes, requiring, with an average of 20 CFU (colony forming units) dish, identification of over 8.000 colonies of microfungi. This pattern would be completely subjective, being the distance between the points a subjective choice.

To reduce the potential work load, we eliminated some sampling points and maintained only those required to sustain the multidirectional characteristics of the design (Fig. 3). This design allows laying sampling units that progressively double their surface. Fig. 4 shows that the imaginary lines, joining the points form sampling units, positioned relative to one another with a 45° rotation, and related by the regular area increase, as the sides of each unit are as long as the diagonal of the preceding one. This pattern of sampling units determines the sampling points, independently of any subjective choice. Moreover, it permits to define sampling areas, even if artificially, to which the number of new species can be related. We describe this as the Surface Increasing Method (SIM) of sampling. It represents an operational "module" (Fig. 5) applicable to study areas of whatever size, provided that they are sufficiently know.

Example

As already mentioned regarding relationships between mycocoenoses and plant communities, we used a study area already subjected to a survey by phytoso-

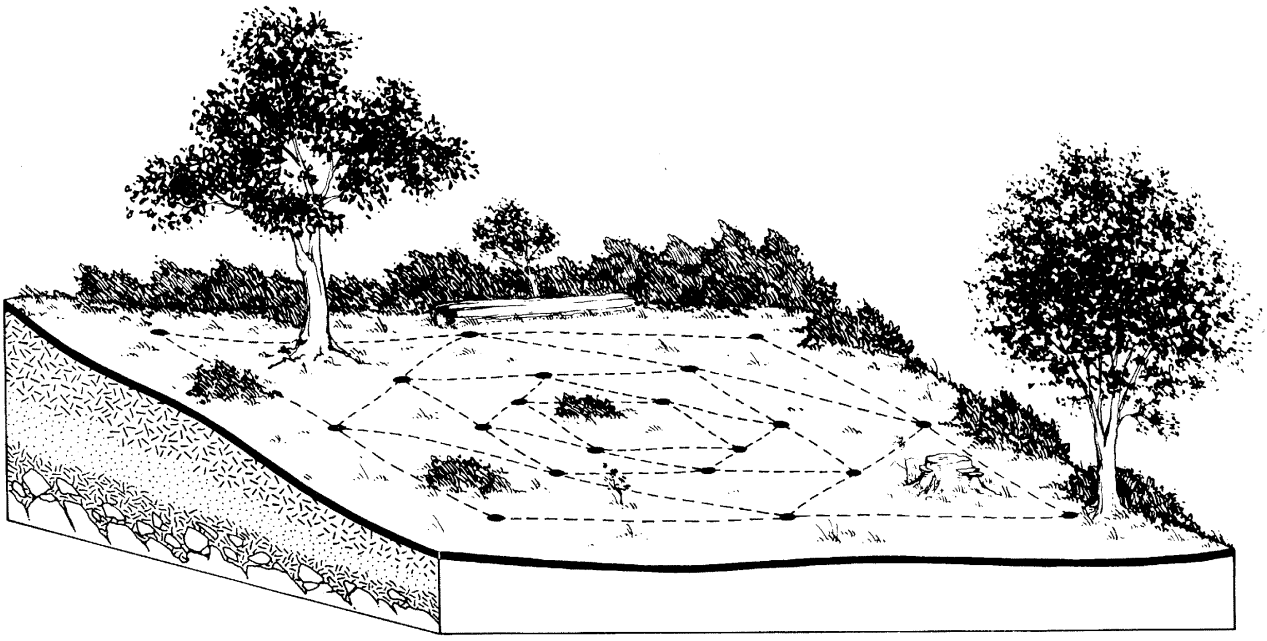

Fig. 5. Example of the arrangement of sampling points in a plant community, using the SIM (Surface Increasing Method) scheme.

ciological relevés. The reasons are several:

1) The relevé is the floristic-sociological and ecological description of a stand (phytocoenose).

2) The main obstacle of soil microfungal investigations is the impossibility of direct access to the micromycocoenoses that represent the very subject of study; it is necessary to avoid this obstacle by making use of a "mediator" or a factorguide.

3) A valid mediator is the plant community. In fact, the more we known about it, the better the approach to a study of the micromycocoenosis in the community.

4) By using a relevé, the mycologist has the advantage of knowing in advance some important aspects about study site (reconnaissance and choice of plots, description of structure and strata, floristic analysis, etc.); the relevé serves as a reference base for comparative studies.

We chose for study an areas within the so-called "average" type, where the relevé depict the floristic composition most similar to that of the phytosociological unit. Regarding the advantages derived from this choice, we are confident that an area where an analysis of the vegetation has been carried out based upon the floristic-sociological approach, and therefore well-known for its floristic, vegetational and environmental characteristics, can be considered better suited for this study than others not so well-known for these characteristics. If we consider that the ecological studies of soil microfungi contain little indications of the main phanerophytes, even when these studies are carried out within well defined vegetational types, we believe the relevé approach represents a step forward. An interesting aspect of this approach is the new "role" of the mycologist as regards the field work and the ecological environment. He is fully awair of the importance of the field work, the real beginning of the investigation, preparing the way for the laboratory phase when the fungi appear and their analysis begins. A limitation is the dependance on floristic-sociological studies, as it can only be carry out after the plant communities have been studied.

The study of the mycocoenoses within sampling units raises two further questions, regarding both floristic homogeneity and the utility of adding fungi to the list of higher plants. As regards homogeneity, an answer can be given after the comparison of the mycocoenoses in different vegetation types. So far it has not been proved that homogeneity in the microfungal flora depends on homogeneity in the total vegetation. The results of Sappa's studies (1955) seem to suggest just the opposite. The addition of fungi to floristic lists is in doubt. Some phytosociologists consider not only fungi, but all Crypthogames, atipical in communities of higher plants and prefer having separate lists. If the aim of the addition of fungi is to increase the information content of the relevé, it would seem correct not to include fungi in the relevé until the significance of the presence of fungi species in a plant community is studied.

We have tested the SIM method based on the phytosociological relevés of *Acer pseudoplatanus* (Aceri-Tilietum), *Fagus sylvatica* (Carici-Fagetum) and *Ostrya carpinifolia* (Buglossoido-Ostryetum) forests; and in *Carex davalliana* (Caricetum davallianae) and *Sphagnum magellanicum* (Sphagnetum magellanici) peat-bog in Northern Italy. In this paper some aspects of these investigations are reported and the application of the new sampling method is evaluated. As regards the floristic data, the isolation method of microfungi, and the spe-

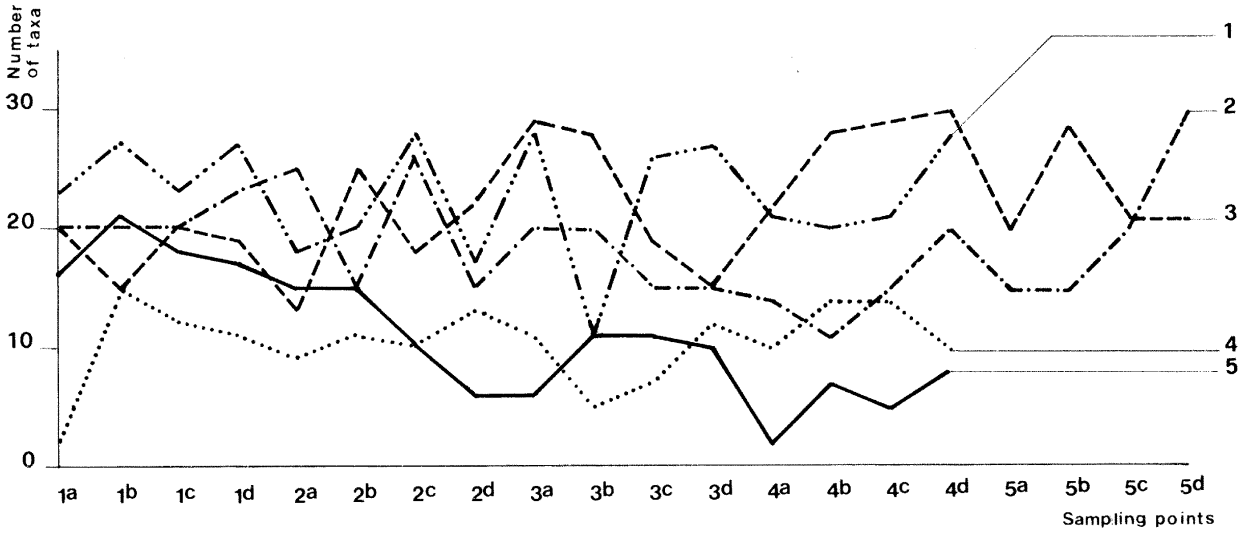

Fig. 6. Graphs of variation in the number of taxa at the different sampling location (see scheme, Fig. 4). The numbers 0 to 30 indicate the number of taxa present at one location. The graph lenght changes with the size of the study site. Legend: 1) Ostryetum, 2) Fagetum, 3) Aceretum, 4) Sphagnetum, 5) Caricetum.

cies composition of the micromycocoenoses, we refer to descriptions in Massari and Piccoli (1982) and Massari and Bartoli (1985). To simplify matters in the text and in the figures, vegetation types are indicated as Aceretum, Fagetum, Ostryetum, Caricetum and Sphagnetum.

Table 1. Sample characteristics in the study.

Vegetation type	Sampling unit size (Relevé area)	Sample size	Total taxa
Aceri-Tilietum	200 m^2	21	108
Carici-Fagetum	200 m^2	21	94
Buglossoido-Ostryetum	100 m^2	17	119
Caricetum davallianae	3200 cm^2	16	73
Sphagnetum magellanici	3200 cm^2	16	51

Results and discussion

Table 1 shows the interdependence between the number of taxa, sampling unit size, and the number of sampling units (sample size) in different vegetation types. The variations of the total number of fungal taxa in the samples (Fig. 4) is shown in Fig. 6. The graphs are rather irregular. The forests present, on an average, a higher number of taxa than the peat-bog. The values do in fact fluctuate between 10 and 30 taxa in forests while those in peat-bog vary between 5 and 15. Regarding the width of the variation, we observe that at two points of the *Carex davalliana* community, a few centimeters from one another, respectively 2 and 21 fungal taxa were isolated. Also, in the peat-bog community there were remarkable differences, sometimes in the number of taxa at the different points of the sampling scheme.

Both bog types have high cover values of higher species. It could be supposed that the sampling points with few fungi could coincide with the bare soil, but there are no bare patches in these communities. In the forests - which incorporate broad areas of bare soil - the cover values of herbs or mosses are low. For Aceretum, Fagetum and Ostryetum these values are 80, 40, 40% for the herbaceous layer and 5, 10, 10% for the moss layer. In these types, perhaps with the exception of the Ostryetum, the variation in numbers of fungal taxa is less irregular over the sampling scheme.

The numbers of taxa are indicated beside each sampling point at which they were isolated (Figs. 7/1, 2, 3, 4, 5). The sampling points with similar numbers of taxa are marked by contours. Based on these, the distribution of taxa appears to be less irregular than Fig. 6 suggestes. In the Caricetum for instance, where in one case 2 and 21 taxa had been isolated a few centimeters apart, a denser group of fungi can in part be observed (Fig. 7/5) in the central part of the sampling site. The marginal areas show much reduced numbers of taxa. Also analyses of the other plant communities show that the fungal distribution is not so irregular as the graphs in Fig. 6 might suggest.

The comparative analyses in this study show that the number of taxa, when given in relation to the design of sampling points, is a relevant source of information. We believe that based on our scheme there is the possibility of proving or disproving that the distribution of soil microfungi is anomalous and cannot fit a uniform pattern.

The species-area curves (Fig. 8) are in accordance with what is predictable based upon known facts about plant communities. In many cases the curves level off, implying that at the given sampling unit (plot) size, where the leveling off begins, full species saturation may have been reached. In other cases, we have found curves that keep rising. It is not possible, at the moment, to establish if these curves indicate that the sampling unit is too small or too large, as both may be a reason (Westoff and van der Maarel, 1973).

The number of new taxa progressively diminish as we pass from the smaller inside quadrats to the larger external ones, as can be seen in Table 2, based on data reported by Massari and Piccoli (1982), Massari (1983), Massari and Bartoli (1985).

The few new taxa isolated in the last larger and more external sampling unit should mean that the mycological composition, with the inevitable limitation deriving from the isolation method, can be used to represent the micromycocoenoses in plant communities. In conclusion we point out that:

1) soil samples should be taken according to the sampling scheme;

2) as many soil samples are analyzed as required by the species-area curves;

3) the sampling is concluded only when the curve starts leveling off;

4) our floristic lists are incomplete, but we believe that we have achived, according to the minimal area concept, a first approximation to the species composi-

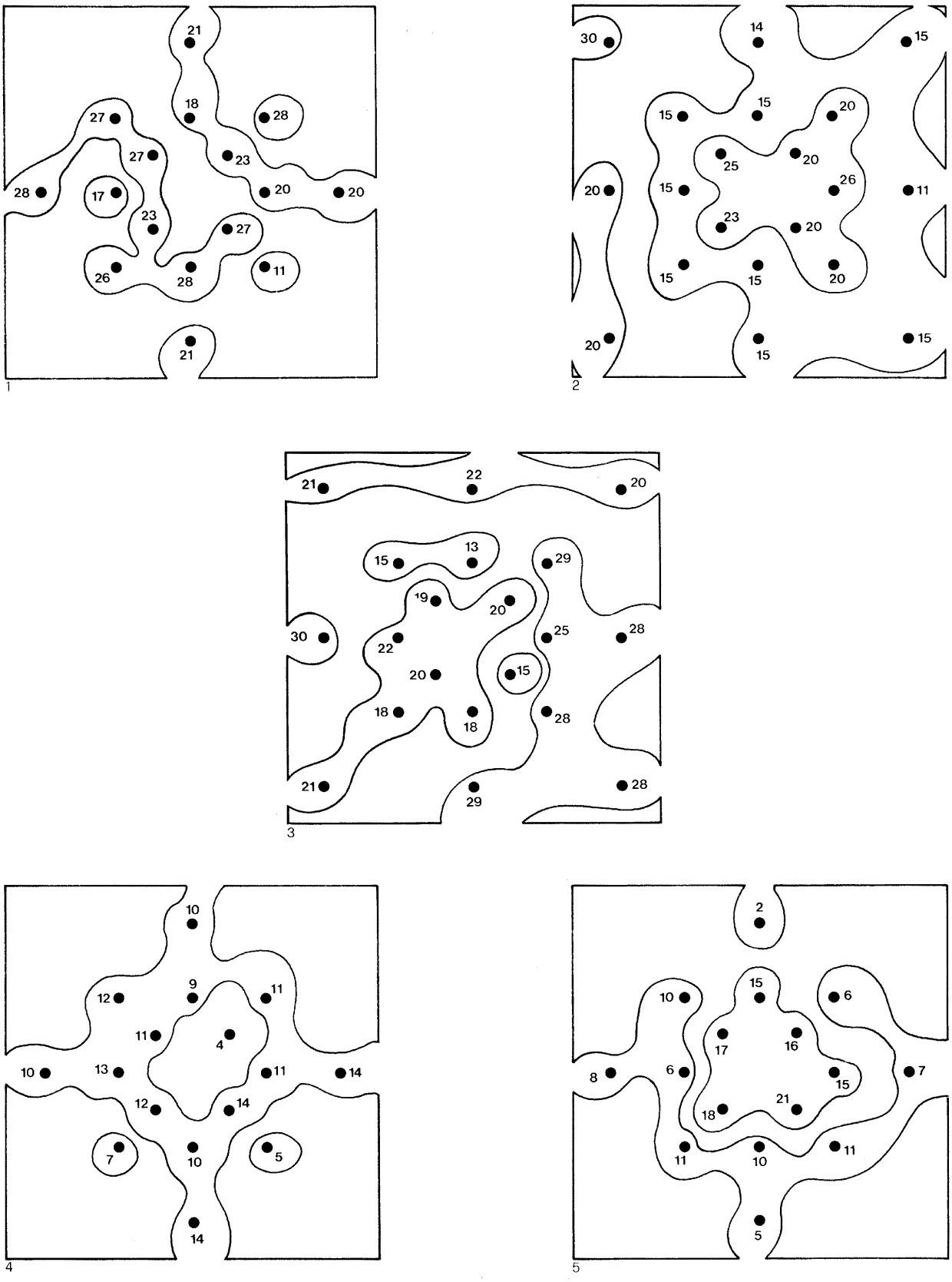

Fig. 7. Distribution of the number of taxa present at the sampling location of the basic scheme (Fig. 4). Contour lines are shown. Legend: 1) Ostryetum, 2) Fagetum, 3) Aceretum, 4) Sphagnetum, 5) Caricetum.

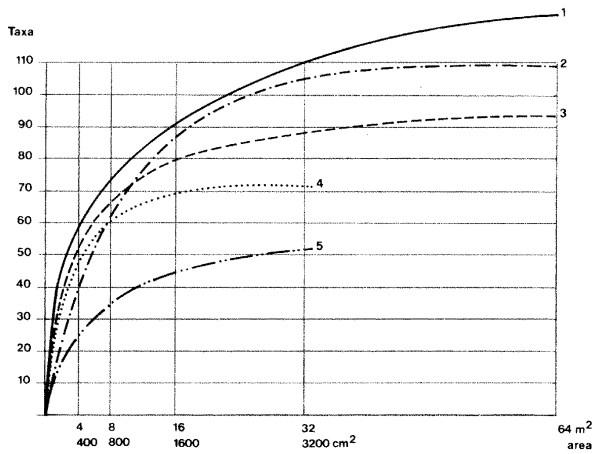

Fig. 8. Species-area curve of microfungi isolated in soils of three forests and in two peat-bogs in Northern Italy. Legend: 1) Ostryetum, 2) Aceretum, 3) Fagetum, 4) Caricetum, 5) Sphagnetum.

tion of the micromycocoenoses;

5) more reliable results will depend on the availability of more reliable isolation techniques and culture media; the sampling technique does not seem to be limiting;

6) we have taken the first step towards understanding the geographical and environmental distribution of soil fungi.

Acknowledgments. We tank A.M. Luppi-Mosca for his suggestion and, in particular, L. Orlòci for reading and commenting on the text. The work was partially supported by an Italian M.P.I. grant (to A. Bartoli).

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Table 2. Increase of new taxa in sampling units (plots and the relationship to progressively increasing size.

Vegetation type	Sampling unit				
	1a1b1c1d	2a2b2c2d	3a3b3c3d	4a4b4c4d	5a5b5c5d
Aceri-Tilietum	46	+ 18 (=64)	+ 22 (= 86)	+ 20 (=106)	+ 2 (=108)
Carici-Fagetum	50	+ 14 (=64)	+ 14 (= 78)	+ 10 (= 88)	+ 6 (= 94)
Buglossoido-Ostryetum	63	+ 26 (=89)	+ 23 (=112)	+ 7 (=119)	—
Caricetum davallianae	48	+ 12 (=60)	+ 10 (= 70)	+ 3 (= 73)	—
Sphagnetum magellanici	25	+ 10 (=35)	+ 10 (= 45)	+ 6 (= 51)	—

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