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The Inhalable Mycobiome of Sawmill Workers: Exposure Characterization and Diversity

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ABSTRACT Exposure to fungal spores has been associated with respiratory symptoms and allergic alveolitis among sawmill workers, but the complexity of sawmill workers' fungal exposure has been poorly studied. We characterized the fungal diversity in air samples from sawmill workers' breathing zones and identified differences in the richness, diversity, and taxonomic composition between companies, departments, wood types, and seasons. Full-shift personal inhalable dust samples ($n = 86$) collected from 11 industrial sawmill, sorting mill, and planer mill companies processing spruce and/or pine were subjected to DNA metabarcoding using the fungal internal transcribed spacer (ITS) region 2. The workers were exposed to a higher total number of operational taxonomic units (OTUs) in summer than in winter and when processing spruce than when processing pine. Workers in the saw department had the richest fungal exposure, followed by workers in the planing department and sorting of dry timber department. Sawmills explained 11% of the variation in the fungal community composition of the exposure, followed by season (5%) and department (3%). The fungal compositions of the exposures also differed between seasons, sawmills, wood types, and departments at the taxonomic level, ranging from the phylum to the species level. The differences in exposure diversity suggest that the potential health effects of fungal inhalation may also be different; hence, a risk assessment based on the fungal diversity differences should be performed. This study may serve as a basis for establishing a fungal profile of signature species that are specific for sawmills and that can be measured quantitatively in future risk assessments of sawmill workers.

IMPORTANCE To gain more knowledge about exposure-response relationships, it is important to improve exposure characterization by comprehensively identifying the temporal and spatial fungal composition and diversity of inhalable dust at workplaces. The variation in the diverse fungal communities to which individuals are exposed in different seasons and sawmills suggests that variations in exposure-related health effects between seasons and companies can be expected. More importantly, the distinct fungal profiles between departments across companies indicate that workers in different job groups are differently exposed and that health risks can be department specific. DNA metabarcoding provides insight into a broad spectrum of airborne fungi that may serve as a basis for obtaining important knowledge about the fungi to which workers are exposed.

KEYWORDS DNA metabarcoding, ITS2, fungal diversity, occupational, exposure characterization

Sawmill workers are exposed to wood dust and multiple wood-associated chemicals and microbiota, including fungi (1–6). An occupational exposure limit (OEL) of 1×10^5 spores/m^3 has been proposed on the basis of the experimental and epidemiological evidence of the lowest-observed-effect level (LOEL) for exposure to general

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fungal spores (7). Exposure to fungal spores has been associated with respiratory symptoms and allergic alveolitis among sawmill workers (8–12). The cultivation of fungal aerosols has shown that *Cladosporium* spp., *Alternaria* spp., *Aspergillus* spp., *Penicillium* spp., *Trichoderma* spp., a *Paecilomyces* sp., *Rhizopus* spp., and *Mucor* spp. are common in sawmills in Canada (13, 14), Poland (15), Italy (16), and Norway (17). Several of these species may provoke immunological and allergic responses, also shown by the presence of specific antibodies in the blood of workers (10, 14, 18). However, protective effects of fungi have also been reported (19, 20), and it is increasingly recognized that it is the fungal compositional distribution in the exposure that is important to health (19–24).

To gain more knowledge about the exposure-response relationships, it is important to improve the exposure characterization by comprehensively identifying the fungal composition and diversity of inhalable dust in workplaces. A lack of specificity makes the identification of airborne fungi by traditional methods, such as microscopy, difficult, and less than 1% of the estimated diversity is assumed to be cultivable under laboratory conditions (25). In contrast, DNA-based methods are more robust in targeting all fungal particle types that contain DNA, living or dead spores, hyphae, and fragments. The use of high-throughput sequencing (HTS)-based DNA metabarcoding technologies to capture a complete picture of the microbial communities present in different environments, including air samples with fractions of different sizes, is gaining wider acceptance (26–29).

HTS methods are still not widespread in occupational settings but have been used to study the microbial diversity in bioaerosols emitted in waste-sorting plants (30), composting plants (31), dairy farms (32), swine confinement buildings (33), and water purification plants (34). The composition of the fungal diversity present in bioaerosols emitted in waste-sorting plants has been shown by 18S ribosomal DNA pyrosequencing to be complex and to be dominated by *Ascomycota*, with the prevailing genera being *Penicillium*, *Aspergillus*, *Rhizopus*, *Wallemia*, and *Hemicarpenteles* (30). An analysis of the fungal diversity in aerosols on 5 dairy farms, performed by the use of Illumina MiSeq sequencing of the internal transcribed spacer 1 (ITS1) region, showed that 6 of 8 fungal classes (*Eurotiomycetes*, *Dothideomycetes*, *Wallemiomycetes*, *Agaricomycetes*, *Sordariomycetes*, and *Tremellomycetes*) were dominant, but their relative abundance varied between farms (32). Differences in the fungal diversity profile in composting materials and aerosols from facilities processing domestic compost and facilities processing pig carcasses were also found by this approach (31).

Although these studies shed unprecedented light into the large diversity of fungal species in aerosols, due to the small number of samples and the use of stationary sampling methods instead of personal sampling, interpretation of the results was limited with regard to the ability to make generalizations about the taxonomic composition and personal exposure assessments. Further, this type of data has so far not been directly coupled to health effect measurements. However, in a case study where work with a batch of seed triggered organic dust toxic syndrome (ODTS), it was shown that the microbial profile of the dust from the problematic batch was dominated by microorganisms that differed from those in dust from seeds without associated health effects (35).

Fungal spore exposure in the Norwegian sawmill industry was recently shown to be highly variable and exceeded the recommended OEL of 1×10^5 fungal spores/m^3 for 38% of the workers (1), suggesting that fungal exposure may contribute to adverse health effects in this industry. The microbial complexity of the exposure, such as variations in fungal species domination, composition, and richness, is, however, unknown. The present study therefore aimed to use metabarcoding of fungal DNA obtained from air samples to investigate the fungi to which sawmill workers are exposed. The objectives were to study the fungal diversity in personal air samples from workers at 11 sawmill and planer mill companies by identifying possible differences in the richness, diversity, and taxonomic composition between companies, departments, wood types, and seasons that may have an impact on health risks.

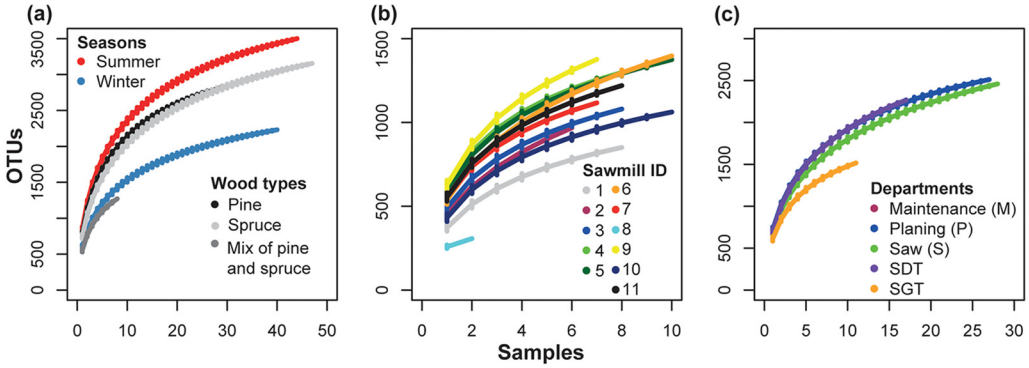

FIG 1 Species accumulation curves for sequencing depth against OTU richness for two seasons (summer and winter), three wood types (pine, spruce, mix) (a), 11 different sawmills (ID, identifier) (b), and five different department (S, saw department; SGT, sorting of green timber department; SDT, sorting of dry timber department; P, planing department; M, maintenance department (c).

RESULTS

Data characteristics and taxonomic composition. The quality-filtered data set, after excluding all replicates, negative-control, and mock community samples, contained 5,078 operational taxonomic units (OTUs) (16,794,279 reads) distributed among 84 sawmill samples. The number of reads per sample in the nonrarefied data set ranged from 3,796 to 928,818 (average = 199,932), and the number per OTU ranged from 1 to 1,119,372 (average = 3,307). *Ascomycota* was the most common phylum detected in the sawmill samples (50.3% of the OTUs and 65.5% of the reads), followed by the *Basidiomycota* (45.6% OTUs and 31.8% reads) and the *Zygomycota* (0.7% of the OTUs and 1.0% reads) (see Fig. S4 and Table S2 in the supplemental material). The *Chytridiomycota*, *Rozellomycota*, and *Glomeromycota* were detected in low proportions (they all made up <0.1% of the reads). The most common orders detected among the ascomycetes were the *Capnodiales* (3.3% of the OTUs and 13.2% of the reads), *Eurotiales* (1.1% of the OTUs and 9.3% of the reads), *Saccharomycetales* (1.7% of the OTUs and 6.8% of the reads), and *Microascales* (0.5% of the OTUs and 6% of the reads), whereas the *Tremellales* (3.4% of the OTUs and 6.9% of the reads), *Sporidiobolales* (1.2% of the OTUs and 3.6% of the reads), *Polyporales* (4.9% of the OTUs and 3.4% of the reads), and *Cystofilobasidiales* (1% of the OTUs and 2.8% of the reads) were the most common among the basidiomycetes.

Diversity analysis. The average OTU richness per sample in the rarefied data set was 540. The OTU accumulation curves clearly showed that the total number of OTUs was higher in summer than in winter (Fig. 1a). Correspondingly, the average number of OTUs per sample varied significantly between seasons (analysis of variance [ANOVA], $P < 0.001$), with higher occurrences being seen during summer (277 ± 101 [standard deviation {SD}] OTUs) than during winter (187 ± 75 [SD] OTUs) (Table 1; Fig. S5). Higher fungal richness was observed during the processing of spruce wood than during the processing of pine (Fig. 1a), but the average OTU richness per sample was not significantly different (ANOVA, $P = 0.583$). Variability in the species recovered was observed among different departments, but the difference was not significant (Table 1). The planing department had the highest species richness, followed by the departments for saw and sorting of dry timber (Fig. 1c). Due to a limited number of samples per sawmill, it is difficult to draw conclusions about the differences among the different sawmills (Fig. 1b). However, some variability in the total species recovered among sawmills was observed, ranging from 157 ± 75 (SD) OTUs in sawmill 10 to 316 ± 87 (SD) OTUs in sawmill 9 (Fig. S5). Significant differences in the Shannon diversity index (Fig. S6) and evenness (Fig. S7) were observed between several sawmills. The total abundances of the 10 most common OTUs varied significantly between seasons, being high during winter and low during summer (Table 1; Fig. S8). The volume of air collected for each sample had no effect on richness or the diversity level (Table 1; Fig.

TABLE 1 Effects of different sawmills, departments, seasons, and wood types being processed during the sampling day on fungal richness, Shannon diversity index, evenness, and abundance of the 10 most common OTUs

Variable	df[a]	*F* value	*P* value[b]
Richness			
Sawmills	10	1.86	0.065
Departments	4	1.65	0.171
Season	1	18.44	**<0.001**
Wood type	2	0.54	0.583
Air volume	1.82	2.33	0.131
Spore count	1.82	0.36	0.549
Dust	1.82	2.05	0.156
Shannon diversity index			
Sawmills	10	2.22	**0.025**
Departments	4	0.48	0.749
Season	1	1.45	0.232
Wood type	2	0.04	0.958
Air volume	1.82	1.29	0.260
Spore count	1.82	3.55	0.063
Dust	1.82	8.69	**0.004**
Evenness			
Sawmills	10	1.99	**0.046**
Departments	4	0.24	0.913
Season	1	0.01	0.921
Wood types	2	0.04	0.964
Air volume	1.82	0.64	0.427
Spore count	1.82	7.70	**0.007**
Dust	1.82	7.83	**0.006**
Abundance of 10 most common OTUs			
Sawmills	10	2.30	**0.021**
Departments	4	1.48	0.216
Season	1	9.92	**0.002**
Wood types	2	1.57	0.215
Air volume	1.82	0.15	0.704
Spore count	1.82	1.95	0.166
Dust	1.82	25.54	**<0.001**

[a]df, number of degrees of freedom.
[b]Statistically significant differences among variables were analyzed using ANOVA and Tukey's HSD *post hoc* test. Boldface indicates significance at a *P* value of <0.05.

S9), but the evenness of the fungal composition declined significantly with increasing spore counts (number of spores per cubic meter of air) and dust masses (in milligrams per cubic meter), analyzed in parallel samples and reported previously (Table 1; Fig. S10 and S11) (1). Further, the Shannon diversity index declined and the abundances of the 10 most common OTUs increased significantly with increased dust exposure.

Fungal community structure and composition. Variation partitioning analysis revealed that different sawmills accounted for most of the variation (11%) in fungal community composition (Fig. 2), followed by seasonal differences (5%) and departments (3%). In contrast to fungal richness, different wood types processed during the day of sampling (spruce, pine, or a mixture of the two wood types) contributed very little (1%) to the total explained variation in fungal community composition. Altogether, 79% of the variation remained unexplained.

Multivariate analysis (permutational analysis of variance [PERMANOVA] and nonmetric multidimensional scaling [NMDS]) resulted in similar patterns and confirmed that most of the variation in fungal community composition appeared between the sawmills (Table 2; Fig. 3). Fungal communities from three sawmills (sawmills 4, 10, and 11) were heterogeneous compared to those from the other sawmills, and sawmill 3 displayed the most distinct fungal community (Fig. 3b). The department, season, and wood type used on the day of sampling were significantly correlated with the fungal community structure (Table 1; Fig. 3a and c). The first NMDS axis (NMDS1) mainly

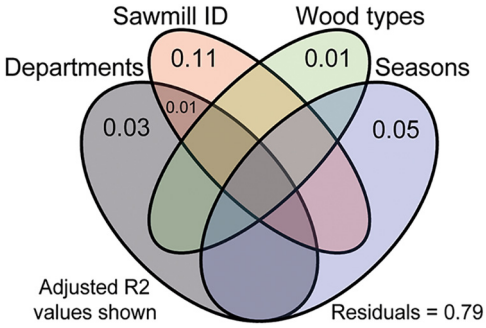

FIG 2 Pure and shared effects of 11 sawmills, five departments, two seasons, and three wood types on the fungal community in personal samples from the breathing zone of sawmill workers derived from variation partitioning analysis. Statistics denote the proportion of explained variation, and values of <0 are not shown.

reflected seasonal variation, while the second NMDS axis (NMDS2) mainly represented the variation associated with the different departments. Samples from workers in the planing and sorting of dry timber departments had distinct ellipses, suggesting exposure to a unique fungal composition in these departments, whereas workers in the saw and the sorting green timber departments were exposed to more similar communities. The fungal community structure was significantly related to the sampled air volume and dust exposure (both $P < 0.05$) (Table 2), and the relationship with spore counts was nonsignificant.

We also observed changes in fungal composition at different taxonomic levels, ranging from the phylum to the species level. During the winter season, the *Ascomycota* dominated, whereas basidiomycetes were more abundant during the summer period (Fig. 4a; Table S2). The ascomycete orders *Capnodiales*, *Saccharomycetales*, and *Microascales* were especially abundant during winter. In contrast, *Eurotiales* (ascomycetes) and *Tremellales* and *Polyporales* (both basidiomycetes) were relatively more common during summer (Fig. 4b; Table S2). Different genera also showed seasonal patterns, where *Mycosphaerella* (a plant pathogen), *Fomitopsis* and *Nakazawaea* (both saprotrophs), and *Cantharellales* (not assigned at the genus level) significantly dominated during summer and were absent during winter (Fig. 5a). In contrast, *Ceratocystis* and *Candida* (both of which are plant pathogens), *Trichoderma* (a saprotroph), and *Herpotrichiellaceae* showed opposite patterns, being more abundant during winter. The most common OTUs also exhibited strong seasonal variations. OTUs with high sequence similarity (>97%) to *Cryptococcus albidus*, *Candida railenensis*, *Aureobasidium melanogenum*, and *Peterozyma xylosa* (all of which are yeasts and saprotrophs) and *Ceratocystis adipose* (a plant pathogen) were more abundant during winter, whereas *Nakazawaea holstii* and *Fomitopsis pinicola* (both of which are saprotrophs) were most common during summer (Fig. 3d; Table 3).

TABLE 2 Relative importance of different sawmills, departments, seasons, wood types, dust exposure, and sampled air volume on airborne fungal community composition[a]

Variable	df	Sum of sq	Mean sq	F Model	R^2	Pr(>*F*)
Sawmills	10	7.699	0.770	2.60	0.23	**<0.001**
Departments	4	2.266	0.567	1.91	0.07	**<0.001**
Season	1	2.160	2.160	7.28	0.07	**<0.001**
Wood types	2	0.840	0.420	1.42	0.03	**0.008**
Dust	1	0.425	0.425	1.43	0.01	**0.032**
Air volume	1	0.409	0.409	1.38	0.01	**0.042**
Residuals	64	18.982	0.297	0.58		
Total	83	32.781	1			

[a]Relative importance was revealed by PERMANOVA. df, number of degrees of freedom; Sum of sq, sum of squares value; Mean sq, mean square value; F Model, F statistic obtained from the model; Pr(>*F*), probability that the value is greater than the *F* value. Boldface indicates significance at a *P* value of <0.05.

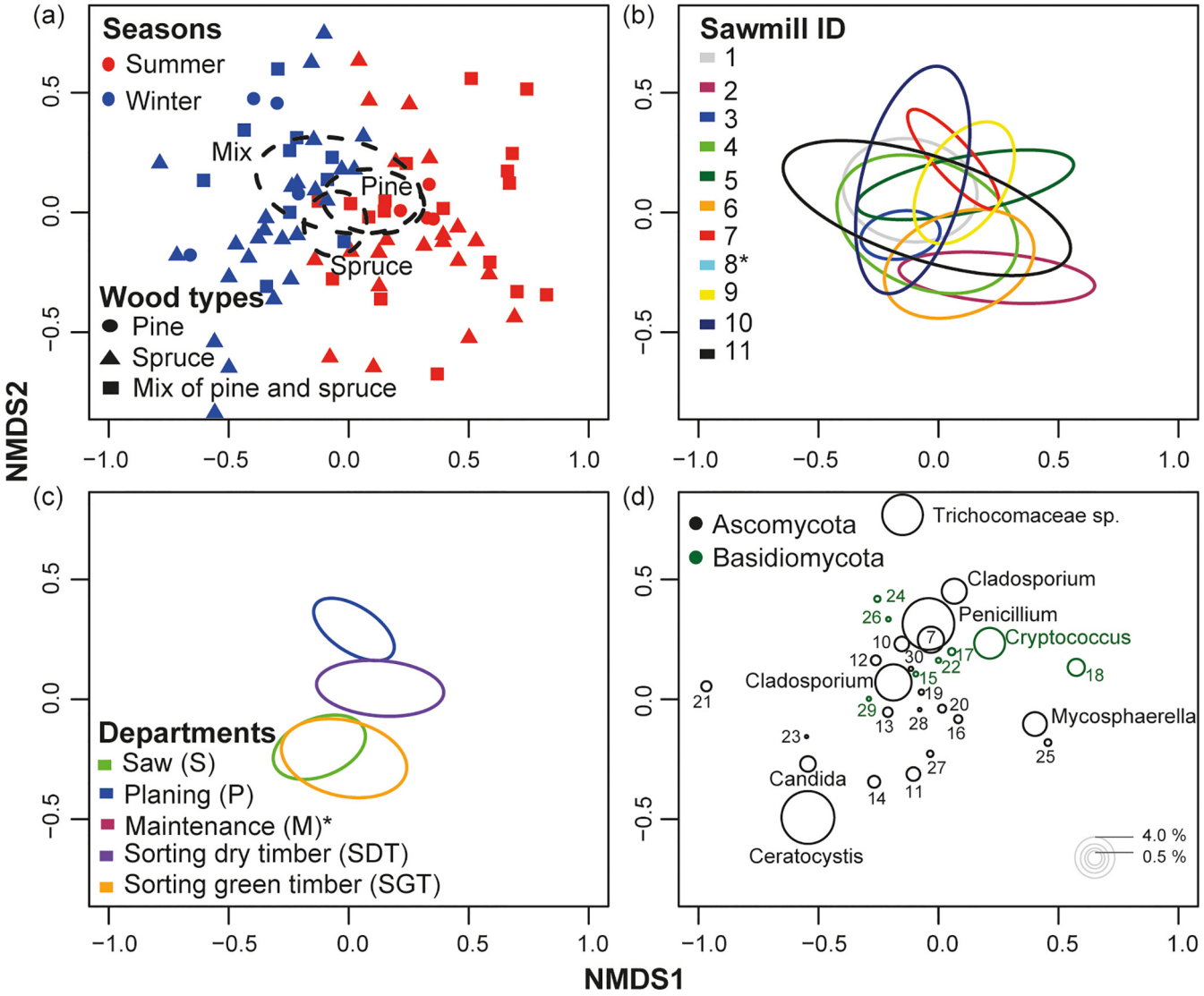

FIG 3 Nonmetric multidimensional scaling (NMDS) ordination analysis of fungal communities in personal air samples from Norwegian sawmills. (a) The plot represents 84 different personal air samples coded by color for the sampling season (summer and winter) and coded by shape for the different wood species (pine, spruce, or a mix of the two wood species) processed on the sampling day. (b) Differently colored ellipses represent the 95% confidence interval around the centroids of individual companies. (c) Differently colored ellipses represent the 95% confidence interval around the centroid for different departments at all sawmills. (d) NMDS ordination analysis showing the fungal operational taxonomic unit (OTU; species) composition from all sawmills. The ordination plot is based on all fungal OTUs present, but only the 30 most common OTUs are shown here. Each of these OTUs represented, on average, at least 0.5% of the total reads. The top 30 OTUs accounted for 49% of the total reads. All factors (season, wood type, sawmill, and department) had significant effects ($P < 0.05$) on the ordination configuration. An asterisk denotes a sawmill or department with too few samples for inclusion in the ordination analysis.

At the phylum level, the relative abundances of the *Ascomycota* and *Basidiomycota* were similar, regardless of whether spruce and pine wood was processed during the day of sampling (Fig. 4a; Table S2). However, we could see a higher dominance of the orders *Capnodiales* and *Polyporales* when pine was processed, whereas the *Saccharomycetales*, *Microascales*, and *Tremellales* dominated when spruce was processed (Fig. 4b; Table S2). The genus *Peterozyma* (a yeast and saprotroph) was strictly associated with the processing of spruce, whereas *Fomitopsis* (saprotrophs causing brown rot) and *Dothideomycetes* were common when pine as well as mixed wood types were processed (Fig. 5b). OTUs with >97% sequence identity to *Ceratocystis adiposa*, *Penicillium bialowiezense*, and *Mycosphaerella tassiana* (all of which are plant pathogens) and *Candida railenensis*, *Peterozyma xylosa*, and *Debaryomyces hansenii* (all of which are yeasts and saprotrophs) were common when spruce was processed, while OTUs with

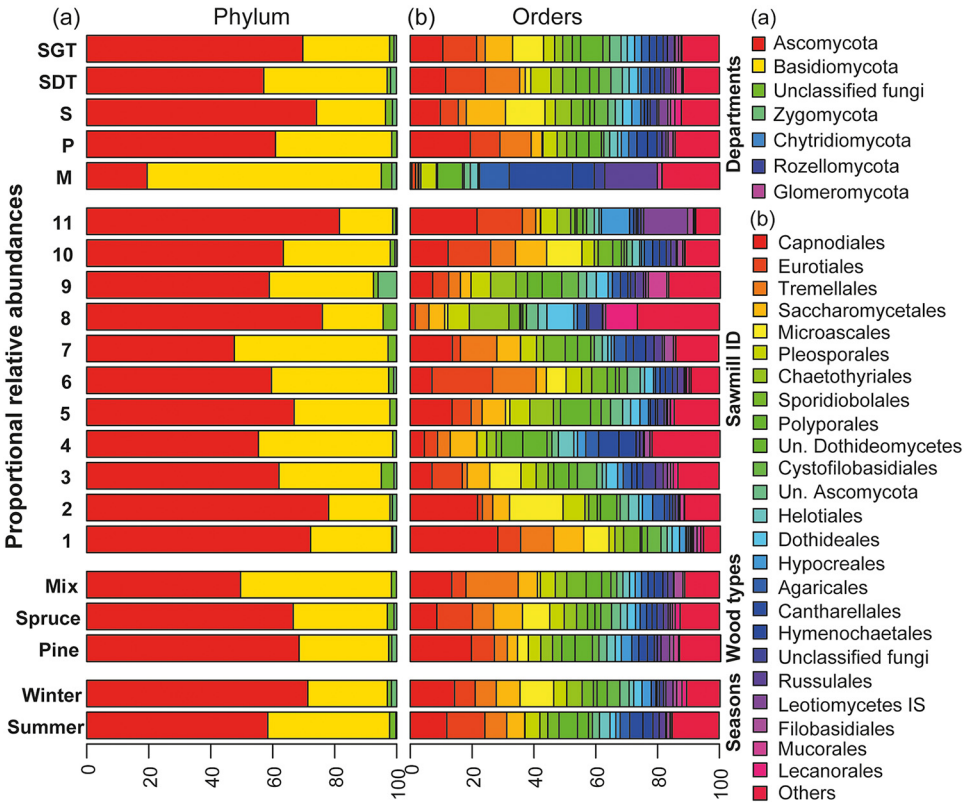

FIG 4 Proportional relative abundances of fungal compositions at the phylum (a) and order (b) levels recovered from personal inhalable samples in Norwegian sawmills. The data represent the average number of reads per sample for individual variables. S, saw department; SGT, sorting of green timber department; SDT, sorting of dry timber department; P, planing department; M, maintenance department; IS, *incertae sedis*.

similarity to *Cryptococcus albidus* (a yeast and saprotroph known to cause infections in animals and humans) were more prevalent when pine was handled (Table 3).

Ascomycota dominated in personal air samples from all sawmills, being relatively more abundant in the saw and the sorting of green timber departments than in the planing and the sorting of dry timber departments (Fig. 4a; Table S3). The proportion of *Ascomycota* was low in the maintenance department group, but due to the small sample size, it was not feasible to make direct comparisons with the other departments. At the level of orders, *Capnodiales* were abundant in the planing department, *Eurotiales* were abundant in the sorting of dry timber and the sorting of green timber departments, *Tremellales* were abundant in the planing and the sorting of dry timber departments, and *Saccharomycetales* were abundant in the saw and the sorting of green timber departments (Fig. 4b; Table S3). Significant departmental differences in the abundance distribution at genus level were also observed (Fig. 5c; Table S3). The saw and the sorting of green timber departments showed very similar compositions at the genus level, with a high abundance of the genera *Ceratocystis*, *Peterozyma*, and *Nakazawaea*.

Variation in the relative abundances of the *Ascomycota* was also observed among different sawmills, with the *Ascomycota* dominating in sawmills 1, 2, 8, and 11, whereas *Basidiomycota* were proportionally higher in sawmill 7 (Fig. 4a; Table S4). Similarly, a strong dominance of the order *Capnodiales* was detected in samples from sawmills 1, 2, and 11, whereas *Tremellales* were more common in sawmills 6 and 7 (Fig. 4b; Table S4). Proportionally, the order *Microscales* dominated in sawmill 2. Sawmills 4 and 8 had rather variable compositions compared to all other sawmills. The genus-level compositional distributions were similar for sawmills 1, 2, and 7, which were dominated by *Mycosphaerella*, *Rhodotorula*, and *Cryptococcus*, and distinct from those for sawmills 4,

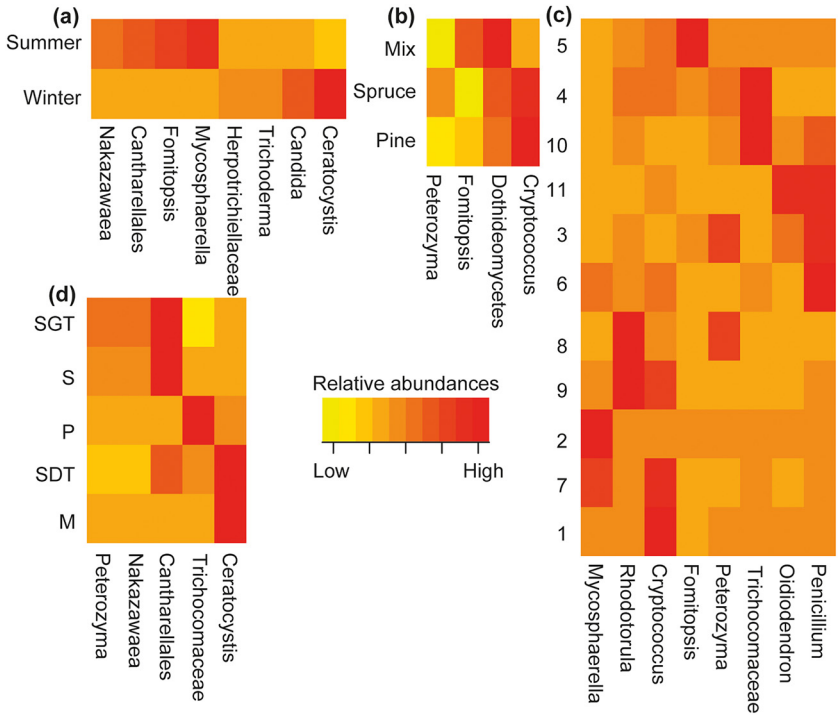

FIG 5 Hierarchical clustering-based heat plots for the proportional abundances of different taxa which varied significantly between two seasons (summer and winter) (a) and among three wood types (pine, spruce, and a mix of the two species) (b), 11 different sawmills (c), and five different departments (d). Only the genera with statistically significant differences (ANOVA and Tukey's HSD *post hoc* test) among the studied factors are shown here.

5, and 10, which were dominated by *Fomitopsis* and *Trichocomaceae*. *Oidiodendron* was the most common in sawmills 3 and 11, whereas *Penicillium* dominated in sawmills 3, 6, 10, and 11 (Fig. 5d; Table 3).

DISCUSSION

In this study, we have studied the fungal community pattern in the personal air samples of workers in five different departments at 11 sawmills, sorting mills, and planer mills processing pine, spruce, or both wood types during summer and winter. A broad spectrum of fungi was found, and the taxonomic groups characteristic of the exposure in sawmills in general were *Capnodiales*, *Microascales*, *Eurotiales*, and *Saccharomycetales* (all ascomycetes) and *Tremellales* (a basidiomycete). The workers' exposures differed in fungal diversity and composition, depending on the sawmill, department, season, and wood type.

Sawmills and departments. Different sawmills accounted for most of the variation in exposure to fungal communities, as indicated by the distinct fungal community structure and composition among the sawmills. This might be related to sawmill-specific factors, such as variations in production volume, production technology, work organization, and wood type. The processed timber may also have different origins that may play a role in the different fungal community characteristics. We have no information about the latter, but it is expected that the fungal communities associated with wood of different origins vary because of both host specificity and biogeographic differences in fungal communities (36). The richness difference observed between sawmills may also be due to some of these factors. The distinct fungal compositional profiles between departments across companies indicate that workers in different job groups are differently exposed. Although the fungal exposure level has previously been shown to differ between departments (1, 37), the large species diversity within each department and the distinct difference in fungal composition between departments

TABLE 3 Taxonomic affinity and occurrences of the 20 most abundant OTUs detected in personal air samples of workers at 11 different sawmills, at five different departments, at two different seasons, and during processing of different wood types[a]

							Total occurrence (%) by[d]:																				
					Total		Season		Wood type			Sawmill no.												Department				
OTU	Top hit	Taxonomic group	Identity[b]	Cov[c]	Occurrence[e]	% of reads[f]	Summer	Winter	Pine	Spruce	Mix	1	2	3	4	5	6	7	8	9	10	11	M	P	S	SDT	SGT
9004	*Cladosporium exasperatum*	*Capnodiales* (A)	97.4	100	34	6.7	38.6	42.5	41.4	40.4	37.5	62.5	50.0	50.0	37.5	40.0	40.0	42.9	50.0	28.6	20.0	37.5	42.9	1.5	2.2	2.9	3.4
4	*Ceratocystis adiposa*	*Microascales* (A)	100.0	100	19	5.9	0.0	47.5	6.9	31.9	25.0	25.0	33.3	75.0	12.5	20.0	40.0	0.0	0.0	0.0	20.0	0.0	0.0	0.7	0.9	2.0	1.1
7	*Penicillium bialowiezense*	*Eurotiales* (A)	100.0	100	34	4.4	29.5	52.5	34.5	44.7	37.5	50.0	16.7	75.0	12.5	20.0	50.0	57.1	0.0	28.6	50.0	50.0	57.1	0.7	1.8	1.0	1.1
15	*Cryptococcus albidus*	*Tremellales* (B)	99.1	100	25	3.9	22.7	37.5	31.0	23.4	62.5	100.0	0.0	12.5	37.5	10.0	30.0	71.4	0.0	28.6	0.0	25.0	71.4	0.4	3.6	0.0	3.4
1	*Mycosphaerella tassiana*	*Capnodiales* (A)	100.0	100	16	2.7	22.7	15.0	13.8	21.3	25.0	12.5	66.7	0.0	0.0	0.0	50.0	57.1	0.0	14.3	0.0	12.5	57.1	0.0	0.4	3.9	0.0
10	*Trichocomaceae*	*Eurotiales* (A)	100.0	100	26	2.6	22.7	40.0	34.5	29.8	25.0	37.5	16.7	25.0	25.0	10.0	40.0	57.1	0.0	42.9	40.0	25.0	57.1	0.4	1.3	1.0	2.3
17	*Dothideomycetes*	*Dothideomycetes* (A)	99.4	100	36	2.3	38.6	47.5	55.2	36.2	37.5	62.5	16.7	37.5	75.0	40.0	30.0	71.4	0.0	42.9	20.0	50.0	71.4	1.5	2.2	1.0	6.8
4287	*Cladosporium langeronii*	*Capnodiales* (A)	99.3	99	40	2.1	36.4	60.0	44.8	44.7	75.0	75.0	33.3	50.0	37.5	60.0	30.0	100.0	0.0	42.9	30.0	37.5	100.0	2.2	2.7	2.0	3.4
22	*Candida railenensis*	*Saccharomycetales* (A)	99.5	100	23	2.0	15.9	40.0	24.1	31.9	12.5	50.0	16.7	12.5	50.0	30.0	30.0	0.0	100.0	28.6	20.0	12.5	0.0	1.1	1.8	1.0	4.5
20	*Pleosporales*	*Pleosporales* (A)	100.0	100	21	1.5	15.9	35.0	27.6	17.0	62.5	62.5	0.0	37.5	0.0	20.0	20.0	57.1	0.0	42.9	0.0	25.0	57.1	0.7	2.2	0.0	0.0
13	*Nakazawaea holstii*	*Saccharomycetales* (A)	100.0	100	24	1.3	34.1	22.5	34.5	27.7	12.5	50.0	33.3	37.5	25.0	30.0	20.0	0.0	100.0	57.1	10.0	12.5	0.0	1.1	1.8	2.0	2.3
21	*Trichoderma trixiae*	*Hypocreales* (A)	100.0	100	30	1.3	22.7	50.0	34.5	40.4	12.5	37.5	33.3	75.0	37.5	10.0	40.0	28.6	50.0	28.6	20.0	50.0	28.6	0.4	1.3	2.0	3.4
19	*Aureobasidium melanogenum*	*Dothideales* (A)	100.0	92	30	1.3	25.0	47.5	27.6	38.3	50.0	87.5	16.7	50.0	50.0	30.0	40.0	28.6	50.0	42.9	0.0	12.5	28.6	1.1	3.1	1.0	4.5
24	*Peterozyma xylosa*	*Saccharomycetales* (A)	98.5	100	34	0.9	34.1	47.5	27.6	48.9	37.5	50.0	33.3	100.0	37.5	50.0	50.0	0.0	50.0	28.6	30.0	12.5	0.0	1.9	1.8	2.0	3.4
33	*Sporidiobolales*	Sporidiobolales (B)	100.0	100	23	0.9	20.5	35.0	24.1	25.5	50.0	75.0	16.7	25.0	0.0	10.0	30.0	71.4	0.0	42.9	0.0	25.0	71.4	0.4	2.7	1.0	0.0
16	*Epicoccum nigrum*	*Pleosporales* (A)	99.4	100	27	0.8	31.8	32.5	31.0	29.8	50.0	37.5	66.7	12.5	12.5	40.0	40.0	57.1	50.0	42.9	10.0	12.5	57.1	1.5	1.3	3.9	1.1
28	*Cryptococcus* sp.	*Tremellales* (B)	100.0	100	37	0.8	38.6	50.0	51.7	34.0	75.0	62.5	50.0	37.5	50.0	40.0	40.0	85.7	50.0	71.4	0.0	25.0	85.7	1.5	2.2	2.9	4.5
37	*Fomitopsis pinicola*	*Polyporales* (B)	100.0	100	27	0.8	59.1	2.5	34.5	27.7	50.0	37.5	0.0	25.0	50.0	60.0	60.0	28.6	0.0	14.3	30.0	0.0	28.6	2.2	1.3	0.0	4.5
27	*Herpotrichiellaceae*	*Chaetothyriales* (A)	100.0	100	19	0.7	15.9	30.0	31.0	19.1	12.5	37.5	16.7	37.5	0.0	30.0	30.0	0.0	0.0	71.4	10.0	0.0	0.0	1.1	1.3	1.0	0.0
67	*Debaryomyces hansenii*	*Saccharomycetales* (A)	100.0	100	10	0.7	11.4	12.5	0.0	17.0	25.0	12.5	0.0	0.0	0.0	10.0	30.0	42.9	0.0	0.0	20.0	0.0	42.9	0.4	0.4	0.0	0.0

[a]The different wood types consisted of pine, spruce, or a mix of the two species. S, saw department; SGT, sorting of green timber department; SDT, sorting of dry timber department; P, planing department; M, maintenance department; A, *Ascomycota*; B, *Basidiomycota*.

[b]The percent similarity of the OTU sequences in a BLASTn search against the UNITE database.

[c]The percent coverage of the OTU sequences in a BLASTn search against the UNITE database.

[d]Total occurrences of the OTUs in samples within each category.

[e]Total occurrence (in percent) of the OTUs across the data set, comprised of 84 different samples.

[f]Proportion of total reads assigned to each OTU.

shown in the present study are striking. There seemed to be a systematic turnover of fungal diversity throughout the process line, from dominating orders of *Saccharomycetales* in the saw department, *Saccharomycetales* and *Eurotiales* in the sorting of green timber department, *Eurotiales*, *Tremellales*, and *Capnodiales* in the planing department, and *Eurotiales* and *Tremellales* in the sorting of dry timber department. The fungal composition in the sorting of green timber department almost completely overlapped that in the saw department, with both being departments handling green timber that has not yet been dried. Logs entering the saw are newly debarked and thus are expected to bring along and release microorganisms related to the bark and the external natural environment. While some of this seemed to carry through to the sorting of green timber, other species were also detected. Although it has not been shown that the community composition in the heartwood and sapwood of *Picea abies* and *Pinus sylvestris* is different (38), fungi growing inside the wood may be released at later stages of the process line. Indeed, the dominating species to which workers were exposed observed in the sorting of dry timber department and the planer department were completely different, with no overlap with other departments. The fungi found in these department may also have occurred on green timber before being kiln dried and during storage of the dried timber and thereafter transported on the surface of the timber planks to the sorting department, where they were aerosolized. As workers in different departments are clearly exposed to distinct fungi, future exposure and risk assessments in the sawmill, sorting mill, and planer mill industry should discriminate between departments.

Seasonal differences. We observed a higher fungal richness in summer than in winter, as expected, due to the more favorable fungal growth conditions and higher rate of spore dispersal in the warm and humid summer season. The species to which the workers were exposed differed between summer and winter, although the seasonal difference in species composition was relatively small (accounting for 3% of the variation). This is in concordance with the findings of earlier studies, where seasonal differences in fungal communities associated with plant leaves (39), roots (40), and soil (41) have been observed. Interestingly, there were systematic seasonal differences in taxonomic composition, with more ascomycetes being observed during winter and more basidiomycetes being observed during summer. This fits into the rough classification of ascomycetes being more tolerant to environmental stress, in this case, harsh winter conditions. Especially, many yeasts, which are mainly decomposers, were relatively more abundant during winter. This corresponds well with the findings from Santalahti and colleagues showing that saprotrophic fungi, which are metabolically active when photosynthesis conditions are less favorable (42), dominate during winter compared with their prevalence during the growth season in boreal Scots pine forests (41). The polypore *Fomitopsis pinicola*, an extremely widespread brown rot fungus in the boreal forest landscape, was relatively more abundant during summer, when it is expected to be vegetatively more active. However, the abundance of the 10 most common OTUs was higher in winter than in summer. This relationship might be related to the evenness of the samples; i.e., if there are fewer species during winter, these will appear to be relatively more abundant. Hence, the summer season most likely represents the most diverse fungal exposure. This was also supported by ordination analyses showing a broader ordination configuration of samples collected in summer than of samples collected in winter. The spore exposure is also shown to be higher in summer than in winter at Norwegian sawmills (A. Straumfors, submitted for publication). Overall, these results highlight that the seasonal shift in fungal communities related to the sawmills may impose variation in occupational exposure.

Differences related to wood type. The workers were exposed to significantly different proportions of several fungal genera depending on the wood type that they were handling. The overall fungal composition of the exposure was, however, only slightly affected by the wood type processed in the sawmills, as shown by the very low contribution (1%) of wood type to the variation in the fungal composition in the

variation partitioning analyses, but it had a significant effect in NMDS ordination analyses. A higher fungal richness was also observed during work with spruce than during work with pine, but the average OTU richnesses per sample were not significantly different between the two wood types, and this difference may have been due to factors in the sawmills that were not related to the wood types. Whereas the levels of exposure to fungi in the phyla *Ascomycota* and *Basidiomycota* were similar when workers were working with spruce or pine, the relative proportion of *Basidiomycota* to which workers were exposed was higher when they were working with both wood types, suggesting either an alternative work organization or different competition in dispersal or growth among fungi when mixed wood types are handled at the sawmills. Another possibility is that mixed wood types were more commonly processed during summer. The differences in diversity (Shannon diversity index, not richness), evenness, and the top 10 OTU abundance between sawmills suggest that sawmill-specific conditions other than different wood types indeed play a role.

Fungal diversity in relation to traditional exposure measurements. Measurements of dust masses and fungal spore counts in microscopy are common methods in occupational exposure assessments and have been published for the same workers evaluated in the present study (1). The HTS technology provides a further, more detailed picture of the exposure, and it is interesting to compare the fungal community pattern (richness, diversity, abundance) of the personal samples with air sample volume, dust masses, and fungal spore counts.

We observed that the abundance of the top 10 OTUs was positively correlated with the dust concentration but not with the spore concentration. This suggests that fungal particles other than spores are present in the dust and are included in the HTS method but not in the spore count analyses in field emission scanning electron microscopy (FESEM). This is not unexpected, and fungal fragments have indeed been shown to be the dominating fungal particles (by number) in the thoracic aerosol fraction in sawmills (37). Additionally, many fungi (especially basidiomycetes) are nonsporulating and *Mycelia sterilia* and sterile fungi do not produce spores. Although fragments are not expected to dominate the fungal mass that is measured by the use of dust gravimetry, the DNA contribution from the fungal mycelium and fragments may explain the positive relationship between the top 10 OTU abundance and dust exposure. A possible explanation for the negative association between fungal diversity measures and spore counts could be that only a few species had sporulated and dominated the spore count.

Job group-specific fungal exposure profiles. The observation that different job groups were exposed to biomes with specific fungal profiles is supported by other studies that utilized HTS technologies for the characterization of microbial diversity. The fungal profile in waste-sorting plants was dominated by the *Ascomycota*, with the prevailing genera being *Penicillium* and *Aspergillus* but also *Rhizopus* and *Wallemia*, as well as by the order *Hemicarpenteles*, with this profile diverging from the general sawmill profile (30). The fungal profile in dairy farms had some similarities with the profile in sawmills, although higher fungal classes were assigned and were dominated by the *Eurotiomycetes*, *Dothideomycetes*, *Wallemiomycetes*, *Agariomycetes*, *Sordariomycetes*, and *Tremellomycetes* (32). Variations in relative abundance between farms were observed, but in contrast to the present sawmill study, in which the profiles could be shown to be statistically significantly different between departments across sawmills, there were too few samples to evaluate the compositional variations between farms. Fungal diversity has also been shown to differ between facilities processing carcass compost, dominated by *Eurotiomycetes*, and facilities processing domestic compost, dominated by *Sordariomycetes* (31). These rough comparisons with the first insights of fungal diversity in different occupations suggest that each job group or occupation has some shared and some unique fungal exposure patterns. The use of HTS technology is important for the spatial and temporal characterization of the fungal diversity in

different occupations. Subsequently, occupation-specific signature profiles may be used in improved exposure and risk assessments.

Health aspects. The exposure difference in fungal diversity between seasons and sawmills suggests that variations in exposure-related health effects between seasons and companies can be expected. More importantly, the distinct fungal profiles between departments across companies indicate that different job groups are differently exposed and that health risks can be department specific. Thus, exposure assessments by department or job group are important for evaluating health risks in sawmills.

The broad spectrum of fungi detected in the present study includes many known human pathogens (43–46). Fungi in the order *Eurotiales*, to which *Paecilomyces* spp. belong, were abundant in several of the departments, whereas *Mucorales* (such as *Rhizopus*), belonging to the *Zygomycetes*, had a low abundance. This is in contrast to previous studies from Norwegian and Swedish sawmills, where *Rhizopus microsporus* and *Paecilomyces variotii* were the dominant fungi, and the presence of IgG antibodies against these species in the blood of exposed workers correlated with the exposure levels (9, 47). Furthermore, these species were reported to be responsible for hypersensitivity pneumonitis in Swedish sawmill workers (48). The use of cultivation versus DNA metabarcoding (this study) and possible shifts in the mycobiome due to changes in process technology over the past decades may account for some of these differences. However, a Canadian sawmill study also recovered very few *Rhizopus* and *Paecilomyces* spp. by cultivation (4).

The hygiene hypothesis proposes that the rising prevalence of allergic diseases is causally related to a reduced exposure to environmental microbes and harmless infections during childhood (21). One of the most important observations that supports the hygiene hypothesis is that children who grow up on traditional farms are less prone to allergic diseases than children who live in the countryside but not on a farm (22). The protection seems to be associated with the exposure to a wide range of microbes provided by the farm environment (19, 20, 23), and the hygiene hypothesis has been reformulated to the biodiversity hypothesis (24). Contact with complex microbial communities early in life is a main driver for the development of a balanced immune system (49) and human microbiota (50). The question is, furthermore, what impact that complex microbial occupational exposures may have on the health of the working population. The first stage in the attempt to answer this question is the characterization of the microbial complexity of occupational exposures. An investigation of the relation between the exposure diversity and inflammatory and anti-inflammatory responses and potential health effects will be a next step. The present study describes the whole spectrum of fungal exposures in the Norwegian sawmill industry. The association between fungal diversity and health effects will be explored as part of a larger longitudinal study of exposure and health in Norwegian sawmills, sorting mills, and planer mills.

Conclusions. This study provides insights into the complete spectrum of fungal inhalable exposure across different sawmills, departments, wood types, and seasons in the Norwegian sawmill industry. The differences in exposure diversity suggest that the potential health effects of fungal inhalation may also be different; hence, a future risk assessment based on the fungal diversity differences should be performed. This study may serve as a basis toward establishing a fungal profile of signature species that are specific for sawmills and that can be measured quantitatively in future risk assessments of sawmill workers. The presence of important species in the most abundant taxonomic groups should be validated with more precise and quantitative methods, such as species-specific PCR. Further exposure assessment using specific methods to measure the signature mycobiome may enable more precise risk assessments, particularly in relation to species pathogenic to humans.

MATERIALS AND METHODS

Sampling strategy. Eleven industrial sawmill, sorting, and planing companies in Norway processing pine (*Pinus sylvestris*), spruce (*Picea abies*), or both pine and spruce were included in the study. Eighty-six

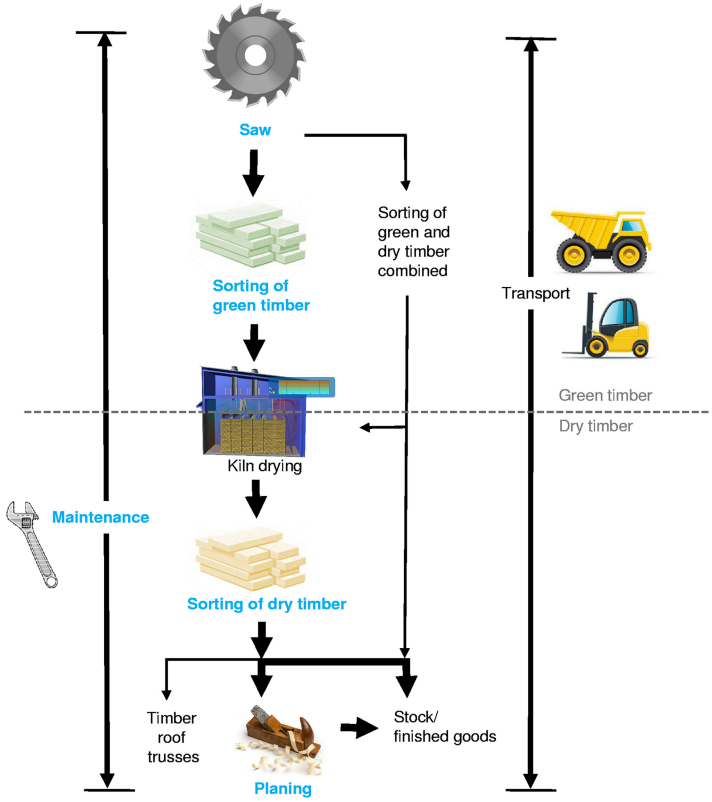

FIG 6 Process overview at sawmills, sorting mills, and planer mills. Arrows show the process direction, and arrows with a greater thickness indicate the parts that are the most common. Maintenance and transport included work along the whole process line. The mycobiomes in personal inhalable samples from workers in the saw department, sorting of green timber department, sorting of dry timber department, planing department, and maintenance group (blue labels) were studied.

full-shift personal inhalable dust samples were collected during the years 2013 and 2014. Two to 10 (mean, 8) samples per company were collected. Sampling was performed both during summer ($n = 46$) and during winter ($n = 40$) when pine ($n = 29$), spruce ($n = 47$), and both pine and spruce ($n = 10$) were being processed. The sampling included exposure measurements in five departments: saw ($n = 28$), planing ($n = 29$), sorting of dry timber ($n = 17$), sorting of green timber ($n = 11$), and maintenance group ($n = 1$). A schematic illustration of the industrial process is given in Fig. 6. The associated job groups have been published previously (1).

Personal sampling of inhalable dust. The samples were collected with 37-mm conical inhalable sampling (CIS) cassettes with conical inlet holes of 8 mm (Casella Solutions, Kempston, UK), using an airflow of 3.5 liters/min. The flow rate was calibrated and recorded using a digital flow meter (Defender; SKC Inc., Eighty-Four, PA, USA) before and after sampling. The workers carried two sampling cassettes loaded with a polycarbonate (PC) filter (pore size, 1.0 μm) in parallel, one for microscopic spore counts in field emission scanning electron microscopy (FESEM), as described previously (1, 37), and one for DNA isolation and sequencing. Other workers carried sampling cassettes with a polyvinylchloride (PVC) filter (pore size, 5 μm) for measurement of the aerosol mass by gravimetric analyses, as described previously (1). The mean of the dust masses by department, season, company, and day was used to allocate representative dust mass values to the workers selected in the present study (see Table S1 in the supplemental material).

Filter elution, DNA extraction, amplification, and sequencing. Exposed filters were carefully transferred to 15-ml tubes, and 5 ml of phosphate-buffered saline with 0.1% Tween 20 (0.1% PBST) was added, followed by careful shaking for 3 min at 400 rpm in a vertical position. The samples were subsequently sonicated for 3 min and mildly and briefly vortexed, followed by a short spin at 2,500 rpm in the centrifuge. The filters were removed, and the filter eluates were centrifuged at 3,500 rpm for 30 min at 4°C. All of the supernatant except for 50 μl was removed, and the pellet was resuspended in the remaining 50 μl and transferred to 1.5-ml Eppendorf tubes. The 15-ml tube was flushed three times with 50 μl cetyltrimethylammonium bromide (CTAB) buffer to ensure complete transfer of the sample.

The samples were then kept on ice until cell lysis was performed by treatment with liquid N_2 for 30 s and direct transfer of the samples to 90°C for 4 min; this procedure was repeated once. After some cooling, the samples were spun down and 400 μl of 0.5-mm silica/zirconia beads (BioSpec Products Inc.,

Bartlesville, OK, USA) was added. The samples were then treated with liquid N_2 for 30 s, followed by bead beating (Mini Beadbeater-96; BioSpec Products Inc.) for 4 min, a process which was repeated once, before 250 μl of CTAB buffer and 4 μl of RNase A were added. Subsequently, the samples were incubated with shaking (300 rpm) for 1 h at 65°C. After a quick spin, 130 μl of P3 buffer from the Qiagen isolation kit (DNeasy plant minikit; Qiagen n.v., Hilden, Germany) was added, the mixture was mixed with the extract, and that mixture was incubated for 5 min on ice before the debris was pelleted by a new spin. The lysate was transferred to a new 1.5-ml Eppendorf tube, and DNA isolation was then completed with DNeasy mini-spin columns according to the manufacturer's protocol. The DNA concentration was measured with the PicoGreen reagent (Quant-iT kit; Invitrogen), and the DNA samples were stored at −20°C. The extracted DNA concentration ranged from 0.5 pg/μl to 900 pg/μl (mean, 125 pg/μl).

Fungi were amplified using the fITS7a and ITS4 (51, 52) primer combination. To achieve demultiplexing, 14-bp multiplex identification DNA tags (MIDs) were attached at the 5′ end of both the forward and the reverse primers. The PCR mixture consisted of 10 μl 2× Kapa polymerase master mix, containing high-fidelity Kapa DNA polymerase (0.5 U at 1×) in a proprietary reaction buffer containing deoxynucleoside triphosphates (dNTPs; 0.3 mM each dNTP at 1×), $MgCl_2$ (2 mM at 1×), stabilizers, 1 μl of each of the reverse and forward primers (0.25 μM), and either 3, 4, or 5 μl of DNA template, depending on the DNA concentration in the samples. The volume was adjusted to 20 μl with Milli-Q water. The PCR conditions were as follows: initial denaturation at 95°C (3 min); amplification with 30 cycles of 98°C (10 s), 54°C (20 s), and 72°C (25 s); and a final cycle of elongation at 72°C for 7 min. The PCR products were cleaned using a ChargeSwitch magnetic PCR cleanup kit (Invitrogen).

The DNA concentration of the amplified samples was determined using the Quant-iT PicoGreen reagent, and 65 ng of each sample was pooled in two libraries. The pools were diluted 7-fold to a final concentration of 10 ng/μl, where the purity and concentration were measured using a NanoDrop spectrophotometer and a Qubit (v2.0) fluorometer (Life Technology, Carlsbad, CA, USA), respectively. In addition to the 86 sawmill samples, 2 negative controls, 9 blank filter controls from the sawmills, and 12 technical controls (which were run with different library tags on the same sequencing run or with identical tags on different sequencing runs) were also included in the sequencing run. To estimate index jumping in the sequencing run, 16 mock community samples comprised of fungal cultures of *Rhizopus microsporus*, *Cladosporium allicinum*, *Penicillium variotii*, *Fusarium graminearum*, *Aspergillus fumigatus*, and *Wallemia sebi* with known genome sizes were also included as single, double, or multiple species. Molecular data were generated using Illumina MiSeq paired-end (PE; 2 × 300 bp) sequencing using two different lanes.

Bioinformatics work flow. The raw reads received from MiSeq sequencing were passed through the error correction tool BayesHammer, based on Bayesian clustering (53). Corrected reads were paired using the PEAR (v0.9.10) program with a minimum overlap of 15 bp and a quality score of 20 (Q20) threshold for trimming the low-quality part of a read (54). Paired reads were passed for quality control using the FASTX-Toolkit (v0.0.14; fastq_quality_filter; http://hannonlab.cshl.edu/fastx_toolkit/index.html) and the following parameter settings: a minimum Phred quality score of 36 and a proportion of bases that must have a minimum quality score of 0.9. The reads were further quality controlled using the VSEARCH (v2.4.3) program (55) to remove reads with an ambiguous base of 0, a length of <100 bp, and a total number of expected errors (E) of >0.5 for all bases. High-quality filtered reads were demultiplexed using the SDM (v1.41) program embedded into the LotuS pipeline (56). Using the FQGREP (v0.4.4) program (https://github.com/indraniel/fqgrep) and FASTX-Toolkit, the reads were oriented in the same direction and the primers were trimmed. The ITS2 region of the reads was extracted using the ITSx (v1.0.11) program (57), and reads of <100 bp were excluded from the data set. We used VSEARCH for dereplication, followed by removal of all the global singletons.

Furthermore, the same program was employed for clustering of the reads at a 97% similarity threshold using the –cluster_size function, and the most abundant sequence of each cluster was designated the representative sequence. Chimera analysis was performed on the representative sequences using the –uchime_denovo algorithm (58), implemented in VSEARCH, with a minimum divergence parameter of 0.8, an abundance skew of 2, and a minimum difference in segment of 3. In order to focus on the most widespread and abundant fungi and to minimize the impact of rare OTUs resulting from sequencing and PCR errors, all OTUs with <10 reads were removed (59). Taxonomic assignment was made by comparing the representative sequence against the sequences in the curated reference database UNITE (v6) (60).

To control for erroneous overestimation of diversity, the OTU table was curated with the postclustering algorithm LULU, which in a predefined manner groups sequence clusters with high sequence similarity and co-occurrence patterns into the same OTU and, hence, corrects for oversplitting (61). Thereafter, OTUs with no hit by BLAST analysis, the highest similarity to the plant kingdom, and <80% query coverage as well as identity were excluded from further analysis. To account for possible leakage or reads among multiplexed samples due to tag switching (62, 63), the number of reads for each OTU across all samples was summed, and a threshold of 0.5% was applied to remove low-frequency occurrences of the respective OTU. This threshold was selected based on the observed leakage level of reads into the positive controls (mock community samples). This data treatment strategy generated an OTU table in which all the control samples (extraction, negative, and field blanks) were perfectly clean with no reads. Furthermore, the mock composition represented by mock communities was per our expectations. This indicated the appropriateness of the data treatment procedure to account for index hopping. As expected, both technical replicate samples displayed more intersample than intrasample variances in community structure (see Fig. S1 in the supplemental material). This suggests the robustness and consistency of the sequencing strategy and the data generated with two different sequencing runs

using different combinations of barcode settings. One representative sample from the replicated samples was randomly picked and included in the final data set. Two samples did not work while sequencing; therefore, the final data set comprised 84 samples.

The rank abundance curve generated from this data set suggests that the reads were not equally distributed among the samples (Fig. S2a) and that the distribution patterns of the reads per OTU were also skewed, with some samples having only a few very abundant OTUs and many samples having very low abundances (Fig. S2b). Per sample rarefaction curves of OTU richness indicated that complete diversity was not captured for the majority of the samples (Fig. S3). Additionally, a positive relationship between OTU richness and sequencing depth was also observed ($R^2 = 0.29$; $P < 0.001$). To overcome this possible sequencing depth bias, we normalized the data set by randomly subsampling down to 3,796 reads per sample. The data were further converted into proportions and, to improve variance homogeneity, arcsine transformed prior to analyses.

Statistical analysis. The statistical analyses were performed in R (v3.5.0) software (64). Richness, Shannon, and evenness diversity measures and the total abundance of the 10 most common OTUs were calculated using the R package vegan. The levels of variation of these most common OTUs across different sawmills ($n = 11$), departments within the sawmills ($n = 7$), the wood species processed on the day of sampling ($n = 3$), and season ($n = 2$) were examined using ANOVA followed by Tukey's honest significant difference (HSD) *post hoc* test from the R package agricolae and visualized using box plots. Differences in the proportional abundances of the different genera (for all factors under investigation, as described above) were also tested using ANOVA (the Benjamini-Hochberg false discovery rate correction was applied), followed by Tukey's HSD *post hoc* test, and are illustrated by heat plots based on hierarchical clustering. To test for the relationship between different diversity measures and air volume, dust mass, and spore counts (logarithm transformed), we used a generalized linear model (GLM). Species accumulation curves for the number of cumulative OTUs for different sawmills, departments, seasons, and the wood types used on the sampling day were calculated using the function specaccum from the R package vegan with 10,000 permutations.

The Bray-Curtis dissimilarity index was used to generate community distance matrices and was used further in all community structure analyses. To address the relative importance of different factors (11 sawmills, 5 departments within each sawmill, 3 different wood types processed on the day of sampling, and 2 seasons) and vectors (air volume, dust mass, and spore counts) on fungal community structure, multivariate permutational analysis of variance (PERMANOVA), implemented in the Adonis function of the vegan package, was used. PERMANOVA was executed using a forward selection practice to improve the final model (65). First, single-variable models were examined, and thereafter, only significant factors were included in the final model in order of their R^2 values. Furthermore, nonmetric multidimensional scaling (NMDS) ordination analyses were used to visualize the effects of the studied variables on the fungal community composition using the metaMDS function of the vegan package (66). Centroids of the factors were fitted into NMDS plots using the function envfit. The ordibar and ordiellipse functions were used to plot the 95% confidence intervals (CI) of the different departments and sawmills, respectively. A significance level consisting of a P value of <0.05 was used throughout the study. To gain further partitioning of the variation of community dissimilarity from different factors (sawmills [$n = 11$], departments within sawmills [$n = 5$], the wood types processed on the day of sampling [$n = 3$], and season [$n = 2$]), redundancy analysis (RDA) in the package vegan (varpart function) was carried out. The R^2 values were adjusted according to each predictor (factor) level.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AEM.01448-19.

SUPPLEMENTAL FILE 1, PDF file, 2.2 MB.

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