
Coprophilous fungi

Closing the loop: improving circularity with manure-loving mushrooms

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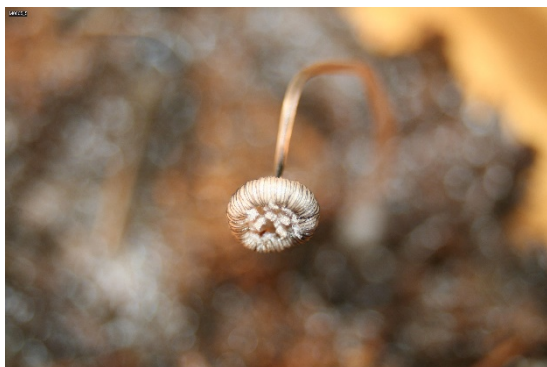
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Within project KB-40-005-008; Closing the loop: improving circularity with manure-loving mushrooms), part of the Investment theme Connected circularity, we have been provided with the opportunity to work on a this topic.

A literature study was performed on the options that coprophilic fungi offer. It focused on the taxonomic and ecological knowledge of coprophilous mushrooms present in the Netherlands and on the threats of fungal diversity on dung. Next to this the literature study focusses on the options that coprophilous fungi offer as a source of secondary metabolites or enzymes. Furthermore it briefly focusses on an overview of genomes available of coprophilous fungi. The literature study is finalized with a brief outlook towards possibilities of using coprophilous mushrooms in a circular agriculture system.

In the second part of the project we able to build a collection of coprophilic basidiomycete strains comprising of 38 strains distributed over at least 23 species. Limited tests of their ability to grow on a small range of types of manure demonstrated growth of 23 strains on chicken manure (ranging from limited growth to abundant growth). A total of 19 strains showed growth on cow manure (again ranging from limited growth to abundant growth). Pig manure was least favorite in our experiments, with only 4 strains showing growth with different abundances.

We believe that this project will provide a starting point for a study of applicability of coprophilic fungi in circular agriculture..

Keywords: coprophilic fungi, coprophilous fungi, manure, dung fungi, manure mushrooms.

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Photo cover: a small mushroom of a coprophilic mushroom species

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Summary

In nature manure is recycled by unique fungi (coprophilic fungi), which are capable of growth on substrates with high nitrogen contents. They bind a lot of the nutrients and in a delayed release they are making these nutrients available for plants, animals and insects, thereby closing nutrient cycles. This may provide opportunities for processing of manure. Within project KB-40-005-008; Closing the loop: improving circularity with manure-loving mushrooms), part of the Investment theme Connected circularity, we have been provided with the opportunity to work on a this topic.

A literature study was performed on the options that coprophilic fungi offer. It focused on the taxonomic and ecological knowledge of coprophilous mushrooms present in the Netherlands and on the threats of fungal diversity on dung. Next to this the literature study focusses on the options that coprophilous fungi offer as a source of secondary metabolites or enzymes. Furthermore it briefly focusses on an overview of genomes available of coprophilous fungi. The literature study is finalized with a brief outlook towards possibilities of using coprophilous mushrooms in a circular agriculture system. In the second part of the project we able to build a collection of coprophilic basidiomycete strains comprising of 38 strains distributed over at least 23 species. Limited tests of their ability to grow on a small range of types of manure demonstrated growth of 23 strains on chicken manure (ranging from limited growth to abundant growth). A total of 19 strains showed growth on cow manure (again ranging from limited growth to abundant growth). Pig manure was least favorite in our experiments, with only 4 strains showing growth with different abundances.

We believe that this project will provide a starting point for a study of applicability of coprophilic fungi in circular agriculture.

1 Introduction

Animal dung is a special substrate for fungi. Fungi growing thereon have been called coprophilous (or sometimes coprophilic). The term is derived from two Greek terms, viz. copros = dung; and philous = having a love for, preferring. (The word fimicolous, to denote the same habitat preference is derived from the Latin fimus or fimum = dung, and cola – inhabiting, however coprophilous is the term most often used in the scientific literature, and this usage is also followed in this essay.) Some fungi occur exclusively on dung, whereas other species occupy broader niches, also occurring on certain forms of organic matter. Most coprophilous fungi are found on dung of herbivores, both wild herbivores and domesticated herbivores like cattle, horses and sheep. Rabbit dung is also rich in coprophilous fungi; as it constitutes a tractable substrate for experimental studies, it has frequently been studied. Rabbit dung can be easily converted into so-called copromes, standardised dung pellets created through collecting, drying, powdering, sterilising and reconstituting these to pellets (Wood & Cooke, 1984). It needs to be assessed whether a similar technique will also be beneficial when studying fungi on dung of larger domesticated herbivores. While copromes have been mainly used in the study of fungal succession on dung, their use could also be beneficial for a range of other questions, e.g. the role that interference competition plays in the upregulation of the production of antimicrobial compounds (Bills *et al.*, 2013), or the role that species mixtures play in enhancing or reducing dung degradation rates.

Compared to the dung of herbivores, pig dung is not known to be rich in coprophilous fungi. Dung of carnivores and dung of birds is generally also (very) poor in these fungi. The most likely explanation is that dung of these organisms contains mostly easily degradable compounds and low amounts of lignin, as a consequence of which coprophilous fungi (and especially coprophilous Basidiomycota) are either outcompeted or do not have sufficient time to complete their life cycle before the dung pellet is degraded. Coprophilous fungi have generally been linked to dung of endothermic (warm-blooded) animals. In its natural habitat animal dung is usually found as smaller to larger individual resources, but due to animal husbandry dung may be collected and, mixed with plant residues, be piled as manure heaps. Both classes of substrates (dung pellets, manure heaps) partly select for different fungi. Manure heaps are likely to heat during the composting process, resulting in a strong selection for a small number of thermophilic or thermotolerant fungi. Dung of different animal species usually harbours its own fungal community. Such differences arise from the different food items that the animals have been consuming (Kruys & Ericson, 2008), from differential selection during gut passage, and from the properties of the dung when excreted. Important recent publications dealing with coprophilous fungi are Krug *et al.* (2004) and Doveri (2004).

Many coprophilous species germinate only after passage through the animal gut. Coprophilous fungi therefore have adaptations that maintain their viability in such hostile environments. Many species have thick and dark walls, while the spores of some species are covered by a gelatinous sheath. After gut passage and deposition of dung, coprophilous fungi develop and form spore-bearing organs, called fruitbodies. From these fruitbodies spores are actively discharged. They often land on nearby vegetation (and the gelatinous sheath likely facilitates attachment), which then increases the chances that the spores are consumed with the vegetation. However, not all species that occur on dung have a life cycle that is dependent on passage through the animal gut. Several grassland fungi also occur on dung and separating true coprophilous fungi from these subcoprophilous fungi (Griffith & Roderick, 2008) is not always easy.

Coprophilous fungi are most common in grasslands (Griffiths & Roderick, 2008), however they can occur in every habitat where large and smaller herbivores defecate, including dunes, heathlands and forests. Several coprophilous fungi have also been reported to have the ability to live as plant endophytes (Herrera *et al.* 2011; Newcombe *et al.* 2016). Occurrence of coprophilous fungi from surface-sterilised plant tissue had been reported before, however it had remained unclear whether these fungi were incidental contaminants that were not killed by alcohol or bleach (which may even have enhanced spore germination, just like gut passage might achieve) or whether these fungi were true endophytes. Newcombe *et al.* (2016) provided evidence for an endophytic life style of *Sordaria fimicola* and also for negative fitness effects on the growth of the grass *Bromus tectorum*. However, Griffith *et al.* (2017) reported that the same fungus was more common on the same grass species under more drought-prone environments, suggesting a possible role of the endophyte in drought tolerance. It has also been reported that *Sordaria fimicola* can reduce symptoms of the cereal disease take-all in rye and wheat (Dewan *et al.*, 1994). The endophytic life style, which likely causes higher fungal selectivity towards certain plant species, could also be an explanation for the positive correlation between the number of plant species foraged by herbivores and the number of coprophilous species found on the dung of these herbivores. Endophytic occurrence has also been reported for subcoprophilous fungi such as *Psilocybe semilanceata* (Keay & Brown, 1990).

Dung is often characterised by its high amounts of nitrogen and also phosphorus; from a stoichiometric perspective dung has a low N:P ratio, much lower than is needed from the perspective of fungal demand (and plant demand as well). There are only few studies that have linked the occurrence of coprophilous fungi to dung C:N ratio. Richardson (2001) listed C:N ratios of dung of five mammal species (sheep, deer, cattle, rabbit and hare) ranging between 20 and 30. It is likely that these dung samples came from animals that were fed with relatively nitrogen-poor and lignin-rich plant material. Cattle that is fed a more nitrogen-rich diet has lower C:N ratios, often ranging 10-15, whereas pig manure has even lower C:N ratios, up to 6. Even though dung is enriched in P,

focus has been on N-content in dung rather than on P-content as an explanation for the fungal specificity or selectivity for dung.

Because of its high content of mineral nutrients, often accompanied by easily degradable carbon compounds, dung is a habitat with intense competition between fungi and bacteria, and between different species of fungi. As a consequence of the saprotrophic life style the dung is degraded – although there do not seem many studies that have assessed the decomposition process of various types of dung (Nagy & Harrower, 1980) and the enzymes that are responsible for the degradation of cellulose and lignin, as some of these could have biotechnological application (see below). After dung has been deposited a succession of fungal species has been observed (Richardson, 2001; Richardson, 2002).

Succession has most often been studied on the basis of the appearance of reproductive structures. The first fungi to appear are members of the Mucoromycota (*Pilaira*, *Pilobolus* – Fig. 1). Species of *Pilobolus* take somewhat more time than *Pilaira*, on average 6.5 compared to 3.5 days, before their fruitbodies are visible (Richardson, 2002).

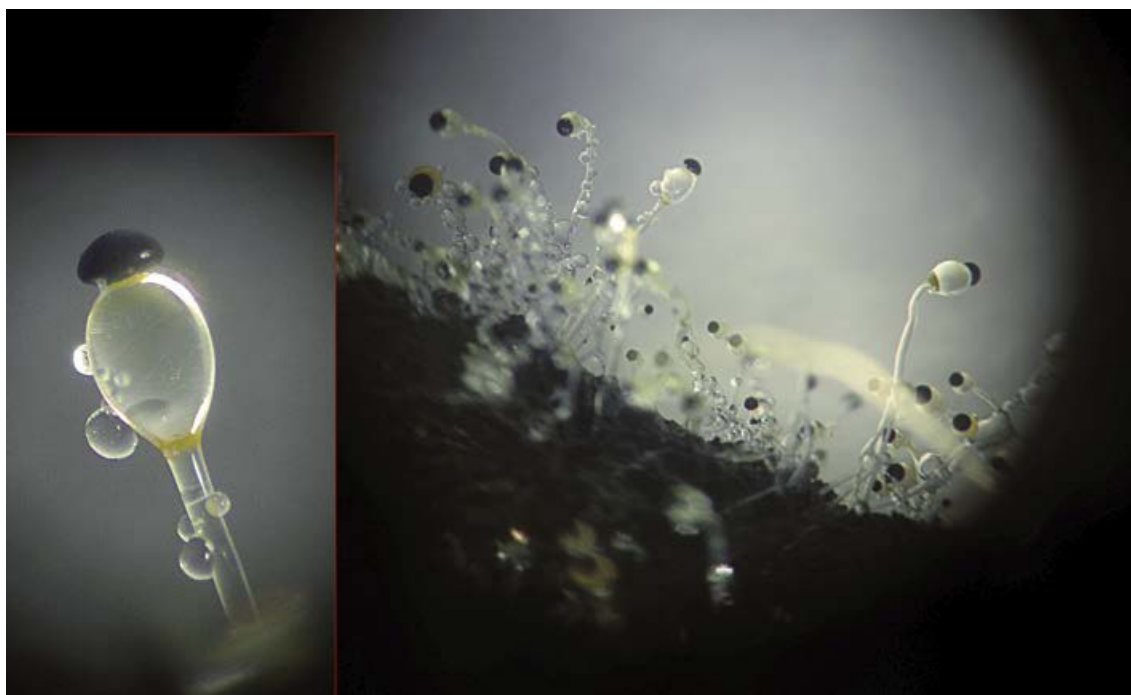


Figure 1. *Pilobolus* spp. From Dam & Kuyper (2016)

These Mucoromycota are followed by members of the Ascomycota and finally members of the Basidiomycota. However, there is substantial overlap in time of first appearance of Ascomycota and Basidiomycota. Various theories have been forwarded to explain this successional process. Some theories focus on the time needed to form reproductive structures, which is related to the growth rate in the mycelial stage, and the size of the reproductive structures, where species with smaller reproductive structures appear first. This size-related successional pattern can be more easily studied on large dung pellets (produced by cattle or horses) than on rabbit pellets. Other theories focus on the use of the various carbon compounds of the substrate, with Mucoromycota first depleting the simple carbohydrates (sugars), followed by the Ascomycota that utilise cellulose and

hemicelluloses, and in the final stage Basidiomycota with ligninolytic activity, even though lignin cannot be used by fungi as the sole carbon source. However, it has recently been shown that ligninolytic activity is not restricted to Basidiomycota (see below). The size-related successional theory is physiological rather than ecological and is consistent with the lack of interaction between fungal species – even under experimental conditions when one species is inoculated on a sterilised coprome, the time needed for formation of reproductive structures would remain unchanged. The nutritional theory on the other hand implies resource competition. In its most simple form there is exploitation competition where more rapidly growing organisms deplete simple substrates and leave substrates that are somewhat more difficult to degrade (and that are degraded with a lower carbon use efficiency) for species that grow more slowly and appear later in succession. Next to exploitation competition interference competition is very important, where species produce substances with a strong antagonistic effect on other species (Sarrocco, 2016). Interference competition can occur at a distance when diffusible secondary metabolites are excreted. Interference can also be a direct process upon hyphal contact. Direct hyphal interference was demonstrated for the fungus *Coprinellus heptemerus* (Basidiomycota) against the species *Pilaira anomala* (Mucoromycota) and *Ascobolus crenulatus* (Ascomycota) resulting in lysis and subsequent mortality of the mycelium of the sensitive species (Harper & Webster, 1964; Ikediugwu & Webster, 1970a). In a subsequent investigation, Ikediugwu & Webster (1970b) noted that hyphal interference was widespread in a much larger range of coprophilous Basidiomycota, including *Clitopilus pinsitus* (possibly *C. passeckerianus?*), *Bolbitius titubans*, *Panaeolus papilionaceus* and *Stropharia semiglobata*. Coprophilous fungi produce antibacterial, antifungal and antihelminthic compounds. In general the ability to produce toxic antimicrobial substances is more strongly developed among the fungal species with lower growth rates. Some of those metabolites are produced constitutively (e.g., copsisin) while other metabolites (e.g. lagopodin; Stöckli *et al.*, 2019) are induced. Production of metabolites that are inducible could allow the fungus to save resources in the absence of competitors; hence testing for such compounds necessitates the use of experimental systems where two or more species are inoculated simultaneously. Below we will discuss this issue and its potential applications more extensively.

In this essay, written as part of the deliverables in the framework of the wild card “Closing the loop: improving circularity with manure-loving mushrooms”, we will deal with the following topics:

1. Taxonomic and ecological knowledge of coprophilous mushrooms in the Netherlands
2. Threats of fungal diversity on dung
3. Using coprophilous fungi for secondary metabolites
4. Using coprophilous fungi as a source of enzymes
5. Mining genomes of coprophilous fungi
6. Outlook: towards a circular agriculture and the role of coprophilous mushrooms

2 Taxonomic and ecological knowledge of coprophilous mushrooms in the Netherlands

Currently around 95 species of Basidiomycota have been reported from dung and dung heaps in the Netherlands (Table 1). These species grow on solid, straw-rich (and hence lignin-rich) dung. Thin animal slurries are unsuitable as a substrate for coprophilous Basidiomycota, and only a few members of the Ascomycota, such as *Cheilymenia granulata* (Fig. 2) is found on somewhat less solid cattle manure in conventionally managed grassland.



Figure 2. *Cheilymenia granulata*, a species that can occur on cattle manure in conventionally managed grasslands. From Dam & Kuyper (2016).

Almost all species of the Basidiomycota belong to the Agaricales, the mushroom group with a cap, stipe and gills (the true toadstools). Many species belong to the families of the Psathyrellaceae and Strophariaceae, both fungal families characterised by coloured, thick-walled spores. Many of these species form small fruitbodies, but a few form large conspicuous fruitbodies such as *Panaeolus semiovatus* and *Stropharia semiglobata* (Figs 3-4).

Much more common are Ascomycota with at least 170 species in the Netherlands. While some of these are fairly large and conspicuous, many other species are small to very small, not easily visible with the naked eye, taxonomically complex and therefore often underreported. This essay will therefore have a focus on the coprophilous Basidiomycota, however the section on antibiotics and enzymes will also include some information on Ascomycota.



Figure 3. *Panaeolus semiovatus*. From Dam & Kuyper (2013).



Figure 4. *Stropharia semiglobata*. From Dam & Kuyper (2013)

The number of coprophilous Mucoromycota on dung in the Netherlands is hardly known, apart from conspicuous species of the genus *Pilobolus*. Coprophilous Mucoromycota are not regularly studied by field mycologists.

Table 1. List of coprophilous Basidiomycota. Nomenclature follows Arnolds & Van den Berg (2013), a somewhat conservative nomenclature compared to names on Mycobank and Index Fungorum. The table contains the scientific name, the Dutch name, a specification of the substrate (Su) and the animal species (Or) that produced the dung, and an indication of its occurrence in the Netherlands (Fr), also taken from Arnolds & Van den Berg (2013).

With respect to Su (substrate code): 1.6 – the species grows on compost heaps, including heaps of composted dung; 7.0 – species grows on dung; 7.3 – species grows on dung; 7.4 – species grows on straw-rich manure heaps. With respect to Or (organism code): 9.0 – Vertebrates (unspecified); 9.2- rabbit and hare; 9.3 – red deer and roe deer; 9.4 – cattle; 9.6- horse; 9.7 – sheep. (Other Or codes are relevant for certain Ascomycota on dung, but are not further treated here).

With respect to commonness: 1 refers to extremely rare species, 9 to very common species. Species with 0 have not been reported from the Netherlands since 2000.

Scientific name	Dutch name	Su	Or	Fr
<i>Agaricus bisporus</i>	Gekweekte champignon	1.6		6
<i>Agaricus subrufescens</i>	Bladhoopchampignon	1.6		2
<i>Agrocybe pediades</i> var. <i>fimicola</i>	Grasleemhoed	7.3	9.0	0
<i>Bolbitius coprophilus</i>	Roze kleeelhoedje	1.6		1
<i>Bolbitius demangei</i>	Kaskleeelhoedje	1.6		0
<i>Bolbitius titubans</i> var. <i>olivaceus</i>	Verkleurend kleeelhoedje	1.6		3
<i>Clitopilus fasciculatus</i>	Toefige molenaar	1.6		0
<i>Clitopilus passeckerianus</i>	Kattenoorzwam	7.4	9.6	1
<i>Conocybe brunneidisca</i>	Paardenvijgbreeksteeltje	7.3	9.6	4
<i>Conocybe daamsii</i>	Kasbreeksteeltje	1.6		0
<i>Conocybe farinacea</i>	Melig breeksteeltje	7.4	9.6	3
<i>Conocybe fimetaria</i>	Wortelend mestbreeksteeltje	7.3	9.0	3
<i>Conocybe fuscimarginata</i>	Compostbreeksteeltje	1.6		4
<i>Conocybe hornana</i>	Beursbreeksteeltje	1.6		1
<i>Conocybe magnispora</i>	Okergeel mestbreeksteeltje	7.3	9.4	2
<i>Conocybe microrrhiza</i> var. <i>microrrhiza</i>	Spitsvoetbreeksteeltje (var. microrrhiza)	1.6		0
<i>Conocybe pubescens</i>	Donzig breeksteeltje	7.3	9.6	6
<i>Conocybe siliginea</i> f. <i>siliginea</i>	Vaal breeksteeltje	7.3	9.0	7
<i>Conocybe siliginea</i> f. <i>rickenii</i>	Bleek breeksteeltje	7.3	9.0	6
<i>Conocybe singeriana</i>	Knolvoetbreeksteeltje	7.3	9.0	1
<i>Conocybe watlingii</i>	Grootsporig mestbreeksteeltje	7.3	9.0	1
<i>Coprinellus bisporus</i>	Tweesporige donsinktzwam	7.3	9.0	4
<i>Coprinellus brevisetulosus</i>	Tengere mestinktzwam	7.3	9.4	4
<i>Coprinellus congregatus</i>	Gezellige donsinktzwam	7.4	9.0	3
<i>Coprinellus curtus</i>	Paardenmestdonsinktzwam	7.3	9.0	3
<i>Coprinellus ephemerus</i>	Vluchtige inktzwam	7.4	9.0	3
<i>Coprinellus heptemerus</i>	Bruine mestinktzwam	7.3	9.0	5
<i>Coprinellus heterosetulosus</i>	Donker mestdwerdje	7.3	9.6	5
<i>Coprinellus marculentus</i>	Hoekigsporige donsinktzwam	7.4	9.0	3
<i>Coprinellus pellucidus</i>	Bleek mestdwerdje	7.3	9.4	5
<i>Coprinellus sassii</i>	Grootsporige donsinktzwam	7.3	9.6	0

Table 1 (continued). List of coprophilous Basidiomycota. Nomenclature follows Arnolds & Van den Berg (2013), a somewhat conservative nomenclature compared to names on Mycobank and Index Fungorum. The table contains the scientific name, the Dutch name, a specification of the substrate (Su) and the animal species (Or) that produced the dung, and an indication of its occurrence in the Netherlands (Fr), also taken from Arnolds & Van den Berg (2013).

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With respect to commonness: 1 refers to extremely rare species, 9 to very common species. Species with 0 have not been reported from the Netherlands since 2000.

Scientific name	Dutch name	Su	Or	Fr
<i>Coprinopsis bicornis</i>	Tweesporig mesthazenpootje	7.3	9.4	1
<i>Coprinopsis candidolanata</i>	Wit hazenpootje	7.3		1
<i>Coprinopsis cardiaspora</i>	Hartjesinktzam	7.3	9.0	3
<i>Coprinopsis cinerea</i>	Wortelende inktzwam	7.4	9.0	6
<i>Coprinopsis cordispora</i>	Korrelige mestinktzam	7.3	9.0	5
<i>Coprinopsis cothurnata</i>	Melige mestinktzam	7.4	9.0	4
<i>Coprinopsis ephemeroides</i>	Geringde korrelinktzam	7.3	9.4	4
<i>Coprinopsis filamentifer</i>	Hoeksporige inktzwam	7.3	9.7	1
<i>Coprinopsis foetidella</i>	Stinkende mestinktzam	7.3	9.6	2
<i>Coprinopsis macrocephala</i>	Stromesthazenpootje	7.4	9.0	4
<i>Coprinopsis narcotica</i>	Bedwelvende inktzwam	1.6		4
<i>Coprinopsis nivea</i>	Witte mestinktzam	7.3	9.6	6
<i>Coprinopsis pachysperma</i>	Vale mestinktzam	7.3	9.4	2
<i>Coprinopsis parvula</i>	Kleine inktzwam	7.0	9.0	1
<i>Coprinopsis patouillardii</i>	Korrelige inktzwam	1.6		5
<i>Coprinopsis poliomalla</i>	Grijs mestdwerge	7.3	9.4	4
<i>Coprinopsis pseudocortinata</i>	Wit mestdwerge	7.3	9.0	2
<i>Coprinopsis pseudonivea</i>	Valse witte mestinktzam	7.3	9.4	3
<i>Coprinopsis pseudoradiata</i>	Klein mesthazenpootje	7.3	9.0	2
<i>Coprinopsis radiata</i>	Pelsinktzam	7.3	9.6	4
<i>Coprinopsis radicans</i>	Grootsporige stinkinktzam	7.3	9.0	2
<i>Coprinopsis sclerotiorum</i>	Knolletjesspechtinktzam	7.3	9.4	1
<i>Coprinopsis scobicola</i>	Kashazenpootje	1.6		0
<i>Coprinopsis stercorea</i>	Kleine korrelinktzam	7.3	9.4	6
<i>Coprinopsis trispora</i>	Driesporige inktzwam	7.3	9.0	2
<i>Coprinopsis tuberosa</i>	Grijze korrelinktzam	7.4	9.0	4
<i>Coprinopsis utrifer</i>	Poederinktzam	7.3	9.0	0
<i>Coprinopsis vermiculifer</i>	Schapenmestinktzam	7.3	9.7	1
<i>Coprinopsis xenobia</i>	Withaarinktzam	7.3	9.4	1
<i>Coprinus sterquilinus</i>	Geringde inktzwam	7.3	9.6	3
<i>Deconica coprophila</i>	Mestkaalkopje	7.3	9.0	6

Table 1 (continued). List of coprophilous Basidiomycota. Nomenclature follows Arnolds & Van den Berg (2013), a somewhat conservative nomenclature compared to names on Mycobank and Index Fungorum. The table contains the scientific name, the Dutch name, a specification of the substrate (Su) and the animal species (Or) that produced the dung, and an indication of its occurrence in the Netherlands (Fr), also taken from Arnolds & Van den Berg (2013).

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Scientific name	Dutch name	Su	Or	Fr
<i>Deconica merdaria</i>	Meststropharia	7.4	9.0	5
<i>Deconica merdicola</i>	Keutelkaalkopje	7.3	9.6	4
<i>Deconica moelleri</i>	Geringd mestkaalkopje	7.3	9.0	2
<i>Deconica subcoprophila</i>	Grootsporig mestkaalkopje	7.3	9.0	5
<i>Entoloma ollare</i>	Bloempotsatijnzwam	1.6		0
<i>Gymnopus luxurians</i>	Compostcollybia	1.6		4
<i>Leucoagaricus americanus</i>	Gebundelde champignonparasol	1.6		2
<i>Leucoagaricus meleagris</i>	Compostchampignonparasol	1.6		2
<i>Leucocoprinus cretaceus</i>	Krijtwitte plooiparasol	1.6		3
<i>Panaeolus antillarum</i>	Tropische vlekplaat	7.4	9.6	3
<i>Panaeolus cinctulus</i>	Gezoneerde vlekplaat	7.4	9.0	6
<i>Panaeolus papilionaceus</i>	Franjevlekplaat	7.3	9.0	7
<i>Panaeolus semiovatus</i>	Geringde vlekplaat	7.3	9.0	6
<i>Panaeolus subfirmus</i>	Grote vlekplaat	7.3	9.4	3
<i>Parasola megasperma</i>	Groot mestplooirokje	7.3	9.0	1
<i>Parasola misera</i>	Klein mestplooirokje	7.3	9.0	6
<i>Parasola schroeteri</i>	Mestplooirokje	7.3	9.0	5
<i>Pholiotina coprophila</i>	Mestbreeksteeltje	7.3	9.0	3
<i>Psathyrella berolinensis</i>	Harige mestfranjehoed	7.3	9.9	1
<i>Psathyrella granulosa</i>	Korrelige mestfranjehoed	7.3	9.0	1
<i>Psathyrella hirta</i>	Vlokkige mestfranjehoed	7.3	9.6	4
<i>Psathyrella saponacea</i>	Paardenmestfranjehoed	7.3	9.6	2
<i>Psathyrella sphaerocystis</i>	Bepoederde mestfranjehoed	7.3	9.4	0
<i>Psathyrella stercoraria</i>	Kleine mestfranjehoed	7.4	9.0	1
<i>Psathyrella waverenii</i>	Dwergmestfranjehoed	7.3	9.4	0
<i>Psilocybe fimetaria</i>	Blauwvoetkaalkopje	7.3	9.6	4
<i>Psilocybe liniformans</i>	Slijmrandkaalkopje	7.3	9.6	4
<i>Psilocybe puberula</i>	Harig kaalkopje	7.3	9.0	4
<i>Stropharia dorsipora</i>	Scheefporige stropharia	7.3	9.6	1
<i>Stropharia luteonitens</i>	Strogele stropharia	7.3	9.0	4
<i>Stropharia semiglobata</i>	Kleefsteelstropharia	7.3	9.0	7
<i>Volvariella volvacea</i>	Tropische beurszwam	1.6		1

3 Threats of fungal diversity on dung

It may seem surprising at first sight, but despite the fact that the Netherlands produce an overload of dung, the actual occurrence of coprophilous fungi has gone down (Arnolds & Veerkamp, 2008; Ozinga *et al.*, 2013). Table 2 indicates the coprophilous Basidiomycota that are currently listed in the Red Data list of rare and threatened fungi in the Netherlands. For the Red Data 67 species of coprophilous Ascomycota and Basidiomycota have been evaluated and 36 (54%) were put on the Red Data list, which is remarkable for a country in which discussions on manure excess are very frequent.

The main cause for decline of coprophilous fungi is the change in physical and chemical constitution and composition of dung. Animal manure is less solid and is often spread on the land as a slurry, and this yields a substrate that is not suitable for these fungi. Also animal feed has changed. Our grasslands are currently monocultures of rye grass (*Lolium perenne*), a species with easily degradable material, resulting in less fibrous dung structure. The use of concentrates as animal feed, currently an issue of a fierce societal debate, also reduces the quality of dung from the perspective of coprophilous fungi. And finally the increasing tendency to keep animals inside has reduced the amount of animal dung pellets in grasslands and thereby contributed to the decline of coprophilous fungi. Species of manure heaps have also declined in the Netherlands, also related to changes in agricultural management.

Whereas in agricultural land there is a strong decline of coprophilous fungi, a spectacular increase of these fungi in nature reserves has been noted. Many nature reserves, be they dunes, grasslands, heathlands or forests, are managed by large herbivores. These vegetation types are often more rich in plant species and the plant litter usually contains more lignin, resulting in dung pellets that provide more opportunities for these coprophilous fungi. However, these gains do not at all compensate for the losses on agricultural land, because the area of grazed nature reserves is much smaller than the area of modern-managed grasslands for cattle. Agricultural management of grassland is a further factor of importance. Hartmann *et al.* (2015) showed that organic farming, where farmyard manure was applied, showed higher abundance of several coprophilous basidiomycete genera such as *Coprinellus*, *Coprinopsis* and *Psathyrella*.

There have been suggestions that a further cause of decline of coprophilous fungi is due to the use of antibiotics in veterinary medicine. The first author of this essay has regularly received emails with the question whether the decline of *Agaricus campestris*, a species that is most common in horse-grazed grasslands, but which is not a coprophilous fungus in a strict sense, is related to the use of ivermectin. These questions were based on observations that despite the increasing numbers of horses in the agricultural landscape, the species is declining. The same suggestion has been made for the species *Poronia*



Figure 5. *Poronia punctata*, a species characteristic for horse dung. Its decline in the United Kingdom has been putatively linked to the use of antihelmintics. From Dam & Kuyper (2016).

punctata (Fig. 5). A study by Edwards (2015) did not find evidence that the antihelmintic pyrantel had a negative effect on this species. She did not study ivermectin, and also did

Table 2. Red Data-listed coprophilous Basidiomycota. From Arnolds & Van den Berg (2013).

Red List category	Species
VN – Disappeared, not recorded since 2000	<i>Conocybe microrrhiza</i> var. <i>microrrhiza</i> ; <i>Psathyrella waverenii</i>
EB – Seriously threatened, very strongly declined and currently very rare	<i>Coprinus sterquilinus</i>
BE – Threatened, strongly declined and currently rare	<i>Coprinellus ephemerus</i> ; <i>Coprinopsis macrocephala</i> ; <i>C. vermiculifer</i> ; <i>Stropharia luteonitens</i>
KW – Vulnerable, declined and currently uncommon	<i>Coprinellus bisporus</i> ; <i>C. brevisetulosus</i> ; <i>C. congregatus</i> , <i>C. curtus</i> ; <i>C. heptemerus</i> ; <i>Coprinopsis cinerea</i> ; <i>C. ephemeroides</i> ; <i>C. pachysperma</i> ; <i>C. poliomalla</i> ; <i>C. radiata</i> ; <i>C. stercorea</i> ; <i>C. tuberosa</i> ; <i>Deconica merdaria</i> ; <i>Panaeolus cinctulus</i> ; <i>Psathyrella hirta</i>
GE – Sensitive, Very rare or rare species, but without evidence of decline	<i>Bolbitius titubans</i> var. <i>olivaceus</i> ; <i>Coprinellus marculentus</i> ; <i>Coprinopsis filamentifer</i> ; <i>C. foetida</i> ; <i>C. pseudoradiata</i> ; <i>Deconica moelleri</i> ; <i>Leucoagaricus americanus</i> ; <i>L. meleagris</i> ; <i>Leucocoprinus cretaceus</i> ; <i>Parasola megasperma</i> ; <i>Pholiotina coprophila</i> ; <i>Psathyrella saponacea</i> ; <i>Stropharia semiglobata</i>

not find studies about the effect of ivermectin, whereas a negative effect had once been suggested (cited in Edwards, 2015). Considering that ivermectin has a negative effect on dung beetles and that many veterinary medicines have negative effects on microbial communities, it could be worthwhile to investigate this effect of ivermectin in coprophilous fungi experimentally. Another class of antihelminthics, the benzimidazoles, have fungitoxic activity (Edgington *et al.*, 1971), although it is not known whether coprophilous fungi are also affected.

4 Using coprophilous fungi for secondary metabolites

As noted in the Introduction dung is an ephemeral nutrient-rich substrate where many microbes compete for resources. Species with low growth rates would be easily outcompeted where it not for the fact that many of these produce antimicrobial compounds. Karwehl & Stadler (2016) even suggested that coprophilous mushrooms are predestined to produce antibiotics. Bills *et al.* (2013) suggested that a search for novel antibiotic substances might be particularly useful among coprophilous fungi. In their paper they concentrated on Ascomycota, and they provided an overview of major secondary metabolites of coprophilous Ascomycota. Their table 1 provides a list of ascomycete orders, families, genera and species with the specific compounds, the biosynthetic family and the biological activity. Many of those compounds are notable as fungal compounds, opening the possibilities that such compounds can be used in control of phytopathogenic fungi (see also Sarrocco, 2016). In both the main biosynthesis pathway and their effects, the Ascomycota differ from the Basidiomycota; in the latter group the terpenoid biosynthesis pathway dominates and secondary compounds are antibacterial rather than antifungal. Some compounds seem to be the result of past processes of horizontal gene transfer. This process was suggested for *Podospora anserina* where the pathway for sterigmatocystin synthesis was derived from *Aspergillus nidulans*, two species that could occur simultaneously in dung (Slot & Rokas, 2011).

By producing such compounds coprophilous fungi can monopolise the dung substrate. That outcome was shown for *Stilbella fimetaria*, a species characteristic for rabbit pellets. The species produces peptaibol antibiotics from which other fungi and bacteria suffer, whereas the species itself is resistant to that toxin. Consequently the species can exclusively occupy individual rabbit pellets (Lehr *et al.*, 2006).

We will concentrate on a few exemplary secondary metabolites with high antibiotic potential among the Basidiomycota. One major example is the coprophilous species *Clitopilus passeckerianus* (Kattenoorzwam; Fig. 6) and also the related *C. fasciculatus* (Toefige molenaar), known for their ability to produce pleuromutilin. It is not clear yet how many species occur in this clade (Figure 7). Depending on cut-off between one and six species could be recognised), and this is currently being investigated (G. Consiglio, personal communication). This phylogenetic tree lists a large number of names (7) in this clade, but it is likely that many collections have been misidentified or have been reported under misapplied names.



Figure 6. *Clitopilus passeckerianus*. Photo N. Dam, from <https://www.verspreidingsatlas.nl/0023090>

In fact, it is the misapplied name *Pleurotus mutilus* that lies at the basis of the name pleuromutilin, a substance with high pharmaceutical potential. However, the phylogenetic tree leaves no doubt that the ability to produce pleuromutilin has only evolved once. *Clitopilus passeckerianus* is frequently reported from mushroom-growing facilities, but it is likely that it does not parasitise on the common button mushroom *Agaricus bisporus*, but just is selected by the same substrate. The strains in that clade under the name *C. cf. scyphoides* were isolated from soil (Thorn *et al.*, 1996) and as cultures produced fruitbodies, they were able to isolate spores, produce monokaryons that were subsequently sampled. The presence of these monokaryons would allow testing a biological species concept. *Clitopilus fasciculatus* was also described as growing on beds of cultivated mushrooms; it is not known from what substrate the sequenced collection came from, although it was likely terrestrial, and the specimen may have been misidentified. CBS strain 455.86 was collected on wood, while the ecology of CBS strain 270.36 is unknown. While strains have been reported to fruit in the laboratory and then to produce pleuromutilin, it is not known whether it is produced in higher amounts if it is

grown in a competitive environments like composted dung. The ability to produce pleuromutilin has also been reported for *Psathyrella conopilus* (sub nomine *Drosophila subatrata*), but the study by Harley *et al.* (2009) failed to confirm that. Currently it seems wise to assume that the substance is only present in part of the *Clitopilus* clade.

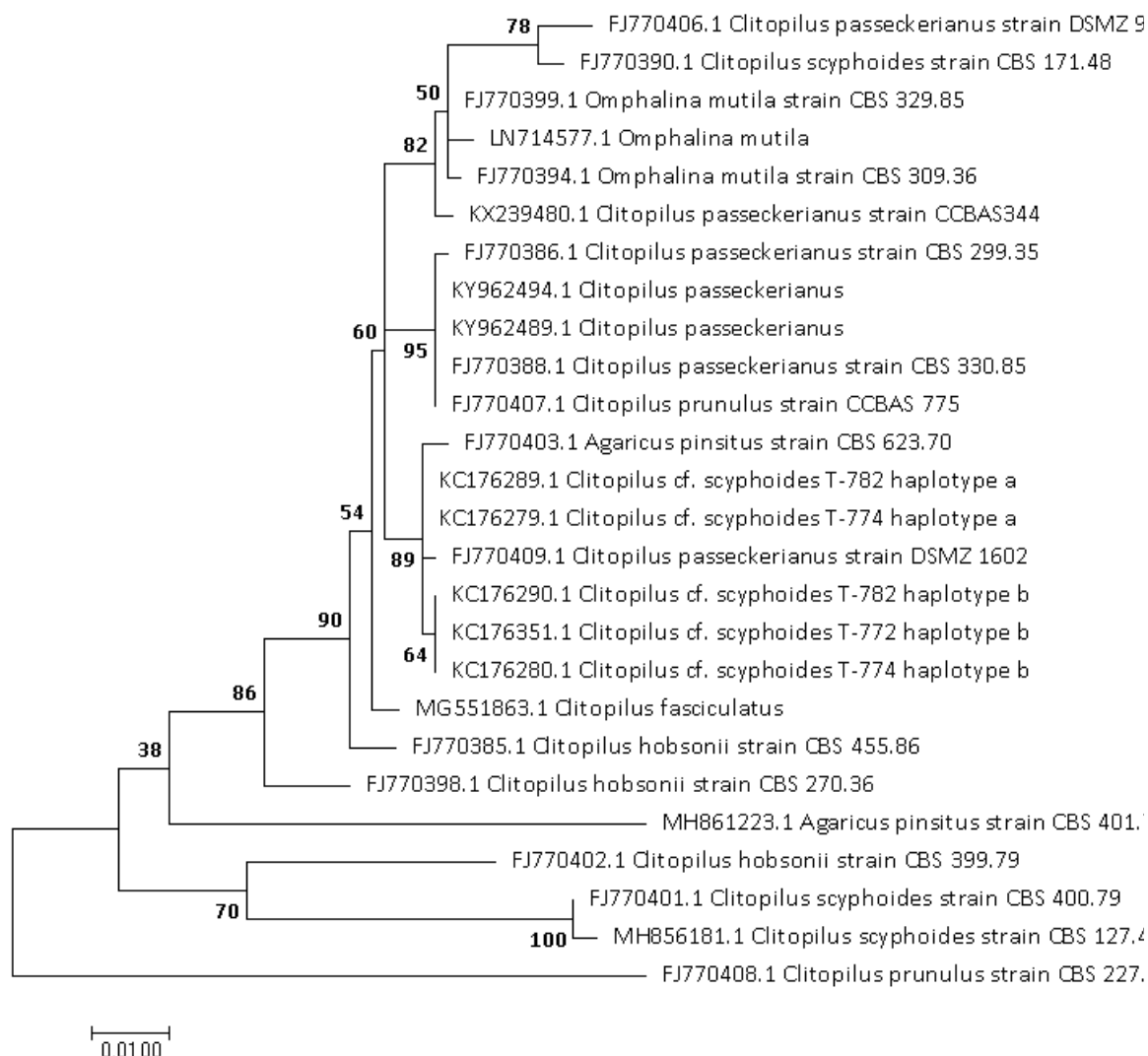


Figure 7. Fig. 7. Phylogenetic tree of (part of) the genus *Clitopilus* (Entolomataceae), based on ITS sequences, updated from Hartley *et al.* (2009). The strain used by Hartley *et al.* is ATCC 34646, which is the same as DMSZ1602. Alignment with Clustal; model Tamura 3-parameter model, gamma distributed; Maximum likelihood with 500 bootstrap replicates. Bootstrap support values are indicated at the branches. Cultures that produce pleuromutilin have been indicated.

Pleuromutilin and its derivatives are antibacterial drugs that inhibit protein synthesis by binding to the peptidyl transferase component of the 50S subunit of ribosomes. The class of semi-synthetic derivatives of this antibiotic includes lefamulin (for systematic use in humans), retapamulin (approved for topical use in humans since 2007), valnemulin and tiamulin (both approved for use in animals) and the investigational drug azamulin. This antibiotic class has not shown cross-resistance with other antibiotics and is considered as a class of antibiotics with great potential. There is increased interest to use pleuromutilin-derived antibiotics for the treatment of multi-drug resistant (MDR)

tuberculosis strains and methicillin-resistant *Staphylococcus aureus* (MRSA). The genetic basis for pleuromutilin production has been elucidated and consists of a cluster of seven genes. The gene cluster can be expressed in other fungi resulting in very substantial increases in production (more than tenfold; Bailey *et al.*, 2016). Such expression is beneficial as yields in the fungus are low and overexpression of the genes ineffective. Recent reports showed that the fungus is able to fruit in the lab and that from cultures monokaryons can be generated, allowing classical breeding approaches for increased yield as well. (De Mattos-Shipley *et al.*, 2017)

A further example is constituted by the coprinoid mushrooms. These are currently divided over two families; most species belong to the Psathyrellaceae and three major coprinoid genera are recognised, viz. *Coprinellus*, *Coprinopsis* and *Parasola*. (The genus *Coprinus* in the Agaricaceae contains very few species, the best known being *C. comatus*, which is not coprophilous although it prefers nitrogen-rich soil.) The three other genera all have coprophilous representatives. Coprinoid mushrooms are well known for their production of potential antibiotics (Badalyan, 2020). The best known are copsin (Essig *et al.*, 2014) and lagopodin. It is plausible that a further search for such metabolite actions in coprinoid fungi will yield further compounds with interesting bio-active properties. Copsin, produced by *Coprinopsis cinerea*, belongs to the class of defensin, cysteine-rich peptides with anti-microbial effects. Copsin can rapidly kill gram-positive bacteria by preventing proper cell wall formation. Through genetic modification copsin variants have been created with enhanced activity against *Staphylococcus aureus* and *Bacillus subtilis* (Franzoi *et al.*, 2017). There are no reports that bacterial resistance has evolved. The peptide can be expressed in yeasts. The fungus also produces further effectors against both Gram-positive and Gram-negative bacteria (Kombrink *et al.*, 2019). The fungal genes that were highly expressed in cultures in confrontation with bacteria suggest that the fungus uses a similar arsenal of effectors both in the presence of Gram-positive and Gram-negative bacteria (Essig *et al.*, 2014). *Coprinopsis cinerea* has also been shown to produce the sesquiterpene lagopodin B (Stöckli *et al.*, 2019; the substance was originally discovered in *Coprinopsis lagopus*) and nematotoxic proteins (Plaza *et al.*, 2015).

A further coprinoid fungus of potential interest is *Coprinellus heptemerus*. The species was reported to produce different diterpenoids, called heptemerones. These compounds showed antifungal activity and could possibly be beneficial in controlling plant pathogenic fungi (Kettering *et al.*, 2005). It is likely that a search for relevant metabolites in further species of coprinoid fungi such as *Coprinopsis nivea* (Fig. 8) will bring additions to the list of metabolites.



Figure 8. *Coprinopsis nivea*. Photo N. Dam, from Dam & Kuyper (2013).

Coprophilous mushrooms also produce compounds against plant pathogenic fungi and against nematodes. In the review by Sarrocco (2016) *Coprinellus heptemerus* is given as the only example of a basidiomycete with antifungal activity (against *Magnaporthe grisea*, the causal agent of rice blast). She provides further examples from coprophilous Ascomycota (*Podospora* – 7 species; *Sporormiella* – 4 species). Most metabolites have reported antifungal activity against other coprophilous fungi but in some cases also against plant pathogens and *Candida albicans*, a yeast that often occurs in immunocompromised patients. In an earlier study Weber *et al.* (2007) showed that of the 167 strains of coprophilous Ascomycota tested, around 15% were active against *Candida*.

Some coprophilous fungi belonging to the genus *Psilocybe* produce tryptophan-derived secondary metabolites such as psilocybin and psilocin. Both compounds are known as hallucinogens and for that reasons the fungi are listed as forbidden according to Dutch law. We will not further dwell on these fungi in this essay.

5 Coprophilous fungi as a source of enzymes

Fungi that grow during the later successional stages on dung have the ability to degrade various recalcitrant plant compounds in the dung. It is often assumed that Ascomycota do not or hardly possess the ability to degrade lignin compounds. The genome of the ascomycete *Podospira anserina* (Paoletti & Saupe, 2008) contains many cellulases and hemicellulases as well as putative ligninolytic enzymes. On the other hand it has a low potential to degrade sucrose, inulin and pectin. It has often been suggested that the ability to degrade lignin is quite common in the Basidiomycota and rare in the Ascomycota, with the exception of members of the Xylariales. However, Van Erven *et al.* (2020) recently published unambiguous evidence that *Podospira anserina*, a species that occurs in late successional stages on dung, possesses ligninolytic activity. That discovery would increase biotechnological interest in this fungus that is also known to produce a large number of carbohydrate-active enzymes such as cellulases and hemicellulases (xylanases). Genomic studies had already suggested several putative ligninolytic enzymes including laccases and H₂O₂-producing oxidoreductases. Mathé *et al.* (2019) suggested a novel class of peroxidases, which they also recorded for *Podospira anserina*, but not in the dung-inhabiting *Ascobolus*. Ligninolytic enzymes have often been derived from wood-degrading fungi (*Phanerochaete chrysosporium*, *Ceriporiopsis subvermispora*), however the expression of these peroxidases is often repressed in the presence of mineral nitrogen. It is likely that ligninolytic enzymes in coprophilous Basidiomycota are not repressed by mineral nitrogen, and they may therefore enrich our arsenal of such enzymes.

Recently, Deltedesco *et al.* (2020) studied the effects of a warming experiment on the soil microbial community structure. Their results indicated that upon soil warming ligninolytic basidiomycetes increased in abundance, possibly related to higher macrofaunal activity. *Coprinopsis cordispora* was noted as the species with a clear positive effect to soil warming.

6 Mining genomes of coprophilous fungi

With current technological developments it becomes cheaper to obtain full genome sequences of many organisms, allowing in silico predictions of useful enzymes and gene clusters for major metabolites. Table 3 provides an overview of coprophilous Basidiomycota whose genomes have been or are currently being sequenced.

Table 3. List of coprophilous Basidiomycota whose genomes have been or are currently being sequenced. From <https://mycocosm.jgi.doe.gov/agaricales/agaricales.info.html> and <https://www.ncbi.nlm.nih.gov/genome/browse#!/eukaryotes/agaricales> (both accessed July 6th, 2020).

Species	Reference
<i>Coprinopsis strossmayeri</i>	Banks <i>et al.</i> 2017
<i>Agrocybe pediades</i>	
<i>Agaricus bisporus</i>	
<i>Bolbitius titubans</i>	
<i>Coprinellus pellucidus</i>	
<i>Coprinopsis cinerea</i>	Stajich <i>et al.</i> , 2010
<i>Panaeolus papilionaceus</i>	

7 Outlook: towards a circular agriculture and the role of coprophilous mushrooms

We propose three possible applications of coprophilous fungi for improved circular agriculture that could be explored further: composting manure, bioremediation of manure and pre-treatment of manure for insects.

Several mushroom species are cultivated on compost. *Agaricus bisporus* (button mushroom) is the most well-known mushroom species that is grown on compost that is prepared from a mix of raw materials containing wheat straw-rich horse manure and chicken manure (Van Griensven 1988). To a lesser extent related species like *Agaricus arvensis* (horse mushroom) are grown on this kind of compost. Also *Coprinus comatus* is grown on a mushroom compost, but as mentioned earlier this species is not coprophilous. Even though *Agaricus bisporus* is a sizable crop (a little over 1.1 million tons produced annually in the European Union (<http://www.infochampi.eu/production-figures/>)) its production does not relieve the problems caused by (over) production of cow and pig manure.

Typical mushroom compost (substrate for *A. bisporus*) is made by aerobic composting of a mixture of straw-rich horse manure, chicken manure, gypsum and water (Van Griensven 1988) in three steps. In the first step, the compost is aerated to control temperatures between 70 and 80°C in the presence of high levels of gaseous ammonia. In this step the carbohydrates present in the compost are made accessible for enzymes (Mouthier *et al.*, 2017). In the second step the compost is aerated in such a way that first a 8-12 hr period of 56-58°C is maintained after which the temperature is lowered to 45°C. In this step, lasting about 5 days, the gaseous ammonia in the process air is slowly removed and a thermophilic microbiota, consisting of bacteria and fungi, develops in the compost. The removal of ammonia is crucial for the viability of *A. bisporus*. In modern composting facilities that are used for the production of mushroom compost, the ammonia in the process air is collected and washed from the process air, using sulphuric acid containing washers. The resulting ammonium sulphate is either returned into the composting process scale or sold as a nitrogen fertilizer. In the third step, grains with *A. bisporus* are mixed in the compost. After this the compost is colonised by *A. bisporus* mycelium for a period of 14-17 days (reviewed in Kabel *et al.*, 2017).

Using composting techniques such as used for *A. bisporus*, but with other coprophilous fungi may help to convert animal manure into fertiliser with added value. Postma *et al.* (2020) have tested mushroom compost as a fertiliser and in these experiments, on top of the fertilising value, the fungal-colonised compost has been shown to be able to suppress soil-borne fungal diseases. Coprophilous fungi could potentially be

beneficial for the preparation of such composts. In contrast to *A. bisporus*, other coprophilous fungi may be able to withstand higher ammonia levels in compost, enabling shorter composting processes, making such composts cheaper to produce while reducing loss of material.

Antibiotics and antiparasitics present in manure (especially manure from animals that are given veterinary care) could potentially pose a problem for the disposal of this manure (Postma *et al.*, 2020). Several studies have demonstrated the degradation of antibiotics during composting processes (Dolliver *et al.*, 2008; Ho *et al.*, 2013; Zhang *et al.*, 2015) although the relative roles of (actino-)bacteria and fungi in antibiotic degradation are still poorly known. Moreover, differences between fungal species in their ability to degrade antibiotics and antiparasitics of various classes have hardly been studied. It would be interesting to explore the potential of coprophilous fungi for degrading such compounds. We propose that composting of manure with coprophilous fungi could be helpful in processing manure that is contaminated with antibiotics and or antiparasitics. The product could be a fungal colonised compost that can be used as a fertiliser. Coprophilic fungi have the advantage that they are adapted to manure and might (more) easily be fitted into this process.

Another approach would be to study the use of coprophilic fungi to treat manure for rearing insects. Black soldier fly (BSF), *Hermetia illucens* (L.) (Diptera: *Stratiomyidae*), larvae can be grown on a wide range of organic materials, manure being one of them. Because of their high protein and lipid content, BSF larvae can be used as an additive in animal feeds and biodiesel production (Scala *et al.*, 2020). Parodi *et al.* (2020) showed that BSF has a preference for manure. However, as shown by Raksasat *et al.* (2020) in their review, the nutritional quality of manure does not enable the larvae to grow at the highest rate nor reach the highest body weights. Raksasat *et al.* (2020) point out the possibility that manure can be pretreated with microorganisms to enhance the nutritional value for the larvae of BSF. Coprophilic fungi would presumably be ideal for such an application, as they degrade recalcitrant lignocellulose components in manure, and together with the surplus of nitrogen convert this into proteins (amongst others). Besides, the combination of coprophilic fungi and BSF might have the added benefit of degrading antibiotics and antiparasitics that are present in manure (Cai *et al.*, 2018).

References

- Arnolds, E. & A. van den Berg 2013. Beknopte Standaardlijst van Nederlandse Paddenstoelen 2013. Nederlandse Mycologische Vereniging, 287 pp.
- Arnolds, E. & M.T. Veerkamp 2008. Basisrapport Rode Lijst paddenstoelen. Nederlandse Mycologische Vereniging
- Badalyan, S.M. 2020. Medicinal coprinoid mushrooms (Agaricomycetes) distributed in Armenia (Review). *International Journal of medicinal Mushrooms* 22: 257-267.
- Bailey, A.M., F. Alberti, S. Kilaru, C.M. Collins, K. de Mattos-Shipley, A.J. Harley, P. Hayes, A. Griffin, C.M. Lazarus, R.J. Cox, C.L. Willis, K. O; Dwyer, D.W. Spence & G.D. Foster 2016. Identification and manipulation of the pleuromutilin gene cluster from *Clitopilus passeckerianus* for increased rapid antibiotic production. *Scientific Reports* 6: article 25202.
- Banks, A.M., G.L.A. Barker, A.M. Bailey & G.D. Foster 2017. Draft genome sequence of the coprinoid mushroom *Coprinopsis strossmayeri*. *Genome Announcements* 5: e00044-17
- Bills, G.F., J.B. Gloer & Z. An 2013. Coprophilous fungi: antibiotic discovery and functions in an underexplored arena of microbial defensive mutualism. *Current Opinion in Microbiology* 16: 549-565.
- Cai, M., S. Ma, R. Hu, J.K. Tomberlin, C. Yu, Y. Huang, S. Zhan, Li. Wu, L. Zheng, Z. Yu & J. Zhang 2018 Systematic characterization and proposed pathway of tetracycline degradation in solid waste treatment by *Hermetia illucens* with intestinal microbiota. *Environmental Pollution* 242: 634-642.
- Dam, N. & T.W. Kuyper 2013. Veldgids Paddenstoelen I. Plaatjeszwammen en boleten. KNNV Uitgeverij.
- Dam, N. & T.W. Kuyper 2016. Veldgids Paddenstoelen II. Beker-, Buik-, Gaatjes-, Kern-, Knots-, Koraal-, Korst-, Stekel- en Trilzwammen. KNNV Uitgeverij.
- De Mattos-Shipley, K.M.J., G.D. Foster & A.M. Bailey 2017. Insights into the classical genetics of *Clitopilus passeckerianus* – the pleuromutilin producing mushrooms. *Frontiers in Microbiology*: 8: article 1056
- Deltedesco, E., K.M. Keiblinger, H.P. Piepho, L. Antonielli, E.M. Pötsch, S. Zechmeister-Boltenstern & M. Gorfer 2020. Soil microbial community structure and function mainly respond to indirect effects in a multifactorial climate manipulation experiment. *Soil Biology & Biochemistry* 142: article 107704.
- Dewan, M.M., E.L. Ghisalberti, C. Rowland & K. Sivasithamparan 1994. Reduction of symptoms of take-all of wheat and rye-grass seedlings by the soil-borne fungus *Sordaria fimicola*. *Applied Soil Ecology* 1: 45-51.
- Dolliver H., S. Gupta & S. Noll 2008. Antibiotic degradation during manure composting. *Journal of Environmental Quality* 37: 1245-1253.
- Doveri, F. 2004. *Fungi fimicoli italiani. Guida al riconoscimento dei Basidiomiceti e degli Ascomiceti che vivono su materia fecale.* Associazione micologica Bresadola, Trento.
- Edgington, L.V., K.L. Khew & G.L. Barron 1971. Fungi toxic spectrum of benzimidazole compounds. *Phytopathology* 61: 42-44.
- Edwards, N. 2015. Does Breckland vegetation and its management influence abundance of *Poronia punctata*. MSc thesis, University of Herfordshire.
- Essig, A., D. Hofmann, D. Münch, S. Gayathri, M. Künzler, P.T. Kallio, H.-G. Sahl, G. Wider, T. Schneider & M. Aebi 2014. Copsin, a novel peptide-based fungal antibiotic interfering with the peptidoglycan synthesis. *The Journal of biological Chemistry* 50: 34953-34964.
- Franzoi, M., Y. van Heuvel, S. Thomann, N. Schürch, P.T. Kallio, P. Venier & A. Essig 2017. Structural insights into the mode of action of the peptide antibiotic copsin. *Biochemistry* 56: 4992-5001.
- Griffith, D.L., B. Larkin, A. Kliskey, L. Alessa & G. Newcombe. 2017. Expectations for habitat-adapted symbiosis in a winter annual grass. *Fungal Ecology* 29: 111-115.
- Griffith, G.W. & K. Roderick 2008. Saprotrophic basidiomycetes in grasslands: distribution and function. In: L. Boddy, J.C. Frankland & P. van West (eds), *Ecology of saprotrophic Basidiomycetes*, p. 277-299. Elsevier, Amsterdam.

- Harper, J.E. & J. Webster 1964. An experimental analysis of the coprophilous fungus succession. *Transactions of the British mycological Society* 47: 511-530.
- Hartley, A.J., K. de Mattos-Shipley, C.M. Collins, S. Kilaru, G.D. Foster & A.M. Bailey 2009. Investigating pleuromutilin-producing *Clitopilus* species and related basidiomycetes. *FEMS Microbiology Letters* 297: 24-30.
- Hartmann, M., B. Frey, J. Mayer, P. Mäder & F. Widmer 2015. Distinct soil microbial diversity under long-term organic and conventional farming. *The ISME Journal* 9: 1177-1194.
- Herrera, J., R. Poudel & H.H. Kidir 2011. Molecular characterization of coprophilous fungal communities reveals sequences related to root-associated fungal endophytes. *Molecular Ecology* 61: 239-244.
- Ho, Y.B., M.P. Zakaria, P.A. Latif & N. Saari 2013. Degradation of veterinary antibiotics and hormone during broiler manure composting. *Bioresource Technology* 131: 476-484.
- Ikeduigwu, F.E.O. & J. Webster 1970a. Antagonism between *Coprinus heptemerus* and other coprophilous fungi. *Transactions of the British mycological Society* 54: 181-204.
- Ikeduigwu, F.E.O. & J. Webster 1970b. Hyphal interference in a range of coprophilous fungi. *Transactions of the British mycological Society* 54: 205-210.
- Kabel, M.A., E. Jurak, M.R. Mäkelä & R.P. de Vries 2017. Occurrence and function of enzymes for lignocellulose degradation in commercial *Agaricus bisporus* cultivation. *Applied Microbiology and Biotechnology*, 101(11): 4363-4369.
- Karwehl, S. & M. Stadler 2016. Exploitation of fungal diversity for discovery of novel antibiotics. *Current Topics in Microbiology and Immunology* 398: 303-338.
- Keay, S.M. & A.E. Brown 1990. Colonization by *Psilocybe semilanceata* of roots of grassland flora. *Mycological Research* 94: 49-56.
- Kettering, M., C. Valdivia, O. Sterner, H. Anke & E. Thines 2005. Heptemerones A-G, seven novel diterpenoids from *Coprinus heptemerus*: producing organism, fermentation, isolation and biological activities. *Journal of Antibiotics* 58: 390-396.
- Kombrink, A., A. Tayyrov, A. Essig, M. Stöckli, S. Micheller, J. Hintze, Y. van Heuvel, N. Dürig, C. Lin, P.T. Kallio, M. Aebi, & M. Künzler. 2019. Induction of antibacterial proteins and peptides in the coprophilous mushroom *Coprinopsis cinerea* in response to bacteria. *The ISME Journal* 13: 588-602.
- Krug, J.C., G.L. Benny & H.W. Keller 2004. Coprophilous fungi. In: G.M. Mueller, G.F. Bills & M.S. Foster (eds), *Biodiversity of fungi: inventory and monitoring methods*, p. 467-499. Elsevier, Amsterdam.
- Kruys, Å. & L. Ericson 2008. Species richness of coprophilous ascomycetes in relation to variable food intake by herbivores. *Fungal Diversity* 30: 73-81.
- Lehr, N.A., A. Meffert, L. Antelo, O. Sterner, H. Anke & R.W. Weber 2005. Antiamoebins, myrocin B and the basis of antifungal antibiosis in the coprophilous fungus *Stilbella erythrocephala* (syn. *S. fimetaria*). *FEMS Microbiology, Ecology* 55: 105-112.
- Mathé, C., N. Fawal, C. Roux & C. Dunand 2019. In silico definition of new ligninolytic peroxidase sub-classes in fungi and putative relation to fungal life style. *Scientific Reports* 9: article 20373.
- Mouthier, T.M.B., B. Kilic, P. Vervoort, H. Gruppen & M.A. Kabel 2017. Potential of a gypsum-free composting process of wheat straw for mushroom production. *PLoS ONE*, 12(10), [e0185901]. <https://doi.org/10.1371/journal.pone.0185901>
- Nagy, L.A. & K.M. Harrower 1980. Coprous and non-coprous decomposition. *Transactions of the British mycological Society* 74: 639-641.
- Newcombe, G., J. Campbell, D. Griffith, M. Baynes, K. Launchbaugh & R. Pendleton 2016. Revisiting the life cycle of dung fungi, including *Sordaria fimicola*. *PLoS One* 11: e0147425.
- Ozinga, W.A., E. Arnolds, P.-J. Keizer & T.W. Kuyper 2013. Paddenstoelen in het natuurbeheer. *Preadvies Paddenstoelen*. Bosschap, 402 pp.
- Paoletti, M. & S.J. Saupe 2008. The genome sequence of *Podospira anserina*, a classic model fungus. *Genome Biology* 9: article 223.
- Parodi, A., K. Van Dijk, J.J.A. Van Loon, I.J.M. De Boer, J. Van Schelt & H.H.E. Van Zanten 2020. Black soldier fly larvae show a stronger preference for manure than for a mass-rearing diet. *Journal of applied Entomology* 144(7): 560-565.
- Plaza, D.F., S.S. Schmieder, A. Lipzen, E. Lindquist & M. Künzler 2015. Identification of a novel nematotoxic protein by challenging the model mushroom *Coprinopsis cinerea* with a fungivorous nematode. *Genes, Genomes, Genetics* 6: 87-98.

- Postma, J., M. Schilder, J. Bloem, J. Visser G. Van Os, K. Brolsma, M. Hoogmoed, R. Postma & G. Korthals 2020. Sturen op bodemweerbaarheid door toediening van organische materialen; TKI-AF-15261. Wageningen Research, Rapport WPR-1024. (Freely available at <https://doi.org/10.18174/536631> <https://doi.org/10.18174/536631>).
- Postma R., P.A.I. Ehler, W. Van Dijk, J. Roefs & L.R. Gollenbeek, 2020. Contaminanten en wetgeving bij mestproducten. Wageningen Livestock Research, rapport 1269 (Freely available at <https://doi.org/10.18174/530719>).
- Raksasat, R., J.W. Lim, W. Kiatkittipong, K. Kiatkittipong, Y.C. Ho, M.K. Lam, C. Font-Palma, H.F. Mohd Zaid & C.K. Cheng 2020. A review of organic waste enrichment for inducing palatability of black soldier fly larvae: Wastes to valuable resources. *Environmental Pollution* 267, 115488
- Richardson, M.J. 2001. Diversity and occurrence of coprophilous fungi. *Mycological Research* 105: 387-402.
- Richardson, M.J. 2002. The coprophilous succession. *Fungal diversity* 10: 101-111.
- Sarrocchio, S. 2016. Dung-inhabiting fungi: a potential reservoir of novel secondary metabolites for the control of plant pathogens. *Pest Management Science* 72: 643-652.
- Scala, A., J.A. Cammack, R. Salvia, C. Scieuzo, A. Franco, S.A. Bufo, J.K. Tomberlin & P. Falabella 2020. Rearing substrate impacts growth and macronutrient composition of *Hermetia illucens* (L.) (Diptera: Stratiomyidae) larvae produced at an industrial scale. *Scientific Reports* 10(1), 19448
- Slot, J.C. & A. Rokas 2011. Horizontal transfer of a large and highly toxic secondary metabolic gene cluster between fungi. *Current Biology* 21: 134-139.
- Stajich J.E., S.K. Wilke, D. Ahrén, C.H. Au, B.W. Birren, M. Borodovsky *et al.* 2010. Insights into evolution of multicellular fungi from the assembled chromosomes of the mushroom *Coprinopsis cinerea* (*Coprinus cinereus*). *Proceedings of the National Academy of Sciences of the United States of America* 107: 11889-11894.
- Stöckli, M., B.I. Morinaka, G. Lackner, A. Kombrink, R. Sieber, C. Margot, C.E. Stanley, A.J. deMello, J. Piel & M. Künzler 2019. Bacteria-induced production of the anti-bacterial sesquiterpene lagopodin B in *Coprinopsis cinerea*. *Molecular Microbiology* 11: 605-619.
- Thorn, R.G., C.A. Reddy, D. Harris & E.A. Paul. 1996. Isolation of saprophytic basidiomycetes from soil. *Applied and Environmental Microbiology* 62: 4288-4292.
- Van Erven G., A.F. Kleijn, A. Patyshakuliyeva, M. De Falco, A. Tsang, R.P. de Vries, W.J.H. van Berkel & M.A. Kabel. 2020. Evidence for ligninolytic activity of the ascomycete fungus *Podospora anserina*. *Biotechnology for Fuels* 13: art. 75.
- Van Griensven L.J.L.D. 1988. The Cultivation of Mushrooms. Rustington, Sussex, England: Darlington Mushroom Laboratories.
- Weber, R.W.S., R. Kappe, T. Paululat, E. Mösker & H. Anke 2007. Anti-Candida metabolites from endophytic fungi. *Phytochemistry* 68: 886-892.
- White, T.J., Bruns, T., Lee, S. & J.W. Taylor 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols: A Guide to Methods and Applications*. Edited by: Innis MA, Gelfand DH, Sninsky JJ, White TJ., New York: Academic Press Inc, 315-322.
- Wood, S.N. & R.C. Cooke 1984. Use of semi-natural resource units in experimental studies on coprophilous succession. *Transactions of the British mycological Society* 82: 337-339.
- Zhang, Z., J. Zhao, C. Yu, S. Dong, D. Zhang, R. Yu, C. Wang & Y. Liu 2015. Evaluation of aerobic co-composting of penicillin fermentation fungi residue with pig manure on penicillin degradation, microbial population dynamics and composting maturity. *Bioresource Technology* 198: 403-409.

Annex 1 Building a collection of coprophilic fungi.

7.1 Obtaining strains from culture collections.

Based on the list of coprophilic basidiomycete species that are present in the Netherlands as shown in the literature review, various culture collections (Westerdijk Institute, DSMZ, CCBAS, BCCM-MUCL, the fungal collection of WUR Plant Breeding) have been searched for strains of these species. When choosing strains to order, a number of selection criteria were checked:

- Permission for research / applications (Nagoya (country of origin known?); other IP regulations)
- Broad range of manure substrates (manure; manure pile; compost (at various levels of degradation))
- Broad range host species (horse, cow, sheep, pig, etc. (no bird manure species were found in collections))
- Different taxonomic groups (different genera)
- Nuclear status (dikaryons usually grow much better than monokaryons; if nuclear status was not identified in collections, two strains were ordered).

Only very few coprophilic species could be found in existing culture collections. Of those that were present in collections, the information on the species was often incomplete. A total of 26 strains could be selected from culture collections. 24 strains were obtained from the Westerdijk Institute. They comprised 6 dikaryons, 8 monokaryons, and 10 strains of unknown nuclear status. Two strains were obtained from CCBAS (one dikaryon and one strain of unknown nuclear status).

7.2 Collection of strains from nature

Pure cultures were made of fungi that grew on various samples of manure. These samples were either found in nature (Figure 9) or were obtained from various farms (rabbit dung, pig manure, cow dung, Figure 10). In total we were able to isolate 12 different strains from the various manure samples.

7.3 Taxonomic identification

Molecular methods were used to taxonomically identify the various strains. Using primers ITS-1F, ITS-4 or ITS-4B (White *et al.*, 1990) a part in the internal transcribed spacer of the ribosomal repeat was amplified by PCR. Subsequently the DNA fragments were sequenced and the sequences were compared



Figure 9. Manure samples and mushrooms found in nature.

with sequences in Genbank. This yielded species names at various levels of success. Results are shown in Table 1. The final collection comprises 38 strains distributed over the species *Agrocybe pediades*, *Bolbitius titubans*, *Clitopilus passeckerianus*, *Conocybe siliginea*, *Conocybe singeriana*, *Coprinellus bisporus*, *Coprinellus congregates*, *Coprinellus heterosetulosus*, *Coprinellus marculentus*, *Coprinopsis cinerea*, *Coprinopsis radiata*, *Coprinopsis sclerotiger*, *Coprinopsis vermiculifer*, *Coprinus sterquilinus*, *Panaeolus fimicola*, *Panaeolus papilionaceus*, *Panaeolus semiovatus*, *Pholiotina coprophila*, *Psathyrella prona* f. *cana*, *Psathyrella stercoraria*, *Stropharia luteonitens*, *Stropharia semiglobata* and 2 not further identified *Coprinopsis* species. In summary, 38 strains distributed over at least 23, possibly 24 species.



Isolation from pig manure



Isolation from cow manure



Isolation from rabbit manure

Figure 10. Fungi and manure samples obtained from farms.

Table 4. List of collected strains of coprophilic basidiomycete species.

Culture	Description	Coll. Nr	Presumed species	Country of origin
CBS 101.39		MES 16210	<i>Agrocybe pediades</i>	
CBS 101815		MES 16205	<i>Stropharia semiglobata</i>	Netherlands
CBS 102729		MES 16206	<i>Stropharia semiglobata</i>	
CBS 114.21		MES 16209	<i>Coprinus sterquilinus</i>	Switzerland
CBS 121199	Identified by CBS as <i>Conocybe pubescens</i>	MES 16207	<i>Conocybe singeriana</i>	Netherlands
CBS 121201	Identified by CBS as <i>Conocybe siliginea</i>	MES 16208	<i>Conocybe ambigua</i>	Netherlands
CBS 132.46	Identified by CBS as <i>Coprinopsis vermiculifer</i>	MES 16213	<i>Coprinopsis vermiculifer</i>	France
CBS 151.38		MES 16220	<i>Coprinopsis radiata</i>	France

Table 4 (continued). List of collected strains of coprophilic basidiomycete species.

Culture	Description	Coll. Nr	Presumed species	Country of origin
CBS 154.39	CBS identified this strain as <i>Coprinellus ephemerus</i>	MES 16211	<i>Coprinopsis</i> sp.	Belgium
CBS 160.46		MES 16214	<i>Pholiotina coprophila</i>	France
CBS 168.72	CBS identified this strain as <i>Psathyrella hirta</i>	MES 16201	<i>Psathyrella stercoraria</i>	Scotland
CBS 169.72	Identified by CBS as <i>Psathyrella hirta</i>	MES 16202	<i>Psathyrella stercoraria</i>	Scotland
CBS 177.49	ITS identification could not distinguish between <i>Coprinopsis marculentus</i> or <i>Coprinellus callinus</i> . CBS identified this strain as <i>Coprinus marculentus</i>	MES 16221	<i>Coprinellus</i> sp.	Denmark
CBS 179.49	CBS identified this strain as <i>Coprinus marculentus</i>	MES 16215	<i>Coprinellus marculentus</i>	Denmark
CBS 183.51	CBS identified this strain as <i>Coprinellus ephemerus</i>	MES 16216	<i>Coprinellus congregatus</i>	Denmark
CBS 184.52	CBS identified this strain as <i>Coprinellus bisporus</i> .	MES 16218	<i>Coprinellus bisporus</i>	Greenland
CBS 187.51	CBS identified this strain as <i>Coprinellus heterosetulosus</i>	MES 16217	<i>Coprinellus heterosetulosus</i>	Norway
CBS 275.39	Identified by CBS as <i>Panaeolus semiovatus</i>	MES 16212	<i>Psathyrella prona</i> f. <i>cana</i>	
CBS 276.39	CBS identified this strain as <i>Panaeolus semiovatus</i>	MES 16224	<i>Panaeolus papilionaceus</i>	
CBS 301.64	CBS identified this strain as <i>Coprinopsis stercorea</i>	MES 16199	<i>Coprinellus bisporus</i>	Netherlands
CBS 356.53	CBS identified this strain as <i>Stropharia luteonitens</i>	MES 16219	<i>Stropharia luteonitens</i>	France
CBS 435.85		MES 16204	<i>Bolbitius titubans</i>	Czechia
CBS 477.70	Identified by CBS as <i>Coprinopsis stercorea</i>	MES 16200	<i>Coprinopsis sclerotiger</i>	Scotland
CBS 618.79	CBS identified this strain as <i>Stropharia luteonitens</i>	MES 16203	<i>Stropharia luteonitens</i>	France
CCBAS 356		MES 16198	<i>Coprinellus bisporus</i>	Czechia
CCBAS 775		MES 16197	<i>Clitopilus passeckerianus</i>	Slovakia
M0005-3A		MES 16175	<i>Coprinopsis</i> sp.	Netherlands

Table 4 (continued). List of collected strains of coprophilic basidiomycete species.

Culture	Description	Coll. Nr	Presumed species	Country of origin
M0005-4		MES 16177	<i>Coprinopsis radiata</i>	Netherlands
M0009-1		MES 16182	<i>Coprinopsis sclerotiger</i>	Nederland
M0011-1		MES 16183	<i>Coprinopsis cinerea</i>	Netherlands
M0013-1		MES 16222	<i>Panaeolus semiovatus</i>	Netherlands
M0013-2		MES 16223	<i>Panaeolus semiovatus</i>	Netherlands
M0026		MES 16187	<i>Panaeolus semiovatus</i>	Netherlands
M0027		MES 16188	<i>Panaeolus semiovatus</i>	Netherlands
M0030-A		MES 16189	<i>Stropharia semiglobata</i>	Netherlands
M0032		MES 16193	<i>Stropharia semiglobata</i>	Netherlands
M0033-A		MES 16194	<i>Stropharia semiglobata</i>	Netherlands
M0034		MES 16196	<i>Panaeolus fimicola</i>	Netherlands

7.4 Testing growth on a number of manure species.

To test growth of the coprophilic strains on various types of manure, samples of chicken manure, cow manure and pig manure were collected at farms. All types of manure were dried at 70°C before being used. Portions of 15 g of dried manure were placed in microboxes (<https://saco2.com>) and wetted with 35 ml of water. After the water was absorbed, the manure was pasteurised for 12 hrs at 70°C. The manure samples were inoculated with the various coprophilic strains and incubated at 24°C. Next to this a number of (non-coprophilic) strains from the fungal collection of WUR Plant Breeding were used for comparison. After 14 days of incubation, mycelium growth was scored on a scale of 0 (no growth) to 10 (abundant growth). As can be seen in Figure 11, the results of the first screening showed good growth for a number of species on chicken manure and cow manure. On pig manure only four strains showed some level of growth. Table 5 provides an overview of the growth of the various strains on the three different types of manure. Twenty three strains grew on chicken manure (ranging from limited growth to abundant growth), a total of 19 strains showed growth on cow manure (again ranging from limited growth to abundant growth) and only 4 strains (as mentioned) showed growth on pig manure with different abundances. *Agaricus bisporus* did not grow well on any of these types of manure.

It prefers degraded manure such as compost. Surprisingly, *Pleurotus eryngii* grew well on the chicken manure sample, although in nature this species is known for its association with cruciferous plants. Potentially, the species that did not grow well in this experiment, may prefer other types of manure substrate (for instance, not preheated) or other cultivation conditions.

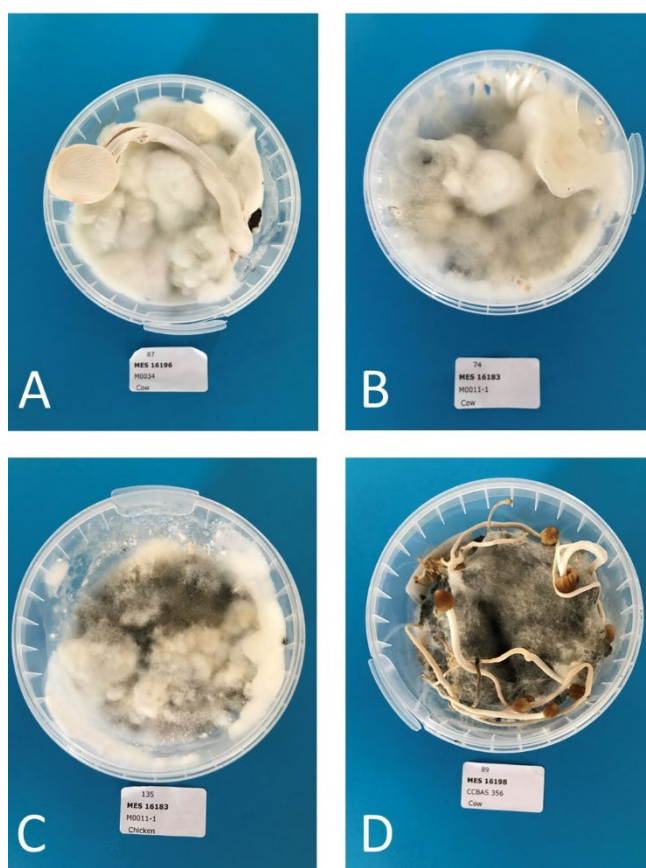


Figure 11. Growth of various coprophilic basidiomycete species on manure samples. *Panaeolus foenisecii* on cow manure (A), *Coprinopsis* sp. on cow manure (B) and on chicken manure (C), and *Coprinellus callinus* on cow manure (D).

Table 5. Overview of growth responses of coprophilic basidiomycete species on three different types of manure samples. Growth was scored on a scale of 0 (no growth) to 10 (abundant growth).

Culture	Collection Nr.	Presumed species name	Type of manure		
			Chicken	Cow	Pig
CCBAS 356	MES 16198	<i>Coprinellus bisporus</i>	10	8	4
CBS 184.52	MES 16218	<i>Coprinellus bisporus</i>	7	9	4
M0011-1	MES 16183	<i>Coprinopsis cinerea</i>	10	10	0
	MES 12379	<i>Clitopilus passeckerianus</i>	10	9	0
M0034	MES 16196	<i>Panaeolus fimicola</i>	5	10	0
M0027	MES 16188	<i>Panaeolus semiovatus</i>	4	10	0
M0005-3A	MES 16175	<i>Coprinopsis</i> sp.	2	1	7

	MES 11565	<i>Pleurotus eryngii</i>	8	1	0
M0032	MES 16193	<i>Stropharia semiglobata</i>	7	1	0
CBS 183.51	MES 16216	<i>Coprinellus congregatus</i>	5	0	0
	MES 00004	<i>Volvariella volvacea</i>	5	0	0
M0005-4	MES 16177	<i>Coprinopsis radiata</i>	1	3	0
M0033-A	MES 16194	<i>Stropharia semiglobata</i>	2	2	0
CBS 276.39	MES 16224	<i>Panaeolus papilionaceus</i>	2	1	0
CBS 356.53	MES 16219	<i>Stropharia luteonitens</i>	3	0	0
CCBAS 775	MES 16197	<i>Clitopilus passeckerianus</i>	1	1	1
M0030-A	MES 16189	<i>Stropharia semiglobata</i>	2	1	0
CBS 101.39	MES 16210	<i>Agrocybe pediades</i>	1	1	0
CBS 114.21	MES 16209	<i>Coprinus sterquilinus</i>	2	0	0
CBS 179.49	MES 16215	<i>Coprinellus marculentus</i>	0	2	0
CBS 477.70	MES 16200	<i>Coprinopsis sclerotiger</i>	1	1	0
M0009-1	MES 16182	<i>Coprinopsis sclerotiger</i>	1	1	0
M0013-1	MES 16222	<i>Panaeolus semiovatus</i>	1	1	0
CBS 101815	MES 16205	<i>Stropharia semiglobata</i>	1	0	0
CBS 102729	MES 16206	<i>Stropharia semiglobata</i>	0	0	0
CBS 121199	MES 16207	<i>Conocybe singeriana</i>	0	0	0
CBS 121201	MES 16208	<i>Conocybe siliginea</i>	0	0	0
CBS 132.46	MES 16213	<i>Coprinopsis vermiculifer</i>	0	0	0
CBS 151.38	MES 16220	<i>Coprinopsis radiata</i>	0	0	0
CBS 154.39	MES 16211	<i>Coprinopsis sp.</i>	0	0	0
CBS 160.46	MES 16214	<i>Pholiotina coprophila</i>	0	0	0
CBS 168.72	MES 16201	<i>Psathyrella stercoraria</i>	0	0	0
CBS 169.72	MES 16202	<i>Psathyrella stercoraria</i>	0	0	0
CBS 177.49	MES 16221	<i>Coprinellus marculentus</i>	0	0	0
CBS 187.51	MES 16217	<i>Coprinellus heterosetulosus</i>	0	0	0
CBS 275.39	MES 16212	<i>Psathyrella prona f. cana</i>	0	0	0
CBS 301.64	MES 16199	<i>Coprinellus bisporus</i>	0	0	0
CBS 435.85	MES 16204	<i>Bolbitius titubans</i>	0	0	0
CBS 618.79	MES 16203	<i>Stropharia luteonitens</i>	0	0	0
M0013-2	MES 16223	<i>Panaeolus semiovatus</i>	0	0	0
M0026	MES 16187	<i>Panaeolus semiovatus</i>	0	0	0
	MES 02058	<i>Agaricus subrufescens</i>	0	0	0
	MES 02121	<i>Lentinula edodes</i>	0	0	0
	MES 03793	<i>Agaricus bisporus</i>	0	0	0
	MES 14997	<i>Agaricus bisporus</i>	0	0	0

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Report WPR-

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