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Abstract

The relationship between litter quality and life strategy of soil microorganisms (copiotrophy vs oligotrophy) is important for understanding soil processes such as decomposition. Yet, whether and how this relationship may vary with the addition of substrates of contrasting quality (i.e., labile vs recalcitrant) has rarely been evaluated for both bacteria and fungi simultaneously. Using a 3-month incubation experiment with either maize leaves (enriched in soluble carbon (C)) or roots (enriched in structural C), we measured changes in litter quality in association with the composition of bacterial and fungal communities assessed *via* pyrosequencing after 0, 15, 35 and 91 days. Overall, leaf addition led to a higher differentiation from the unamended soil for bacterial and early-decomposers fungal communities compared with root addition. This finding clearly indicates that the differentiation of microbial communities strongly depends on substrate quality for both bacterial and fungal communities. Further, the differentiation of bacterial communities after litter addition remained relatively similar throughout the incubation period. This suggests that many bacterial taxa are more adapted to complex C compounds than previously thought. Finally, our study underscores the limits of the copiotroph–oligotroph model at the phylum level and the necessity to work at a finer taxonomic resolution.

Keywords

Soil, pyrosequencing, decomposition, succession dynamics, microbial community structure.

In recent decades, the traditional view regarding the shift from bacteria to fungi as decomposition progresses has been challenged by several studies (Rousk and Frey, 2015; Purahong et al., 2016). At the same time, the ecological attributes of both bacterial and fungal decomposers have been increasingly used in studies to better assess changes in carbon- (C) and nutrient-use efficiencies during litter decomposition: early decomposers (often assimilated to copiotrophs) have high nutritional requirements and preferentially consume rich and soluble substrates, whereas late-decomposers (often assimilated to oligotrophs) exhibit low growth rates and consume poor and complex C compounds (Fanin & Bertrand, 2016; Ho et al., 2017). Most substrates used to assess the validity of the copiotroph–oligotroph model are synthetic (*e.g.*, glucose and simple carbohydrates) or relatively labile (*e.g.*, leaf litter) (Goldfarb et al., 2011; Purahong et al., 2016; Tláskal et al., 2016). However, whether the addition of complex substrates naturally found across various ecosystems (as roots enriched in structural C compounds) has similar effects on the microorganisms' assemblages (copiotrophs vs. oligotrophs) during microbial succession is still an open question. To assess the importance of substrate quality on the differentiation of both bacterial and fungal communities in terms of copiotrophs/oligotrophs successions, we performed a controlled microcosm incubation experiment with two litters varying strongly in their physicochemical characteristics (maize leaves and roots), an unamended control (without litter) and followed microbial community structure by pyrosequencing at four sampling dates (0, 15, 35 and 91 days). We hypothesized that (i) microbial communities will undergo the copiotrophs/oligotrophs successions for both litters, and (ii) within these successions, copiotrophic and oligotrophic taxa will be more favored by the amendment with maize leaves and roots, respectively.

63 The soil used for the experiment was a silty loam agricultural soil collected at
64 an experimental research station (Estrees-Mons, northern France). The soil
65 contained 8.70 mg C g^{-1} soil and a mineral N content high enough to prevent N
66 limitation for the decomposition of both litters (Sauvadet et al., 2016). Microcosms
67 were built with soil sampled at a depth of 0-20 cm, with either no litter or 1 cm pieces
68 of maize leaves or roots that were mixed into the soil at a depth of 0-5 cm and a rate
69 of 4.8 g C kg^{-1} soil mimicking a common crop residue quantity (about 7 t ha^{-1}) after
70 the growing season (e.g. Hiel et al., 2018). The microcosms were incubated at 15°C
71 for 3 months, after which they were destructively sampled at four successive dates:
72 0, 15, 35 and 91 days later (three replicates by treatment and sampling date). Carbon
73 mineralization was measured in all the incubated microcosms using a CO_2 trap (1 M
74 NaOH) titrated by continuous flow colorimetry using an autoanalyzer (TRAACS 2000,
75 Bran and Luebbe). The litter was hand-sorted then washed in water and dried for one
76 week at 37°C before soluble, cellulose, hemicellulose and the Klason lignin contents
77 were measured (Sauvadet et al., 2016). The soil bacterial and fungal community
78 structures were determined on soil alone (after leaves and roots were separated) at
79 each sampling date by 454 pyrosequencing using 16S rRNA and 18S rRNA genes,
80 respectively, as described by Terrat et al. (2012). Diversity analyses were performed
81 on 5000 and 7500 reads for the 16S and 18S rRNA gene sequences of the bacterial
82 and fungal communities, respectively, using the GnS-PIPE pipeline developed for the
83 GenoSol platform (Institut National de la Recherche Agronomique [INRA], Dijon,
84 France).
85 The microbial communities were then analyzed using the Bioconductor suite
86 package and R software (https://www.bioconductor.org). Dissimilarities between the
87 bacterial and fungal communities of each treatment at each sampling date were first

88 calculated using the Poisson distance at the genus level (after exclusion of genera
89 who were unique across all samples), and then depicted graphically with nonmetric
90 dimensional scaling (NMDS). Significant differences in Poisson distances between
91 amended and unamended treatments were tested by analysis of variance (ANOVA)
92 followed by post hoc tests using Fisher's least significant difference (LSD). In a
93 second phase, we focused on the genera that were the most affected by litter
94 amendments. To do so, the data were log-transformed (rlog function of the DESeq2
95 R package) to obtain normality and minimize the differences between taxa with low
96 abundances. We then performed a differential expression analysis (DESeq function
97 of the DESeq2 R package) between soil communities that did not receive litter
98 (unamended soil) and soil communities that did receive either leaves or roots at the
99 corresponding sampling date. In this analysis, we considered only the genera that
100 differed significantly in relative abundance from the community in unamended soils
101 (P-value < 0.05 adjusted with Benjamini-Hochberg corrections), which could be
102 distinguished as either enriched (greater abundance than that in the unamended
103 community) or depleted (lower abundance than that in the unamended community).
104 More details of the DESeq analyses were reported by Edwards et al. (2015).
105 The leaf and root additions influenced the structure of both the bacterial and
106 fungal communities at all sampling dates relatively to the unamended soils (Fig. 1a
107 and 1b). Poisson distances between unamended and amended treatments were
108 higher with leaves than roots addition for fungal communities at the earliest stage of
109 decomposition (i.e., before 400 mg C kg^{-1} soil mineralized, Fig. 1d) and for bacterial
110 communities at all sampling dates (Fig. 1c). These results highlight that the structure
111 of bacterial and early-decomposers fungal communities depends directly on
112 substrate quality (Table S1), and confirm previous reports demonstrating that many

bacterial and fungal taxa are strongly dependent upon C availability (van der Wal et al., 2013; Purahong et al., 2016; Ho et al., 2017). This further suggests that taxa feeding on recalcitrant C substrates are closer to communities living in unamended soils than opportunistic taxa feeding on freshly added labile C substrates. On the other hand, fungal community differentiation peak after 15 days of leaf decomposition, suggesting that these early-decomposers (presenting copiotrophic characteristics) could only develop when litter soluble content was high enough (Table S1). Interestingly, only fungal community presented such a peak; we could assume that bacterial differentiation occurred earlier (between 3 and 7 days in Tardy et al., 2015), yet our results support that fungi, and not only bacteria, takes an active part in early decomposition process. Further, the constant differentiation level of amended bacterial communities from their unamended counterpart supports the emerging idea that bacterial taxa can be better able to increase with the proportion of complex C compounds than previously thought (Rousk and Bååth, 2007; Rousk and Frey, 2015). However, as expected, high level of soluble C favor the differentiation of bacterial taxa which may also feed on products originating from fungal degradation (Purahong et al., 2016; Tláskal et al., 2016).

When studying microbial community composition, we found that only 6 out of 59 genera (e.g., *Arthrobacter* or *Variovorax* for bacterial community, Table S2) and 11 out of 57 genera (e.g., *Ascobulus* or *Heterobasidion* for fungal community, Table S3) were impacted by both litter types (Fig. 2).The idea that many microbial genera depend upon substrate quality is reinforced by the low redundancy between microbial genera at different sampling dates (Fig. 2); only a few bacterial genera remained enriched at all sampling dates (e.g., *Pseudomonas* during both leaves and roots decomposition, Table S2) and no fungal genus was consistently enriched or depleted

over time (Table S3). These results highlight the strong specificity of microbial genera to both litter quality and decomposition stage, which is partly overlooked when focusing only at the phylum level. Indeed, the bacterial genera impacted during litter decomposition belonged mainly to the three same phyla (i.e., Proteobacteria, Bacteroidetes and Actinobacteria), which are often regarded as copiotrophic in the literature (Ho et al., 2017). However, most phyla as Proteobacteria, had both enriched (e.g., *Variovorax*) and depleted genera (e.g., *Geobacter*) after litter additions, and this regardless of litter quality or sampling date (Table S2). This clearly indicates that different taxa within a phylum may present contrasting metabolic status. This further underscores the limits of the copiotroph–oligotroph model at the phylum level and the necessity to work at a finer taxonomic resolution (Philippot et al., 2010; Purahong et al., 2016; Ho et al., 2017).

We conclude that high quality substrate induces greater differentiation of early decomposers fungal communities and bacterial communities than low quality substrate. Further, both early- and late-decomposers showed a strong specificity to litter quality and decomposition stage at the genus level. Our results challenge the traditional concept of bacterial/fungal shift during decomposition and underline the necessity to identify more accurately life strategies of soil microbes at different taxonomic levels in order to improve our understanding of structure-function relationships and the role of microbial communities in ecosystem functions such as C mineralization and decomposition rates.

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Figure captions

Fig. 1. Microbial community structure during the experiment represented by NMDS plots (a, b) and Poisson distances from the unamended community (c, d) using bacterial (a, c) or fungal (b, d) data. All figures are based on the Poisson distance calculated at the genus level. Abscissa axis of Fig. 1c and 1d are expressed as the cumulated amount of C mineralized, presented in Table S1. Significant differences between all sampling dates and litters were tested by ANOVA followed by post hoc Fisher tests and are represented by different letters for each graph.

Fig. 2. Phylum identification of the enriched and depleted genera with maize leaf or root addition relative to the unamended soils for bacteria (a) and fungi (b). Abundance changes are expressed as log_2fold (i.e., log_2[sample] - log_2[unamended community]). Only the genera which exhibit significant differences with the unamended community are considered (P-values < 0.05).

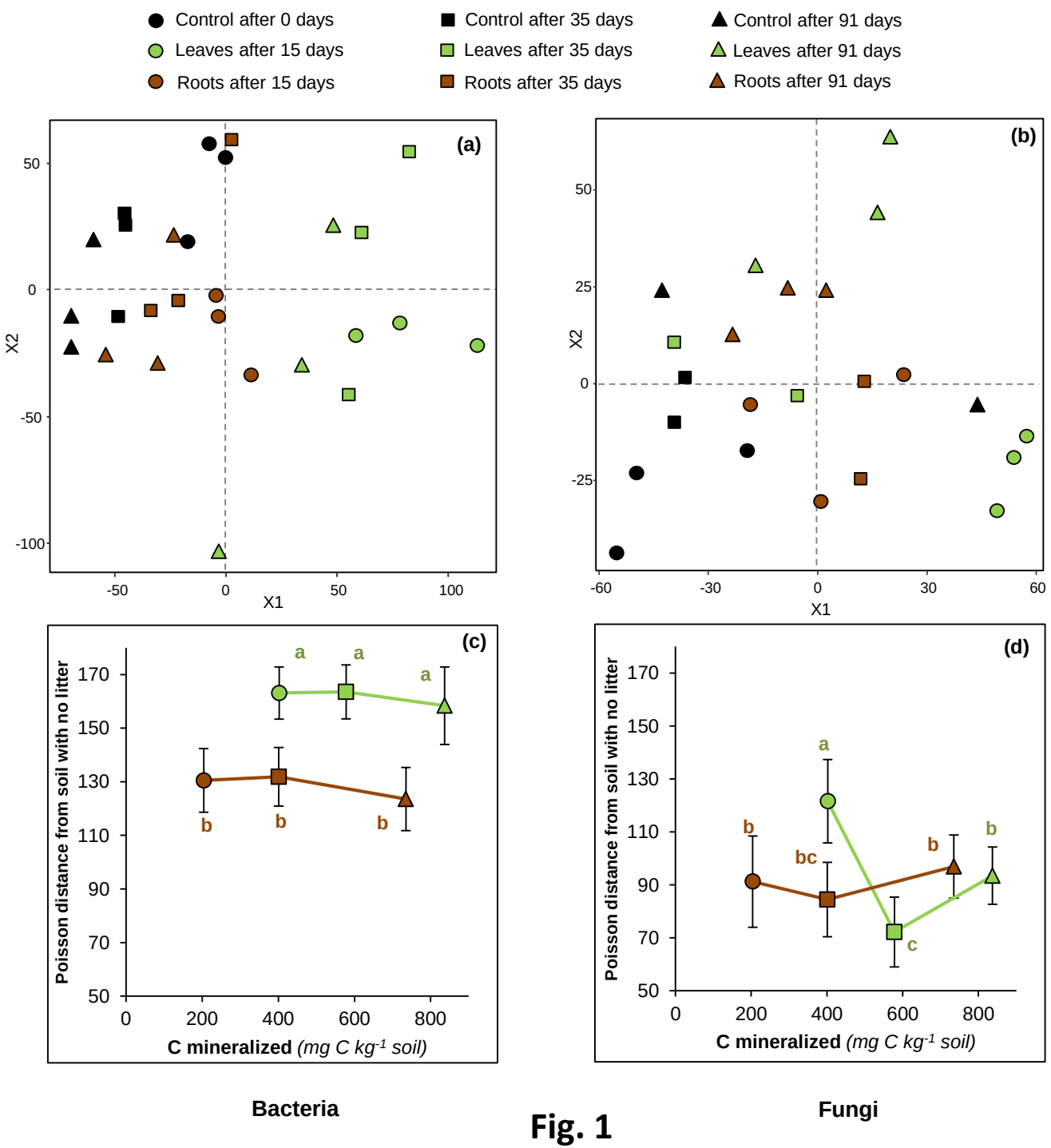
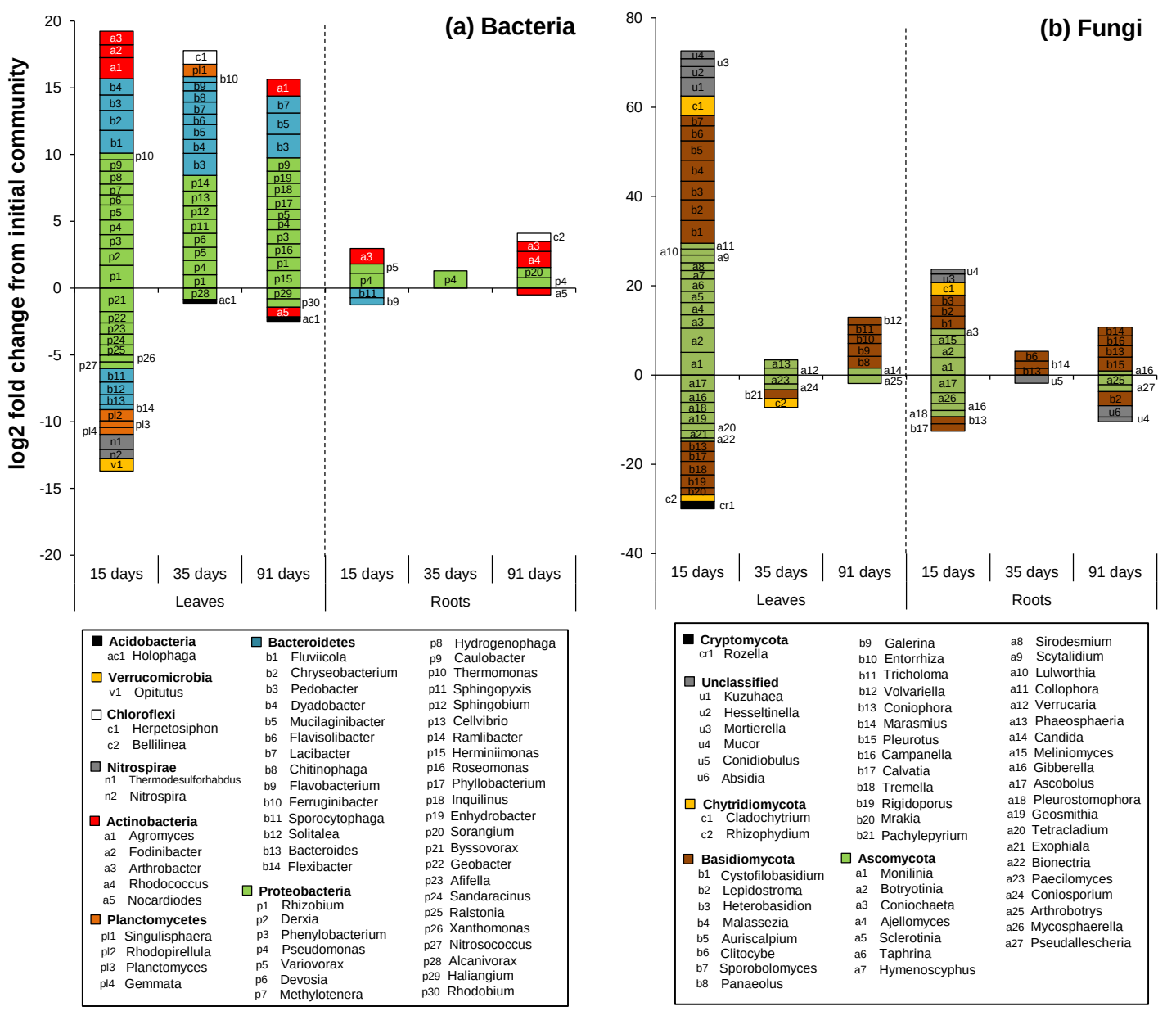

Control after 0 days
Leaves after 15 days
Roots after 15 days
Control after 35 days
Leaves after 35 days
Roots after 35 days
Control after 91 days
Leaves after 91 days
Roots after 91 days
(a)
(b)
(c)
(d)
X1
X2
Poisson distance from soil with no litter
C mineralized (mg C kg-1 soil)
Bacteria
Fungi

Fig. 1



Acidobacteria
ac1 Holophaga

Verrucomicrobia
v1 Opitutus

Chloroflexi
c1 Herpetosiphon
c2 Bellilinea

Nitrospirae
n1 Thermodesulforhabdus
n2 Nitrospira

Actinobacteria
a1 Agromyces
a2 Fodinibacter
a3 Arthrobacter
a4 Rhodococcus
a5 Nocardioides

Planctomycetes
pl1 Singulisphaera
pl2 Rhodopirellula
pl3 Planctomyces
pl4 Gemmata

Bacteroidetes
b1 Fluviicola
b2 Chryseobacterium
b3 Pedobacter
b4 Dyadobacter
b5 Mucilaginibacter
b6 Flavisolibacter
b7 Lacibacter
b8 Chitinophaga
b9 Flavobacterium
b10 Ferruginibacter
b11 Sporocytophaga
b12 Solitalea
b13 Bacteroides
b14 Flexibacter

Proteobacteria
p1 Rhizobium
p2 Derxia
p3 Phenylobacterium
p4 Pseudomonas
p5 Variovorax
p6 Devosia
p7 Methylotenera
p8 Hydrogenophaga
p9 Caulobacter
p10 Thermomonas
p11 Sphingopyxis
p12 Sphingobium
p13 Cellvibrio
p14 Ramlibacter
p15 Herminiimonas
p16 Roseomonas
p17 Phyllobacterium
p18 Inquilinus
p19 Enhydrobacter
p20 Sorangium
p21 Byssovorax
p22 Geobacter
p23 Afifella
p24 Sandaracinus
p25 Ralstonia
p26 Xanthomonas
p27 Nitrosococcus
p28 Alcanivorax
p29 Haliangium
p30 Rhodobium

Cryptomycota
cr1 Rozella

Unclassified
u1 Kuzuhaea
u2 Hesseltinella
u3 Mortierella
u4 Mucor
u5 Conidiobulus
u6 Absidia

Chytridiomycota
c1 Cladochytrium
c2 Rhizophydium

Basidiomycota
b1 Cystofilobasidium
b2 Lepidostroma
b3 Heterobasidion
b4 Malassezia
b5 Auriscalpium
b6 Clitocybe
b7 Sporobolomyces
b8 Panaeolus
b9 Galerina
b10 Entorrhiza
b11 Tricholoma
b12 Volvariella
b13 Coniophora
b14 Marasmius
b15 Pleurotus
b16 Campanella
b17 Calvatia
b18 Tremella
b19 Rigidoporus
b20 Mrakia
b21 Pachylepyrium

Ascomycota
a1 Monilinia
a2 Botryotinia
a3 Coniochaeta
a4 Ajellomyces
a5 Sclerotinia
a6 Taphrina
a7 Hymenoscyphus
a8 Sirodesmium
a9 Scytalidium
a10 Lulworthia
a11 Collophora
a12 Verrucaria
a13 Phaeosphaeria
a14 Candida
a15 Meliniomyces
a16 Gibberella
a17 Ascobolus
a18 Pleurostomophora
a19 Geosmithia
a20 Tetracladium
a21 Exophiala
a22 Bionectria
a23 Paecilomyces
a24 Coniosporium
a25 Arthrobotrys
a26 Mycosphaerella
a27 Pseudallescheria

Fig. 2