

MASTERARBEIT | MASTER'S THESIS

Titel | Title

Biodiversity and morphological characterization of coprophilous fungi in cattle
and sheep dung from Austria during the year 2024

verfasst von | submitted by

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angestrebter akademischer Grad | in partial fulfilment of the requirements for the degree of

Master of Science (MSc)

Wien | Vienna, 2025

Studienkennzahl lt. Studienblatt |
Degree programme code as it appears on the
student record sheet:

UA 066 832

Studienrichtung lt. Studienblatt | Degree pro-
gramme as it appears on the student record
sheet:

Masterstudium Botany

Betreut von | Supervisor:

ao. Univ.-Prof. Mag. Dr. Irmgard Greilhuber

Abstract

Dung is a very complex substrate and serves as a habitat for many fungi species. Coprophilous fungi play an important role in the nutrient cycle, as fungi are capable of breaking down lignin and making nutrients available again to the environment. Although coprophilous fungi are a vital part of the ecosystem, they have been studied very little in Austria. This study, which is part of the project “AustroDung” funded by the Austrian Biodiversity Fund, aims to create a species list of coprophilous fungi from 42 selected sampling locations across Austria. To investigate different fungal communities, both sheep and cattle dung were sampled and their influencing factors analysed. Using morphological methods and metabarcoding, more than 400 fungi species were identified, including several rumen fungi that are often difficult to record in purely morphological analyses. In addition to the 42 sampling locations, other factors such as deworming practices, animal husbandry, and environmental variables were considered. The results indicated that cattle dung exhibited a higher fungal diversity but also more fungal genera compared to sheep samples. In addition, the metabarcoding method resulted in a higher species diversity, compared to the morphological analyses. Species of the families Ascobolaceae and Pilobolaceae were found to be the most common in dung.

Coprophilous fungi, Metabarcoding, Ascomycota, Basidiomycota, Dung

Kurzzusammenfassung

Dung ist ein sehr komplexes Substrat und dient als Lebensraum für viele Pilzarten, Koprophile Pilze spielen eine wichtige Rolle im Nährstoffkreislauf, da die Pilze in der Lage sind, Lignin abzubauen und der Umwelt diese wieder zur Verfügung zu stellen. Obwohl koprophile Pilze ein wichtiger Bestandteil des Ökosystems sind, wurden sie in Österreich bislang nur wenig untersucht. Diese Arbeit, die Teil des Projektes „AustroDung“ des Österreichischen Biodiversitätsfonds, ist, hat zum Ziel, eine Artenliste koprophiler Pilze von 42 ausgewählten Standorten aus Österreich zu erstellen und die Methodik untereinander zu vergleichen. Um verschiedene Pilzgemeinschaften zu untersuchen, wurden sowohl Schaf- als auch Rinderdung beprobt. Mittels morphologischer Methoden und Metabarcoding wurden mehr als 400 Pilzarten identifiziert, darunter auch mehrere Pansenpilze, die mit rein morphologischen Analysen schwer erfassbar sind. Zusätzlich zu den 42 Probenahmestellen wurden auch andere Faktoren wie Entwurmung, Tierhaltung und Umwelteinflüsse berücksichtigt. Die Ergebnisse zeigen, dass Rinderdung im Vergleich zu Schafproben eine höhere Pilzvielfalt aber auch mehr Pilzgattungen aufweist. Es konnte ein erheblicher Unterschied zwischen der Artenvielfalt, der beiden Methoden festgestellt werden. Arten aus den Familien Ascobolaceae und Pilobolaceae waren im Dung am häufigsten vertreten.

Koprophile Pilze, Metabarcoding, Ascomycota, Basidiomycota, Dung, Dungpilze

Acknowledgements

I would like to express my heartfelt gratitude to Univ.-Prof. Mag. Dr. Irmgard Greilhuber, who supported me throughout this thesis with her continuous help, expertise and encouragement. My gratitude also goes to everyone involved in the AustroDung project, whose contributions were invaluable to the success of this work.

A special thank you to my friends, who accompanied me across Austria and always listened to my problems.

Most of all, I thank my mother, who sadly could not witness this moment but always believed in me and nurtured my love for nature from an early age.

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Introduction

Animal dung serves as an essential habitat for coprophilous fungi. These fungi can be categorized into facultative coprophilous fungi, which grow both on dung and other organic materials and strict coprophilous fungi, which exclusively occur on dung. The life cycle of coprophilous fungi already begins during the grazing of their host animal. Some fungi require passage through the digestive system for germination, while others can germinate directly without this process. During grazing coprophilous and other fungi are ingested, and most of the other fungi die in the gastrointestinal tract due to the gastric juices and high temperatures (Bell, 1983; Richardson, 2002; Newcombe et al., 2016). To survive the digestive tract of animals and protect their spores, many coprophilous fungi developed thicker cell walls and/or a gelatinous sheaths (Kuyper et al., 2021; Jasim et al., 2021). After getting digested, fruiting bodies develop on the dung and their spore are discharged. These spores then stick to the surrounding vegetation, which is then consumed by the animals (Newcombe et al., 2016).

Dung is a habitat characterized by intensive competition between fungi and bacteria, due to its high concentration of mineral nutrients and easily degradable carbon compounds (Kuyper et al., 2021). The production of bioactive secondary metabolites is involved in these interactions (Srivastava 2021).

The community composition of coprophilous fungi often reflects the diet of the host animals. For instance, different species are typically found on dung from herbivores compared to carnivores (Kruys & Ericson, 2008; Kuyper et al., 2021). However, closely related herbivores tend to host similar fungal communities (Richardson, 2001). *Pilobolus* species from the phylum Mucoromycota are among the first dung colonizers. They are followed by members of Ascomycota and Basidiomycota, though their appearance can overlap (Kuyper et al., 2021). Certain fungi, such as Basidiomycota and *Podospora anserina* are known for their ability to degrade lignin (Paoletti & Saupe, 2008), which could be a reason for their later appearance on the dung, because initially more easily degradable carbohydrates are broken down (Hudson, 1968; Harley, 1978; Kuyper et al., 2021).

Coprophilous fungi play a crucial role in nutrient cycling by storing and releasing nutrients into their environment, thus providing essential resources for plants and animals (Srivastava 2021). This process is however highly threatened. In the Netherlands some coprophilous Basidiomycota are already on the Red Data list even though they have an overload of manure. Dung from

cows or horses is often spread on fields as a slurry, which causes a decline in coprophilous fungi. Furthermore, natural habitats such as grasslands and forests offer more favourable conditions for the growth of coprophilous fungi compared to agricultural land. This is largely due to the higher lignin content in dung and a higher plant diversity found in these ecosystems. Also the use of antibiotics further contributes to the decline of coprophilous fungi and negatively impacts their populations (Kuyper et al., 2021).

Despite their important ecological role, studies on coprophilous fungi are rare. Knowledge about their occurrence and distribution is insufficient in Austria. Few observations have been conducted so far, highlighting the significance of the present thesis in contributing to the biodiversity research of coprophilous fungi in Austria. Schweiger's dissertation (1985) reported 93 coprophilous fungal species in Austria and a study in Montafon (Vorarlberg) by Welt & Heine (2006) lists 29 species. A comprehensive documentation of coprophilous fungi in Austria was conducted by Greilhuber and her team in the years 2021-2023 by recording 199 different fungal species on Heck cattle dung in the Lainzer Tiergarten (Vienna) (Greilhuber 2023).

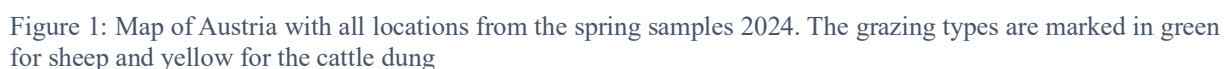
This thesis aims to further investigate and document coprophilous fungi species in all federal states of Austria. Efforts were made to select a wide range of sampling sites during the project “AustroDung” of the Austrian Biodiversity Fund, which addresses dung fungi and dung beetles. Both organic and conventional livestock farms were included. Besides morphological observation, the use of barcoding and metabarcoding enables detection of dung fungi that are not currently or not at all forming fruiting bodies and thereby leads to higher number of registered coprophilous fungi. Which is also the goal of this study, to expand the knowledge about the diversity of coprophilous fungi in Austria.

Coprophilous fungi

Several Mycoromycota, Basidiomycota and Ascomycota are growing on dung, so-called fimicolous or coprophilous fungi. Concerning classification, especially the phylum Ascomycota is undergoing vast changes due to new findings made possible by molecular genetic methods. Some fungi remain in an uncertain position (*Incertae sedis*) due to a lack of further knowledge (Dougoud et al., 2024). Among the most prominent fimicolous Ascomycota found on herbivore dung are the genera *Ascobolus*, *Saccobolus*, *Coprotus*, *Zygopleurage*, *Podospora*, *Schizoth-*

cium and *Lasiobolus*. In contrast, fimicoulous Basidiomycota are less diverse, with notable examples including ink caps such as *Coprinellus* and *Coprinopsis* species (Greilhuber 2023), (Schweiger 1985). The study by Calaca et al. (2023) clearly illustrates that the current observation of dung inhabiting fungi is still insufficient compared to other fungi groups. It also highlights that coprophilous fungi are increasingly being recognized for their relevance across various field. These fungi are not merely organisms adapted to their life in dung. They play an important role in medicine, environmental monitoring and even palaeontological studies. Their ecological significance underlines the necessity for further targeted research in this area (Calaca et al., 2023; Miranda et al., 2020).

A total of 42 locations were tested in spring and 43 in autumn in all nine federal states in Austria. The exact locations and sampling time are listed in Figure 1. The aim was to sample dung from two sheep and two cattle farms per federal state, but in spring, only two locations in Vorarlberg were sampled and in autumn, three. In Lower Austria six locations were sampled. The locations were carefully chosen based on information from dung beetle studies in Austria as well as the ecological characteristics of the respective areas and the willingness of the farmers.



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Figure 2: Size difference between sheep (left) and cattle dung (right)

Morphological characterization

For morphological characterization one of the three samples per location was plated onto sterilized petri dishes ensuring the dish was fully coated with dung. Due to the size of the dung, two petri dishes were filled. The petri dishes were then placed in high humidity chambers. In these chambers, moistened Seramis was placed at the bottom covered with paper. The boxes were kept at room temperature (19-24°C). During the summer months the temperature was slightly higher. Every few days, the boxes were moistened to ensure the dung from drying out and an air circulation was installed to circulate filtered air through an air hole automatically every 15 minutes.



Figure 3: Humidity chambers (left) and some of the dung samples (right)

Identification

The samples were checked daily visually for fungal growth under the stereo microscope (Leica Zoom 2000). Detailed examinations and photographic documentation was done with a Keyence VHK 6000 and a Zeiss Axio Imager.A1 with the software package Zeiss ZEN BlueEdition. The fungus was carefully removed from the dung with tweezers and placed on a microscope slide with a drop of water. It was gently pressed with a coverslip and further observed and photographed. Magnifications of 10x, 40x and 100x were used. To show the microchemical reactions (e.g. amyloidity or dextrinoidity) of certain structures, staining agents such as Lugol and Melzers were applied.

Morphological identification of the Ascomycota followed the book “Fungi Fimicoli Italici” by F. Doveri (2004). For the genus *Pilobolus* specialized literature of M. Reul (2020) and M. C. Greaves (2014) were used. For identifying ink cap mushrooms, the website “Bender-Biotop” (<https://www.bender-coprinus.de/anfang/startseite.html>, accessed on 08.05.2025) was helpful.

For almost every identified fungal species a fungarium sample was prepared. By removing the fungi from the dung, placing them in Eppendorf tubes and drying them at room temperature for several days before sealing. These vouchers are stored in the herbarium WU-Mycologicum.

Molecular Analysis

Sanger sequencing

Within the scope of this work, it was not possible to isolate and cultivate dung fungi on a larger scale and due to the difficult isolation of the fungi directly from the dung petri dishes, Sanger sequencing was only done for the samples of Lainzer Tiergarten (D31) from spring together with Chiara Öhlinger (Öhlinger, 2024).

When morphological identification failed, the fungi were prepared for sequencing. The fruiting body was carefully removed from the dung sample as cleanly as possible and placed on a malt extract agar plate with antibiotics.

For the agar plates, 10 g of malt extract and agar was weighted into a 500 ml flask and filled with 500 ml deionized water. This was carefully shaken and autoclaved at 1.5 bar and 121°C. To prevent as much as possible contaminations, antibiotics were added to the agar medium. As soon as the autoclave was below 70°C, 100 mg of streptomycin and penicillin G was added to the flask. To dissolve the antibiotics the flask was again carefully shaken and poured in sterile

plates. To make agar malt extract plates without antibiotics, the last step was skipped and the medium was removed from the autoclave and poured onto plates.

These steps were performed under a sterile laminar hood to avoid contamination. The plates were then sealed with parafilm and kept at room temperature to allow the species to grow. If multiple species started to grow on the plates, they were as many times as needed transferred to fresh malt extract plates without antibiotics, until only one species remained per plate and three replicates were done as backups.

For further processing the hyphae from one plate were scraped off as clean as possible and transferred into 2 ml Eppendorf tubes that were previously filled with 2% liquid malt extract.

For the 2% liquid malt extract medium, 50 ml deionised water and 1 g of malt extract was added to a 50 ml Schott bottle. This was then autoclaved at 1.5 bar and 121°C.

After adding the hyphae to the liquid malt extract, the tubes were then incubated on a shaker for 1-3 days until cloudiness in the medium was observed. Once a sufficient amount of material had grown, it was centrifuged, frozen at -21°C and subsequently lyophilized (Alpha 1-4 LDplus, Christ).

DNA Extraction

DNA extraction was done with the DNeasy Plant Mini Kit (Qiagen) with smaller modifications following a protocol by Michael Barfuss (unpublished data) and the help of Chiara Öhlinger (Öhlinger, 2024):

1. Put 5 glass beads into the freeze dried samples
2. Shake for 4 min at 30 1/s in the TissueLyser II.
3. Add 500 µl of AP1
4. Vortex and centrifuge
5. Add 5 µl RNase and vortex
6. Incubate the samples for 15 min at 65°C and centrifuge afterwards
7. Add 5 µl proteinase K and vortex
8. Incubate the samples for 15 min at 65°C and centrifuge again
9. Add 250 µl P3 and vortex
10. Incubate the samples on ice for 5 min and centrifuge
11. Add 125 µl Chloroform/isoamyl alcohol (must be done under the hood)

12. Shake the samples and again incubate on ice for 5 min
13. Centrifuge for 10 min at 11,000 g at 4°C
14. Transfer the upper phase to the QIAshredder spin column and centrifuge for 2 min
15. Transfer 650 µl to a new 2 ml Eppendorf tube
16. Add 1300 µl of AW1
17. Transfer the liquid to the mini column tubes and centrifuge at 6000 g for 1 min
18. Discard the liquid and repeat it 3 times
19. Transfer the column to a collection tube
20. Add 500 µl of AW1 onto the column and centrifuge at 8000 g for 1 min
21. Discard the liquid on the bottom
22. Add 500 µl of AW2 onto the column and centrifuge again at 8000 g for 1 min
23. Discard the liquid and repeat the step once more
24. Centrifuge at 10,000 g for 4 min.
25. Transfer the column to a 2 ml microtube
26. Elute the DNA by adding 80 µl AE buffer directly onto the column
27. Wait for 5 min
28. Centrifuge at 11,000 g for 1 min
29. Repeat the last 3 steps
30. Check the isolated DNA using a Nanodrop Spectrometer
31. Freeze the DNA until further processing.

PCR (polymerase chain reaction)

To test whether everything went smoothly during the DNA extraction and to continue with preparation for sequencing, a PCR (polymerase chain reaction) was performed. Amplification of the ITS and LSU regions, see Table 1 was done using the primers ITS4/ITS5 and LR5/LROR (White et al., 1990). The nuclear ribosomal internal transcribed spacer (ITS) region is often used as a marker in fungal environmental samples (Köljalg, et al. 2013).

Table 1: Primer sequences for Sanger sequencing

Primer	Sequence	Gen
ITS4	TCCTCCGCTTATTGATATGC	ITS
ITS5	GGAAGTAAAAGTCGTAACAAGG	ITS
LR5	TCCTGAGGGAACTTCG	LSU
LROR	ACCCGCTGAACTTAAGC	LSU

A Mastermix (Table 2) was prepared and 10 μ l of it was transferred to each 2 mL PCR tube and vortexed. Furthermore 2 μ l DNA was added and sealed afterwards.

Table 2: Mastermix for the PCR (Sanger sequencing)

Mastermix	
2X Phusion Green HS II (Thermo Scientific)	2,5 μ l
2X Phusion Plus Green (Thermo Scientific)	2,5 μ l
Trehalose (Vazyme)	2 μ l
BSA	0,1 μ l
ITS5 (5 μ M)	1 μ l
LR5 (5 μ M)	1 μ l

The 10 tubes were put into the thermocycler with the PCR program as shown in Table 3.

Table 3: Thermocycler program for Sanger sequencing

Temperature ($^{\circ}$ C)	Time (sec.)	Number of cycles
98	Preheat	
98	30	
98	15	35x
55	15	
72	60	
72	300	
8	∞	

Success of the PCR reaction was checked by gel electrophoresis. For the gel electrophoresis a 1.5% agarose gel was prepared and 1 μ l of the DNA were pipetted into the pockets and a 100 bp ladder was added.

The DNA reaction was then purified by adding 1 μ l FastAP to the PCR tubes to remove the dNTPs. This mixture was incubated for 45 min at 30 $^{\circ}$ C.

A new Mastermix (Table 4) was prepared for the sequencing reaction and all four primers were added separately.

Table 4: Second Mastermix for PCR (sanger sequencing)

Mastermix	
PCR water	2,7 µl
Seq. Buffer	1,7 µl
Trehalose (Vazyme)	2 µl
Primer	1 µl
Big Dye (Thermo Fisher)	0,6 µl

The mix was transferred into a well plate and 2 µl of the last PCR product was added and put into a new thermocycler program (Table 5).

Table 5: Thermocycler PCR program for the cycling reaction (Sanger sequencing)

Temperature (°C)	Time (sec.)	Number of cycles
96	Preheat	
96	60	
96	10	
50	5	35x
60	240	
8	∞	

After completing the cycling reaction, the products were further processed by Michael Barfuss, who carried out the Sanger sequencing with an 3730xl Genetic Analyser; Applied Biosystems.

Barcoding – Metabarcoding

For each location three subsamples were taken and filled separately in 2 ml Eppendorf tubes and stored at -18°C until further processing. The samples were then lyophilized at -20°C and 1 mbar for around 24 hours in a freeze dryer (Alpha 1-4 LDplus, Christ).

DNA Extraction

For DNA extraction the MagAttract PowerSoil Pro EP kit (Qiagen) was used following the protocol specified.

In PowerBead DNA Plates 100 mg lyophilized dung samples were weighed into and 150 µl PCR-water was added. Furthermore, 800 µl CD1 and 4 µl RNase A were pipetted. After finishing these steps the plate was sealed and placed into the FastPrep96 to homogenize the samples. The plate was carefully removed and centrifuged at 4000 rpm for 15 min at room temperature to produce a supernatant, from which 500 µl was carefully removed and transferred to a clean 1 ml Collection Plate. 300 µl CD2 was added and the samples were put into the centrifuge for another 15 min.

Then, 450 µl supernatant was transferred to a clean 96-well-plate without disturbing the residual pellet. The next washing steps were carried out by the robot and also the dilutions of the finished DNA.

PCR (polymerase chain reaction)

The extracted DNA was diluted 10× and 100× and a control PCR was carried out by Primer sets (Table 6) not only for fungi but also for nematodes and eukaryotes.

Table 6: Primer sets for metabarcoding

Organism	Forward primer	Reversed primer
Fungi	FQ-F	FQ-R
Nematodes	NemF	18Sr2b
Eukaryotes	NF 1	18Sr2b

A Mastermix (Table 7) was prepared by mixing the specific primer as well as PCR water and GoTagGreen and lastly 1 µl isolated DNA was added. So that in total 15 µl material was prepared in the tubes.

Table 7: Mastermix for PCR (metabarcoding)

Mastermix	
GoTagGreen	7,5 µl
PCR Water	3,5 µl
Forward Primer (10 µM)	1,5 µl
Reversed Primer (10 µM)	1,5 µl

For the polymerase chain reaction the program shown in Table 8 was followed.

Table 8: Thermocycler program (metabarcoding)

Temperature (°C)	Time (sec.)	Number of cycles
80	Preheat	
95	150	
94	30	35x
54	30	
72	45	
72	5	
4	∞	

To check for a successful DNA extraction, a fungus-specific PCR was performed using the FungiQuant primer (Liu et al., 2000). After the PCR, a 2% agarose gel was prepared for gel electrophoresis. Eight µl of the PCR product were loaded into each chamber and a 100 bp marker was used.

After a successful PCR the samples were prepared for sequencing. The primers were pipetted separately into Eppendorf tubes and diluted DNA was added. The aliquots were then sent to LGC Genomics in Berlin for high-throughput sequencing using Illumina sequencing (MiSeq platform, PE300).

Due to the time consuming progress, only the barcoding data from the spring samples will be discussed in this thesis.

Statistical analysis

The finished sequences of the sanger sequencing were subjected to the Basic Local Alignment Search Tool (BLAST).

After the completed Illumina sequencing the data was further processed by Harald Berger (Austrian Institute of Technology, Tulln), following the method described by Gorfer et al. (2021). Low-quality and very short sequences were removed and the sequences were merged afterwards. Sequences were then clustered into Operational Taxonomic Units (OTUs) with a maximum of 3% sequence difference and chimeric sequences were filtered out afterwards (Gorfer, et al. 2021).

Taxonomic affiliation was performed using the UNITE 10.0 database, to improve phylogenetic accuracy the data was manual edited. The OTUs were ecological guild mapped into different functional groups with the help of the following scientific literature: Riley et al., (2014), Hibbett (2006), Hibbett & Donoghue (2001), Binder & Hibbett (2006); Larsson et al., (2006); Miller, et al. (2006), Thorn et al., (2000), Slippers et al., (2013), Jaklitsch et al., (2015), Zhang et al., (2011), Landvik et al., (1998), Bender, et al. (2013), Kruys et al., (2014), Tedersoo & Smith (2013), Doveri (2011), Baschien et al., (2013), Krah et al., (2018), Rinaldi et al., (2008), Brundrett (2008), Diederich & Serusiaux (2000), Serusiaux et al., (2004). This mapping was conducted at the genus level of the fungi (Deltedesco et al., 2020; Gorfer et al., 2021).

Further processing of the successfully sequenced data was carried out in R version 4.3.1 (cran.r-project.org) by myself. The following packages were used for data analysis: phyloseq (McMurdie & Holmes 2013), metagMisc (R suite, Doi: 10.5281/zenodo.597622, unpubl.), vegan (Oksanen, et al. 2025) and microViz (Barnett, et al., 2021). The dataset was normalized and tested at 100, 200, 500 and 1000 reads per sample (rarefy). Since 126 out of 129 samples remained at 1000 reads per sample this threshold was used for further data evaluation.

For each sampling site additional information was collected regarding the farming methods, weather conditions, deworming, elevation, breed and climatic provinces.

Results and Discussion

Table 9 lists all identified fungal species. This includes both facultative and obligate coprophilous fungi, as well as rumen fungi, which were mostly found in cattle dung. Since the metabarcoding results for the autumn samples are missing, the table includes only spring data from both metabarcoding and morphological identification and autumn data based solely on morphological results. Further results will be incorporated and presented in the “AustroDung” project.

Table 9: List of coprophilous fungi: The species names follow the nomenclature of Mycobank. The table contains the federal state where the substrate was collected, the scientific species names, the livestock type of the substrate, the method used for the identification of the fungi and the person who identified the fungi. MB =Metabarcoding, MA = Morphological analyses, B = Burgenland, NÖ = lower Austria, W = Vienna, K = Carinthia, OÖ = upper Austria, V = Vorarlberg, T = Tyrol, STMK = Styria, S = Salzburg, MG = Markus Gorfer, HB = Harald Berger, EN = Elisabeth Nachtmann, CÖ = Chiara Öhlinger, MK = Matthaeus Koncilja

Species	Federal state	Cattle	Sheep	Method	Identified by
<i>Agaricus litoralis</i>	B	1	0	MB	MG, HB
<i>Agaricus pseudo-pratensis</i>	B; NÖ	2	1	MB	MG, HB
<i>Agaricus purpur-lesquameus</i>	W	1	0	MB	MG, HB
<i>Arnium irregulare</i>	B; K; OÖ	3	0	MA	MK
<i>Ascobolus albidus</i>	K	0	1	MA	EN
<i>Ascobolus elegans</i>	W	1	0	MA	EN, CÖ
<i>Ascobolus hawaiiensis</i>	W	1	0	MA	EN, CÖ
<i>Ascobolus immersus</i>	B; K; NÖ; OÖ; S; STMK; W; T; V	22	16	MA/MB	EN, CÖ, MK, MG, HB
<i>Ascobolus lineolatus</i>	K	1	0	MB	MG, HB
<i>Ascobolus michaudii</i>	NÖ; B; S	1	2	MA	EN, MK
<i>Ascobolus saccoboloides</i>	K; NÖ; OÖ; STMK	2	4	MA	EN, MK
<i>Ascobolus stercorarius</i>	OÖ; S; T; NÖ; W; B	8	4	MA/MB	EN, CÖ, MK, MG, HB
<i>Bolbitius titubans</i>	NÖ; OÖ; STMK; K; V	4	2	MA/MB	EN, MG, HB
<i>Candolleomyces sub-cacao</i>	W	0	1	MB	MG, HB
<i>Cephalotrichum telluricum</i>	B; OÖ; W; K	3	1	MB	MG, HB
<i>Chaetomidium cephalothecioides</i>	B	0	1	MA	MK
<i>Chaetomium elatum</i>	B	0	1	MA	EN
<i>Chaetomium globosum</i>	K	0	1	MA	EN
<i>Cheilymenia stercorea</i>	W	1	0	MB	MG, HB

<i>Cladorrhinum foecundissimum</i>	NÖ; OÖ; W; K; S	3	4	MB	MG, HB
<i>Cleistothelebolus nipigonensis</i>	K; NÖ; STMK; T; OÖ; S; W	6	13	MB	MG, HB
<i>Conocybe apala</i>	K; W	1	2	MB	MG, HB
<i>Conocybe macrospora</i>	K; V	2	0	MB	MG, HB
<i>Conocybe ochrostriata</i>	K	1	0	MB	MG, HB
<i>Conocybe pilosella</i>	B	1	0	MB	MG, HB
<i>Conocybe tenera</i>	B; W; V	2	1	MB	MG, HB
<i>Copranophilus spinuliformis</i>	NÖ; OÖ	1	1	MA	MK
<i>Coprinellus bulleri</i>	B	1	1	MA	EN
<i>Coprinellus curtus</i>	T	1	0	MA	EN
<i>Coprinellus disseminatus</i>	W	1	1	MB	MG, HB
<i>Coprinellus flocculosus</i>	OÖ; K; W; NÖ	1	3	MB	MG, HB
<i>Coprinellus hep-temerus</i>	B; K; OÖ; S; STMK; V; W; T; NÖ	13	18	MA/MB	EN, MK, MG, HB
<i>Coprinellus micaceus</i>	B; K; NÖ; V; OÖ; S; W	4	3	MB	MG, HB
<i>Coprinellus pusillulus</i>	NÖ; STMK	0	4	MA	MK
<i>Coprinellus xanthothrix</i>	W	1	1	MB	MG, HB
<i>Coprinopsis argentea</i>	B; W	2		MB	MG, HB
<i>Coprinopsis asiaticophlyctidospora</i>	B	1	0	MB	MG, HB
<i>Coprinopsis candidolanata</i>	NÖ; OÖ; S; STMK; W	0	6	MB	MG, HB
<i>Coprinopsis cinerea</i>	V; K; OÖ; S	1	5	MB	MG, HB
<i>Coprinopsis fluvialis</i>	B	1	1	MB	MG, HB
<i>Coprinopsis foetidella</i>	B; K; W; S; STMK	4	3	MA	EN
<i>Coprinopsis lagopus</i>	K; OÖ	0	2	MB	MG, HB
<i>Coprinopsis lilacina</i>	K; NÖ; OÖ; S; STMK; V; T	12	10	MB	MG, HB
<i>Coprinopsis narcotica</i>	K	0	1	MA	EN
<i>Coprinopsis nivea</i>	K; NÖ	2	1	MB	MG, HB
<i>Coprinopsis poli-omalla</i>	NÖ; OÖ; S; STMK	4	1	MA/MB	MK, MG, HB
<i>Coprinopsis pseudo-cortinata</i>	NÖ	2	1	MA	MK
<i>Coprinopsis pseudoradiata</i>	STMK; T	2	0	MA	EN
<i>Coprinopsis radiata</i>	K; NÖ; OÖ; V; W; B; S; STMK; T	11	17	MA/MB	MK, MG, HB
<i>Coprinopsis sclerotiorum</i>	B; K; OÖ; S; V; T; NÖ; W	8	10	MB	MG, HB
<i>Coprinopsis semitalis</i>	K; NÖ	0	2	MB	MG, HB
<i>Coprinopsis stercorea</i>	K; NÖ; W; OÖ; T; B	8	2	MA	EN, MK

<i>Coprinopsis utrifera</i>	S	0	1	MB	MG, HB
<i>Coprinopsis xenobia</i>	W; T	2	0	MB	MG, HB
<i>Coprinus comatus</i>	B	0	1	MB	MG, HB
<i>Coprobria granulata</i>	OÖ	0	1	MA	MK
<i>Coprotus aurora</i>	OÖ	0	1	MA	EN
<i>Coprotus disculus</i>	B; K; NÖ; OÖ; S; W; T; STMK	13	10	MA	EN, MK
<i>Coprotus glaucellus</i>	B; K; NÖ; OÖ; STMK; V; W; S	11	12	MB	MG, HB
<i>Coprotus granuliformis</i>	NÖ; OÖ	2	1	MA	MK
<i>Coprotus sexdecimsporus</i>	K; NÖ; W	3	3	MA	EN, MK
<i>Deconica crobula</i>	K; W	0	2	MB	MG, HB
<i>Deconica merdaria</i>	W	1	0	MA	EN, CÖ
<i>Echria gigantospora</i>	K	0	1	MB	MG, HB
<i>Enterocarpus grenotii</i>	S; W; K	2	1	MB	MG, HB
<i>Ephemerocybe brevisetulosa</i>	NÖ; OÖ; S; W; STMK; T; B	11	6	MA/MB	EN, CÖ, MK, MG, HB
<i>Ephemerocybe chris-tianopolitana</i>	B; W	0	3	MB	MG, HB
<i>Ephemerocybe ephemera</i>	B; OÖ; W; S; V	3	2	MB	MG, HB
<i>Ephemerocybe heterosetulosa</i>	OÖ; W; NÖ	2	1	MA	EN, CÖ, MK
<i>Ephemerocybe pellucida</i>	K; NÖ; OÖ; S; STMK; V; W	11	8	MA	EN, CÖ, MK
<i>Ephemerocybe sclerocystidiosa</i>	B; K; OÖ; W; NÖ; T; S; STMK; V	7	12	MB	MG, HB
<i>Gamsia columbina</i>	STMK	1	0	MB	MG, HB
<i>Helicocarpus griseus</i>	S	1	0	MB	MG, HB
<i>Hormographiella verticillata</i>	B	0	3	MB	MG, HB
<i>Iodophanus carneus</i>	K; OÖ; STMK; NÖ	3	3	MA	MK
<i>Iodophanus testaceus</i>	S; STMK	0	2	MB	MG, HB
<i>Kernia geniculotricha</i>	OÖ; W; NÖ	3	2	MB	MG, HB
<i>Kernia nitida</i>	K; NÖ; B; T	3	5	MA	EN, MK
<i>Lasiobolidium orbiculoides</i>	K; NÖ; T; B; W; V	3	5	MB	MG, HB
<i>Lasiobolus cuniculi</i>	NÖ	1	1	MA	MK
<i>Melanospora zamiae</i>	STMK	0	1	MA	MK
<i>Mucor roridus</i>	NÖ; B; K; OÖ; STMK; T; W	2	9	MA	EN, MK
<i>Narcissea cordispora</i>	NÖ	1	0	MA	MK
<i>Oedocephalum glomerulosum</i>	NÖ; W	1	1	MA	MK
<i>Orbilbia oligospora</i>	NÖ	1	0	MA	MK
<i>Ovatospora medusarum</i>	B	0	1	MA	EN

<i>Panaeolus foenisecii</i>	B; K; V; OÖ; S	3	5	MB	MG, HB
<i>Panaeolus papilionaceus</i>	B; K; S; V; NÖ; OÖ	7	5	MA/MB	EN, MG, HB
<i>Parasola misera</i>	NÖ	1	0	MA	MK
<i>Peziza arvernensis</i>	OÖ	0	1	MB	MG, HB
<i>Peziza fruticosa</i>	NÖ	0	1	MB	MG, HB
<i>Peziza scatigena</i>	OÖ	1	0	MA	MK
<i>Pilaira anomala</i>	B	1	1	MB	MG, HB
<i>Pilaira moreaui</i>	B	0	1	MB	MG, HB
<i>Pilobolus crystallinus</i>	OÖ; STMK; V; W; T; B; K; S; NÖ	15	20	MA/MB	EN, MK, MG, HB
<i>Pilobolus heterosporus</i>	S; V; NÖ; K; OÖ	3	3	MA	EN, MK
<i>Pilobolus kleinii</i>	B; K; NÖ; OÖ; S; STMK; V; W; T	20	19	MA/MB	EN, CÖ, MK, MG, HB
<i>Pilobolus lentiger</i>	NÖ; B; K; OÖ; S; STMK; V; T	14	12	MA/MB	EN, MK, MG, HB
<i>Pilobolus longipes</i>	T; K	2	1	MA/MB	EN, MG, HB
<i>Pilobolus pullus</i>	S; STMK; W; K; NÖ; V	3	4	MB	MG, HB
<i>Pilobolus umbonatus</i>	V; W; OÖ; K; STMK	3	5	MB	MG, HB
<i>Podospora anserina</i>	NÖ; STMK; B	2	4	MA	EN, MK
<i>Podospora australis</i>	B	0	1	MA	EN
<i>Podospora austrohemisphaerica</i>	K; OÖ; STMK; W	0	4	MB	MG, HB
<i>Podospora bulbillosa</i>	K; OÖ; STMK; W; T; B; NÖ; S	7	6	MB	MG, HB
<i>Podospora communis</i>	K; OÖ; STMK; B; NÖ; W; V	5	12	MA	EN, MK
<i>Podospora dacryoidea</i>	T; OÖ; V	1	3	MB	MG, HB
<i>Podospora fimiseda</i>	NÖ; W; OÖ; STMK	6	0	MA	EN, MK
<i>Podospora flexuosa</i>	OÖ	0	1	MB	MG, HB
<i>Preussia africana</i>	B; K; NÖ; OÖ; T; S; STMK	6	7	MB	MG, HB
<i>Preussia dubia</i>	W; NÖ	1	2	MB	MG, HB
<i>Preussia flanaganii</i>	B; K; OÖ; S; STMK; V; W; NÖ; T	13	16	MB	MG, HB
<i>Preussia fleischhakii</i>	K	1	0	MA	MK
<i>Preussia minipascua</i>	K; S; V; T; OÖ; STMK	5	6	MB	MG, HB
<i>Preussia octomera</i>	T	1	0	MB	MG, HB
<i>Preussia persica</i>	B; K; S; OÖ; STMK; T; NÖ; V; W	20	21	MB	MG, HB
<i>Preussia terricola</i>	B	1	0	MA	EN
<i>Protostropharia semiglobata</i>	K; V; OÖ	2	1	MB	MG, HB
<i>Psathyrella microrhiza</i>	W	1	0	MB	MG, HB

<i>Psathyrella panaeoloides</i>	B	1	1	MB	MG, HB
<i>Psathyrella piluliformis</i>	B	1	0	MB	MG, HB
<i>Psathyrella potteri</i>	OÖ; W; V	2	2	MB	MG, HB
<i>Pseudoechria curvicollella</i>	V; STMK	1	1	MA	EN
<i>Pseudoechria longicollis</i>	K; OÖ; W; STMK	2	5	MB	MG, HB
<i>Pyxidiophora petchii</i>	NÖ	1	0	MA	MK
<i>Rhyphophila decipiens</i>	B; NÖ; STMK; T; S; K	6	2	MA	EN, MK
<i>Rhyphophila myriospora</i>	B	0	1	MA	EN
<i>Rhyphophila pleiospora</i>	W; NÖ	1	1	MA/MB	EN, CÖ, MG, HB
<i>Saccobolus citrinus</i>	B; K; NÖ; OÖ; S; W; STMK; T	13	10	MA	EN, CÖ, MK
<i>Saccobolus depauperatus</i>	NÖ; STMK	0	3	MA	MK
<i>Saccobolus glaber</i>	K; NÖ; STMK; T	1	4	MA	EN, MK
<i>Saccobolus minimus</i>	B; NÖ; STMK; T; V	0	7	MA	EN
<i>Saccobolus ovibovinus</i>	K; NÖ; W	1	3	MA	MK
<i>Saccobolus succineus</i>	V	1	1	MA	EN
<i>Saccobolus truncatus</i>	B; K	2	0	MA	EN
<i>Saccobolus versicolor</i>	NÖ; STMK	0	2	MA	EN
<i>Schizothecium aloides</i>	NÖ; STMK; W	2	2	MA	EN, MK
<i>Schizothecium carpinicola</i>	B; K; NÖ; OÖ; S; STMK; V; W; T	18	19	MB	MG, HB
<i>Schizothecium conicum</i>	K; NÖ; OÖ; W; T; B; STMK	10	7	MA	EN, CÖ, MK
<i>Schizothecium curvuloides</i>	K; S; V; OÖ; NÖ; STMK	4	8	MB	MG, HB
<i>Schizothecium fimicola</i>	OÖ; V; W; NÖ	3	2	MB	MG, HB
<i>Schizothecium glutinans</i>	STMK	0	1	MA	EN
<i>Schizothecium miniglutinans</i>	W; OÖ; S	2	1	MA	EN
<i>Schizothecium tetrasporum</i>	W; B	1	1	MA	EN, CÖ
<i>Schizothecium vesticola</i>	B; STMK	0	3	MA	EN
<i>Selinia pulchra</i>	NÖ; STMK; W	0	4	MA	EN, MK
<i>Sordaria fimicola</i>	B; NÖ; OÖ; S; V; W; STMK; T; K	8	11	MA/MB	MK, MG, HB
<i>Sordaria inaequalis</i>	OÖ	0	1	MA	EN
<i>Sphaerobolus ingoldii</i>	K; W	1	3	MB	MG, HB
<i>Sphaerobolus stellatus</i>	B; S	1	1	MB	MG, HB

<i>Sporormia fimetaria</i>	NÖ; STMK; T	3	0	MB	MG, HB
<i>Sporormiella intermedia</i>	B; K; NÖ; OÖ; S; STMK; V; W; T	12	12	MB	MG, HB
<i>Sporormiella irregularis</i>	NÖ; W	2	0	MB	MG, HB
<i>Sporormiella leporina</i>	NÖ	1	0	MB	MG, HB
<i>Sporormiella megaspora</i>	B; K; NÖ; OÖ; STMK; T	9	6	MB	MG, HB
<i>Sporormiella minima</i>	K; NÖ; OÖ; S; V; STMK; B; W	10	10	MA	EN, MK
<i>Sporormiella pulchella</i>	NÖ	1	0	MB	MG, HB
<i>Sporormiella similis</i>	B; K; NÖ; W; STMK	6	7	MB	MG, HB
<i>Thecotheus inaequilateralis</i>	OÖ	0	1	MA	MK
<i>Thecotheus pelletieri</i>	NÖ; S; W	5	0	MA	EN, CÖ, MK
<i>Thelebolus globosus</i>	B; K; NÖ; OÖ; S; STMK; V; W; T	20	22	MB	MG, HB
<i>Thelebolus stercoreus</i>	STMK; OÖ	1	1	MA	EN
<i>Triangularia arizonensis</i>	B; NÖ; OÖ; STMK; W	7	4	MA/MB	EN, CÖ, MK, MG, HB
<i>Triangularia longicaudata</i>	K; OÖ; W; T; S; STMK; V; NÖ	6	10	MB	MG, HB
<i>Triangularia pauciseta</i>	NÖ; OÖ; S; STMK	5	3	MA	MK
<i>Triangularia pauciseta</i>	NÖ; OÖ; S; W; STMK; B; K	8	10	MA/MB	MK, MG, HB
<i>Triangularia phialophoroides</i>	T	1	0	MB	MG, HB
<i>Triangularia setosa</i>	OÖ; STMK; NÖ; T; W	2	3	MA	EN, MK
<i>Unguiculella tityrii</i>	NÖ	1	0	MA	MK
<i>Westerdykella nigra</i>	B; NÖ	1	1	MB	MG, HB
<i>Yunnania carbonaria</i>	OÖ	1	0	MB	MG, HB
<i>Zopfiella pleuropora</i>	V	1	0	MB	MG, HB
<i>Zygopleurage zygospora</i>	B; K; NÖ; S; W; OÖ	7	0	MA	EN, MK

Lundqvist (1972) mentioned that some species prefer specific dung substrates. To follow up this idea I summarized all species that were found exclusively on one substrate type to identify possible patterns. The following species were analysed exclusively on cattle dung

<i>Agaricus litoralis</i>	<i>Coprinopsis xenobia</i>	<i>Psathyrella piluliformis</i>
<i>Agaricus purpurlesquameus</i>	<i>Deconica merdaria</i>	<i>Pyxidiophora petchii</i>
<i>Arnium irregulare</i>	<i>Gamsia columbina</i>	<i>Saccobolus truncatus</i>
<i>Ascobolus elegans</i>	<i>Helicocarpus griseus</i>	<i>Sporormia fimetaria</i>
<i>Ascobolus hawaiiensis</i>	<i>Narcissea cordispora</i>	<i>Sporormiella irregularis</i>
<i>Ascobolus lineolatus</i>	<i>Orbilina oligospora</i>	<i>Sporormiella leporina</i>
<i>Cheilymenia stercorea</i>	<i>Parasola misera</i>	<i>Sporormiella pulchella</i>
<i>Conocybe macrospora</i>	<i>Peziza scatigena</i>	<i>Thecotheus pelletieri</i>
<i>Conocybe ochrostriata</i>	<i>Podospora fimiseda</i>	<i>Triangularia phialophoroides</i>
<i>Conocybe pilosella</i>	<i>Preussia fleischhakii</i>	<i>Unguiculella tityrii</i>
<i>Coprinellus curtus</i>	<i>Preussia octomera</i>	<i>Yunnanica carbonaria</i>
<i>Coprinopsis asiaticiphlyctidospora</i>	<i>Preussia terricola</i>	<i>Zopfiella pleuropora</i>
<i>Coprinopsis pseudoradiata</i>	<i>Psathyrella microrhiza</i>	<i>Zygopleurage zygospora</i>

While most of these species were detected only once, it is quite difficult to make a conclusion about their substrate preferences. However, *Podospora fimiseda*, *Zygopleurage zygospora*, *Ascobolus stercorarius* and *Thecotheus pelletieri* were analysed at five or more cattle sites, which may indicate a stronger association with cattle dung.

Table 10: Species growing and analysed exclusively on sheep dung

<i>Ascobolus albidus</i>	<i>Coprobria granulata</i>	<i>Podospora australis</i>
<i>Candolleomyces subcacao</i>	<i>Coprotus aurora</i>	<i>Podospora austrohemisphaerica</i>
<i>Chaetomidium cephalothecoides</i>	<i>Deconica crobula</i>	<i>Podospora flexuosa</i>
<i>Chaetomium elatum</i>	<i>Echria gigantospora</i>	<i>Rhypophila myriospora</i>
<i>Chaetomium globosum</i>	<i>Ephemerocybe christianopolitana</i>	<i>Saccobolus depauperatus</i>
<i>Coprinellus pusillulus</i>	<i>Hormographiella verticillata</i>	<i>Saccobolus minimus</i>
<i>Coprinopsis candidolanata</i>	<i>Iodophanus testaceus</i>	<i>Saccobolus versicolor</i>

<i>Coprinopsis lagopus</i>	<i>Melanospora zamiae</i>	<i>Schizothecium glutinans</i>
<i>Coprinopsis narcotica</i>	<i>Ovatospora medusarum</i>	<i>Schizothecium vesticola</i>
<i>Coprinopsis semitalis</i>	<i>Peziza arvernensis</i>	<i>Selinia pulchra</i>
<i>Coprinopsis utrifera</i>	<i>Peziza fruticosa</i>	<i>Sordaria inaequilateralis</i>
<i>Coprinus comatus</i>	<i>Pilaira moreaui</i>	<i>Thecotheus inaequilateralis</i>

Most of the fungi in Table 10 were found and identified in one sample of sheep dung. The species *Coprinopsis candidolanata*, *Selinia pulchra*, *Saccobolus minimus*, *Tulosesus christianopolitanus* and *Hormographiella verticillata* were found more than four times, which could indicate a potential substrate preference. Nevertheless, to draw reliable conclusions about substrate specificity further studies across a wider range of dung types are necessary. Schweiger (1985) already explored fungal growth on different substrates in more detail, yet he too was unable to determine clear substrate preferences. The online forum <https://www.pilzforum.eu> (accessed on 19.04.2025) regularly features new reports on coprophilous fungi. However, most observations originate from Germany and a thorough review of these postings would be required.

Morphological results

The morphological analyses resulted in 113 fungi species, including mostly coprophilous species but also saprotroph and nematode parasites. The analysis led to the detection of 96 coprophilous species, see Figure 4.

14 species remained unidentified. Many of these were inkcap species, which have a very short growth phase and often lack key identification features. Another challenge during identification was the presence of many fungi in asexual stages, which also made it difficult to determine them further.

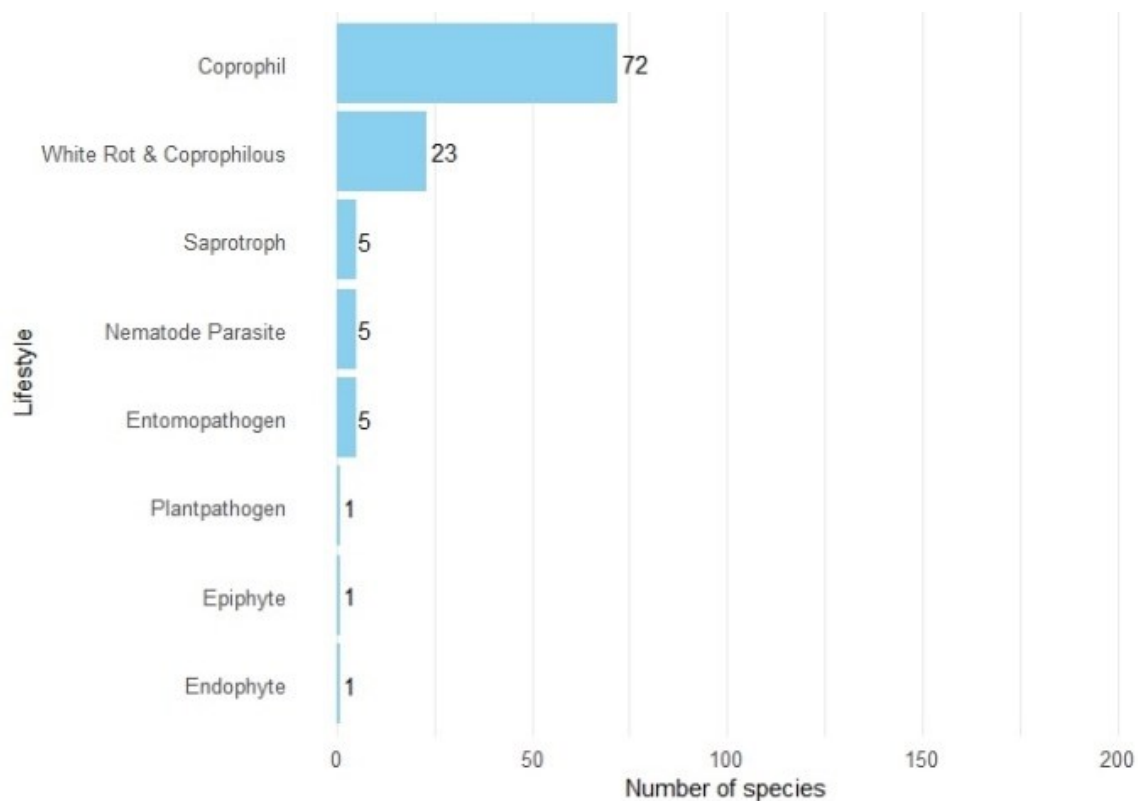


Figure 4: Classification of fungi based on their lifestyle. In this figure all fungi which were identified on morphological features are included

Species occurrence

Since the exact number of fungal fruiting bodies was not counted, the data in Figure 5 refers to the number of dung samples on which each fungi species was found. Although *Pilobolus kleinii* stood out in terms of frequency compared to other fungi, several other species also appeared

quite often, mostly representatives of Ascomycota. The species *Coprotus disculus* and *Saccobolus citrinus* were each found on 23 dung samples, while *Ascobolus immersus* was found on 22. Among the ten most common coprophilous fungi, only one Basidiomycete, *Tulosesus pellucidus*, is present, occurring on 19 samples. This result was expected, as Ascomycota are particularly common on dung (Richardson 2001). *Ascobolus immersus* and *Coprotus disculus* were already frequently detected in studies conducted between 2021 and 2023 (Greilhuber, 2023).

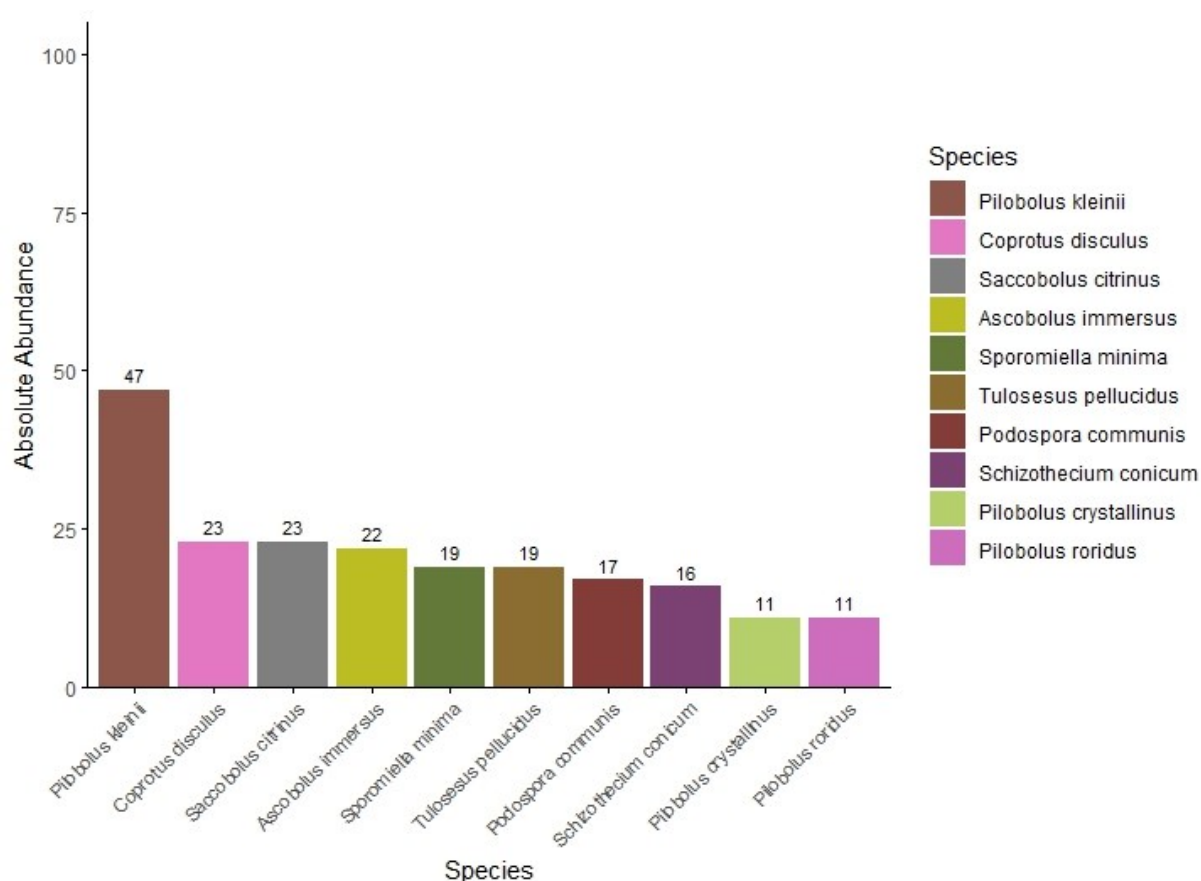


Figure 5: The 10 most common found coprophilous fungi on dung, during the morphological analyses. The percentage was calculated by on how many dung samples the fungi grew and not the quantity of the individual fungi

Seasonal occurrence

Within the morphological analyses, the phylum Ascomycota was the most prominent group of fungi found in the dung samples (see Figure 6). Among them, the genera *Saccobolus* (11.3%), *Ascobolus* (7.6%) and *Podospora* (7.6%) were especially common. In addition to Ascomycota, representative of the phyla Basidiomycota and Mucoromycota were also detected. This composition is typical for dung (Richardson 2001).

The proportion of Ascomycota remained relatively stable at around 60%. One of my hypotheses was that more Basidiomycota would appear in autumn, as this season is generally favourable for fungal growth. However, this assumption could not be confirmed, in addition the mean temperature in spring was 13.7°C, while in autumn it was at 14.2°C. It seems that the season does not significantly affect the distribution of fungal phyla.

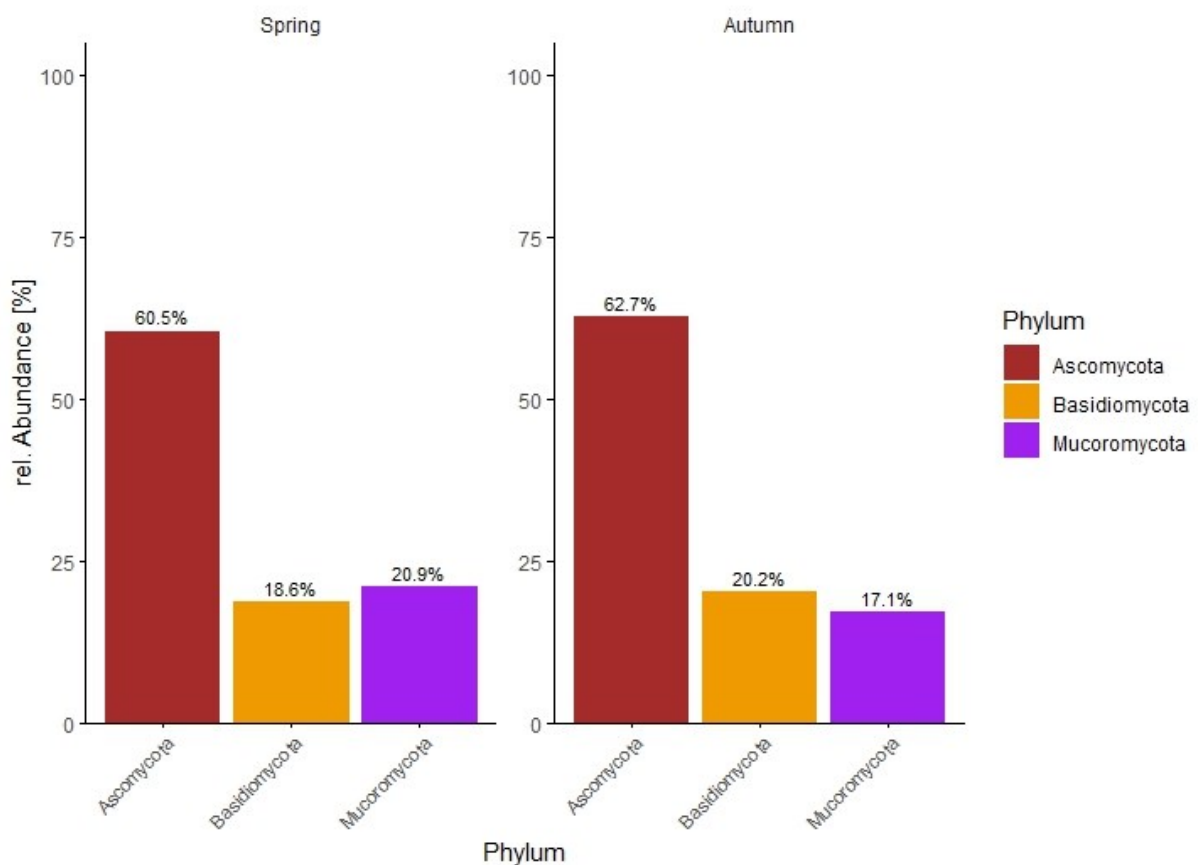


Figure 6: Rel. Abundance of fungi on Phylum level. Comparison between spring and autumn samples



Figure 7: *Ascobolus immersus*

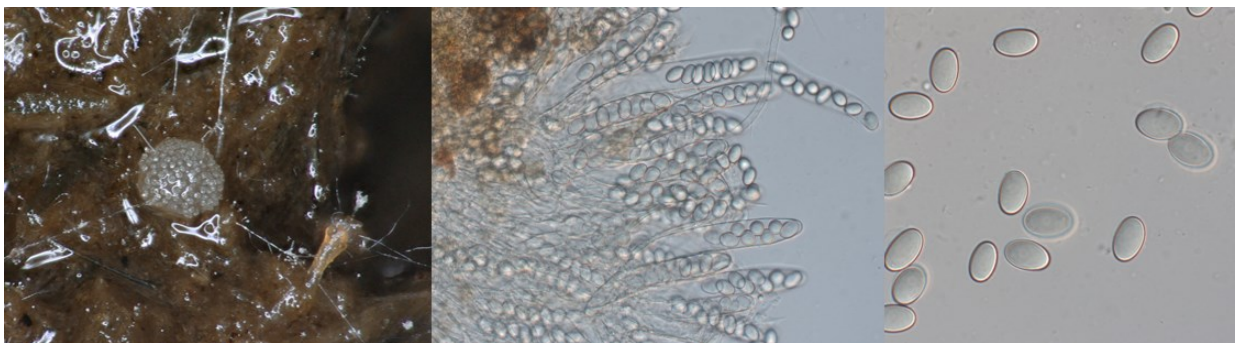


Figure 8: *Coprotus disculus*



Figure 9: *Saccobolus citrinus*

However, a total of 10 fungal genera (Figure 10) were exclusively found in the autumn samples, including species of the genera *Chaetomium*, *Peziza* and *Ascodesmis*. In contrast, 15 genera were found only in spring, among them *Arnium*, *Bolbitius* and *Deconica*. Seven out of these fungi are not considered to be obligate coprophilous fungi and many of them are known to occur also in autumn.

In the study by Richardson (2001), certain fungi species were also observed to grow more frequently in winter samples. However, the frequency curves for both seasons (summer and winter) were nearly identical. This suggests that the environmental conditions, rather than the season itself, might be more important in determining fungal growth. In this study, the average perception for five days prior to sampling was calculated. In spring it was 21.8 mm, while in autumn it was only 8.7 mm. This supports the assumption that the environmental condition in spring were more favourable for fungi development, with both temperature and moisture level being more optimal for fungi growth.

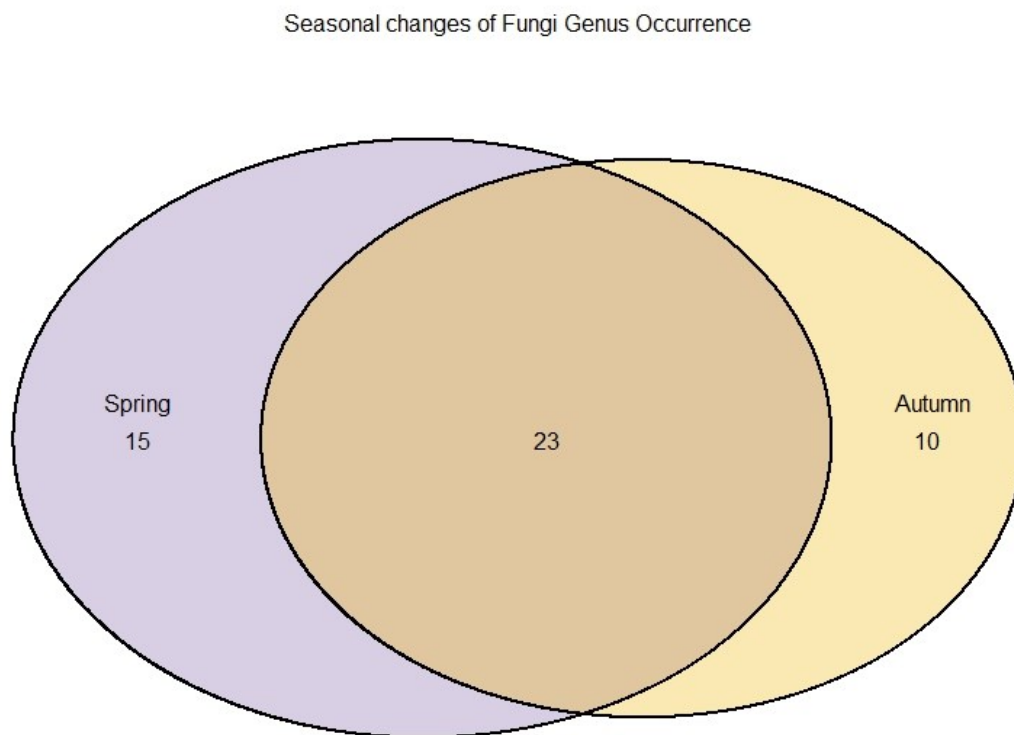


Figure 10: Comparison of spring and autumn genera

Substrate comparison

The most prominent coprophilous fungi identified in the morphological analyses belong to the genus *Pilobolus*, which makes up around 22%, followed by *Saccobolus* at 15.9% and Psathyrelaceae at 11.9% in cattle samples and 6.5% in sheep samples. The composition between cattle and sheep is not as different as expected (Figure 11). This could be because both animals are herbivores and the fungal families do not appear to distinguish significantly between them. In addition to the coprophilous fungi there were also other fungi growing on the dung, including *Parasola misera*, *Pyxidiophora petchii*, *Mucor racemosus*, *Copranophilus spinuliformis*, *Orbilia oligospora*, *Chaetomium elatum* and *Chaetomium globosum*.

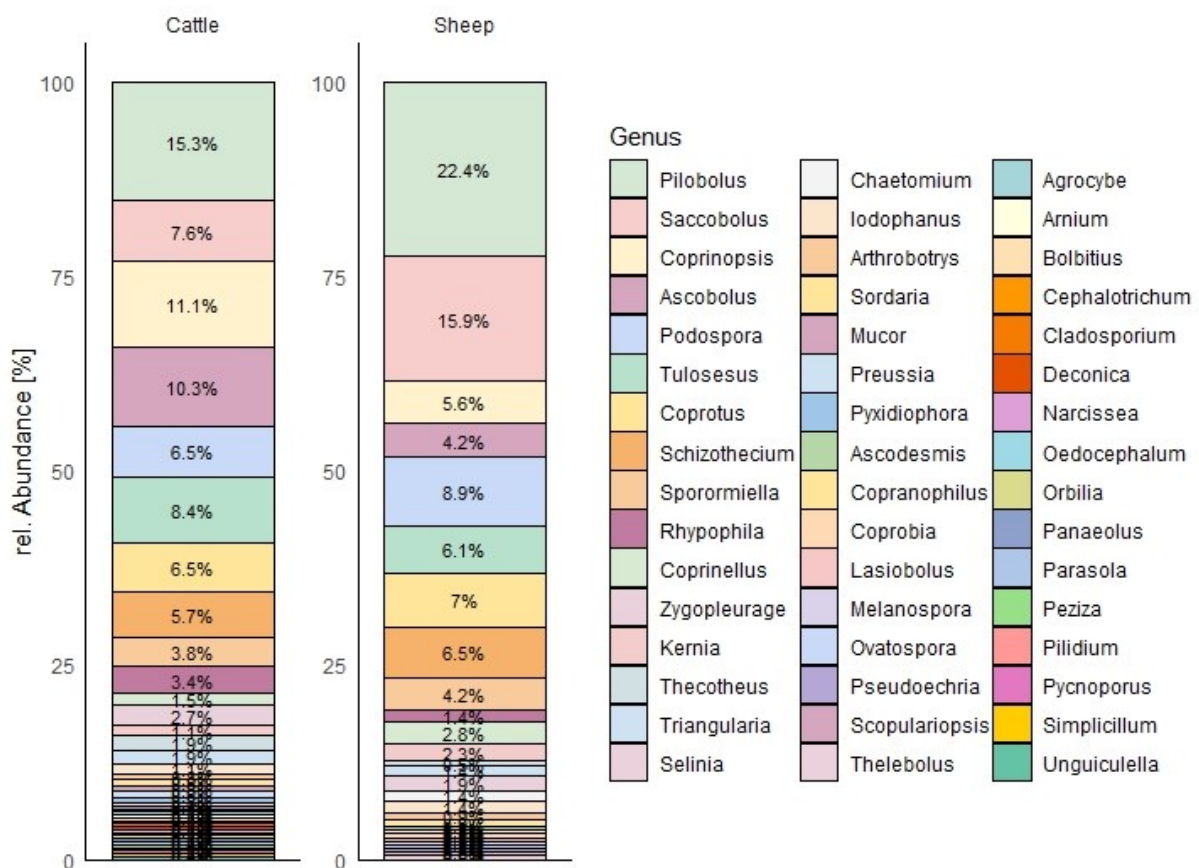


Figure 11: Rel. Abundance of fungi based on their genus level for cattle and sheep dung

It was observed that in both cattle and sheep dung, Ascomycota accounted for more than 60% of the fungal community, see Figure 12. In cattle dung, Basidiomycota followed with 23.7% and Mucoromycota with 18%. In contrast, in sheep dung, Mucoromycota ranks second with

22.4%, followed by Basidiomycota at 14.5%. This indicates a slight difference in the composition of fungal phyla between the two herbivorous animal species. However, after performing a t-test, the difference was not statistically significant ($p=0.69$), suggesting that the observed variation may have occurred by coincidence.

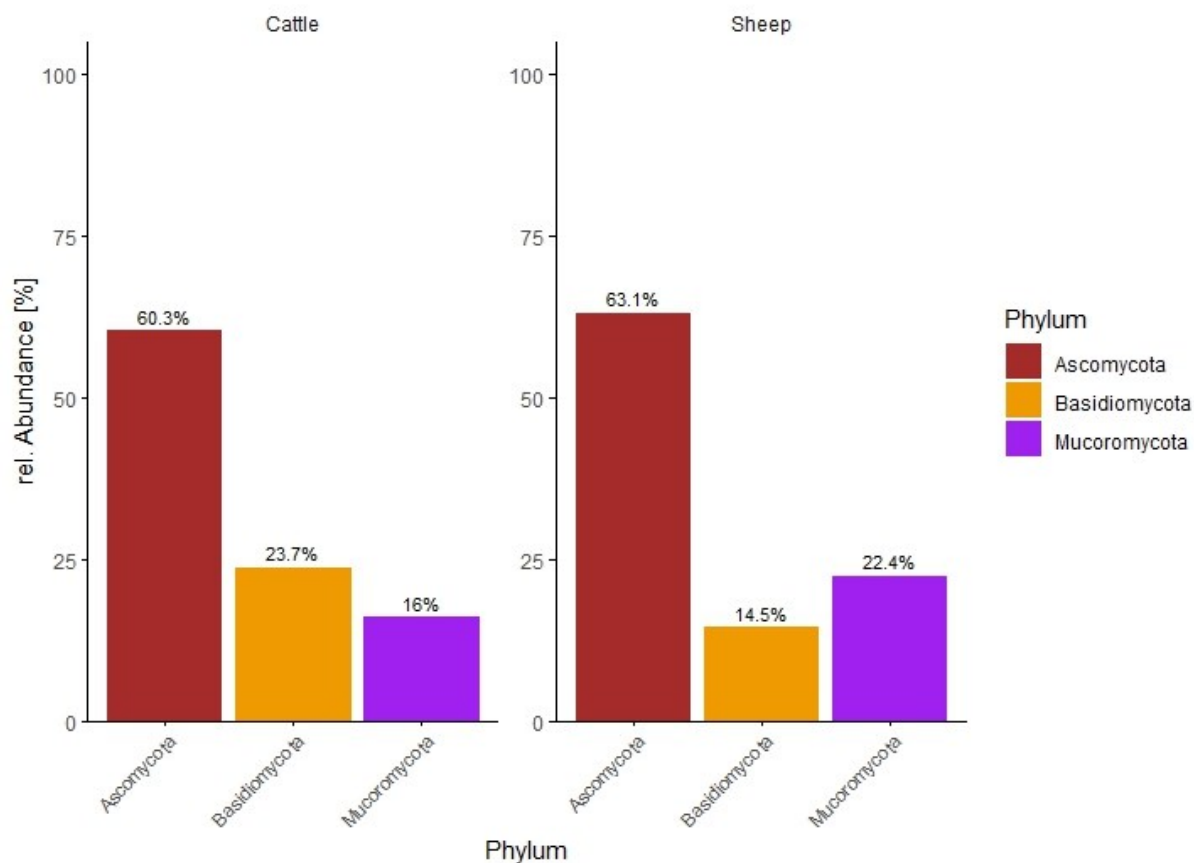


Figure 12: Rel. Abundance of fungi on Phylum level. Comparison between cattle and sheep dung samples

Genus *Pilobolus*

The most dominant fungi species growing on herbivore dung belong to the *Pilobolaceae* family. This group of fungi is characterized by their unbranched sporangiophores, which stand upright and grow towards the light. At the tips of the sporangiophores are the sporangia, which contain the spores. These spores are forcibly discharged from the sporangia under high pressure to ensure effective dispersal (Mahardhika et al., 2024).

Piloboli were one of the fastest growing fungi on dung. It usually took around 2 – 5 days until the first sporangiophores were visible. A total of 11 *Pilobolus* species were identified. Among them, an interesting finding, a presumed *Pilobolus kleinii* variation. This species was identified

by Matthaeus Koncilia with the literature of Reul (2020) and Viriato (2008) and originated from the site in Thalgau (sample D_51) in Salzburg. The family Pilobolaceae was found in both cattle (8.4%) and sheep dung (10%). Some species showed a higher frequency occurrence than others. This pattern has already been noted by Nyberg & Persson (2002) and Richardson (2001). *Pilobolus kleinii* was particularly prominent, growing on 47 out of 85 dung samples (spring and autumn in total). *P. kleinii* is characterized by its large spores, typically over 10 μm in length, yellowish and ellipsoid in shape (Figure 13). Also clearly visible is a constriction between the spore capsule and the subsporangial vesicle (Reul, 2020). In Contrast, *Pilobolus lentiger* (Figure 14) has variably sized, round to subglobose spores and lacks the constriction seen in *P. kleinii*. The spores of *P. lentiger* can reach up to 18 μm in size (Reul, 2020).



Figure 13: *Pilobolus kleinii*



Figure 14: *Pilobolus lentiger*

Genus *Pyxidiophora*

An interesting finding was that fungi of the genus *Pyxidiophora* were observed growing on some of the dung plates. In total, two species were identified: *Pyxidiophora petchii* and *Pyxidiophora spinuliformis* (Figure 15). This genus is typically parasitic and not limited to dung; it has also been found on other fungi, vascular plants, and myxomycetes (Blackwell & Malloch, 1989). The study by Blackwell and Malloch (1989) suggests that *P. spinuliformis* is associated with dung beetles and mites, which serve as dispersal agents for the fungal spores. Mites are considered particularly important, as they are faster and often more abundant on dung, allowing for more efficient spore dispersal (Blackwell & Malloch 1989). I was only able to identify the genus *Pyxidiophora* in the autumn samples, which aligns with the findings of Blackwell and Malloch (1989), who state that *Pyxidiophora* species prefer moist conditions. These conditions were indeed more prevalent at these sampling sites in autumn compared to May/June.

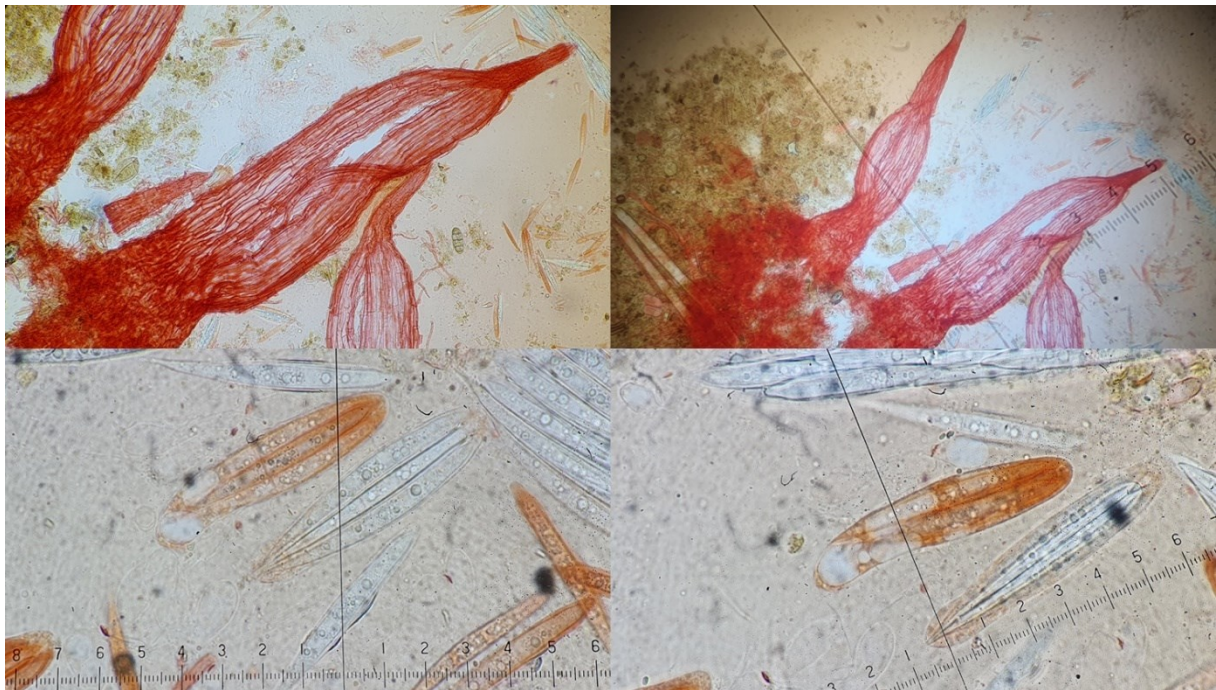


Figure 15: *Pyxidiophora spinuliformis*, photographed and identified by Matthaeus Koncjlia

Other Fungi specimens

Some fungi were also observed growing near dung samples in the field. These specimens were identified by Irmgard Greilhuber. Whether these fungi have any influence on the coprophilous fungi is unclear. However, in order to consider all possible factors during sample collection, I documented and recorded any fungal species found in the surrounding environment at every sampling site. Most of the sites did not have any growing fungi, in the near surrounding areas. At the location St. Martin bei Gmünd in Lower Austria I found several fungal species including *Agrocybe pediades*, *Panaeolus papilionaceus* (= coprophilous), *Pycnoporus cinnabarinus* and *Lentinus substrictus*.

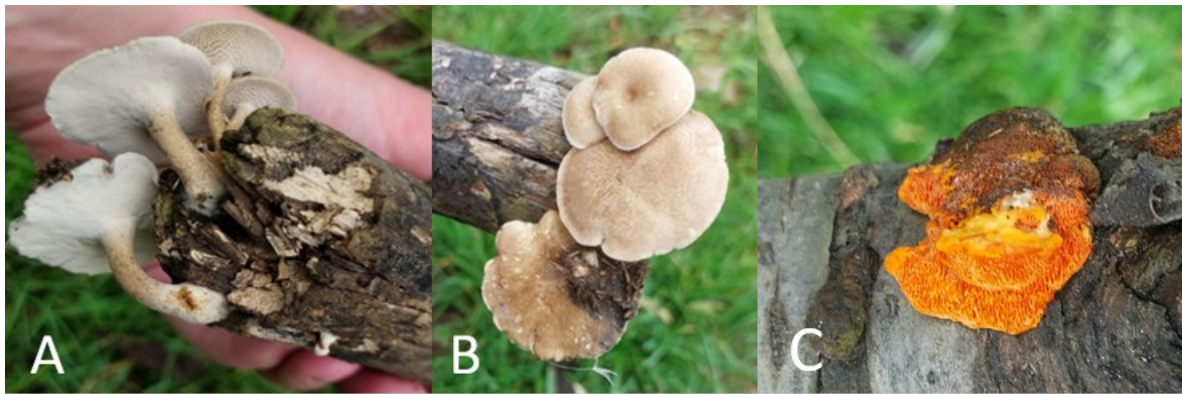


Figure 16: A/B *Lentinus substrictus* and C *Pycnoporus cinnabarinus*

Sanger sequencing analysis

These results were previously presented in the bachelor thesis of Chiara Öhlinger (2024). Therefore, they will only be briefly mentioned here and not discussed in detail.

A total of 10 fungal species from the Lainzer Tiergarten site (taken during spring) were analysed. Only 5 of them could be identified on the species level, see Table 11. The results obtained from the ITS region proved to be more precise than those from the LSU region. This is likely due to the fact that the ITS region has received more attention in fungal research and has therefore been extensively studied. Foos et al. (2010) mentions that the nuclear large subunit 28S ribosomal RNA (LSU) is useful for distinguishing genera but often lacks the resolution required for species level identification.

Table 11: Isolated fungi from the sample location „Lainzer Tiergarten“. The abbreviations listed in the left column (Strain ID), refer to the agar plates on which the fungi were cultivated.

STRAIN ID	ITS	LSU
B	<i>Simplicillium lanosoniveum</i>	<i>Simplicillium lanosoniveum</i>
BY	<i>Pilidium lythri</i>	<i>Pilidium lythri</i>
C	<i>Kernia columnaris</i>	<i>Kernia columnaris</i>
M	<i>Cladosporium herbarum</i> agg.	<i>Cladosporium herbarum</i> agg.
N	<i>Mucor hiemalis</i>	<i>Mucor hiemalis</i>

Metabarcoding

The evaluation of the samples conducted by metabarcoding resulted in 692 different fungi species. This includes saprotrophic species, plant pathogens and coprophilous fungi species, see Figure 17.

Figure 17 shows a broad ecological diversity of fungi, ranging from decomposer to symbionts and parasite, which were identified in this study. In total 21 different ecological lifestyles were documented. However, 22.3 % of the fungi could not be assigned to a specific ecological role and some lifestyles were resented by only a few species. Coprophilous fungi made up most of the dung, in total 65%, followed by saprotrophs, rumen fungi and lichens.

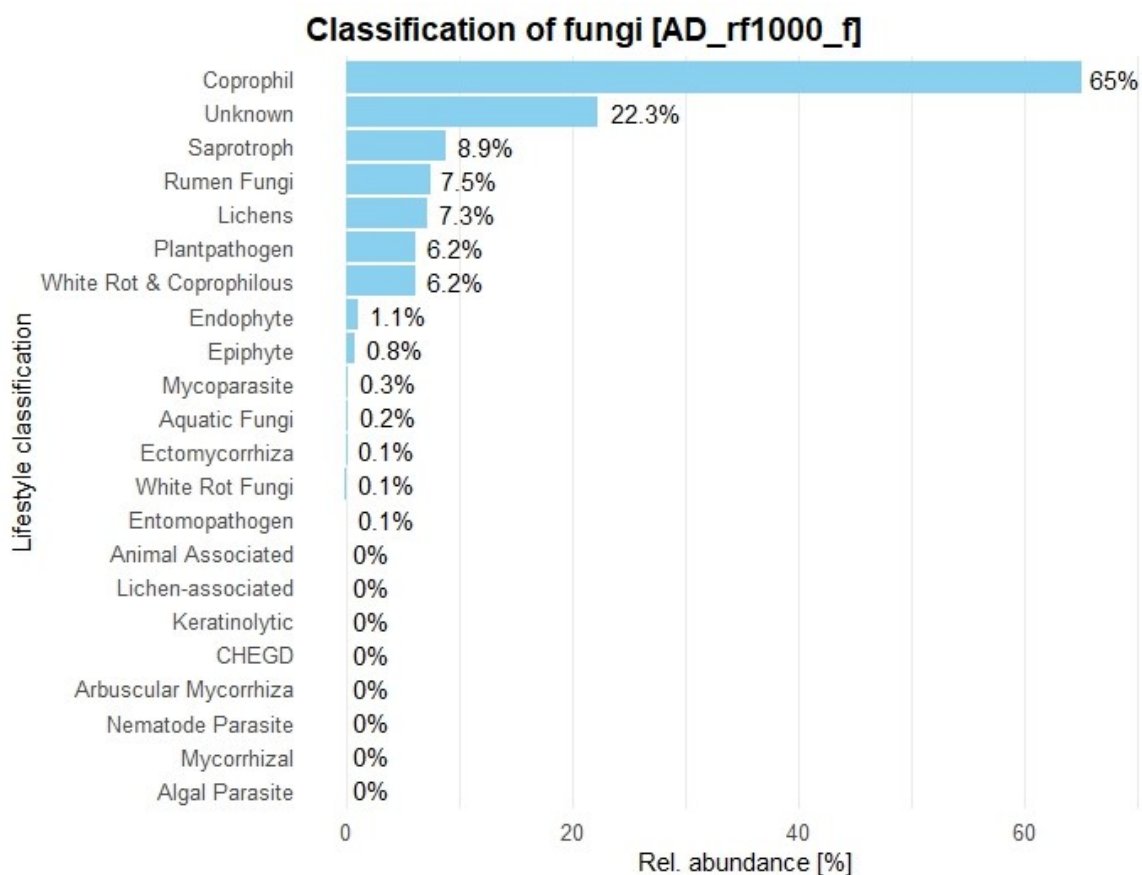


Figure 17: Classification of fungi groups based on their environmental lifestyle. The last eight lifestyle groups only have very few individuals and make up only 0.001 % on their own. For the sake of completion the eight groups were left in the plot.

Fungal community

In total 448 fungal genera were identified, highlighting the ecological richness and complexity of dung as a habitat. In Figure 18 the category “others” include all genera representing less than 1% of the total diversity. The most species rich genus is *Ascobolus* accounting for 14.9% of all identified taxa, followed by *Thelebolus* (12.8%) and *Pilobolus* (11%). This confirms that obligate coprophilous fungi are among the most detected fungi genera. Interestingly, not only coprophilous fungi were detected but also a genus of lichens, representing 5.7% of the dataset. Furthermore, *Ustilago*, a plant pathogenic fungus, represents 1.3% of the total abundance.

Additionally, some gut fungi were found, including the genera *Caecomyces* (1.7%) and *Piromyces* (1.2%). Several fungi of the family Neocallimastigaceae could not be identified beyond the family level. Overall, 1.8% of the sequences could only be assigned to the kingdom fungi and not to a more specific taxonomic level. A particularly interesting finding is the detection of the genus *Antarctomyces*, which comprises only two known species endemic to Antarctica. These fungi are highly specialized for cold environments and are classified as psychrophilic.

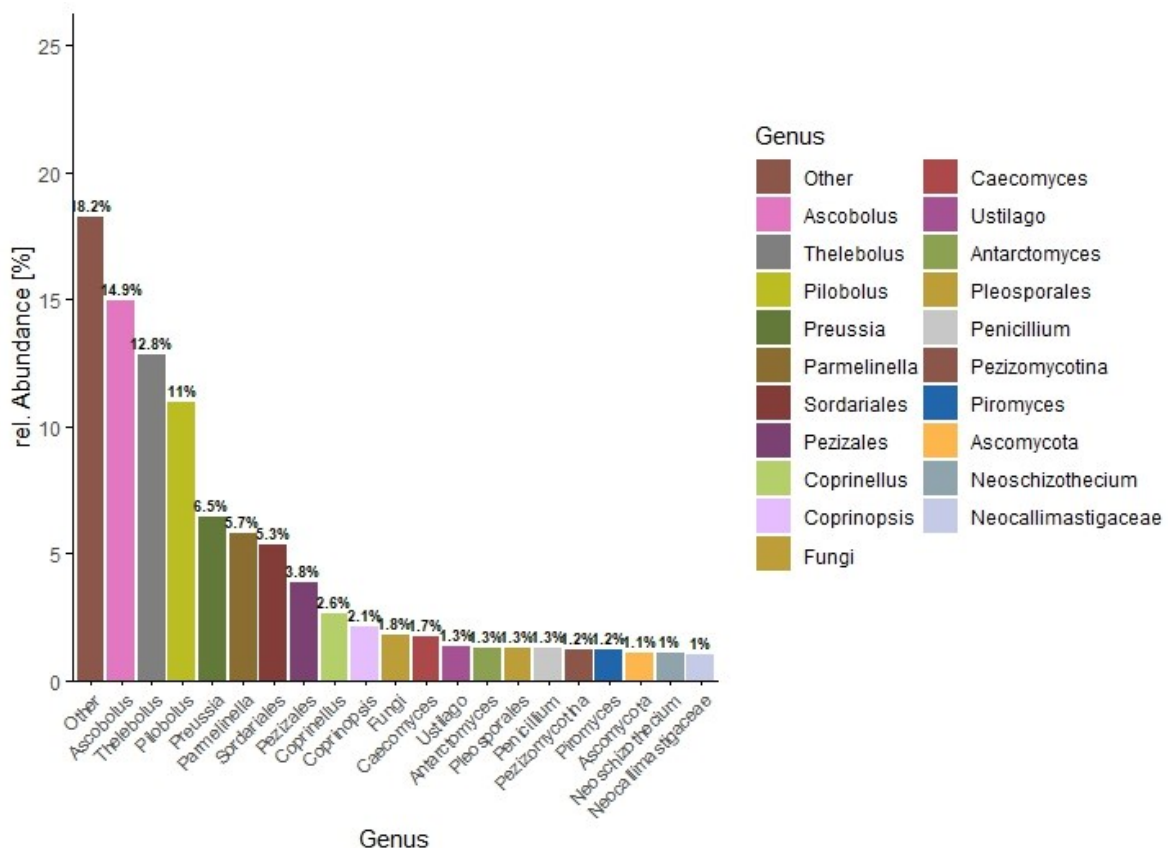


Figure 18: Relative abundance of fungal genera from the spring data. All fungi with a relative abundance below 1 were merged as “other”.

Comparison of sheep and cattle dung

The calculation of the Shannon Index showed that cattle samples had slightly higher diversity compared to sheep samples (t-test $p = 0.01703$), see Figure 19.

This could have several reasons. Cattle dung has a much larger volume than sheep dung. Sheep dung also dries out faster because of its small size. Davis (1987), van Geel et al. (2003), Davis & Shafer (2006) and Baker et al. (2012) speak of an assumption that larger herbivores also have higher fungi biomass, due to their bigger herbivore biomass. All these factors could contribute to the higher diversity found in cattle dung.

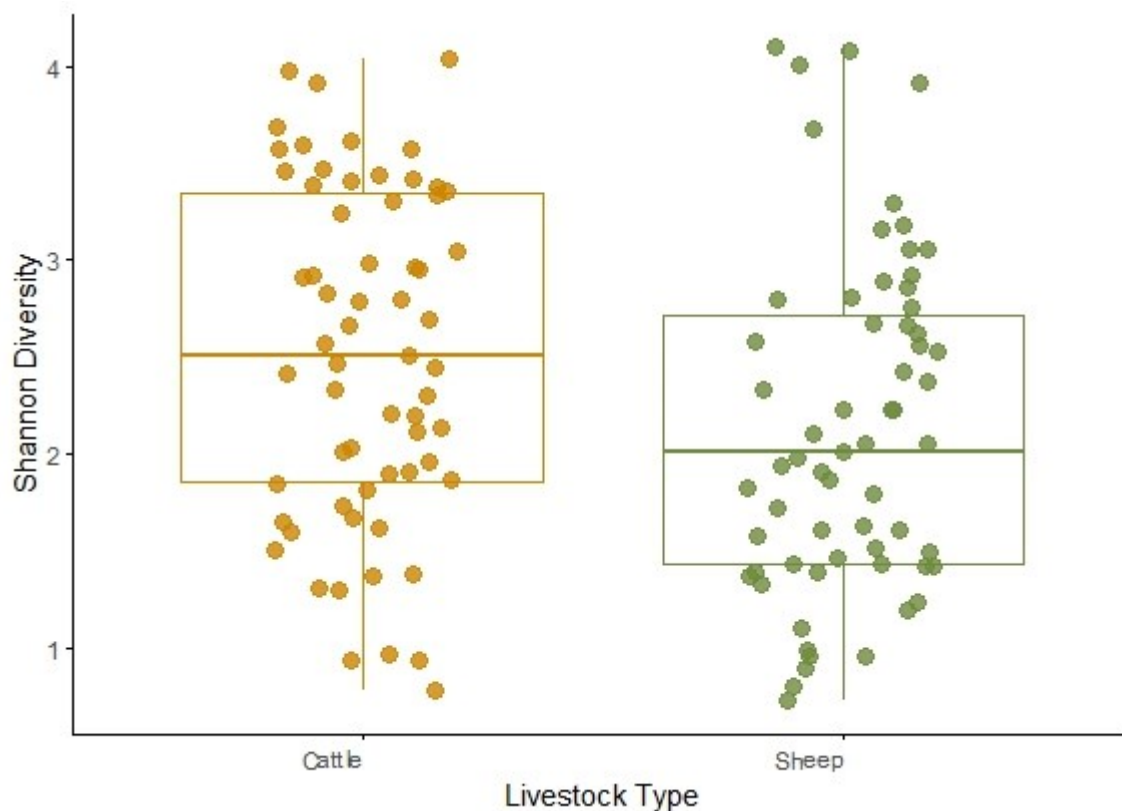


Figure 19: Comparison of sheep and cattle dung by their fungal diversity

A comparison of the fungal genera between the two host animals revealed that the most frequently occurring genera are quite similar in both dung types, see Figure 20. These includes *Ascobolus*, *Thelebolus*, *Preussia*, *Sordariales*, *Pilobolus* and the lichen genus *Parmelinella*. Notably, *Parmelinella* was much more frequently detected in cattle dung (9.7%) compared to

sheep dung (1.8%). Additionally, members of the family Neocallimastigaceae, a group of obligate anaerobic gut fungi, were found predominantly in cattle dung. This is likely due to the larger digestive system of cattle. In contrast, *Pilobolus* species appeared more frequently in sheep dung. *Coprinellus* and *Coprinopsis*, two coprophilous Basidiomycota, were also more common in sheep dung, accounting for less than 1% in cattle dung samples.

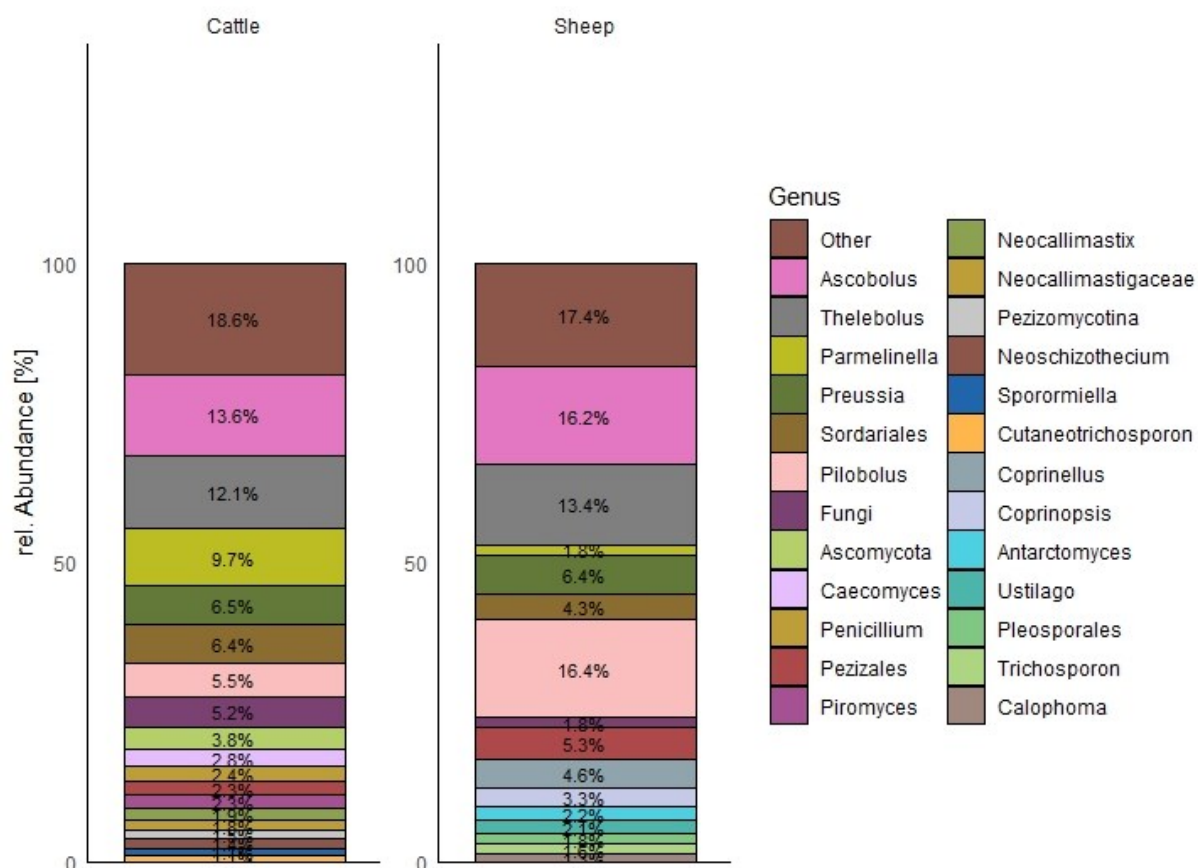


Figure 20: Comparison of cattle and sheep dung and presentation of the most common genera. Relative Abundance calculated for each animal dung

Sampling sites with sheep and cattle dung

Since the sampling included three locations where sheep and cattle were on the same pasture or very close together, I examined these sites more closely. The sampling sites are shown in Figure 21. In Donnerskirchen, the animals were only a few meters apart on the sample hill site, yet the results show differences in the composition of the fungi. Mostly rumen fungi were found in the cattle samples, along with some coprophilous and saprotrophic fungi. In contrast, over 50% of the fungi in the sheep samples could not be identified, followed by coprophilous fungi and PP (plantpathogen).

At the Kamptal sampling site, the animals grazed on the same pasture alongside other animals like donkeys and horses. The sample composition here is very similar, with over 50% belonging to *Ascobolus*, followed by other coprophilous fungi.

In St. Martin bei Gmünd, the animals were only a few meters apart on different pastures. The composition of fungi is quite similar, with *Ascobolus* and *Sodariales* being dominant in both types of dung. In cattle dung, *Parmelinella*, which is a lichenized fungus, was also dominant.

These results show that even though, the animals graze very near, the composition of the coprophilous fungi can differ.

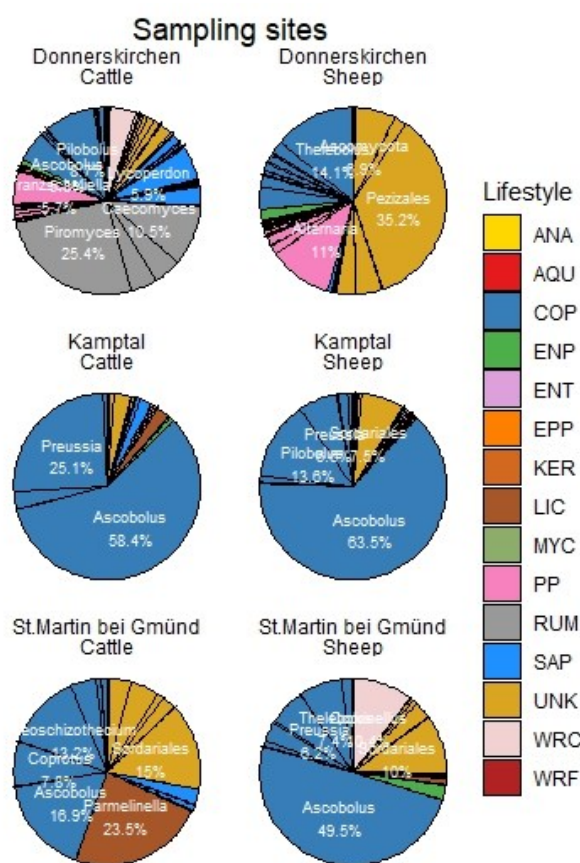


Figure 21: Three Sample locations with cattle and sheep pasture. The fungi in this figure were classified based on their ecological lifestyle. The percentages are only given for the most common fungi genera. ANA = animal associated, AQU = aquatic fungi, COP = coprophilous, ENP = endophyte, ENT = entomopathogen, EPP = epiphyte, KER = keratinolytic, LIC = lichens, MYC = mycoparasite, PP = plant pathogen, RUM = rumen fungi, SAP = saprotroph, UNK = unknown, WRC = white rot & coprophilous, WRF = white rot fungi

Deworming vs no deworming

At most locations the animals were not dewormed at all in 2024. Only a few farmers treated their animals one to three times. In Figure 22 the category “Deworming >0.5” includes all cases from 0.5 treatments, meaning only a few animals were dewormed, up to 3 deworming treatments in 2024. There appear no clear differences and it seems that dewormed animals have no significant effect on the fungal community composition in dung. However, due to the limited amount of data, no clear conclusions can be drawn. Further studies would be necessary to determine whether the frequency and dosage of deworming treatment have an impact. Also the specific type of deworming agent or the timing of samples collection could also play an important role, especially due to the rapid excretion of these substances and should therefore be considered in future studies (Demuth & Müntener 1997).

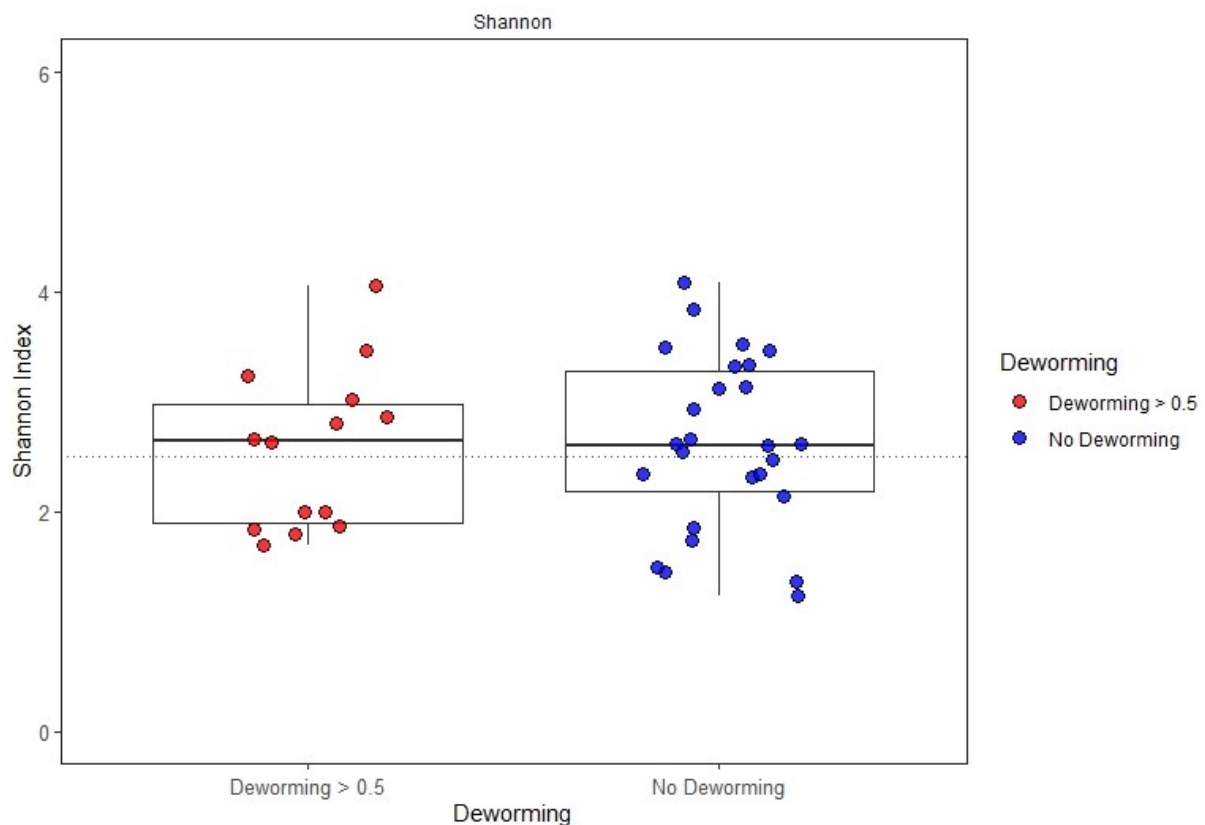


Figure 22: Comparison of dewormed and not dewormed animal dung by their shannon diversity

Comparison of conventional and organic farms

The farming methods include both conventional and organic animal farms. Conventional farms show slightly higher fungal diversity than organic farms, see Figure 23. This difference could be coincidental. The t-test is not significant, with a p-value of 0.1114. Additionally, there are more organic farms than conventional ones in the dataset, which could have influenced the result. In the bachelor's thesis of Wohletz (2023), a difference between bio and conventional farm was observed. It was shown that samples from the organic farm and the Lainzer Tiergarten site exhibited similar levels of fungal growth, whereas samples from conventional farming systems showed a significantly reduced presence of fungi. Wohletz collected samples both from stables and directly from the fields, which could have contributed to the observed differences. In this study, samples were exclusively collected from fields.

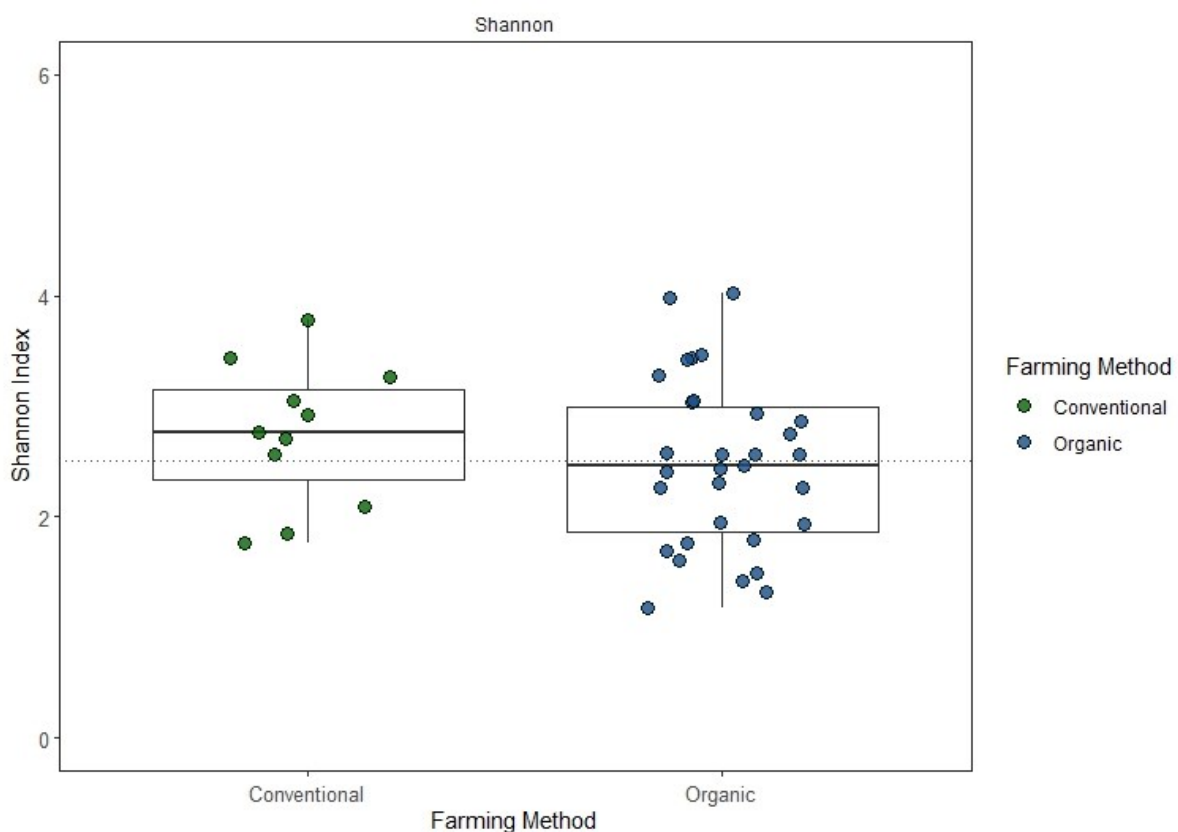


Figure 23: Comparison of conventional and organic farming methods by their Shannon diversity

Climatic provinces in Austria

Austria can be divided into four climatic provinces: Alpine, Illyrian, transitional central European climatic zone and Pannonian climate. Figure 24 shows that the Illyrian province has the lowest fungal diversity, followed by the transitional central European and Alpine zone. The Pannonian province shows the highest diversity compared to the others. This is especially interesting because the Pannonian zone includes the federal state of Burgenland and the Wiener Becken. The climate there is very dry and hot (Fally et al., 2015).

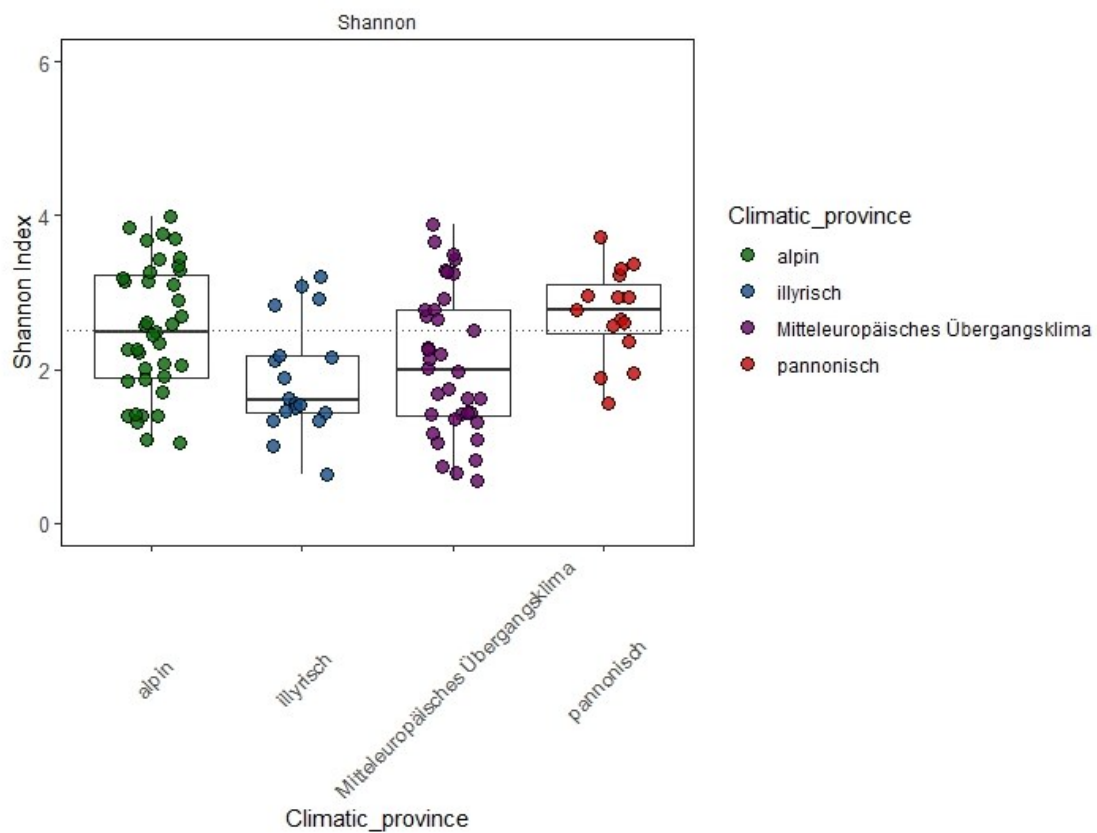


Figure 24: Comparison of the four climatic provinces in Austria by their fungal diversity. Illyrisch = Illyrian province, Mitteleuropäisches Übergangsklima = transitional central European zone, pannonisch = Pannonian province

Species Richness

Some samples exhibited notably higher species richness than others, see Figure 25. The samples D_49 (Lembach im Mühlkreis, sheep) and D_31 (Lainzer Tiergarten, cattle) showed the highest fungal diversity, each with 261 detected species. The sample D_42 (Jaukenalm, sheep) followed closely with 233 different species. In contrast, sample D_47 (Gaaden bei Großmittel, sheep) had the lowest richness with only 12 species identified. This sample stood out significantly from the other. Only four of the 12 species were coprophilous fungi, while the rest were mainly plant pathogens and yeasts. Dominant species included *Ustilago nuntica*, *Vishniacozyma dimennae* (syn. *Cryptococcus dimennae*) and *Paraphoma ledniceana*. This may indicate a suppression of typical coprophilous fungi. Samples D_55 (Galtür, sheep) and D_56 (Stubaital, sheep) each contained 35 species. On average, there were 110 different species in one sample.

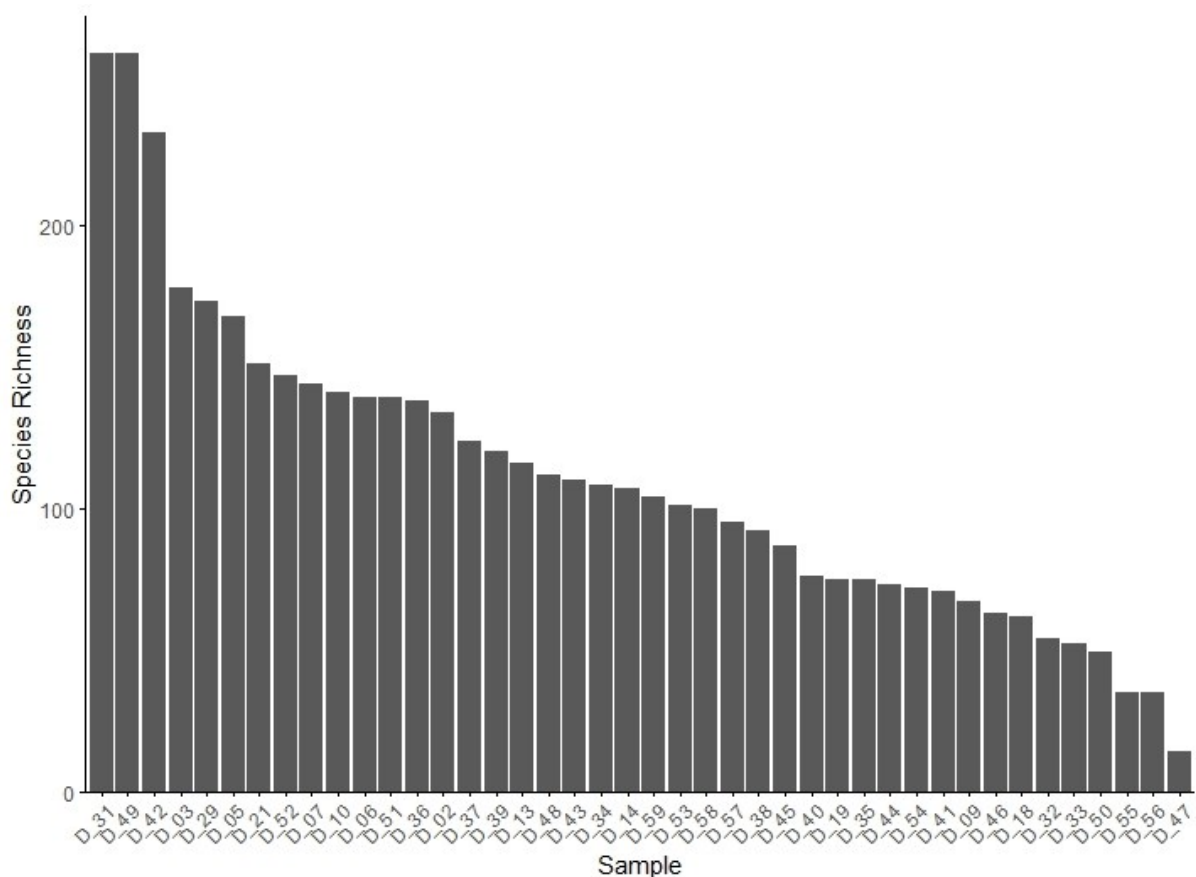


Figure 25: Comparison of the species richness for all samples

Morphological vs Metabarcoding analyses

Out of the identified fungi genera, 43 were found in both the metabarcoding and the morphological analysis, see Figure 26. A total of 238 genera were only detected through metabarcoding data, while only 19 appeared in the morphological methods, which included the genera: *Orbilina*, *Rhypophila*, *Candolleomyces*, *Narcissea*, *Coprobia*, *Thecotheus*, *Unguiculella*, *Lasiobolus*, *Melanospora*, *Oedocephalum*, *Ovatospora*, *Zygopleurage*, *Zopfiella*, *Chaetomidium*, *Copranophilus*, *Echria*, *Gamsia*, *Saccobolus*, *Yunnanina*.

This comparison is important because it shows that neither method alone is enough to identify all fungi genera. It makes sense to combine metabarcoding and morphological analysis. Identifying Basidiomycota was especially difficult. They had to be examined at very specific times, spores and basidia were often not visible at the same time. Several individuals had to be observed to identify them properly but on many samples, only one or two similar individuals were growing. That made it hard to identify them at the species level. Due to that, some Basidiomycota are probably missing from the morphological results. It also has to be considered that identification errors can occur during morphological analysis. This can lead to differences in the species list. To minimize the risk of misidentification of the species, the comparison between metabarcoding and those identified morphologically was conducted at the genus level. This approach was chosen because morphological characteristics at the genus level could be reliably distinguished.

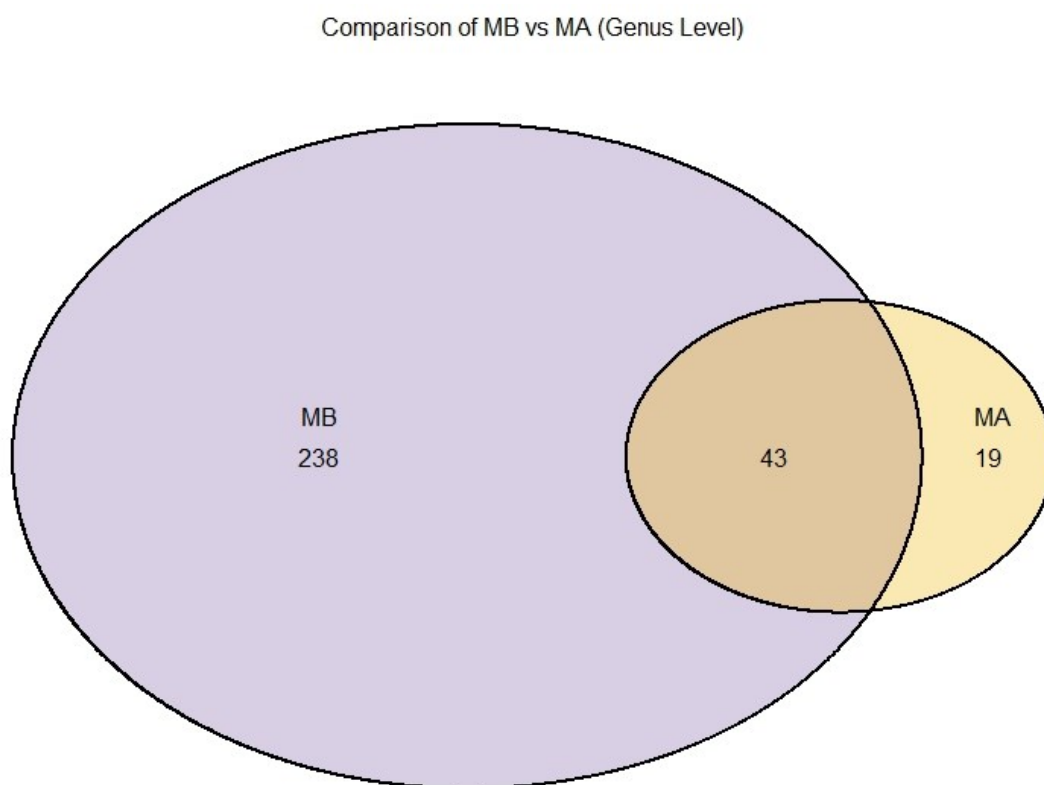


Figure 26: Comparison of the fungal genera number between Metabarcoding (MB) and Morphological analyses (MA)

Interestingly, there was no detection of the genus *Saccobolus* in the metabarcoding samples, even though it was very prominent in the morphological observations. The diversity is also quite high in the dung (Mungai et al., 2012). This is likely due to the limited number of reference sequences available for this genus. Only a few *Saccobolus* species have reference sequences available and none of them were found in either the morphological or the metabarcoding results. In the fungi list, many unidentified Ascobolaceae fungi were found, which could possibly belong to *Saccobolus*. This is a good example showing that more research on coprophilous fungi is still needed. Some fungi were only identified at a broad taxonomic level as “fungi” due to their unclear classification (Incertae sedis) and the lack of comprehensive reference data.

In both methods, the overall phylum composition was very similar, with Ascomycota being the dominant fungal group. However, the use of molecular techniques allowed for the detection of not only coprophilous fungi but also many other interesting groups, including lichens. One interesting group was the gut fungi, which exclusively inhabit the digestive tract of animals. Additionally, pathogens, parasitic fungi and mycorrhiza groups that are difficult to detect without DNA sequencing, were identified. As seen in sample D_49, these fungi groups can also play a significant role, as their presence may help explain differences in fungal growth.

Conclusion

In conclusion, this thesis provides a comprehensive overview of the various factors that can influence fungal communities in herbivore dung. While an effort was made to consider all relevant variables, no single factor could be identified as the sole driver of the differences in fungal composition. Dung remains a complex habitat, shaped by numerous environmental and biological factors, to which coprophilous fungi are remarkably well adapted. It also became evident that the presence of other fungal groups can suppress coprophilous fungi and limit their growth. Nonetheless, sheep and cattle dung appear to host very similar fungal communities.

For Austria, this study successfully compiled an extensive species list based on dung from sheep and cattle, using both morphological and molecular techniques. The results clearly demonstrate that neither method alone is sufficient for identifying coprophilous fungi. A combined approach seemed to be highly effective and should be applied in future studies. This integrated method helps to overcome challenges such as reliance on subtle morphological traits and the current lack of comprehensive sequence data, for instance within the genus *Saccobolus*.

For future research, it would be valuable to establish an experimental design aimed at isolating and better understand specific influencing factors. Repeated sampling of the same animals across different seasons would help to control variables such as breed, dung type and farming practice. Additionally, investigating other animal species in Austria would further expand the species list and deepen our understanding of coprophilous fungal diversity.

Appendix

Table A 1: Information of the sample location, name of the samples and the date of taking the samples

Plotcode	Date	Sampling_Location	Ycoord	Xcoord
D02-1_Cattle_Spring	07.05.2024	Zurndorf	47.9616820	17.01258199999998
D02-2_Cattle_Spring	07.05.2024	Zurndorf	47.9616820	17.01258199999998
D02-3_Cattle_Spring	07.05.2024	Zurndorf	47.9616820	17.01258199999998
D38-1_Sheep_Spring	07.05.2024	Zurndorf bei Neudorf	47.99683900000001	16.87415800000001
D38-2_Sheep_Spring	07.05.2024	Zurndorf bei Neudorf	47.99683900000001	16.87415800000001
D38-3_Sheep_Spring	07.05.2024	Zurndorf bei Neudorf	47.99683900000001	16.87415800000001
D03-1_Cattle_Spring	29.06.2024	Donnerskirchen	47.90175200000002	16.640089
D03-2_Cattle_Spring	29.06.2024	Donnerskirchen	47.90175200000002	16.640089
D03-3_Cattle_Spring	29.06.2024	Donnerskirchen	47.90175200000002	16.640089
D39-1_Sheep_Spring	07.05.2024	Donnerskirchen	47.90175200000002	16.640089
D39-2_Sheep_Spring	07.05.2024	Donnerskirchen	47.90175200000002	16.640089
D39-3_Sheep_Spring	07.05.2024	Donnerskirchen	47.90175200000002	16.640089
D40-1_Sheep_Spring	07.05.2024	Tadten	47.760106171084097	16.984139128434901
D40-2_Sheep_Spring	07.05.2024	Tadten	47.760106171084097	16.984139128434901
D40-3_Sheep_Spring	07.05.2024	Tadten	47.760106171084097	16.984139128434901

D06-1_Cat- tle_Spring	16.05.20 24	Brückl-Süd	46.72217663	14.53067222
D06-2_Cat- tle_Spring	16.05.20 24	Brückl-Süd	46.72217663	14.53067222
D06-3_Cat- tle_Spring	16.05.20 24	Brückl-Süd	46.72217663	14.53067222
D41- 1_Sheep_Spri ng	16.05.20 24	Brückl-Süd, Kappel am Krappfeld	46.842412000000 003	14.483541000000 001
D41- 2_Sheep_Spri ng	16.05.20 24	Brückl-Süd, Kappel am Krappfeld	46.842412000000 003	14.483541000000 001
D41- 3_Sheep_Spri ng	16.05.20 24	Brückl-Süd, Kappel am Krappfeld	46.842412000000 003	14.483541000000 001
D07-1_Cat- tle_Spring	30.06.20 24	Jaukenalm	46.694426569999 997	13.06702786
D07-2_Cat- tle_Spring	30.06.20 24	Jaukenalm	46.694426569999 997	13.06702786
D07-3_Cat- tle_Spring	30.06.20 24	Jaukenalm	46.694426569999 997	13.06702786
D42- 1_Sheep_Spri ng	30.06.20 24	Jaukenalm	46.634845701978 2	13.364903987758 3
D42- 2_Sheep_Spri ng	30.06.20 24	Jaukenalm	46.634845701978 2	13.364903987758 3
D42- 3_Sheep_Spri ng	30.06.20 24	Jaukenalm	46.634845701978 2	13.364903987758 3
D05-1_Cat- tle_Spring	30.06.20 24	Dobratsch	46.59404524	13.70547243
D05-2_Cat- tle_Spring	30.06.20 24	Dobratsch	46.59404524	13.70547243
D05-3_Cat- tle_Spring	30.06.20 24	Dobratsch	46.59404524	13.70547243
D43- 1_Sheep_Spri ng	16.05.20 24	Velden	46.631929962206 54	14.014389118921 967
D43- 2_Sheep_Spri ng	16.05.20 24	Velden	46.631929962206 54	14.014389118921 967
D43- 3_Sheep_Spri ng	16.05.20 24	Velden	46.631929962206 54	14.014389118921 967
D44- 1_Sheep_Spri ng	21.05.20 24	St.Martin bei Gmünd	48.670822000000 001	14.831848000000 001

D44-2_Sheep_Spring	21.05.2024	St.Martin Gmünd bei	48.670822000000001	14.831848000000001
D44-3_Sheep_Spring	21.05.2024	St.Martin Gmünd bei	48.670822000000001	14.831848000000001
D32-1_Cattle_Spring	21.05.2024	St.Martin Gmünd bei	48.670436000000002	14.831068999999999
D32-2_Cattle_Spring	21.05.2024	St.Martin Gmünd bei	48.670436000000002	14.831068999999999
D32-3_Cattle_Spring	21.05.2024	St.Martin Gmünd bei	48.670436000000002	14.831068999999999
D45-1_Sheep_Spring	10.05.2024	Kamptal	48.582264539471097	15.6541462755553
D45-2_Sheep_Spring	10.05.2024	Kamptal	48.582264539471097	15.6541462755553
D45-3_Sheep_Spring	10.05.2024	Kamptal	48.582264539471097	15.6541462755553
D33-1_Cattle_Spring	10.05.2024	Kamptal, Zitternberg	48.582264539471097	15.6541462755553
D33-2_Cattle_Spring	10.05.2024	Kamptal, Zitternberg	48.582264539471097	15.6541462755553
D33-3_Cattle_Spring	10.05.2024	Kamptal, Zitternberg	48.582264539471097	15.6541462755553
D09-1_Cattle_Spring	24.05.2024	Puchenstuben/Hollenstein	47.965287029999999	15.265881139999999
D09-2_Cattle_Spring	24.05.2024	Puchenstuben/Hollenstein	47.965287029999999	15.265881139999999
D09-3_Cattle_Spring	24.05.2024	Puchenstuben/Hollenstein	47.965287029999999	15.265881139999999
D46-1_Sheep_Spring	24.05.2024	Puchenstuben/Hollenstein	47.956595999999998	15.202292
D46-2_Sheep_Spring	24.05.2024	Puchenstuben/Hollenstein	47.956595999999998	15.202292
D46-3_Sheep_Spring	24.05.2024	Puchenstuben/Hollenstein	47.956595999999998	15.202292
D10-1_Cattle_Spring	08.05.2024	Großmittel, Eggen-dorf	47.878008999999999	16.286687000000001
D10-2_Cattle_Spring	08.05.2024	Großmittel, Eggen-dorf	47.878008999999999	16.286687000000001
D10-3_Cattle_Spring	08.05.2024	Großmittel, Eggen-dorf	47.878008999999999	16.286687000000001

D47-1_Sheep_Spring	15.04.2024	Gaaden bei Großmittel	47.829417132610097	16.085346079143498
D47-2_Sheep_Spring	15.04.2024	Gaaden bei Großmittel	47.829417132610097	16.085346079143498
D47-3_Sheep_Spring	15.04.2024	Gaaden bei Großmittel	47.829417132610097	16.085346079143498
D48-1_Sheep_Spring	27.05.2024	NP Kalkalpen_Bodinggraben	47.794206000000003	14.391622
D48-2_Sheep_Spring	27.05.2024	NP Kalkalpen_Bodinggraben	47.794206000000003	14.391622
D48-3_Sheep_Spring	27.05.2024	NP Kalkalpen_Bodinggraben	47.794206000000003	14.391622
D34-1_Cattle_Spring	27.05.2024	NP Kalkalpen_Zaglbauernalm	47.798434	14.37372
D34-2_Cattle_Spring	27.05.2024	NP Kalkalpen_Zaglbauernalm	47.798434	14.37372
D34-3_Cattle_Spring	27.05.2024	NP Kalkalpen_Zaglbauernalm	47.798434	14.37372
D13-1_Cattle_Spring	13.05.2024	Hansberg	48.491223092679775	14.051317599221678
D13-2_Cattle_Spring	13.05.2024	Hansberg	48.491223092679775	14.051317599221678
D13-3_Cattle_Spring	13.05.2024	Hansberg	48.491223092679775	14.051317599221678
D49-1_Sheep_Spring	13.05.2024	Lembach im Mühlkreis (bei Hansberg)	48.489182	13.928364999999999
D49-2_Sheep_Spring	13.05.2024	Lembach im Mühlkreis (bei Hansberg)	48.489182	13.928364999999999
D49-3_Sheep_Spring	13.05.2024	Lembach im Mühlkreis (bei Hansberg)	48.489182	13.928364999999999
D14-1_Cattle_Spring	13.05.2024	Taufkirchen and der Trattnach	48.251615020000003	13.757664999999999
D14-2_Cattle_Spring	13.05.2024	Taufkirchen and der Trattnach	48.251615020000003	13.757664999999999
D14-3_Cattle_Spring	13.05.2024	Taufkirchen and der Trattnach	48.251615020000003	13.757664999999999

D50-1_Sheep_Spring	13.05.2024	Taufkirchen and der Trattnach, Wäizenkirchen	48.326808	13.818016
D50-2_Sheep_Spring	13.05.2024	Taufkirchen and der Trattnach, Wäizenkirchen	48.326808	13.818016
D50-3_Sheep_Spring	13.05.2024	Taufkirchen and der Trattnach, Wäizenkirchen	48.326808	13.818016
D18-1_Cattle_Spring	19.06.2024	Thalgau	47.836289620000002	13.2732758
D18-2_Cattle_Spring	19.06.2024	Thalgau	47.836289620000002	13.2732758
D18-3_Cattle_Spring	19.06.2024	Thalgau	47.836289620000002	13.2732758
D51-1_Sheep_Spring	19.06.2024	Thalgau	47.847855474798997	13.2175355489087
D51-2_Sheep_Spring	19.06.2024	Thalgau	47.847855474798997	13.2175355489087
D51-3_Sheep_Spring	19.06.2024	Thalgau	47.847855474798997	13.2175355489087
D19-1_Cattle_Spring	01.07.2024	Mitterkleinarl-Moosalm	47.296480000000003	13.33572
D19-2_Cattle_Spring	01.07.2024	Mitterkleinarl-Moosalm	47.296480000000003	13.33572
D19-3_Cattle_Spring	01.07.2024	Mitterkleinarl-Moosalm	47.296480000000003	13.33572
D52-1_Sheep_Spring	18.06.2024	Mitterkleinarl - Goldegg	47.3259626927562	13.063105524559401
D52-2_Sheep_Spring	18.06.2024	Mitterkleinarl - Goldegg	47.3259626927562	13.063105524559401
D52-3_Sheep_Spring	18.06.2024	Mitterkleinarl - Goldegg	47.3259626927562	13.063105524559401
D35-1_Cattle_Spring	11.06.2024	Leibnitz, Frötsch	46.84	15.459833
D35-2_Cattle_Spring	11.06.2024	Leibnitz, Frötsch	46.84	15.459833
D35-3_Cattle_Spring	11.06.2024	Leibnitz, Frötsch	46.84	15.459833
D53-1_Sheep_Spring	11.06.2024	Pößnitz, Langeegg	46.656067999999998	15.51998

D53-2_Sheep_Spring	11.06.2024	Pößnitz, Langeegg	46.656067999999998	15.51998
D53-3_Sheep_Spring	11.06.2024	Pößnitz, Langeegg	46.656067999999998	15.51998
D21-1_Cattle_Spring	17.05.2024	Hauereck/Waldheimat	47.505656999999999	15.688698
D21-2_Cattle_Spring	17.05.2024	Hauereck/Waldheimat	47.505656999999999	15.688698
D21-3_Cattle_Spring	17.05.2024	Hauereck/Waldheimat	47.505656999999999	15.688698
D54-1_Sheep_Spring	17.05.2024	Lafnitz	47.355767860601397	16.0182743086195
D54-2_Sheep_Spring	17.05.2024	Lafnitz	47.355767860601397	16.0182743086195
D54-3_Sheep_Spring	17.05.2024	Lafnitz	47.355767860601397	16.0182743086195
D55-1_Sheep_Spring	18.06.2024	Galtür	46.956980000000001	10.281180000000001
D55-2_Sheep_Spring	18.06.2024	Galtür	46.956980000000001	10.281180000000001
D55-3_Sheep_Spring	18.06.2024	Galtür	46.956980000000001	10.281180000000001
D36-1_Cattle_Spring	18.06.2024	Galtür	46.957639999999998	10.19097
D36-2_Cattle_Spring	18.06.2024	Galtür	46.957639999999998	10.19097
D36-3_Cattle_Spring	18.06.2024	Galtür	46.957639999999998	10.19097
D56-1_Sheep_Spring	18.06.2024	Stubaital	47.128430000000002	11.28439
D56-2_Sheep_Spring	18.06.2024	Stubaital	47.128430000000002	11.28439
D56-3_Sheep_Spring	18.06.2024	Stubaital	47.128430000000002	11.28439
D37-1_Cattle_Spring	18.06.2024	Stubaital	47.129130000000004	11.30786

D37-2_Cat- tle_Spring	18.06.20 24	Stubaital	47.129130000000 004	11.30786
D37-3_Cat- tle_Spring	18.06.20 24	Stubaital	47.129130000000 004	11.30786
D31-1_Cat- tle_Spring	01.03.20 24	Lainzer Tiergarten	48.165906172841 9	16.250288205573 298
D31-2_Cat- tle_Spring	01.03.20 24	Lainzer Tiergarten	48.165906172841 9	16.250288205573 298
D31-3_Cat- tle_Spring	01.03.20 24	Lainzer Tiergarten	48.165906172841 9	16.250288205573 298
D57- 1_Sheep_Spri ng	29.06.20 24	Salzwiese	48.219648999999 997	16.226486000000 001
D57- 2_Sheep_Spri ng	29.06.20 24	Salzwiese	48.219648999999 997	16.226486000000 001
D57- 3_Sheep_Spri ng	29.06.20 24	Salzwiese	48.219648999999 997	16.226486000000 001
D29-1_Cat- tle_Spring	19.06.20 24	Fraxern- Übersaxen	47.2509527	9.7069184
D29-2_Cat- tle_Spring	19.06.20 24	Fraxern- Übersaxen	47.2509527	9.7069184
D29-3_Cat- tle_Spring	19.06.20 24	Fraxern- Übersaxen	47.2509527	9.7069184
D58- 1_Sheep_Spri ng	19.06.20 24	Fraxern Übersaxen	- 47.261545885903 5	9.6713113877381 84
D58- 2_Sheep_Spri ng	19.06.20 24	Fraxern Übersaxen	- 47.261545885903 5	9.6713113877381 84
D58- 3_Sheep_Spri ng	19.06.20 24	Fraxern Übersaxen	- 47.261545885903 5	9.6713113877381 84
D59- 1_Sheep_Spri ng	06.05.20 24	Perchtoldsdorf	48.099989647132 04	16.224836557279 744
D59- 2_Sheep_Spri ng	06.05.20 24	Perchtoldsdorf	48.099989647132 04	16.224836557279 744
D59- 3_Sheep_Spri ng	06.05.20 24	Perchtoldsdorf	48.099989647132 04	16.224836557279 744
D02-1_Cat- tle_Autumn	23.08.20 24	Zurndorf	47.961682000000 003	17.012581999999 998
D02-2_Cat- tle_Autumn	23.08.20 24	Zurndorf	47.961682000000 003	17.012581999999 998

D02-3_Cat- tle_Autumn	23.08.20 24	Zurndorf	47.961682000000 003	17.012581999999 998
D38- 1_Sheep_Au- tumn	23.08.20 24	Zurndorf bei Neu- dorf	48.015396706683 497	16.932603954601 198
D38- 2_Sheep_Au- tumn	23.08.20 24	Zurndorf bei Neu- dorf	48.015396706683 497	16.932603954601 198
D38- 3_Sheep_Au- tumn	23.08.20 24	Zurndorf bei Neu- dorf	48.015396706683 497	16.932603954601 198
D03-1_Cat- tle_Autumn	23.08.20 24	Donnerskirchen	47.901752000000 002	16.640089
D03-2_Cat- tle_Autumn	23.08.20 24	Donnerskirchen	47.901752000000 002	16.640089
D03-3_Cat- tle_Autumn	23.08.20 24	Donnerskirchen	47.901752000000 002	16.640089
D39- 1_Sheep_Au- tumn	23.08.20 24	Donnerskirchen	47.901752000000 002	16.640089
D39- 2_Sheep_Au- tumn	23.08.20 24	Donnerskirchen	47.901752000000 002	16.640089
D39- 3_Sheep_Au- tumn	23.08.20 24	Donnerskirchen	47.901752000000 002	16.640089
D40- 1_Sheep_Au- tumn	23.08.20 24	Tadten	47.758575290492 999	16.986048450447 001
D40- 2_Sheep_Au- tumn	23.08.20 24	Tadten	47.758575290492 999	16.986048450447 001
D40- 3_Sheep_Au- tumn	23.08.20 24	Tadten	47.758575290492 999	16.986048450447 001
D06-1_Cat- tle_Autumn	19.09.20 24	Brückl-Süd	46.72217663	14.53067222
D06-2_Cat- tle_Autumn	19.09.20 24	Brückl-Süd	46.72217663	14.53067222
D06-3_Cat- tle_Autumn	19.09.20 24	Brückl-Süd	46.72217663	14.53067222
D41- 1_Sheep_Au- tumn	19.09.20 24	Brückl-Süd, Kappel am Krappfeld	46.842412000000 000	14.483541000000 001
D41- 2_Sheep_Au- tumn	19.09.20 24	Brückl-Süd, Kappel am Krappfeld	46.842412000000 000	14.483541000000 001

D41-3_Sheep_Autumn	19.09.2024	Brückl-Süd, Kappel am Krappfeld	46.842412000000000	14.483541000000001
D07-1_Cattle_Autumn	20.09.2024	Jaukenalm	46.380540000000003	13.21537
D07-2_Cattle_Autumn	20.09.2024	Jaukenalm	46.380540000000003	13.21537
D07-3_Cattle_Autumn	20.09.2024	Jaukenalm	46.380540000000003	13.21537
D42-1_Sheep_Autumn	20.09.2024	Jaukenalm	46.6348457019782	13.3649039877583
D42-2_Sheep_Autumn	20.09.2024	Jaukenalm	46.6348457019782	13.3649039877583
D42-3_Sheep_Autumn	20.09.2024	Jaukenalm	46.6348457019782	13.3649039877583
D05-1_Cattle_Autumn	20.09.2024	Dobratsch	46.617341	13.747493
D05-2_Cattle_Autumn	20.09.2024	Dobratsch	46.617341	13.747493
D05-3_Cattle_Autumn	20.09.2024	Dobratsch	46.617341	13.747493
D43-1_Sheep_Autumn	19.09.2024	Velden	46.632413594227900	14.0143108645554
D43-2_Sheep_Autumn	19.09.2024	Velden	46.632413594227900	14.0143108645554
D43-3_Sheep_Autumn	19.09.2024	Velden	46.632413594227900	14.0143108645554
D44-1_Sheep_Autumn	24.09.2024	St.Martin bei Gmünd	48.670822000000001	14.831848000000001
D44-2_Sheep_Autumn	24.09.2024	St.Martin bei Gmünd	48.670822000000001	14.831848000000001
D44-3_Sheep_Autumn	24.09.2024	St.Martin bei Gmünd	48.670822000000001	14.831848000000001
D32-1_Cattle_Autumn	24.09.2024	St.Martin bei Gmünd	48.680231999999997	14.818344
D32-2_Cattle_Autumn	24.09.2024	St.Martin bei Gmünd	48.680231999999997	14.818344
D32-3_Cattle_Autumn	24.09.2024	St.Martin bei Gmünd	48.680231999999997	14.818344

D45-1_Sheep_Autumn	08.10.2024	Kamptal	48.5268629999999	15.682285
D45-2_Sheep_Autumn	08.10.2024	Kamptal	48.5268629999999	15.682285
D45-3_Sheep_Autumn	08.10.2024	Kamptal	48.5268629999999	15.682285
D33-1_Cattle_Autumn	08.10.2024	Kamptal, Zitternberg	48.582264539471097	15.6541462755553
D33-2_Cattle_Autumn	08.10.2024	Kamptal, Zitternberg	48.582264539471097	15.6541462755553
D33-3_Cattle_Autumn	08.10.2024	Kamptal, Zitternberg	48.582264539471097	15.6541462755553
D09-1_Cattle_Autumn	23.09.2024	Puchenstuben/Hollenstein	47.96696	15.26332
D09-2_Cattle_Autumn	23.09.2024	Puchenstuben/Hollenstein	47.96696	15.26332
D09-3_Cattle_Autumn	23.09.2024	Puchenstuben/Hollenstein	47.96696	15.26332
D46-1_Sheep_Autumn	23.09.2024	Puchenstuben/Hollenstein	47.9086783307826	15.2338833374345
D46-2_Sheep_Autumn	23.09.2024	Puchenstuben/Hollenstein	47.9086783307826	15.2338833374345
D46-3_Sheep_Autumn	23.09.2024	Puchenstuben/Hollenstein	47.9086783307826	15.2338833374345
D10-1_Cattle_Autumn	27.08.2024	Großmittel, Eggen-dorf	47.8780089999999	16.286687000000001
D10-2_Cattle_Autumn	27.08.2024	Großmittel, Eggen-dorf	47.8780089999999	16.286687000000001
D10-3_Cattle_Autumn	27.08.2024	Großmittel, Eggen-dorf	47.8780089999999	16.286687000000001
D47-1_Sheep_Autumn	27.08.2024	Gaaden bei Großmittel	47.829417132610097	16.085346079143498
D47-2_Sheep_Autumn	27.08.2024	Gaaden bei Großmittel	47.829417132610097	16.085346079143498
D47-3_Sheep_Autumn	27.08.2024	Gaaden bei Großmittel	47.829417132610097	16.085346079143498
D48-1_Sheep_Autumn	23.09.2024	NP Kalkalpen_Bodinggraben	47.791395000000001	14.394978

D48-2_Sheep_Autumn	23.09.2024	NP Kalkalpen_Bodinggraben	47.79139500000001	14.394978
D48-3_Sheep_Autumn	23.09.2024	NP Kalkalpen_Bodinggraben	47.79139500000001	14.394978
D34-1_Cattle_Autumn	23.09.2024	NP Kalkalpen_Zaglbauernalm	47.798434	14.37372
D34-2_Cattle_Autumn	23.09.2024	NP Kalkalpen_Zaglbauernalm	47.798434	14.37372
D34-3_Cattle_Autumn	23.09.2024	NP Kalkalpen_Zaglbauernalm	47.798434	14.37372
D13-1_Cattle_Autumn	29.08.2024	Hansberg	48.47117910000001	14.13144493000001
D13-2_Cattle_Autumn	29.08.2024	Hansberg	48.47117910000001	14.13144493000001
D13-3_Cattle_Autumn	29.08.2024	Hansberg	48.47117910000001	14.13144493000001
D49-1_Sheep_Autumn	29.08.2024	Lembach im Mühlkreis (bei Hansberg)	48.489182	13.92836499999999
D49-2_Sheep_Autumn	29.08.2024	Lembach im Mühlkreis (bei Hansberg)	48.489182	13.92836499999999
D49-3_Sheep_Autumn	29.08.2024	Lembach im Mühlkreis (bei Hansberg)	48.489182	13.92836499999999
D14-1_Cattle_Autumn	29.08.2024	Taufkirchen and der Trattnach	48.25161502000003	13.75766499999999
D14-2_Cattle_Autumn	29.08.2024	Taufkirchen and der Trattnach	48.25161502000003	13.75766499999999
D14-3_Cattle_Autumn	29.08.2024	Taufkirchen and der Trattnach	48.25161502000003	13.75766499999999
D50-1_Sheep_Autumn	29.08.2024	Taufkirchen and der Trattnach, Wizenkirchen	48.326808	13.818016
D50-2_Sheep_Autumn	29.08.2024	Taufkirchen and der Trattnach, Wizenkirchen	48.326808	13.818016
D50-3_Sheep_Autumn	29.08.2024	Taufkirchen and der Trattnach, Wizenkirchen	48.326808	13.818016
D18-1_Cattle_Autumn	23.09.2024	Thalgau	47.83402999999998	13.27236000000001

D18-2_Cat- tle_Autumn	23.09.20 24	Thalgau	47.834029999999 998	13.272360000000 001
D18-3_Cat- tle_Autumn	23.09.20 24	Thalgau	47.834029999999 998	13.272360000000 001
D51- 1_Sheep_Au- tumn	23.09.20 24	Thalgau	47.847855474798 997	13.217535548908 7
D51- 2_Sheep_Au- tumn	23.09.20 24	Thalgau	47.847855474798 997	13.217535548908 7
D51- 3_Sheep_Au- tumn	23.09.20 24	Thalgau	47.847855474798 997	13.217535548908 7
D19-1_Cat- tle_Autumn	29.08.20 24	Mitterkleinarl- Moosalm	47.293686999999 998	13.335704
D19-2_Cat- tle_Autumn	29.08.20 24	Mitterkleinarl- Moosalm	47.293686999999 998	13.335704
D19-3_Cat- tle_Autumn	29.08.20 24	Mitterkleinarl- Moosalm	47.293686999999 998	13.335704
D52- 1_Sheep_Au- tumn	29.08.20 24	Mitterkleinarl - Goldegg	47.325962692756 2	13.214318
D52- 2_Sheep_Au- tumn	29.08.20 24	Mitterkleinarl - Goldegg	47.325962692756 2	13.214318
D52- 3_Sheep_Au- tumn	29.08.20 24	Mitterkleinarl - Goldegg	47.325962692756 2	13.214318
D35-1_Cat- tle_Autumn	28.08.20 24	Leibnitz, Frötsch	46.84	15.459833
D35-2_Cat- tle_Autumn	28.08.20 24	Leibnitz, Frötsch	46.84	15.459833
D35-3_Cat- tle_Autumn	28.08.20 24	Leibnitz, Frötsch	46.84	15.459833
D53- 1_Sheep_Au- tumn	28.08.20 24	Pößnitz, Langeegg	46.656067999999 998	15.51998
D53- 2_Sheep_Au- tumn	28.08.20 24	Pößnitz, Langeegg	46.656067999999 998	15.51998
D53- 3_Sheep_Au- tumn	28.08.20 24	Pößnitz, Langeegg	46.656067999999 998	15.51998
D21-1_Cat- tle_Autumn	28.08.20 24	Hauereck/Wald- heimat	47.505656999999 999	15.688698
D21-2_Cat- tle_Autumn	28.08.20 24	Hauereck/Wald- heimat	47.505656999999 999	15.688698

D21-3_Cat- tle_Autumn	28.08.20 24	Hauereck/Wald- heimat	47.505656999999 999	15.688698
D54- 1_Sheep_Au- tumn	28.08.20 24	Lafnitz	47.355767860601 397	16.018274308619 5
D54- 2_Sheep_Au- tumn	28.08.20 24	Lafnitz	47.355767860601 397	16.018274308619 5
D54- 3_Sheep_Au- tumn	28.08.20 24	Lafnitz	47.355767860601 397	16.018274308619 5
D55- 1_Sheep_Au- tumn	28.08.20 24	Galtür	46.971424	10.194527
D55- 2_Sheep_Au- tumn	28.08.20 24	Galtür	46.971424	10.194527
D55- 3_Sheep_Au- tumn	28.08.20 24	Galtür	46.971424	10.194527
D36-1_Cat- tle_Autumn	28.08.20 24	Galtür	46.971367999999 998	10.200716
D36-2_Cat- tle_Autumn	28.08.20 24	Galtür	46.971367999999 998	10.200716
D36-3_Cat- tle_Autumn	28.08.20 24	Galtür	46.971367999999 998	10.200716
D56- 1_Sheep_Au- tumn	27.08.20 24	Stubaital	47.128430000000 002	11.28439
D56- 2_Sheep_Au- tumn	27.08.20 24	Stubaital	47.128430000000 002	11.28439
D56- 3_Sheep_Au- tumn	27.08.20 24	Stubaital	47.128430000000 002	11.28439
D37-1_Cat- tle_Autumn	27.08.20 24	Stubaital	47.129130000000 004	11.30786
D37-2_Cat- tle_Autumn	27.08.20 24	Stubaital	47.129130000000 004	11.30786
D37-3_Cat- tle_Autumn	27.08.20 24	Stubaital	47.129130000000 004	11.30786
D31-1_Cat- tle_Autumn	26.08.20 24	Lainzer Tiergarten	48.165906172841 9	16.250288205573 298
D31-2_Cat- tle_Autumn	26.08.20 24	Lainzer Tiergarten	48.165906172841 9	16.250288205573 298
D31-3_Cat- tle_Autumn	26.08.20 24	Lainzer Tiergarten	48.165906172841 9	16.250288205573 298

D57-1_Sheep_Autumn	26.08.2024	Steinhof	48.21964899999997	16.22648600000001
D57-2_Sheep_Autumn	26.08.2024	Steinhof	48.21964899999997	16.22648600000001
D57-3_Sheep_Autumn	26.08.2024	Steinhof	48.21964899999997	16.22648600000001
D29-1_Cattle_Autumn	28.08.2024	Fraxern Übersaxen -	47.2615458859035	9.671311387738184
D29-2_Cattle_Autumn	28.08.2024	Fraxern Übersaxen -	47.2615458859035	9.671311387738184
D29-3_Cattle_Autumn	28.08.2024	Fraxern Übersaxen -	47.2615458859035	9.671311387738184
D58-1_Sheep_Autumn	28.08.2024	Fraxern Übersaxen -	47.261481000000003	9.671132999999993
D58-2_Sheep_Autumn	28.08.2024	Fraxern Übersaxen -	47.261481000000003	9.671132999999993
D58-3_Sheep_Autumn	28.08.2024	Fraxern Übersaxen -	47.261481000000003	9.671132999999993
D60-1_Cattle_Autumn	28.08.2024	Garfrescha	47.004019599999999	9.98005380000000003
D60-2_Cattle_Autumn	28.08.2024	Garfrescha	47.004019599999999	9.98005380000000003
D61-1_Cattle_Autumn	08.09.2024	Rauris	47.065223000000003	6.522300000000003
D61-2_Cattle_Autumn	08.09.2024	Rauris	NA	NA
D61-3_Cattle_Autumn	08.09.2024	Rauris	47.066437000000001	13.009835000000001

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