



Communities of mold fungi in moisture damaged building materials studied with molecular methods

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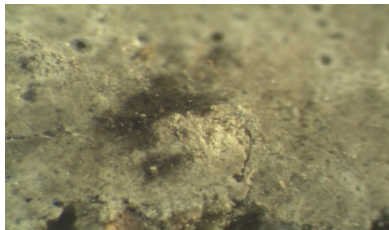
VTT Technical Research Centre of Finland

- Microbial growth in moisture damaged buildings cause problems to occupants and building structures
- Critical conditions for mold and decay development have been modeled (e.g., Adan 1994, Abe et al 1996, Hukka and Viitanen 1999, Sedlbauer 2001, Viitanen *et. al.* 2010, 2011, Ojanen *et. al.* 2011)
- Current knowledge of microbes in moisture damaged buildings relies mostly on culture based methods
- More advanced methods are needed to study the complex microbial populations in moisture damage situations



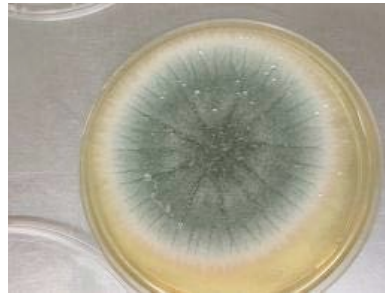
Methods to analyse fungi in moisture damaged building materials

Microscopy



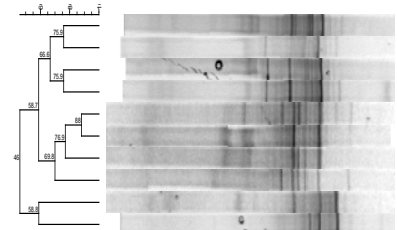
- Fast
- Observation of growth
- Limited identification of species

Cultivation



- Slow
- Detects only fungi growing on chosen media
- Identification of species

Molecular methods



- Fast
- Detects all fungal population
- Identification of species

Goal of this study

- Optimize DNA extraction method for different building materials
- Identify fungal species in moisture damaged building materials
- Study the impact of moisture load and building material type to fungal community structure

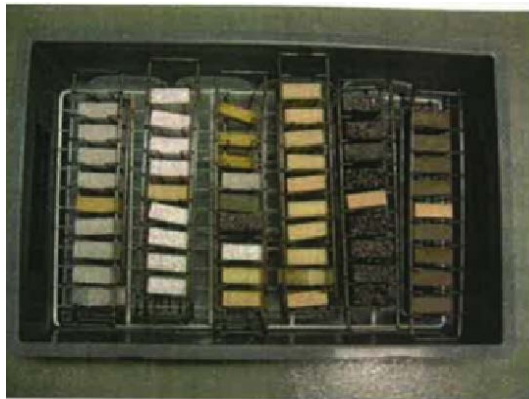


New tools to understand the complicated relationship between moisture, material, microbes and indoor air quality

Experiment set up

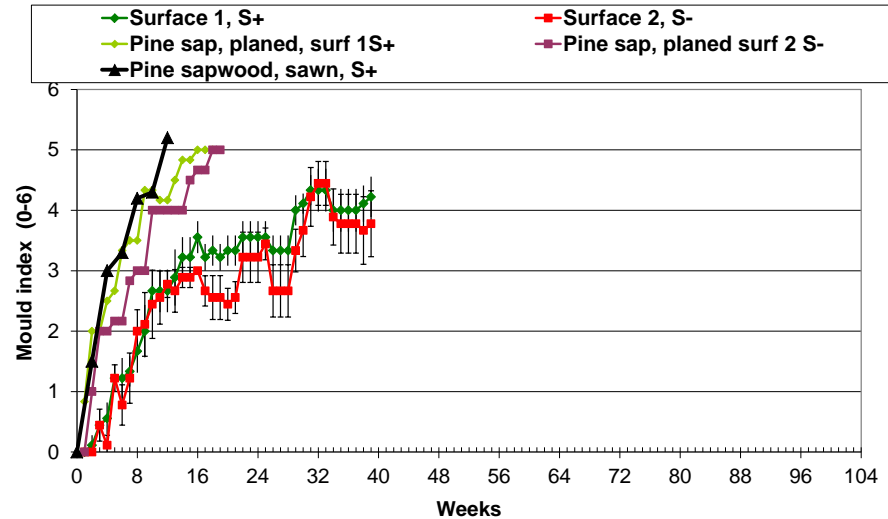
- Pine sapwood
- Spruce, edge glued
- Light weight concrete
- Mineral wool
- Polyester fibre insulant

+ 22 °C ↓ RH 97% /
 RH 90%

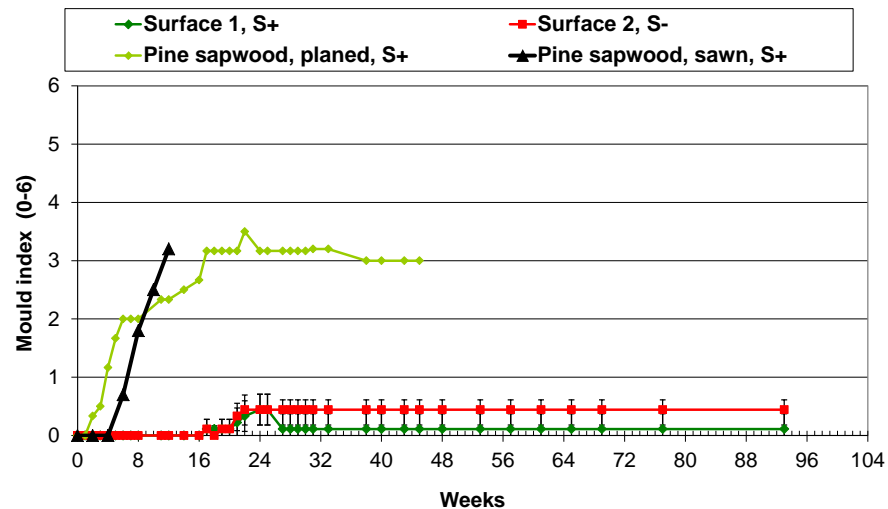


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A) Edge glued spruce board and pine sapwood, RH 97%, 22 C (1)

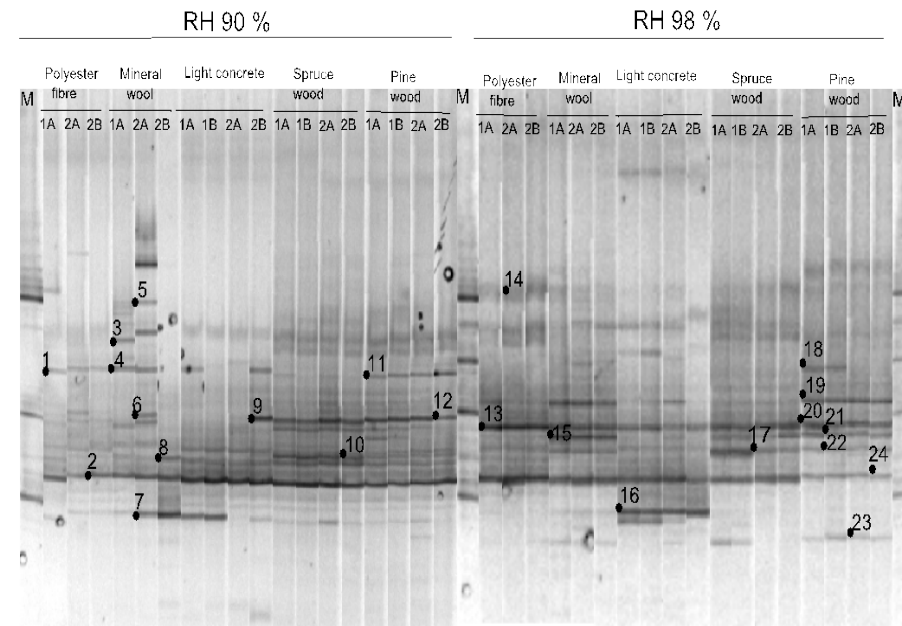
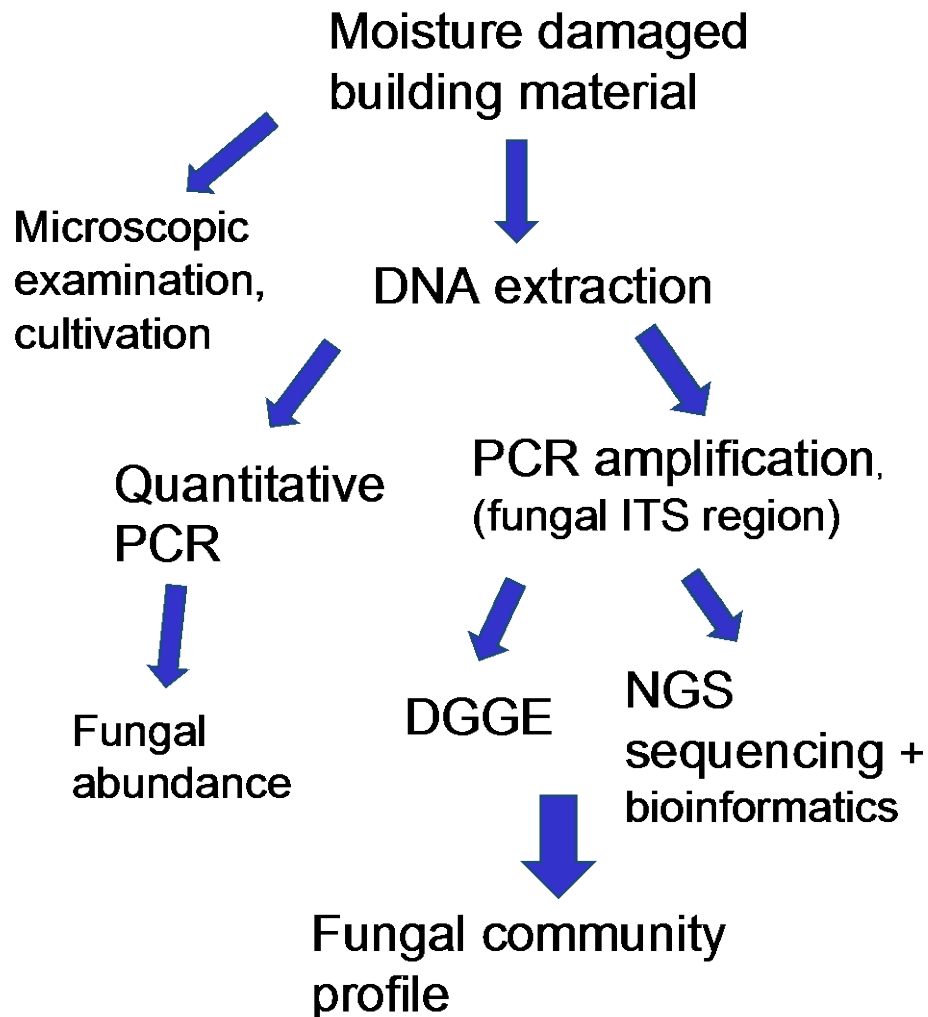


A) Edge glued spruce board and pine sapwood, RH 90%, 22 C (7)



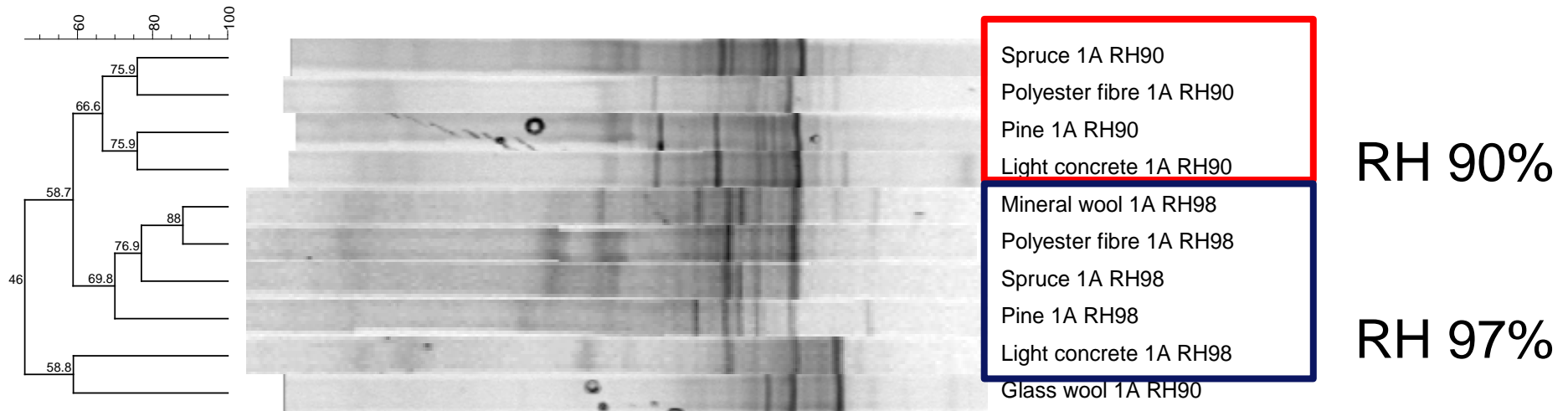
Development of mold index in laboratory exposure at RH 97 and 90 % and 22 °C

Molecular methods used



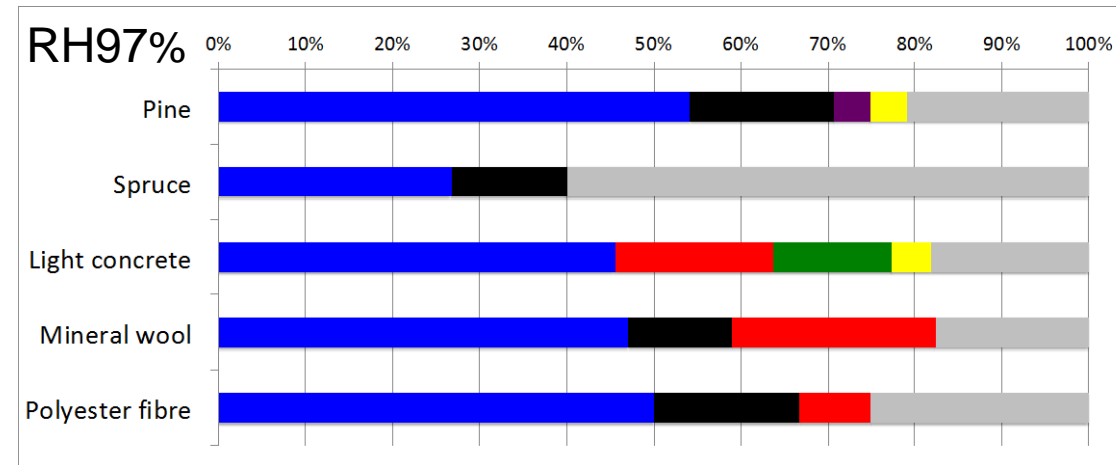
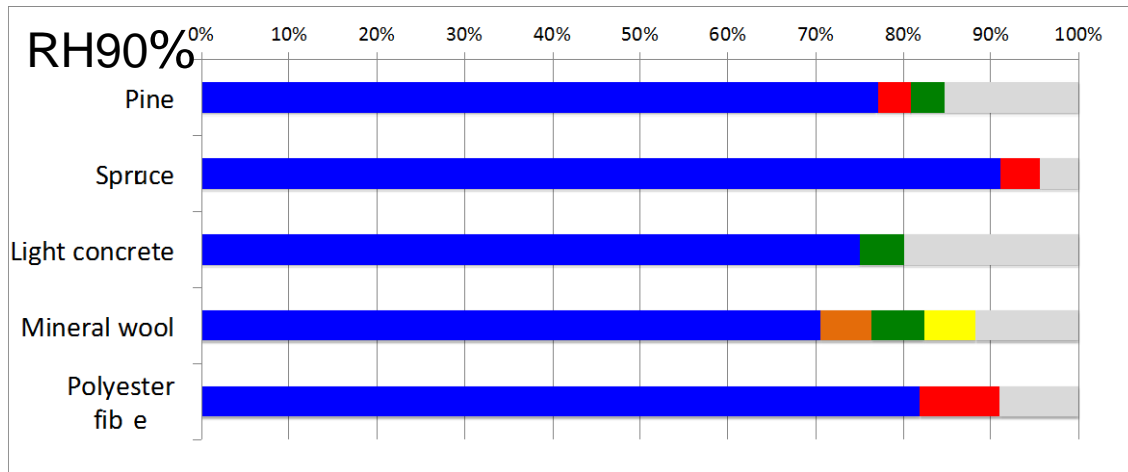
Fungal community profile of different materials

Fungal community similarity analysis



- Similarity analysis clustered together DGGE-band profiles of RH 90% samples (similarity 66-76%)
- RH 97% samples formed their own cluster (similarity 70-88%)
- This suggests that fungal community structure depends more on humidity conditions than building material type

Fungal communities in different building materials



■ Eurotiomycetes
 ■ Dothideomycetes
 ■ Lecanoromycetes
 ■ Sordariomycetes

■ Leotiomycetes
 ■ Agaricomycetes
 ■ Unidentified Ascomycota
 ■ Unidentified fungi

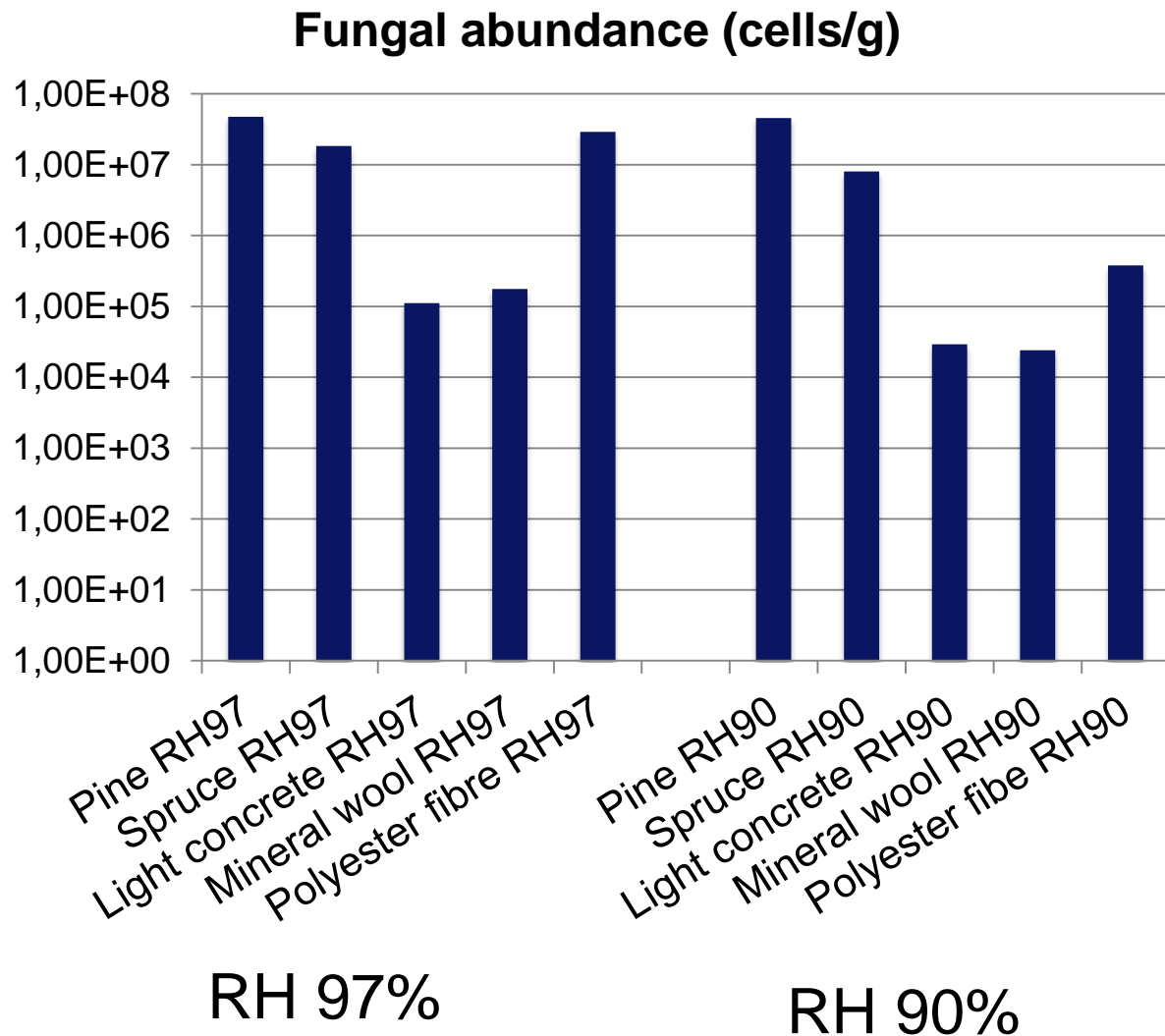
- Fungal communities more diverse in high humidity conditions
- Differences between building material types

Fungal genera detected: cultivation vs. PCR-DGGE



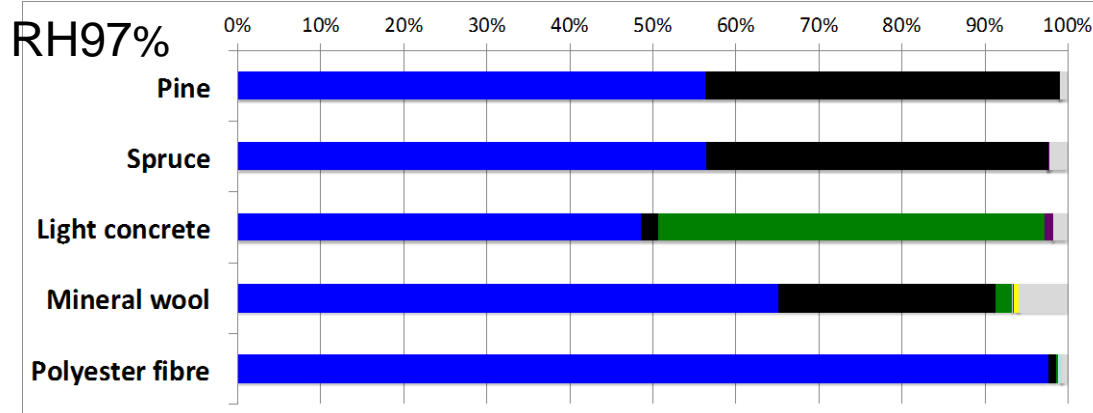
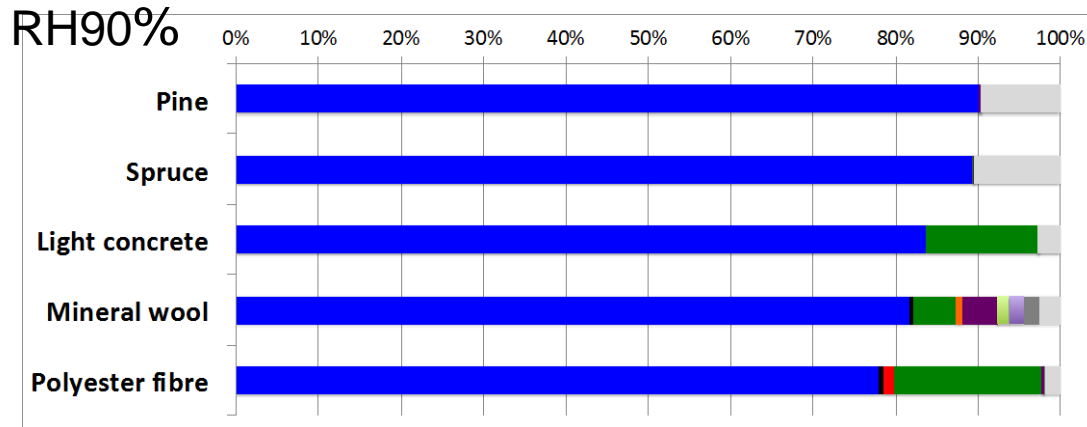
Material type RH 97%	Cultivation	PCR-DGGE
Pine sapwood	<i>Aspergillus</i> , <i>Penicillium</i>	<i>Aspergillus</i> , <i>Penicillium</i> , <i>Talaromyces</i> , <i>Oidiodendron</i> , <i>Mycosphaerellaceae</i> , <i>Thelephoraceae</i>
Spruce, edge glued	<i>Aspergillus</i> , <i>Penicillium</i>	<i>Aspergillus</i> , <i>Penicillium</i> , <i>Mycosphaerallaceae</i>
Light weight concrete	<i>Aspergillus</i> , <i>Penicillium</i> , <i>Stachybotrys</i>	<i>Aspergillus</i> , <i>Eurotium</i> , <i>Phaeophysica</i> , <i>Punctelia</i> , <i>Nectria</i> , <i>Stachybotrys</i>
Mineral wool	<i>Aspergillus</i> , <i>Penicillium</i>	<i>Aspergillus</i> , <i>Penicillium</i> , <i>Mycosphaerella</i> , <i>Phaeophysica</i> , <i>Punctelia</i>
Polyester fibre	<i>Aspergillus</i> , <i>Penicillium</i>	<i>Aspergillus</i> , <i>Mycosphaerella</i> , <i>Heterodermia</i> , <i>Pleosporaceae</i>

Fungal abundance



- Not much variation in the fungal abundance between two moisture conditions
- More differences between building material type
- Similar results with quantitative PCR and cultivation

454 Pyrosequencing



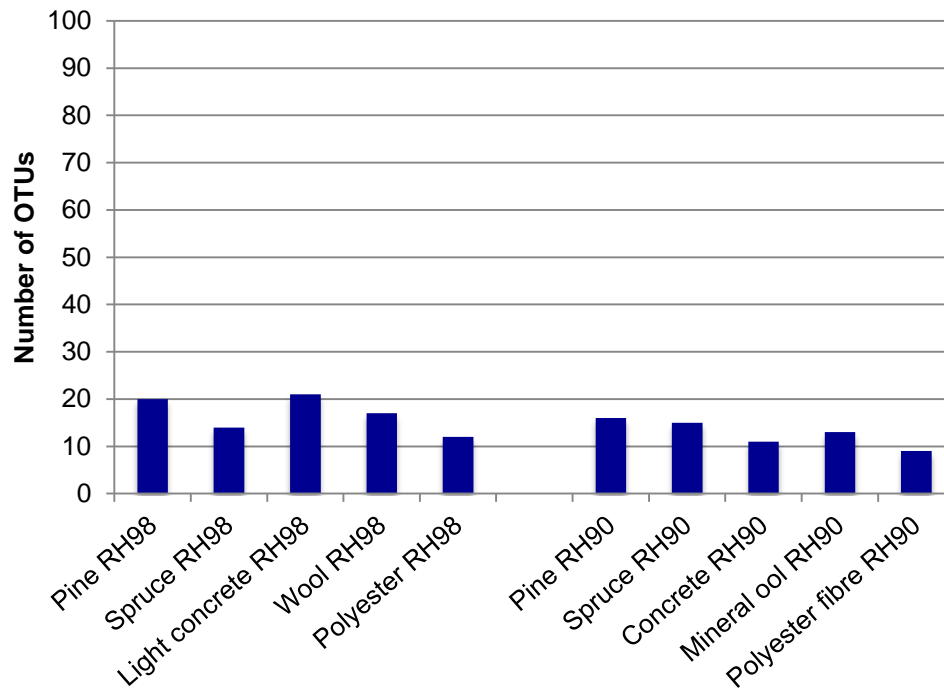
- Same dominant fungal groups detected with pyrosequencing and DGGE
- In addition more minor groups detected with pyrosequencing

➔ more fungal diversity detected with pyrosequencing

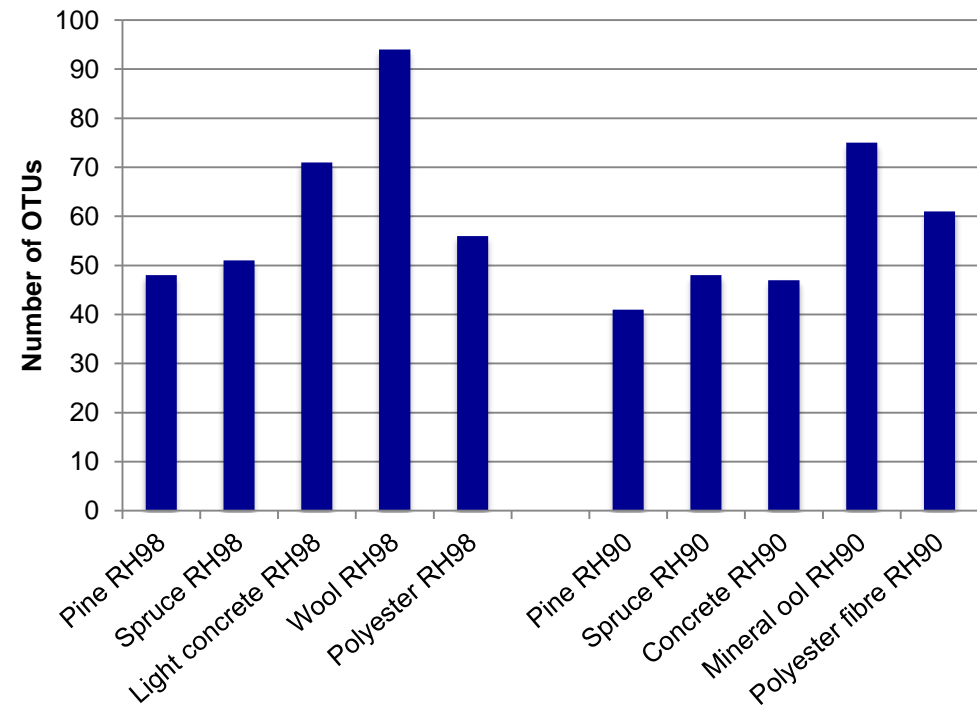
■ Eurotiomycetes ■ Dothideomycetes ■ Lecanoromycetes ■ Sordariomycetes ■ Leotiomycetes
 ■ Ascomycota_Incertae_sedis ■ Unidentified Ascomycota ■ Agaricomycetes ■ Exobasidiomycetes
 ■ Microbotryomycetes ■ Basidiomycota_unidentified ■ Unidentified fungi

DGGE vs. 454 pyrosequencing

Observed OTUs DGGE



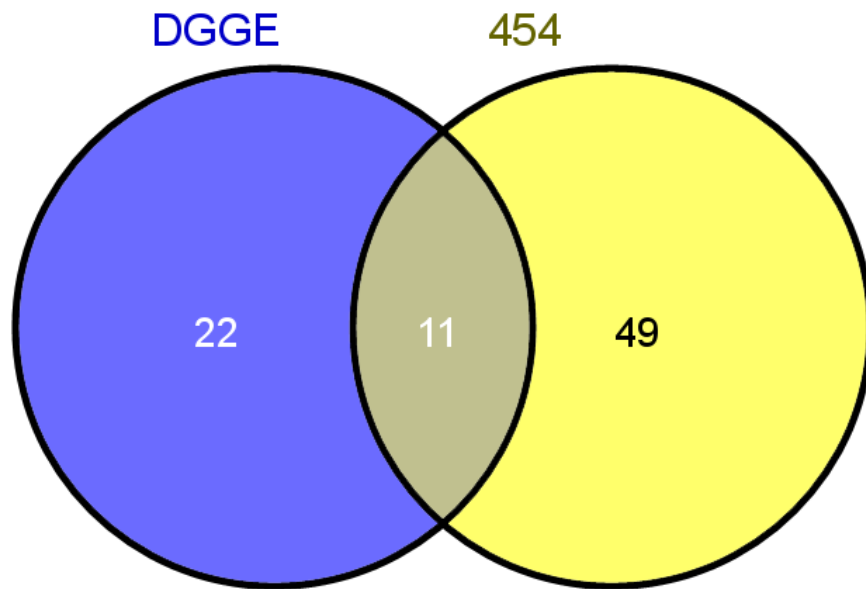
Observed OTUs 454



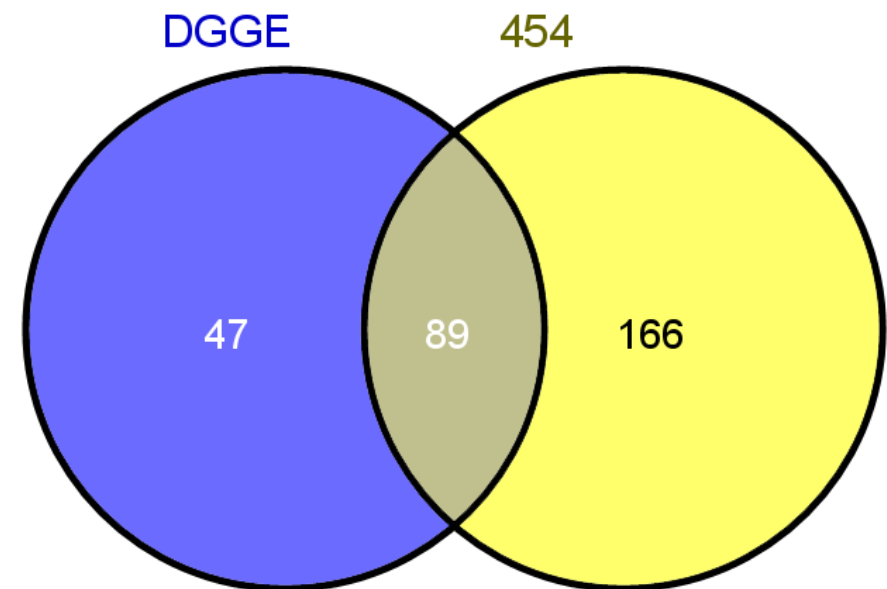
OTU=Operational taxonomic unit

DGGE vs. 454 pyrosequencing

- Pyrosequencing identified more fungal species and OTUs



Venn diagram of shared and unique fungal species



Venn diagram of shared and unique fungal OTUs

Conclusions

- More diverse fungal populations at higher humidity conditions
- Material type influences fungal community structure
- Only small difference in fungal abundance between RH 90% and RH 97 %
- PCR-DGGE reveals dominant fungal species well
- NGS methods detect more fungal species and also minor fungal groups

Why DNA-based methods?

- Molecular methods can be optimized to study fungal populations in moisture damaged building materials
- DNA-based methods provide a deeper and more extensive picture of the fungal communities
- No single method to “solve the problem”: combination of visual and technical inspection, moisture content measurements and microbiological analysis needed to identify mold problems in buildings
- Deeper knowledge achieved with DNA-based methods can reveal new information about the cause and outgrowth of mold problems and give information for repair solutions

Acknowledgments:

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- COST FP1303

