This is the accepted version of the following article:

Nunez, M., & Hammer, H. (2014). **Microbial specialists in below-grade foundation walls in Scandinavia**. Indoor air, 24(5), 543-551, which has been published in final form at http://dx.doi.org/10.1111/ina.12095.

Abstract

Below grade foundation walls are often exposed to excessive moisture by water infiltration, condensation, leakage, or lack of ventilation. Microbial growth in these structures depends largely on environmental factors, elapsed time, and the type of building materials and construction set-up. The ecological preferences of Actinomycetes (Actinobacteria) and the moulds *Ascotricha chartarum*, *Myxotrichum chartarum* (Ascomycota), *Geomyces pannorum*, and *Monocillium* sp. (Hyphomycetes) have been addressed based on analyses of 1764 samples collected in below grade spaces during the period of 2001-2012. Our results show a significant correlation between these taxa and moist foundation walls as ecological niches. Substrate preference was the strongest predictor of taxa distribution within the wall, but the taxa's physiological needs, together with gradients of abiotic factors within the wall structure, also played a role. Our study describes for the first time how the wall environment affects microbial growth.

Key words: moulds, Actinomycetes, building ecology, water damage, below grade foundation walls, microbial ecology.

Practical implications

The field of building ecology is a novel branch of microbial ecology, with possible impact on residents' well-being. We have characterized below grade foundation walls as ecological niches for microorganisms. Knowledge about substrate preferences and physiological needs of the most common taxa in these structures allows us to infer how environmental factors determine taxa distribution through the wall cavity.

Introduction

Foundation walls are the boundary that separates the indoor from the outdoor in below grade spaces, and water damage associated with building practices is common. This is especially relevant in conditioned rooms, as moisture within wall cavities is difficult to detect. The damage scope in these structures depends partly on abiotic parameters, such as moisture content and temperature, but also on building design and the physiological needs of the microorganisms involved.

Traditionally, Scandinavian basements have been used as storage spaces. Below grade foundation walls are usually made of either granite stone sealed with cement, or different types of concrete (Frøstrup, 1993). From the1960's, it was popular to rehabilitate old basements, by insulating them from the inside. This was done by placing an air barrier (usually asphalt-impregnated building paper) on the concrete surface, a timber frame with mineral fibre insulation, and wood panelling or gypsum boards as interior finishes (Frøstrup, 1993). Until the 1990's it was recommended that a damp barrier was placed behind interior finishes. This caused massive microbial growth in wall cavities, as moisture could not dry towards the room. Nowadays, water- and thermal barriers on the outside provide a more robust structure (Rolstad and Blom, 2007). Still, water and water vapour penetrating foundations through joints, cracks or imperfections, besides condensation, make these structures very vulnerable to persistent microbial growth. The bottom part of the wall eventually becomes saturated with water, and drying potential is often limited (Ueno and Lstiburek, 2010). Microbial communities in this ecological niche have not been characterized before.

Growth versus presence

The presence of mould spores as background “noise” is not considered atypical in indoor environments. However, moisture damage and subsequent microbial growth are problematic for indoor air quality (Bornehag et al., 2001). Remediation of infested areas has proved to reduce homeowners complains (Pitkäranta et al., 2011). Since there is no practical way to eliminate all mould spores from the indoor air, preventive measures during the building design phase should focus on: 1) controlling the moisture that is essential for mould growth, and 2) avoiding substrates that promote *in situ* growth (autochthonous growth).

The prerequisites for microbial growth with regard to temperature and nutrients are usually present in below grade foundation walls, at least for long periods of the year. Paper-based building paper, gypsum boards, soiled concrete, and wood products are favourite nutrient sources. The critical factors for growth are moisture and elapsed time. Most microbial species thrive between relative humidity (RH) 75% and 100%. In order to sustain growth and massive sporulation, the substrate must be wetted for more than 48-72 hours and the temperature must be above 5 °C (Rowan et al., 1999; Viitanen and Ojanen, 2007). As RH rises after moisture damage, an ecological succession starts on this ecological niche, typically with *Aspergillus* and *Penicillium* species as pioneers. In the late phase, tertiary colonizers, typically cellulolytic species and species with sexual spore formation, become established. (Grant et al., 1989). Microscopic invertebrates such as mites and Collembola feed on the microbial community, and the ecosystem reaches a dynamic maturity (Gams, 1992). Knowing the ecological role of

microorganisms in the wall structure is essential to disclose the processes that promote microbial damage and further spore spreading to the indoor air.

The importance of direct observation techniques in ecological studies

Until recently, most of our knowledge on microbial ecology derived from culture studies in the laboratory. To a certain extent, laboratory results can be extrapolated to the building environment. However, laboratory tests are usually carried out on standard media, axenic conditions, and static temperature and humidity (de Hoog et al., 2000). In the building ecosystem, microorganisms encounter fluctuation of abiotic factors, competition against other microorganisms, and raid by invertebrates (Yang, 1996).

Substrate preferences of indoor microorganisms have been widely addressed (Pasanen et al., 1992; Gravesen et al., 1999; Hyvärinen et al., 2002; Andersen et al., 2011), but few studies focus on building structures as ecological niches for autochthonous populations (Andersson et al., 1999; Pessi et al., 2002). Likewise, mathematical models of growth requirements and substrate preferences are designed for moulds as a group (Rowan et al., 1999; Sedlbauer, 2001; Viitanen, 1997), without taking into account specific species requirements and interactions.

In the last few years, molecular techniques have been widely used to characterize indoor microorganisms in settled dust. Pitkäranta et al. (2008) compared results obtained from both cultural and molecular techniques, and found that common indoor genera such as *Aspergillus* and *Penicillium* were hardly detected by DNA sequencing. Moreover, only 41% of the OTUs (Operational Taxonomic Units) obtained by DNA sequencing could be identified to species, and over 75% of the OTUs from one building were found in only one sample. Similar results have been obtained in later studies

(Rintala et al., 2008; Amend et al., 2010; Adams et al., 2013). As a result, the identity and ecology of most of the OTUs found indoors still remains obscure, partly because sequence-based species identification is not straight-forward, but also because DNA detection does not distinguish between accidentally present microorganisms and those that are part of an active ecological community (Klein, 2007). Recently, rRNA and the ITS region have been used as markers of fungal activity in wood decomposition studies (Rajala et al., 2011). The challenges and uncertainties surrounding this technique have been discussed by the authors.

Our aim in this work was to characterize functional microbial communities by direct observation techniques. Observations are limited to the most abundant below-grade specialists, as they are major contributors to spore production over time in damaged foundation walls. Their effect on the residents' health conditions remain to be addressed by quantification and characterization of fragments and spores that settle elsewhere in the building, away from their originating point.

Materials and methods

In order to reveal the most common taxa growing in below grade spaces, we first analysed 13,620 samples from a database of building control assessments in Scandinavia, mainly from South and Central Norway, after moisture damage. We have not made any distinction among building types in our study, since this is not relevant in microbial distribution indoors (Lee and Jo, 2006; Amend et al., 2010). The database was compiled over an 8-year period (2001–2009), and included taxa that were found at least once in below grade spaces. Samples were collected in different room types such as attics, bathrooms, kitchens, warm rooms (including bedrooms, living rooms, offices,

classrooms, etc.), besides basements and/or crawlspaces. The type of substrate and building structure was known for all samples.

Taxa were identified to either genus or species level, and ranked by decreasing occurrence in below grade spaces. Those with over 50% of their samples found in below grade spaces were chosen for further analyses. Taxa that occurred in less than 1% of the samples were removed from the dataset.

Additional records of the target taxa collected during 2010–2012 were added to our dataset. Ultimately, a total of 1764 samples of the target taxa collected in below grade spaces (basements and crawlspaces) over an 11-year period (2001-2012) were further analysed regarding ecological niche specialization (Devictor et al., 2010).

The sampling purpose was to document microbial growth on different building substrates and structures in below grade spaces after mould or moisture complaints. Sampling was undertaken on both hidden and exposed substrate surfaces where either discolouration or elevated moisture levels were detected during building inspection.

Samples were taken by means of 12 cm long, transparent tape lifts of standard width. At least one tape lift was taken from each building material within the target structure. Tapes were cut to 6 cm long pieces in the laboratory, and observed under an Olympus BX 45 optical microscope at 400–1000 magnification. One drop of Lactophenol blue solution (© Merck) was added to a microscope slide, and the tape was used as cover glass.

The presence of microbial sporulating structures, such as conidiophores of *Geomyces pannorum* and *Monocillium* sp, ascomata of *Ascotricha chartarum* and *Myxotrichum chartarum*, or filamentous growth of Actinomycetes was the criterion for

growth documentation. Species of *Aspergillus* and *Penicillium*, the most common genera growing in buildings (Andersen et al., 2011; Pitkäranta et al., 2008), were excluded, since these are primary colonizers (Górny, 2004) and do not show special preference for basements or crawlspaces as ecological niches. Fungal species were identified by optical microscopy following Gams (1971) and de Hoog et al. (2000). Actinomycetes were not further identified.

Substrates that accounted for less than 1% of the samples, such as mineral insulation, plastic, or ceramic tiles, were removed from the dataset. The remaining substrates were merged to four categories, namely concrete (including painted concrete and vinyl/linoleum-covered concrete slabs), gypsum board, building paper, and wood (including fibreboards, chipboards, OSB plates, and plywood), as we mainly found the same taxa growing on concrete, painted concrete, and vinyl/linoleum-covered concrete slabs, or on fibreboards, chipboards, plywood, and wood respectively (data not shown). This decision was supported statistically by the multinomial regression analyses presented in the results. Merging the substrates to wood and concrete improved the regression model in the sense that the Akaike information criterion (Claeskens and Hjort, 2008) was reduced from 3363 to 3353 and 3358, respectively.

As for type of building structure, the majority of the samples in crawlspaces were taken from ceilings, since it was in such locations that microbial damage was most obvious under building control assessments (Blom, 2006). In basements, samples were taken from both insulated and non-insulated ceilings, and below grade floors and foundation walls.

Statistical analyses

Data were statistically analysed in order to reveal ecological trends with respect to microorganisms by means of inductive reasoning.

Contingency tables counted the number of samples in each combination of two variables, out of our variables 'taxon', 'building structure', and 'substrate'. Central in the analysis was to test dependence (inhomogeneity) between taxa and building structures. Dependence tests were based on the Fisher exact test, which computes the exact probability of observing the given contingency table under the assumption of independence. The computations were based on the hypergeometric distribution and a small probability, say $p < 0.01$, indicated dependence (Agresti 1992). If inhomogeneity was observed, we tested which taxa were overrepresented on a particular structure. E.g. to test if Actinomycetes were significantly overrepresented on foundation walls for the substrate concrete, we computed a 2x2 contingency table with two taxa categories: 'Actinomycetes' and 'other taxa', and two building structure categories: 'foundation wall' and 'other structures' and ran an one sided exact Fisher test. A significant result meant that for concrete, Actinomycetes were overrepresented on foundation walls compared to the other taxa.

We run this test for all combinations of taxa and structures (data not shown). The significance level in each 2x2 contingency test was chosen so that we were over 99% sure that we had not defined any taxa as overrepresented on a structure when it was really not. This is called multiple comparisons testing, and we followed the procedure of controlling the false discovery rate (Benjamini & Hochberg 1995).

The main properties of the correlations between different room locations, structures, substrates, and taxa were summarized using principal component analysis (Afifi et al., 2011). Principal component analysis converts the original variables into a set of uncorrelated variables where each variable is a linear combination of the original variables. The first principal component explains the largest possible amount of the variance in the data, the second component the same, but under the constraint that it is uncorrelated with the first component, and so on. Consequently the first few principal components summarize the main variability in the original data set.

Multinomial regression was performed with the different taxa as the dependent variable. The multinomial regression model used information about building structure and substrate in order to compute probabilities with regard to which taxon was collected in a given sample. The parameters in the regression model could be used to interpret taxa preferences with respect to building structures and substrates. Using multinomial regression, a few requirements must be fulfilled (Afifi et al., 2011).

- Number of samples: A rule of thumb in logistic/multinomial regression is that one should have at least ten samples per independent variable. We did not have enough samples from crawlspaces to include those data in the regression model. For the basement samples our basic independent variables were the different building structures and substrates. We had enough samples to safely include these variables in the model. Further it was natural to assume that different structures had different effects on different substrates. Such properties could be included in the regression model using interactions between the independent variables. We did not have enough data to include all second-order interactions to

the model. Especially we lacked data to include interactions between the structure ceiling and some of the substrates. All second-order interactions, with enough samples, were considered, but none improved the regression model based on the Akaike information criterion.

- Multicolinearity: correlations between the independent variables larger than 0.4 in absolute value indicate multicolinearity. Computing the correlation between all substrates and building structures, no strong correlations were observed, the highest being -0.23 between wood and foundation wall.

We used the regression model to test different hypotheses, as we did with contingency tables. E.g. to test if any taxa have a significant preference for concrete, we estimated the parameters of the regression model with two substrate factors: 'concrete' and 'other substrates', including all building structures as independent variables as well. We performed this procedure for all structures and substrates. As for contingency tables, the p-values had to be adjusted, since we performed several tests.

All statistical analyses were performed using the statistical software R (R development Core Team 2013).

Results

Taxa preference for below grade spaces

Table 1 shows sample percentages of taxa growing in below grade spaces (%), number of samples from below grade spaces ($N_{Below\ grade}$), and total numbers including above-grade rooms (N_{Total}). Between 99.5% and 62.3% of *Monocillium* sp., *Ascotricha chartarum*, *Geomyces pannorum*, *Myxotrichum chartarum*, and Actinomycetes samples

were collected in below grade spaces. For the other taxa, occurrence in below grade spaces declined to less than 33.5%.

Table 1 Taxa sampled in below grade spaces[1].

Taxa	%	$N_{Below\ grade}$	N_{Total}
Monocillium sp.	99.5	204	205
Ascotricha chartarum	96.5	251	260
Geomyces pannorum	73.5	122	166
Myxotrichum chartarum	70.1	110	157
Actinomycetes	62.3	929	1492
Eurotium spp.	33.3	51	153
Aspergillus spp.	27.8	413	1483
Aspergillus versicolor	27.1	48	177
Acremonium spp.	25.8	191	739
Cladosporium spp.	24.3	578	2377
Stachybotrys spp.	18.1	122	672
Stachybotrys chartarum	18.0	63	350
Penicillium spp.	17.9	350	1960
Ulocladium spp.	12.6	84	667
Chaetomium spp.	6.5	68	1052
Chaetomium globosum	4.5	14	309

[1] %: percentage of samples below grade, $N_{Below\ grade}$: number of samples below grade, N_{Total}: total number of samples.

Table 2 shows the first three principal components for the correlations between all room locations, building structures, substrates, and taxa. The three principal components explained 22, 14, and 10% of the total variation in the dataset. Variables that both had the same sign, and clearly separated from zero in a principal component, tended to occur in the same samples. Room locations 'basement' and 'crawlspace', substrates 'concrete' and 'building paper', and our target taxa, had all clear negative values in the first principal component (Table 2). Based on results from Tables 1 and 2, further

analyses focused on *Monocillium* sp., *Ascotricha chartarum*, *Geomyces pannorum*, *Myxotrichum chartarum*, and Actinomycetes.

Table 2 The first three principal components from the correlations in the full data set.[1]

	Comp.1	Comp.2	Comp.3
Room location			
Attic	0.03	**0.47**	-0.03
Basement	**-0.48**	-0.10	-0.05
Bathroom	0.04	-0.03	**0.41**
Crawlspace	**-0.19**	0.17	-0.01
Kitchen	0.05	0.00	**0.30**
Warm room	0.37	**-0.21**	**-0.28**
Building structure			
Below grade foundation wall	**-0.46**	-0.15	-0.05
Ceiling	0.00	**0.50**	-0.05
Floor	0.00	0.01	0.08
Outer wall	**0.30**	**-0.23**	**-0.36**
Wall against wet room	0.12	-0.11	**0.51**
Substrate			
Building paper	**-0.17**	-0.07	-0.06
Concrete	**-0.23**	-0.08	-0.08
Gypsum board	0.10	**-0.25**	**0.20**
Wall paper	0.13	-0.17	-0.19
Wood	0.13	0.41	0.06
Taxa			
Acremonium sp.	0.03	0.01	-0.03
Actinomycetes	**-0.21**	-0.07	-0.03
Ascotricha chartarum	**-0.14**	-0.06	0.01
Aspergillus sp.	0.01	-0.03	-0.03
Aspergillus versicolor	0.01	-0.05	-0.05
Chaetomium globosum	0.07	-0.04	0.08
Chaetomium sp.	0.09	0.00	**0.25**
Cladosporium sp.	0.02	**0.21**	-0.18
Eurotium sp.	-0.01	-0.01	-0.02
Geomyces pannorum	**-0.11**	0.04	-0.03
Monocillium sp.	**-0.15**	-0.05	-0.02
Myxotrichum chartarum	**-0.13**	-0.02	-0.02
Penicillium sp.	0.09	0.10	-0.05
Stachybotrys chartarum	0.04	-0.09	0.07
Stachybotrys sp.	0.03	-0.09	**0.23**
Ulocladium sp.	0.08	-0.11	-0.07
Percentage of variance	22.2 %	14.2 %	10.1 %

[1] Values above 0.1 in absolute value are shown in bold face.

Distribution of the target taxa

The sampling variables are shown in Figure 1. Figure 1 shows that most of the samples from below grade spaces were collected in basements, that foundation walls were the constructions with the highest number of cases of microbial growth after moisture damage, and that the most common taxon growing in these spaces was Actinomycetes. Most samples were collected on concrete, wood, and building paper. As mentioned before, further analyses were only based on basements, as we did not have enough samples from crawlspaces to perform statistical analyses.

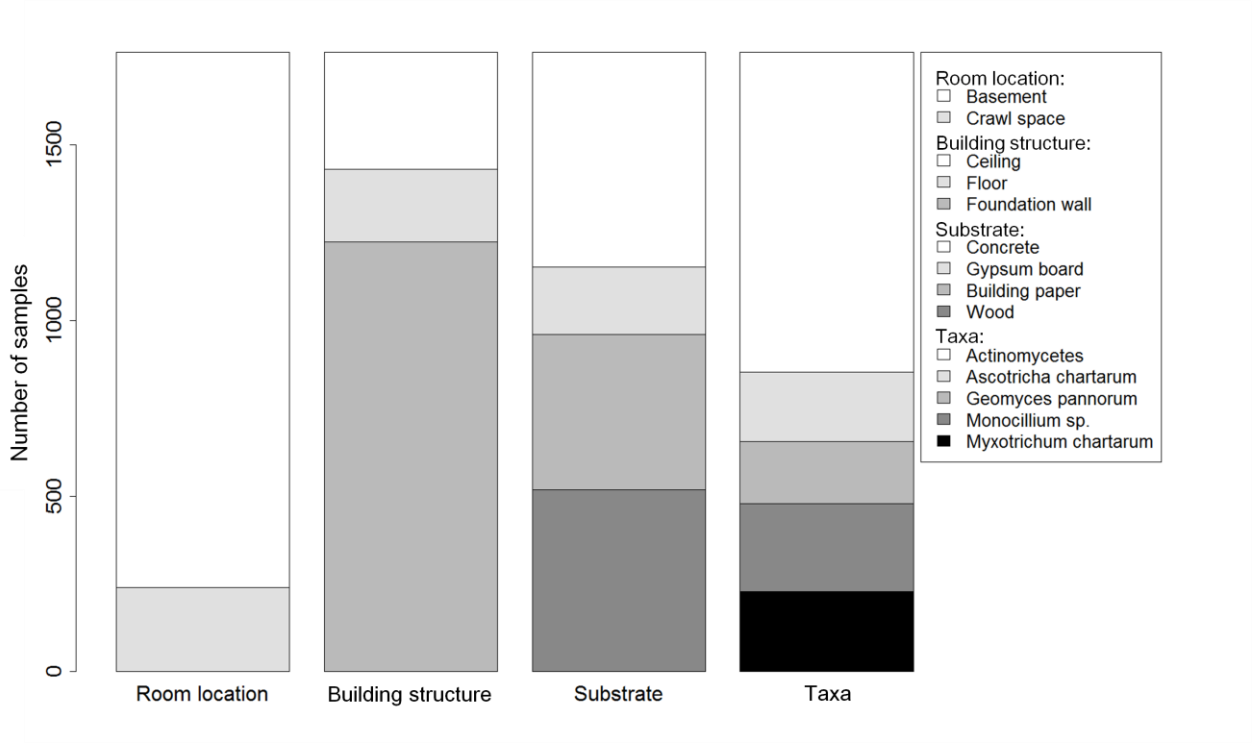

Figure 1 Number of samples for the different variables.

Preference for building structures in basements

When basements are insulated, all analysed substrates can be found in ceilings, walls, and floor structures. We tested whether the taxa studied showed a significant preference for any specific building structure using contingency tables (Table 3).

In Table 3 and further in this section, 'wall' refers to below grade foundation walls.

Table 3 Number of samples of taxa on different structure-substrate combinations in basements[1].

		Actinomycetes	***Ascotricha chartarum***	***Geomyces pannorum***	***Monocillium* sp.**	***Myxotrichum chartarum***	**Total**	
	Ceiling	6 (75)	0 (0)	0 (0)	1 (13)	1 (13)	8	
Concrete	Floor	90 (85)	3 (3)	4 (4)	3 (3)	6 (6)	106	Inhomogeneity:
	Wall	438(91)	11 (2)	5 (1)	23 (5)	6 (1)	***483***	p-value= 0.013
	Ceiling	1 (7)	1 (7)	1 (7)	5 (33)	**7** (47)	15	
Gypsum	Floor	0 (0)	5(100)	0 (0)	0 (0)	0 (0)	5	Inhomogeneity:
Board	Wall	29 (18)	81(52)	0 (0)	36(23)	11 (7)	***157***	p-value< 0.001
	Ceiling	6 (35)	0 (0)	0 (0)	3 (18)	8 (47)	17	
Building	Floor	4 (17)	5(21)	2 (8)	7 (29)	6 (25)	24	Inhomogeneity:

Paper	Wall	65 (20)	25(8)	50(15)	95(28)	95(28)	***330***	p-value= 0.103
	Ceiling	30 (41)	0 (0)	**29**(39)	9 (12)	5 (7)	73	
Wood	Floor	22 (37)	11(18)	15(25)	5 (8)	7 (12)	60	Inhomogeneity:
	Wall	125(51)	29(12)	36(15)	31(13)	26(11)	***247***	p-value< 0.001

[1] Values in bold face show taxa that are significantly overrepresented on a given structure compared to other taxa. Values in bold italic show structures that are significantly overrepresented. Values in parentheses are row percentages.

Our results showed that, for all substrate categories, all taxa were significantly overrepresented on walls as building structure compared to their presence on other structures. The total number of records on walls was at least twice those on ceilings and floors combined; for instance, for concrete a total of 483 samples were sampled on walls compared to only 8 and 106 on ceilings and floors, respectively. The tests were performed assuming that the total number of samples on each substrate-structure combination was Poisson distributed.

Based on the exact Fisher test, there was also significant dependence between taxa and structures for the substrate categories 'gypsum board' and 'wood' (p-values < 0.001). For 'gypsum board', *M. chartarum* had a significant preference for ceilings compared to other structures. For 'wood', *G. pannorum* also had a significant preference for ceilings.

Substrate preferences

Table 4 shows the results of the multinomial regression analysis. The results are presented as odds ratios. For instance, the odds ratio for concrete to Actinomycetes was 4.53, meaning that the odds that the taxon was Actinomycetes was 4.53 times higher if

the substrate was concrete than if it was any of the other analysed substrates. The odds ratio for gypsum board to Actinomycetes, on the other hand, was 0.36, meaning that the odds that the taxon was Actinomycetes was 0.36 times higher if the substrate was gypsum board than if it was any of the other analysed substrates. In other words, Actinomycetes had little preference for gypsum board.

Table 4 Results from the regression analysis given as odds ratios[1].

	Actinomy-cetes	***Ascotricha chartarum***	***Geomyces pannorum***	***Monocillium* sp.**	***Myxotrichum chartarum***
Ceiling	0.89	0.03	2.33 *	1.06	1.71
Floor	0.54	3.12 * *	1.07	0.72	1.11
Wall	1.50	1.37	0.54	1.14	0.68
Gypsum board	0.36 * * *	**10.8 * * ***	0.03 *	1.22	0.48 *
Concrete	**4.53 * * ***	0.90	0.23	0.16	0.53
Building paper	0.38 * * *	0.27 * * *	1.24	**2.24 * * ***	**4.03 * * ***
Wood	**3.14 * * ***	0.47 * * *	**2.29 * * ***	0.42 * * *	0.36 * * *

[1] The most significant odds ratios greater than one are shown in boldface. Signif. codes: p-value < 0.001:***, p-value < 0.01:**, p-value < 0.05:*

According to our analyses, substrates were clearly the strongest predictors for the different taxa, with many strongly significant predictors (Table 4). 'Concrete' and 'wood' significantly increased the odds for Actinomycetes. Similarly, 'gypsum board' was a strong predictor for *Ascotricha chartarum*, 'wood' for *Geomyces pannorum* and 'building

paper' for *Monocillium* sp. and *Myxotrichum chartarum.* We also had some significant building structures as predictors. 'Ceiling' and 'floor' were significant predictors for *Geomyces pannorum* and *Ascotricha chartarum*, respectively.

Figure 2 summarizes taxa preferences for building substrates within the wall assembly observed in the current study, together with potential gradients of abiotic factors through the wall structure in Scandinavia. Relative humidity is higher outside and declines towards the inner part of the wall assembly, while temperature is higher on the room side than outside. A salinity and pH gradient can occasionally develop depending on the composition of both the soil and the concrete element.

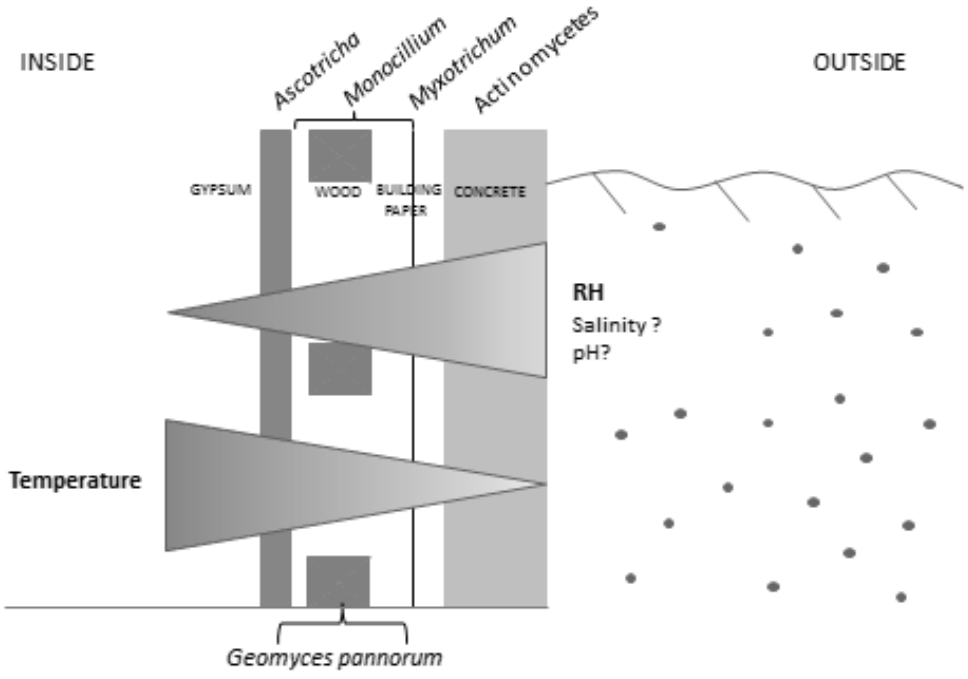

Figure 2 Taxa preference for substrates in below grade foundation walls in Scandinavia.

Discussion

Our results are based on multinomial regression and contingency tables, which are relatively robust tools for analysing data with sampling biases. For instance, taxa which are generally easier to detect than others independent of building substrate, structure, or room location, will not yield biases in the interpretation of the regression parameters (except for the intercept) and contingency tables results.

Further, observation errors that depend on a combination of taxa and another variable (substrate, structure, room location) may yield biases in our results. As an example, taxa with the same colour as the substrate may be overlooked during sampling. This effect is neutralized as moisture measurement, and not substrate discolouration, is the criterion for taking samples. Our results showed for example that Actinomycetes were significantly abundant on concrete, even though they were not visible to the naked eye on this substrate.

Below grade foundation walls as ecological niches

Our results suggest that Actinomycetes, *Ascotricha chartarum*, *Myxotrichum chartarum*, *Geomyces pannorum*, and *Monocillium* sp. are ecological specialists (Devictor et al., 2010) in below grade foundation walls. Their distribution in the wall cavity is determined by gradual changes of abiotic parameters, specific physiological requirements, and species competition. A stochastic element in niche colonization, called “the advantage of the first occupant” (Gams, 1992) or “assembly history” (Fukami et al., 2010), may also play a role.

Below grade foundation walls constitute a distinct microenvironment. In Scandinavia, external insulation of these structures became common in the 2000’s. Thus, for the

majority of the building mass, substrates on the outer part of the structure (i.e. concrete, eventually building paper) remain cold and subject to seasonal weather fluctuations, while substrates on the insulated room side (timber frame and interior finishes) are directly affected by a warmer indoor temperature. Bulk water entering foundation walls carries water-soluble salts through the wall components which crystalize to the interior (Ueno and Lstiburek, 2010). We suggest that, besides RH and temperature gradients, a salinity gradient and in some cases a pH gradient are probably generated in the wall cavity, depending on the chemical composition of both the soil and the concrete component.

An insight into the taxa's physiological requirements further explains their distribution within the wall cavity: we have found **Actinomycetes** growing abundantly on both non-insulated and insulated, wet concrete walls and slab surfaces (Tables 3, 4). This correlates well with previous reports of Actinomycetes as colonizers of wet surfaces (RH above 98%) with basic or neutral pH subject to periodical desiccation (Górny, 2004). Our studies suggest that tolerance to basic pH and periodical desiccation make Actinomycetes specialists on concrete foundation walls with drainage problems or seasonal condensation.

Myxotrichum chartarum was mainly found growing on building paper directly on concrete surfaces (Tables 3, 4), that is on the cold side of foundation walls, and on basement ceilings made of gypsum board (significant value, Table 3). This fungus is a cellulolytic, psychrophilic Ascomycete that can grow at temperatures as low as 5-7 °C, and requires RH above 98% (Tribe and Weber, 2002). Under these conditions, numerous ascomata discharging high numbers of ascospores are often visible to the

naked eye. Salinity preferences are not known, but because of its placement in the wall structure, this fungus is probably halotolerant.

***Monocillium* sp.** also grew significantly on building paper, like *M. chartarum* (Table 4). The genus *Monocillium* is the asexual state of *Niesslia* (Ascomycota). *Niesslia* is a weak cellulose decomposer that has been found in salt marshes (Moustafa and Sharkas, 1982), suggesting that some species have high salinity tolerance. In below grade foundation walls, alkaline water can filtrate through concrete and moisten building paper. *Niesslia* ascomata have never been found in buildings. We hypothesize that *Monocillium* sp. can compete with *M. chartarum* in cases when RH is too low for ascomata formation.

Ascotricha chartarum dominated on gypsum boards in below grade foundation walls (Tables 3, 4), that is on the warm side of these structures. Again, physiology explains this fact, as this species is cellulolytic and mesophilic (optimal growth at 25°C). The species is also alkalotolerant, that is, capable of growing at pH values above 9 or 10, but with optimum growth rates at around neutrality (Muthukrishnan et al., 2012). Both ascomata and asexual state, *Dicyma ampullifera* (deHoog et al., 2000), have been found indoors, growing on cardboard, paper, plaster, linoleum etc. *Ascotricha chartarum* has been characterized as very rare in the building environment (Li and Yang, 2004). Like *Myxotrichum chartarum*, *Ascotricha chartarum* needs an RH level above 98% in order to develop ascomata. The asexual state, *Dicyma ampullifera*, probably grows at lower RH than its sexual state.

It appears that *Myxotrichum chartarum* is outcompeted by *Ascotricha chartarum* on gypsum boards in foundation walls, even if *Myxotrichum chartarum* grows on gypsum

boards in basement ceilings (Table 3, significant result). We suspect that at higher temperatures, *Ascotricha chartarum* is a stronger competitor on gypsum boards than *Myxotrichum chartarum.* Higher temperature tolerance could also explain the specific preference of *Ascotricha chartarum* for gypsum board floors (Table 4). In order to test this possibility, temperature measurements must be included in systematic sampling.

Geomyces pannorum dominated on wooden ceilings in our dataset, but was also common within the wall structure (Table 3). This species is cellulolytic and psychrotolerant, growing in a temperature range of 5–30 °C, with optimal temperatures at 15-20 °C. As opposed to other taxa described here, *G. pannorum* is xerophilic, growing well at 71% RH (Kuthubutheen and Pugh, 1979). This fungus grows typically on wooden ceilings in crawlspaces with relatively low and stable RH and temperature over long periods (Nunez et al., 2012). When the bottom of the foundation wall is not saturated with water, or can partially dry towards the room, *G. pannorum* probably outcompetes other cellulolytic species that require higher RH. In Figure 2, *G. pannorum* is placed separately from the other taxa because of its preference for significantly lower RH than other below-grade specialists.

Sampling method evaluation

Microscopic observation of tape lifts is an effective, cheap, and direct tool for assessing microbial growth indoors. By minimizing disturbance of sporulating structures, this method allows both documentation of growth, and direct taxa identification.

Allochthonous microorganisms whose growth requirements are not fulfilled in a particular environment can still be detected as fragments or spores, but will simply not grow or sporulate *in situ*. Spore accounts of these species will be low and metabolic

activity non-existent (Rintala et al., 2012). This is for example the case for basidiospores of ectomycorrhizal fungi, such as *Russula* and *Amanita*, whose spores are commonly found indoors (Pitkäranta et al., 2008), but growth has never been documented indoors.

Culture- and microscopy-dependent methods as tools for understanding indoor microbial ecology have received recent criticism for being labour-intensive, and because many species are unable to be cultured (Scott, 2012). However, molecular techniques alone can neither identify all species (Li and Yang, 2012), nor provide an understanding of all species in an ecological framework (Pitkäranta et al., 2011). To us, the best proof that microorganisms thrive in a building ecosystem is that they actually grow, proliferate, and interact with other organisms and the environment. This is the ecological definition of microbial communities (Devictor et al., 2010).

In order to understand indoor dynamics as well as emission sources of microorganisms, several analytical techniques have to be combined, and indoor researchers from different disciplines should join efforts, as suggested earlier (Corsi et al., 2012).

Acknowledgements

We thank Walter Gams and Cony Decock for taxonomic identification of *Monocillium* sp., and all our Mycoteam colleagues for collection, isolation, identification, and discussions on the indoor environment.

References

Adams, R., Miletto, M., Taylor, J.V. and Bruns, T.D. (2013) Dispersal in microorganisms: Fungi in indoor air are dominated by outdoor air and show dispersal limitation at short distances, *ISME J.*, **7**(4), 1–12.

Afifi, A., May, S. and Clark, V.A. (2011) *Practical Multivariate Analysis, Fifth Edition,* Chapman & Hall.

Agresti, A. (1992) A survey of exact inference for contingency tables, *Stat. Sci.*, **7**, 131–153.

Amend, A.S., Seifert, K., Samson, R. and Bruns, T.D. (2010) Indoor fungal composition is geographically patterned and more diverse in temperate zones than in the tropics, *Proc. Natl. Acad. Sci. USA*, **107**, 13748–13753.

Andersen, B., Frisvad, J.C., Søndergaard, I.S., Rasmussen, S. and Larsen, L.S. (2011) Associations between fungal species and water-damaged building materials, *Appl. Environ. Microbiol.*, **77**, 4180–4188.

Andersson, M.A., Tsitko, I., Vuorio, R. and Salkinoja-Salonen, M.S. (1999) Mycobacteria and related genera are major colonizers of a wall in a children's day care center. In: *Bioaerosols, Fungi and Mycotoxins: Health Effects, Assessment, Prevention and Control*, Vol 1, pp. 396– 402.

Benjamini, Y. and Hochberg, Y. (1995) Controlling the false discovery rate: A practical and powerful approach to multiple testing, *J. R. Stat. Soc. Series B Stat. Methodol*., **57**, 289–300.

Blom, P. (2006) *Fuktskader i Kryperom. Årsaker og Utbedringsmetoder,* Oslo, Byggforskserien 721.211.

Bornehag, C.G., Blomquist, G., Gyntelberg, F., Järvholm, B., Malmberg, P., Nordvall, L. Nielsen, A., Pershagen, G. and Sundell, J. (2001) Dampness in buildings and health, *Indoor Air*, **11**, 72–86.

Claeskens, G. and Hjort, N.L. (2008) *Model Selection and Model Averaging,* Cambridge University Press.

Corsi, R.L., Kinney, K.A. and Levin, H. (2012) Microbiomes of built environments: 2011 Symposium highlights and workgroup recommendations, *Indoor Air*, **22**, 171–172.

de Hoog, G.S., Guarro, J., Gené, J. and Figueras, M.J. (2000) *Atlas of Clinical Fungi*, Utrecht, CBS.

Devictor, V., Clavel, J., Julliard, R., Lavergne, S., Mouillot, D., Thuiller, W., Venail, P., Villéger, S. and Mouquet, N. (2010) Defining and measuring ecological specialization, *J. Appl. Ecol*., **47**, 15-25.

Frøstrup, A. (1993) *Rehabilitering. Konstruksjoner i Tre*, Universitetsforlaget, Oslo.

Fukami, T., Dickie, I.A., Wilkie, J.P., Paulus, B.C., Park, D., Roberts, A., Buchanan P.K. and Allen, R.B. (2010) Assembly history dictates ecosystem functioning: Evidence from wood decomposer communities, *Ecol. Lett.* **8**, 1283–1290.

Gams, W. (1971) *Cephalosporium-artige Schimmelpilze (Hyphomycetes)*, Stuttgart, Gustav Fischer.

Gams, W. (1992) The analysis of communities of saprophytic microfungi with special reference to soil fungi, *Handbook of vegetation science* **19**, 183–223.

Górny, R.L. (2004) Filamentous microorganisms and their fragments in indoor air – A review, *Ann. Agric. Environ. Med*., **11**, 185–197.

Grant, C., Hunter, C.A., Flannigan, F. and Bravery, A.F. (1989) The moisture requirements of moulds isolated from domestic dwellings, *Int. Biodeterior. Biodegradation*, **25**, 259–284.

Gravesen, S., Nielsen, P.A., lversen, R. and Nielsen, K.F. (1999) Microfungal contamination of damp buildings - Examples of risk constructions and risk materials, *Environ. Health Perspect*., **107**(Suppl 9), 505–508.

Hyvärinen, A., Meklin, T., Vepsäläinen, A. and Nevalainen, A. (2002) Fungi and actinobacteria in moisture-damaged building materials: Concentrations and diversity, *Int. Biodeterior. Biodegradation*, **49**, 27**–**37.

Klein, D.A. (2007) Microbial communities in nature: A postgenomic perspective, *Microbe,* **2**, 591–595.

Kuthubutheen, A.J. and Pugh, G.J.F. (1979) Effects of temperature and fungicides on *Geomyces pannorum* (Link) Hughes, *A. Van Leeuw. J. Microb.,* **45**, 65-79.

Lee, J.H. and Jo, W.K. (2006) Characteristics of indoor and outdoor bioaerosols at Korean high-rise apartment buildings, *Environ. Res.*, **101**, 11–17.

Li, D.W. and Yang, C.S. (2004) Notes on indoor fungi I: New records and noteworthy fungi from indoor environments, *Mycotaxon*, **89**, 473–488.

Li, D.W. and Yang, C.S. (2012) What does the development of fungal systematics mean to DNA-based methods for indoor mold investigations. In: *Proceedings 6th International Bioaerosol Scientific Conference*, Vol 1, pp. 236–245.

Moustafa, F. and Sharkas, M.S. (1982) Fungi associated with cellulose decomposition in the tidal mud-flats of Kuwait, *Mycopathologia*, **78**, 185–190.

Muthukrishnan, S., Sanjayan, K.P. and Jahir, H.K. (2012) Species composition, seasonal changes and community ordination of alkalotolerant micro fungal diversity in a natural scrub jungle ecosystem of Tamil Nadu, India, *Mycosphere*, **3**, 92–109.

Nunez, M., Sivertsen, M.S. and Mattsson, J. (2012) Growth preferences on substrate, construction, and room location for indoor moulds and Actinomycetes. In: *Proceedings of Healthy Buildings* '2012, Vol 1, 5H.3.

Pasanen, A.L., Juutinen, T., Jantunen, M.J. and Kalliokoski, P. (1992) Occurrence and moisture requirements of microbial growth in building materials, *Int. Biodeterior. Biodegradation*, **30**, 273–283.

Pessi, A.M., Suonketo, J., Pentti, M., Kurkilahti, M., Peltola, K. and Rantio-Lehtimäki, A. (2002) Microbial growth inside insulated external walls as an indoor air biocontamination source, *Appl. Environ. Microbiol*., **68**, 963–967.

Pitkäranta, M., Meklin, T., Hyvarinen, A., Paulin, L., Auvinen, P., Nevalainen, A. and Rintala, H. (2008) Analysis of fungal flora in indoor dust by ribosomal DNA sequence analysis, quantitative PCR, and culture, *Appl. Environ. Microbiol*., **74**, 233–244.

Pitkäranta, M., Meklin, T., Hyvärinen, A., Nevalainen, A., Paulin, L., Auvinen, P. and Rintala, H. (2011) Molecular profiling of fungal communities in moisture damaged buildings before and after remediation – A comparison of culture-dependent and culture-independent methods, *BMC Microbiology*, **11**, 235–251.

R Development Core Team (2013) *R: A Language and Environment for Statistical Computing*, R Foundation for Statistical Computing, Vienna, Austria. Available at http://www.R-project.org/.

Rajala, T., Peltoniemi, M., Hantula, J., Mäkipää, R. and Pennanen, T. (2011) RNA reveals a succession of active fungi during the decay of Norway spruce logs, *Fungal Ecol*., **4**, 37–48.

Rintala, H., Pitkäranta, M., Toivola, M., Paulin, L. and Nevalainen, A. (2008) Diversity and seasonal dynamics of bacterial community in indoor environment, *BMC Microbiol*., **8**, 56–69.

Rintala, H., Pitkäranta, M. and Täubel, M. (2012) Microbial communities associated with house dust, *Adv. Appl. Microbiol*., **78**, 75–120.

Rolstad, A.N. and Blom, P. (2007) *Yttervegger mot Terreng. Varmeisolering og Tetting,* Oslo, Byggforskserien 523.111.

Rowan, N.J., Johnstone, C.M., McLean, R.C., Anderson, J.G. and Clarke, J.A. (1999) Prediction of toxigenic fungal growth in buildings by using a novel modelling system, *Appl. Environ. Microbiol*., **65**, 4814–4821.

Scott, J. (2012) An Evolving Architecture: The Past, Present and Future of Indoor Microbiology. In: *IAQA 15th Annual Meeting and Indoor Air Expo*, Vol 1.

Sedlbauer, K. (2001) *Prediction of Mould Fungus Formation on the Surface of and Inside Building Components,* Ph.D. thesis, University of Stuttgart.

Tribe, H.T. and Weber, R.W.S. (2002) A low-temperature fungus from cardboard, *Myxotrichum chartarum*, *The Mycologist*, **16**, 3–5.

Ueno, K. and Lstiburek, J. (2010) Bulk water control methods for foundations, *Building Sci. Res. Report*, **1015**, 1–34.

Viitanen, H.A. (1997) Modelling the time factor in the development of mould fungi. The effect of critical humidity and temperature conditions on pine and spruce sapwood, *Holzforschung,* **51**, 6–14.

Viitanen, H.A. and Ojanen, T. (2007) Improved Model to predict Mold Growth in Building Materials. In: *ASHRAE Buildings X Conference*, Vol 1, pp. 1–8.

Yang, C.S. (1996) Fungal Colonization of HVAC Fiber-glass Air-Duct Liner in the USA. In: *Proceedings of Indoor Air* ’96, Vol 3, pp. 173–177.