Computational analysis of Bioaerosol:

The analysis of molecular genetic datasets in context of environmental factors

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Table of Contents

Abstract

From an ecological perspective, primary biological aerosol particles (PBAP) play a central role in the spread and reproduction of many organisms. Many pathogens of plants and humans, as well as allergens, spread through the atmosphere. They can be highly efficient ice nuclei, potentially influencing the hydrological cycle and global energy budget. Despite the far-reaching influences in ecology, agriculture, human health and climate, the spatial and temporal dynamics in composition and abundancy of PBAP are not well characterised.

In this thesis, the atmospheric diversity and abundancies of heterogeneous types of PBAP are investigated. For this, data science methods are utilised to programmatically process and analyse complex data structures within a database system. The focus is on Sanger, NGS and quantitative PCR datasets of the highly allergenic plants, ragweed and mugwort, plant pathogenic oomycetes, fungi and prokaryotic archaea.

A novel insight into the fine fraction (<3 µm) concentrations of mugwort and ragweed revealed higher concentrations of ragweed despite it being far less abundant in the local flora. Wind back trajectory analysis point to pollen-rupture during long-range transport from the Mediterranean, which also may explain the higher allergenic potential of ragweed compared to mugwort.

Both fungi and oomycetes diversity displayed high seasonal dynamics. Average temperature, more than close temporal vicinity, led to similar compositions. Similar tendencies in the abundancies of different oomycetes taxa indicate the same factors influencing atmospheric occurrence. For fungi independent communities were identified on coarse and fine filter samples. Patterns in the temporal and size fraction occurrences of the most abundant taxa allowed the identification of sporulation strategies amongst fungi with similar lifestyles.

Comparison of Archaea datasets from continental and marine air samples revealed higher proportions of the soil-associated Thaumarchaeota in continental samples (86% vs 60%) and increase of Euryarchaeota in marine samples (40% vs 14%). Thaumarchaeota and methanogenic Euryarchaeota peaked late in the year, presumably due to increased emissions from bare soil and fertilisation on agricultural land.

These results provide new information and insights into the dynamics of PBAP which can be beneficial for diverse fields such as atmospheric, agricultural and medical sciences.

Zusammenfassung

Aus ökologischer Sicht spielen Primäre Biologische Aerosolpartikel (PBAP) eine zentrale Rolle bei der Verbreitung und Vermehrung vieler Organismen. Viele Pflanzen- und Humanpathogene, wie auch Allergene, sind in der Atmosphäre präsent. Als effiziente Eiskeime könnten PBAP einen Einfluss auf den Wasserzyklus und globalen Energiehaushalt haben. Trotz der weitreichenden potenziellen Einflüsse, sind die räumlichen und zeitlichen Dynamiken in Zusammensetzung und Vielfalt nur unzureichend charakterisiert.

In dieser Arbeit werden atmosphärische Diversität und Abundanzen heterogener Typen von PBAP untersucht. Hierbei werden Methoden der data science eingesetzt um programmatisch komplexe Datenstrukturen innerhalb eines Datenbanksystems zu prozessieren und analysieren. Der Fokus liegt auf Sanger- NGS- und quantitativen PCR Datensätzen von Oomyceten, Fungi, Archaea und den Allergenen Pflanzen Ambrosia und Artemisia.

Neue Einblicke in die Feinstaubkonzentrationen (<3 µm) von A. vulgaris und A. artemisiifolia zeigen höhere Konzentration von A. artemisiifolia, obwohl es in der regionalen Pflanzenwelt weit weniger häufig ist. Wind Rückwärtstrajektorien lassen einen Langstreckentransport und dadurch bedingtes Platzen der Pollen vermuten, welches das hohe Allergiepotenzial erklären könnte.

Die Diversität von Fungi und Oomyceten zeigt hohe saisonale Dynamik. Die mittlere Temperatur, mehr als die zeitliche Nähe, führte zu ähnlichen Zusammensetzungen. Ähnliche Tendenzen in der Konzentration verschiedener Oomycetentaxa deuten auf die gleichen Faktoren die das atmosphärische auftreten beeinflussen. In Grob- und Feinstaub wurden voneinander unabhängige Gemeinschaften der Fungi identifiziert. Muster im zeitlichen Auftreten, innerhalb der zwei Größenfraktionen, erlaubten die Identifikation von Sporulationsstrategien der Taxa mit ähnlichen Lebensweisen.

Der Vergleich von Archaea aus kontinentalen und marinen Luftproben, zeigte einen höheren Anteil erdassoziierter Thaumarchaeota in kontinentalen Proben (86 % zu 60 %) und einen höheren Anteil Euryarchaeota in den marinen Proben (40 % zu 14 %). Die Thaumarchaeota und die methanogenen Euryarchaeota erreichten ihre höchste Vielfalt spät im Jahr, vermutlich aufgrund nackter Erde und Düngemitteleinsatz auf Agrarflächen.

Die Ergebnisse liefern neue Informationen und Einblicke in die Dynamiken von PBAP welche Nutzen für diverse Disziplinen wie z.B. atmosphärische, Agrar-, und medizinische Wissenschaften haben können.

1 Introduction

In recent decades the recognition of the fragility of planet earth's climate and the influence of anthropogenic emissions thereupon, has led to the atmospheric sciences becoming one of the most prominent fields in modern day science. Within atmospheric research, the effects of aerosols, which are generally defined as liquid or solid particles suspended in a gas, are one of the central topics (Boucher et al., 2013; Pöschl, 2005). They play roles in a multitude of atmospheric chemical and physical processes, such as the global hydrological cycle, where they act as cloud condensation or ice nuclei and directly and indirectly influence the earth's energy budget through absorption and reflection of solar radiation (Seinfeld & Pandis, 2016).

The expanding field of aerosol research also led to renewed interest into the influence of natural emissions from the biosphere in the atmosphere and earth system as a whole. Primary biological aerosol particles (PBAP) are defined as aerosols of biological origin released in the particle phase from the biosphere into the atmosphere (Deepak, 1991; Després et al., 2012; Fröhlich-Nowoisky et al., 2016; Matthias-Maser, Bogs, & Jaenicke, 2000). They are a highly diverse and complex group of aerosols and include anything from prokaryotes such as bacteria and archaea, eukaryotes such as fungal spores or plant pollen, viruses and even cellular components, fragments or excretions of organisms, all with differing physico-chemical properties. Different types of PBAP can span the entire aerosol size range, which per definition is from roughly 1 nm, limited by rapid particle aggregation, to 100 µm, limited by rapid sedimentation (Seinfeld & Pandis, 2016), with viruses and cellular components such as proteins in the nanometre size range up to the upper boundary seen for many species of plant pollen and fungal spores. However, typical viable and abundant PBAP, such as bacteria, fungal spores and pollen are, almost exclusively, found in the super-micron size range at the upper end of the aerosol size spectrum (Fröhlich-Nowoisky et al., 2016).

The atmospheric composition of PBAP in both diversity and abundancy are temporally and spatially highly variable (Fröhlich-Nowoisky et al., 2012). As major groups of viable PBAP, such as fungal spores, bacteria or pollen, are quite large from an aerosol perspective, they are removed relatively quickly and efficiently through dry and wet deposition, i.e. removal by sedimentation or precipitation (Iribarne & Cho, 1980). This leads to the near-surface boundary layer composition being strongly influenced by emissions from the surrounding local to regional biosphere (Aylor, 1999; Bowers et al., 2013; Gregory, 1961). Furthermore, temporally, both in

abundancy and composition, emissions can be highly variable due to diverse factors, such as an influence of environmental factors, seasonal emissions like pollination or plant pathogens aiming at different annual developmental stages of the host vegetation (Bowers et al., 2013; Franzetti, Gandolfi, Gaspari, Ambrosini, & Bestetti, 2011; Fröhlich-Nowoisky, Pickersgill, Després, & Pöschl, 2009).

From an atmospheric sciences standpoint, compared to other groups of aerosols, the contribution of PBAP in atmospheric processes is not well understood. The uncertainty is illustrated by the annual global emission estimates for PBAP in the Fifth Assessment Report of the Intergovernmental Panel on Climate Change being 50-1000 Tg a^{-1}, spanning three orders of magnitude, while estimates of other major groups are more confined, such as mineral dust, sea spray and total natural secondary organic aerosol estimated at 1000-4000 Tg a^{-1}, 1400-6800 Tg a^{-1} and 470-1140 Tg a^{-1}, respectively (Boucher et al., 2013). This huge emission span should translate to an impact between rather low and potentially high, especially as many species of PBAP display ice-nucleating abilities far more efficient than non-biological aerosols and could therefore, dependent on concentrations, significantly influence cloud formation and thus the global hydrological cycle and energy budget (Fröhlich-Nowoisky et al., 2015, 2016; Pöschl, 2005; Richard, Martin, & Pouleur, 1996). This clearly illustrates a need for a better understanding of emission patterns and abundancy.

Although the influence on the climate is still uncertain, PBAP play a central role in the ecosystem. Many species of microorganisms, such as bacteria or fungi, utilise the airways as a primary means to spread throughout the environment. They can spread to new substrates after depletion of an energy source or, on larger scale, overcome natural barriers, allowing the colonisation of new habitats. This allows genetic exchange between populations and habitats, as well as geographic shifts of biomes (Fröhlich-Nowoisky et al., 2016). Moreover, genetic variability is of central importance for the evolutionary plasticity, and therefore survival, of species (Ingold, 1971). Atmospheric dispersal also plays a central role in the sexual reproduction of many organisms. Many land plants, including agriculturally important crops such as maize, wheat and rye, are wind pollinating, which allows the genetic exchange between spatially separated sessile organisms (Shukla, Vijayaraghavan, & Chaudhry, 1998). Furthermore, PBAP have an immense and well-documented influence on agriculture and forestry (Strange & Scott, 2005). The total annual global losses caused by PBAP - pre- and postharvest - are nearly impossible to assess and only few attempts have been made. Oerke (Oerke, 2006) estimates that amongst six important crop plants (wheat, rice, maize, potatoes, soy beans, and cotton),

PBAP, including fungi and the less severe bacterial pathogens, lead to pre-harvest losses alone of ~10 %, despite substantial preventative measures, with, for example, the global fungicide market, estimated at 18.5 billion dollars (Sparks, Hahn, & Garizi, 2017). Furthermore, pesticide resistance is a major problem facing agriculture today (Hollomon, 2015; Sundin, Castiblanco, Yuan, Zeng, & Yang, 2016). Many agriculturally-relevant pathogens also have a very broad spectrum of host plants that can be part of the natural endemic flora (Dean et al., 2012). This makes the assessment of potential emission sources, and subsequent spread, a challenge.

Next to agricultural sciences, the influence of PBAP on public health is a prominent subject in medical research. It is estimated that 5-30% of the population of industrialised countries suffer from pollen-induced allergies (Cecchi, 2013) and 6% of the general population display a fungal spore-induced respiratory allergy (Denning, O'Driscoll, Hogaboam, Bowyer, & Niven, 2006; Horner, Helbling, Salvaggio, & Lehrer, 1995). Furthermore, climate change is influencing the distribution, abundancy and potency of allergenic plants (D'Amato et al., 2007) with effects that are difficult to assess, as is the case for highly allergenic invasive *Ambrosia artemisiifolia* in northern Europe (Buters et al., 2015).

Therefore, understanding the changing emission patterns of PBAP and factors like seasonality and the influence of meteorology on emission and transport, along with a deeper understanding of their atmospheric dynamics, could have many benefits. More efficient, sustainable and cost-effective practices in agriculture could be developed. Also, human health-related issues, such as allergy early warning systems or even indoor hospital hygiene, could be improved.

The spatio-temporal variability in PBAP composition and abundancy, along with complex short and long-term influences of environmental factors on emissions and atmospheric residence (Ayerst, 1969; Jones & Harrison, 2004), pose a challenge in respect to the analysis of environmental sampling datasets. Therefore, this thesis investigated novel database-based methods for PBAP dataset handling and analysis. The aim was to automate processing and analyses, thereby improving speed and depth, while at the same time improving the quality of the results by reducing human error and increasing analytical methods. The developed methods have given novel insights into a broad spectrum of PBAP classes from eukaryotic fungi and oomycetes, the allergenic plants *Ambrosia artemisiifolia* and *Artemisia vulgaris* to prokaryotic archaea and bacteria. The improved speed and depth of analysis also allowed for a broad range of subjects to be addressed. Occurrence patterns of airborne fungi were compared to information in literature revealing lifestyle-dependent characteristics in seasonality, size and

meteorological correlations. Quantitative PCR analyses of *A. artemisiifolia* and *A. vulgaris* gave novel insights into the sub-pollen particle fraction and back trajectory analyses were employed to confine potential emission sources of the allergenic weeds which pointed to a long-range transport from southern Europe. The comparison of an oomycetes sanger dataset with quantitative PCR abundancies of selected taxa, gave new insights into the temporal dynamics in both diversity and sequence abundancy. Anthropogenic influences on PBAP composition could be hypothesised on the basis of fungal and archaea datasets, be it from climate change and agricultural activities to the emissions from fractured landscapes and populated areas. Finally, correlation analysis also gave insight into the potential influence of meteorology on atmospheric occurrences of the investigated PBAP.

2 Motivation and Research Objectives

2.1 Methodological Objectives:

To advance and expand analysis options and quality, it is of primary importance to understand the complex data-types and data-structures found in PBAP datasets and associated information (i.e. DNA sequences and analysis methods, taxonomic hierarchy, sampler types, spatial data or meteorological data). Therefore, the methodological objectives were:

1. Create a bioaerosol database system and analysis framework (BADB).
2. Create and refine a flexible and expandable database structure that accepts all relevant PBAP, sampling, meteorological and literature information needed for analyses.
3. Automate DNA sequence processing, analysis and database import, improving speed and reproducibility
4. Automate BADB analysis.

For points 1-4: see results presented in Appendix C1-4: (Lang-Yona et al., 2018, Müller-Germann et al., 2017; Pickersgill et al., n.d., Fröhlich-Nowoisky et al., 2014)

2.2 Research Objectives:

1. Compare the DNA concentrations of the allergenic weeds *A. artemisiifolia* and *A. vulgaris* in coarse and fine particulate matter in continental boundary air samples (Appendix C1 (Müller-Germann et al., 2017).

2. Investigate the temporal dynamics in diversity and abundancy of airborne oomycetes. Identify potential influences, thereupon (Appendix C2 (Lang-Yona et al., 2018)).
3. Identify patterns in the temporal dynamics and size fraction occurrences of airborne fungi in the dataset presented in (Fröhlich-Nowoisky et al., 2009). On the basis of the most abundant identified taxa in combination with additional information, such as, meteorological data, nutritional and life-style preferences, fruiting form and spore dimensions, identify possible constraints and influences on the temporal and size fraction occurrences (Appendix C3, (Pickersgill et al., n.d.)).
4. Gain insight into the seasonal dynamics in relative abundance and diversity of archaea in the continental boundary layer sampled in Mainz (Appendix C3 and C4(Fröhlich-Nowoisky et al., 2014; Wehking et al., 2018)). Compare the diversity to marine boundary layer composition sampled in Cape Verde (Appendix C3, (Fröhlich-Nowoisky et al., 2014). Gain a novel insight into seasonal variation in fine particulate matter fraction (Appendix C4, (Wehking et al., 2018)

3 Major Results and Conclusions

1. The DNA concentrations of the locally abundant *A. vulgaris* outweighed fivefold the neophyte and far less abundant *A. artemisiifolia* in the pollen grain bearing coarse fraction during their respective pollination periods. However, in the fine particle fraction, the ratio between the species was reversed, with *A. artemisiifolia* fivefold as abundant as *A. vulgaris*. The most viable explanation for this observation is pollen rupture during long-range transport from southern European hotspots, such as Hungary where *A. artemisiifolia* emissions are reported to make up to approximately 50% of all pollen, annually. Furthermore, back trajectory analyses of wintertime samples containing high *A. artemisiifolia* concentrations, showed that air masses originated from southern France and Hungary, strengthening the hypothesis of a potential long-range transport. The high fine fraction *A. artemisiifolia* concentrations may also explain the high allergenic potential of *A. artemisiifolia*, as small fine particles can penetrate deeper into the respiratory tract, accumulate and perhaps induce sensitisation more efficiently than coarse particles or intact pollen. See appendix C1, (Müller-Germann et al., 2017).
2. A novel insight was gained into the temporal dynamics of the airborne oomycetes community and the rRNA gene abundancy of the oomycetes and selected genera. In

total 55 different hypothetical species (operational taxonomic units (OTU)), of which 54 were plant pathogens (uncertain in the case of one OTU), were identified. Principal coordinate analysis of the species composition revealed three community clusters with a dependence on ambient temperature rather than a seasonal clustering. The abundance of oomycetes rRNA genes was low in winter and enhanced during spring, summer, and fall, with a dominance of Phytophthora, reaching a maximum concentration of ~$1.6 * 10^6$ rRNA genes per cubic meter of sampled air in summer. Common tendencies in the relative atmospheric concentrations and relatively constant proportions of the taxa to the total concentration of oomycetes point to similar passive influences on atmospheric presence. These could be meteorological factors influencing emission and deposition (e.g. splash dispersal by precipitation) or dispersal mechanisms (e.g., attached to soil particles or plant fragments) rather than differing species-specific factors.

3. A comparison of the composition of the airborne fungal community, identified on coarse and fine air-filter samples, revealed that the composition of the airborne fungal community is temporally highly dynamics, however, not constant throughout the investigated 13-month period. There was a high compositional identity of wintertime samples to spring and summer samples during the extremely warm winter 06/07, which points to a very early initial spore dispersal due to mild conditions and is a potential indication of an influence of global warming. Subsequent principal coordinates analysis of the Bray Curtis indices revealed an ambient-temperature-dependent grouping of the samples and also revealed a separation of the coarse and fine fraction communities (cutoff 3 µm) The two size-dependent communities displayed independent temporal dynamics as no discernible common patterns were identified between the coarse and fine filter samples.

   A deeper insight into the identified occurrence patterns was gained by forming a subset of the 25 most abundant taxa in the dataset. The subset was analysed by incorporating additional information on the taxa including known nutritional and lifestyle preferences, fruiting form and spore dimensions. This allowed the identification of traits in the temporal and size fraction occurrences, correlations to meteorology which were common amongst taxa with similar lifestyle preferences and point to lifestyle specific sporulation strategies. These included more distinct seasonalities (temporally-confined emissions) amongst non-ligninolytic fungi which was especially obvious amongst

pathogenic fungi, as these fungi are bound to the annual vegetative cycle of plants. The pathogenic ligninolytic fungi (tree pathogens), were amongst the most abundant taxa and mostly showed continuous occurrences, with single to multiple annual peaks, this strategy could reflect the relatively low inoculation probability of wood pathogens needing susceptible or injured hosts. Patterns in the size fraction occurrences, revealed a concentration of plant surface inhabitants and non-ligninolytic pathogens in the coarse fraction, adapted to surface impaction on hosts, forest dwelling ligninolytic fungi were concentrated around the sampler cutoff (3 µm), adapted to calm conditions beneath the canopy, and a fine fraction dominance to even distribution amongst the cosmopolitan mould fungi, reflecting the unspecific nature of the fungi producing vast numbers of spores that can rely on sedimentation on numerous potential energy sources. See Appendix C3,(Pickersgill et al., n.d.)

4. Insights were gained into the seasonal dynamics of airborne archaea, along with a comparison of diversity in the continental and marine boundary layer (Mainz and Cape Verde). The marine boundary layer displayed an increase in the proportion of the extremophile (salt- and heat-tolerant) Euryarchaeota, where they made up 40% of the identified OTUs, whilst only 14% in Mainz.

   The seasonal diversity in Mainz peaked in autumn and winter, with linear regression analysis showing a negative correlation to temperature and a positive correlation to relative humidity. Large areas of bare soil in agricultural land, in combination with humid and cold conditions influencing the microclimatic conditions of the topsoil, may be leading to an increased emission of the soil-associated Thaumarchaeota. See appendix C3 (Fröhlich-Nowoisky et al., 2014).

5. An NGS-16S-Amplicon approach of prokaryotic 16S rDNA gave a novel insight into the fine fraction composition, due to the comparatively sensitive nature of the method. The OTU composition pointed to a potential influence of marine sources due to the presence of euryarchaeotal Marine group II and the thaumarcheotal genus *Nitrosopumilus,* which is known from marine water samples. Also, the highest abundancy of methanogenic Euryarchaeota was found in autumn, presumably due to increased fertilisation activity. This is a further indication that the composition of archaea seems to be anthropogenically influenced by agricultural activities. Furthermore, the comparison of the ratio of archaea to bacteria with the ratios from other habitats reported in literature showed the highest similarity to the ratio found in

the phyllosphere. Indicating either that the phyllosphere is a significant emission source of prokaryotes, or that the phyllospheric composition is strongly influenced by atmospheric microorganisms. See appendix C3 (Wehking et al., 2018).

The presented work is relevant for numerous scientific, medical and agricultural disciplines and sheds light on many aspects of the highly complex spatial and temporal dynamics of PBAP. However, it also clear that science has a far from complete understanding of any PBAP-related processes, whether it be factors governing emission, dispersal or impacts on humans, foodstuff or the biosphere. With mankind's increasing impact on the planet, the effects especially on the natural world become ever more challenging to predict and, therefore, lessen or avert. Future focus is needed on the influence of climate change on the temporal and spatial distributions of plant pathogenic PBAP, especially in view of increasing pesticide resistance and a growing global population. The development of sustainable methods in agricultural practices will be one of the central challenges in the coming decades.

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Appendix A: Abbreviations

BADB ..... *Bioaerosol Database*

DNA ..... *Deoxyribonucleic Acid*

NGS ..... *Next Generation Sequencing*

OTU ..... *Operational Taxonomic Unit*

PBAP ..... *Primary Biological Aerosol Particles*

PCR ..... *Polymerase Chain Reaction*

Appendix B: List of Publications and Contributions

- Müller-Germann, I., Pickersgill, D. A., Paulsen, H., Alberternst, B., Pöschl, U., Fröhlich-Nowoisky, J., & Després, V. R. (2016). „Allergenic Asteraceae in air particulate matter: Quantitative DNA analysis of mugwort and ragweed", *Aerobiologia, 1–21.* https://doi.org/10.1007/s10453-017-9485-3

  Contribution: First author. Performed parts of the analysis and co-wrote the manuscript. Led the revision process.

- Lang-Yona, N., Pickersgill, D.A., Maurus, I., Teschner, D., Wehking, J., Thines, E., Pöschl, U., Després, V.R., & Fröhlich-Nowoisky J. (submitted for review). "Diversity, abundance, and seasonal dynamics of plant-pathogen Oomycetes in continental air", *Frontiers of Microbiology, special edition: Atmospheric Microbiome.*

  Contribution: First author. Performed large parts of the analysis and co-wrote the manuscript.

- Pickersgill, D. A., Wehking, J., Paulsen, H., Thines, E., Pöschl, U., Fröhlich-Nowoisky J. Weber B. & Després, V. R. (to be submitted). "On the constraints and influences on the temporal and size fraction occurrences of airborne fungi".

  Contribution: First author. Performed the analysis and wrote the manuscript

- Fröhlich-Nowoisky, J., Ruzene Nespoli, C., Pickersgill, D. A., Galand, P. E., Müller-Germann, I., Nunes, T., Gomes Cardoso, J., Almeida, S. M., Pio, C., Andreae, M. O., Conrad, R., Pöschl, U. & Després, V. R. (2014). „Diversity and seasonal dynamics of airborne archaea", *Biogeosciences*, *11*(21), 6067–6079. https://doi.org/10.5194/bg-11-6067-2014

  Contribution: Performed parts of the analysis created the plots and contributed sections of the manuscript.

- Wehking, J., Pickersgill, D. A., Bowers, R. M., Teschner, D., Pöschl, U., Fröhlich-Nowoisky, J., & Després, V. R. (2018). "Community composition and seasonal changes of archaea in coarse and fine air particulate matter", *Biogeosciences*, *15*, 4205–4214. https://doi.org/10.5194/bg-15-4205-2018
  Contribution: Aided in the analysis and writing of the manuscript

- Engel, T., Pickersgill, D. A., Kunz, A., Kunkel, D., Brill, S., Müller, H., & Després, V. R. (2017). „Methods for determining the potential spatial origin of bioaerosols". *gis.Science - Die Zeitschrift Fur Geoinformatik*, *3*.
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- Pöhlker, C., Walter, D., Paulsen, H., Könemann, T., Rodríguez-Caballero, E., Moran-Zuloaga, D., Brito, J., Carbone, S., Degrendele, C., Després, V. R., Ditas, F., Holanda, B. A., Kaiser, J. W., Lammel, G., Lavrič, J. V., Ming, J., Pickersgill, D., Pöhlker, M. L., Praß, M., Ruckteschler, N., Saturno, J., Sörgel, M., Wang, Q., Weber, B., Wolff, S., Artaxo, P., Pöschl, U., and Andreae, M. O. (2018) "Land cover and its transformation in the backward trajectory footprint region of the Amazon Tall Tower Observatory", *Atmos. Chem. Phys. Discuss.*, https://doi.org/10.5194/acp-2018-323.
- Contribution: Aided in the analysis contributed a small section to the manuscript

- Wehking, J., Pickersgill, D.A., Teschner, D., Paulsen, H., Bowers B. Fröhlich-Nowoisky J., Pöschl U., Després V.R. (in preparation). "Particle sizes cut off drives diversity patterns of airborne bacteria".
  Contribution: Aided in the analysis and writing of the manuscript

- Pickersgill, D. A., Huyen C. D. D., Krüger M., Vef M. A., Brill S., Pöschl U., Müller-Germann, I., Fröhlich-Nowoisky, J. & Després, V.R. (in preparation). „Indications of specific temperature patterns inducing pollen release of Ragweed and Mugwort."

  Contribution: Oversaw and developed the project and data analysis

- Priyamvada, H., Pickersgill, D. A., Wehking, J., Després, V.R., & Gunthe, S. (in preparation). "High diversity of bioaerosols in the southern tropical Indian region".

  Contribution: Aided in the experimental setup, analysis and writing of the manuscript

Appendix C: Selected Publications

Apendix C1:

Allergenic Asteraceae in air particulate matter: Quantitative DNA analysis of mugwort and ragweed

Müller-Germann, I., Pickersgill, D. A., Paulsen, H., Alberternst, B., Pöschl, U., Fröhlich-Nowoisky, J., & Després, V. R.

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Aerobiologia

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ORIGINAL PAPER

Allergenic Asteraceae in air particulate matter: quantitative DNA analysis of mugwort and ragweed

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Abstract Mugwort (*Artemisia vulgaris*) and ragweed (*Ambrosia artemisiifolia*) are highly allergenic Asteraceae. They often cause pollen allergies in late summer and fall. While mugwort is native to Europe, ragweed reached Europe as a neophyte from North America about 150 years ago and continued spreading ever since. To understand possible relationships between the spread of ragweed, its abundance in air, and to judge possible health risks for the public, we quantified ragweed DNA in inhalable fine as well as in coarse air particulate matter. Mugwort was chosen for comparison, as it is closely related to ragweed and grows in similar, though mainly not identical, habitats but is native to Germany. The DNA quantification was performed on atmospheric aerosol samples collected over a period of 5 years in central Europe. The DNA concentrations were highest during the characteristic pollination periods but varied greatly between different years. In the inhalable fine particle fraction, ragweed exceeds the mugwort DNA concentration fivefold, while the coarse particle fraction, bearing intact pollen grains, contains more mugwort than ragweed DNA. The higher allergenic potential of ragweed might be linked to the humidity or long-range transport-induced bursting of ragweed pollen into smaller allergenic particles, which may reach the lower airways and cause more intense allergic reactions. Airborne ragweed DNA was detected also outside the local pollination periods, which can be explained by atmospheric long-range transport. Back-trajectory analyses indicate that the air masses containing ragweed DNA during winter had originated in regions with milder climate and large ragweed populations (Southern France, Carpathian Basin).

Electronic supplementary material The online version of this article (doi:10.1007/s10453-017-9485-3) contains supplementary material, which is available to authorized users.

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Keywords Bioaerosol · DNA · Real-time PCR · *Ambrosia artemisiifolia* · *Artemisia vulgaris*

1 Introduction

Mugwort (*Artemisia vulgaris*) and common ragweed (*Ambrosia artemisiifolia*) are highly allergenic weeds belonging to the Asteraceae family. In general, 10–14% of the already atopic individuals in Europe suffer from mugwort-caused pollinosis (Spieksma et al. 1980; Table ESM1). For ragweed, the numbers are even higher: Up to 50% of the atopic individuals in the North American and Canadian population are additionally allergic to ragweed pollen (Wopfner et al. 2005). Within Europe, the prevalence of ragweed sensitization among atopic individuals varies widely over a range of ~2% in Finland, ~15% in the Netherlands and Germany, and up to ~50% in Hungary (Burbach et al. 2009).

Mugwort is native to Europe and parts of Asia and grows in the temperate and humid zones of the northern hemisphere and the Mediterranean Basin (Barney and Di Tommaso 2003; Gadermaier et al. 2004; Wopfner et al. 2005). In Germany, mugwort is a widespread plant, which often grows at ruderal sites like footpaths, field edges, or at dumping grounds (Sebald et al. 1996) but avoids heavily disturbed sites. The surroundings of the sampling location in this study are no exception, with mugwort belonging to the very abundant plants of the region. The main pollination season in Central Europe is generally from July to August, although some pollen can already be observed as early as June and as late as September (D'Amato and Spieksma 1991; Grewling et al. 2012). The threshold which is sufficient to cause first allergic symptoms against mugwort has been reported to range from daily averages of 4–30 pollen per m^3 of air, depending on the area (De Weger et al. 2013 and references therein).

In contrast to native mugwort, common ragweed originates from the North American plains and Canada and has colonized several countries of Southern, Central, and Eastern Europe since the second half of the nineteenth century (Dahl et al. 1999; Mandrioli et al. 1998). Currently, common ragweed is quite rare in most parts of Germany, except for some regions such as the area around the city of Cottbus where the species has become abundant (Alberternst et al. 2006; Brandes and Nitsche 2007; Buters et al. 2015; Lemke 2013; Nawrath and Alberternst 2010). As for the region surrounding our sampling site, reliable data on the abundancy of ragweed are hard to find. From our own experience, ragweed can be found growing in the region. According to Cunze et al. (2013), the habitat suitability of common ragweed under current climatic conditions in our study area is quite high and Buters et al. (2015) shows that all counties to north and east, in southern Hesse, reported ragweed populations of >100 individual plants, between 2000 and 2010 and furthermore, they show that in 2014 our sampling site lay in the region they classify as having an extensive ragweed population.

The pollination of ragweed plants in Central Europe starts in late summer. The first airborne pollen can be detected in July and has their highest concentrations in August and September, and pollen release can last until the first onset of frost (Alberternst et al. 2009; Dahl et al. 1999; Gadermaier et al. 2004) as the plant dies at temperatures under −5 °C (Dahl et al. 1999).

The increasing problem of sensitization to ragweed pollen in Europe has stimulated many studies on ragweed (Alberternst et al. 2006, 2009; Asero 2007; Brandes and Nitsche 2006; Burbach et al. 2009; Buttenschøn et al. 2009; Dahl et al. 1999; Fumanal et al. 2007; Jäger 2000; Kaczinczi et al. 2008; Kaminski et al. 2010; Kasprzyk et al. 2011; Makra et al. 2004; Mandrioli et al. 1998; Otto et al. 2008; Peternel et al. 2005, 2008; Piotrowska and Weryszko-Chmielewska 2006; Poppendieck von 2007; Smith et al. 2008; Stach et al. 2007; Ziska et al. 2007). In countries with extensive ragweed populations leading to an allergological relevant pollen charge in the air, the rate of sensitization is often high, e.g., up to ~50% in Hungary where large ragweed stocks are located (Burbach et al. 2009). In the north of Italy, for example, ragweed pollen allergy has become the second highest of all allergies in the past few decades, with an increase from no documented cases in the years before 1997 up to ~20% in 2007 (Asero 2007). Worldwide the prevalence for ragweed allergy has doubled in 20 years from 15% to 30% (Arbes et al. 2005). Studies, which elucidate the abundance, dispersion, and possible dependence of ragweed pollen distribution and load in the air on meteorological factors, are therefore needed. With the help of such studies, we can better understand possible future health impacts as well as more precisely predict the influence of climate change.

For the quantification of DNA contained in airborne plant material, this study concentrates on a molecular approach. Pollen determination under the light microscope is often not precise enough to go down to the genus or even species level (De Weger et al. 2013). More importantly, fragments of pollen and other plant material which also cause allergic symptoms cannot be identified with a microscopic approach (Otto et al. 2008; Wright 1963), and this is one of the main benefits of this studies approach as insight is gained into concentrations found in the fine particulate matter fraction, which can penetrate deep into the respiratory tract.

The aim of this study was to quantify and compare the concentrations of mugwort and ragweed DNA, which can originate from either pollen or plant tissue, over a 5-year period in Mainz, Germany, from both the coarse and fine particulate size fractions. Air filter samples from the plant-specific flowering seasons and from the non-flowering seasons in spring and winter were analyzed with ragweed- and mugwort-specific quantitative real-time PCR (qPCR) as already established in an exploratory study for birch DNA in Müller-Germann et al. (2015). In addition, qPCR results were statistically tested for correlation to meteorological parameters and wind back trajectories were interpreted. Furthermore, while the sensitization rate of the population seems to be continuously increasing, this study investigates whether this phenomenon may correspond to a simultaneous increase in aerosolized allergenic material over time. The cross-reactivity of mugwort and ragweed might be an important factor for sensitive individuals and thus underlines the need for a comparative study dealing with both allergenic weeds.

2 Materials and methods

All the applied laboratory methods are described in more detail in Müller-Germann et al. (2015). Here, we present a more brief overview and focus on the methodological and analytical differences to Müller-Germann et al. (2015).

2.1 Aerosol sampling

The aerosol sampling procedure has been described in detail in Fröhlich-Nowoisky et al. (2009) and is summarized as follows: Aerosol samples were collected on glass fiber filters (Pall Corporation, Type A/A, 102 mm diameter; sterilized at 500 °C for 12 h) over a 5-year period in Mainz, Germany (130 m above sea level (a.s.l.), March 2006–December 2010). The sampling station was located about 20 m above ground level, on the campus of the University of Mainz (49.99°N, 8.23°E). For the sampling, a high-volume dichotomous sampler (self-built as described in Solomon et al. (1983)) was used to separate and collect coarse and fine aerosol particles on a pair of glass fiber filters with a nominal cutoff at 3 µm. The sampler was operated with a rotary vane pump (Becker VT 4.25) at a total flow rate of $\sim$300 L min^{-1}. The sampled air masses represent a mix of urban and rural continental boundary layer air in central Europe. The sampling period was generally $\sim$7 days, corresponding to a sampled air volume of $\sim$3000 m^3. A few samples were collected over shorter periods (1–5 days, $\sim$400–2000 m^3 air). Loaded filters were packed in aluminum foil (pre-baked at 500 °C) and stored at −80 °C until DNA extraction.

As listed in Table 1, for ragweed 67 filter pairs, comprised of a coarse (labeled a) and a fine (labeled b) particle filter, were analyzed. For mugwort, 89 particle filter pairs were analyzed. For both plants, filter samples from their pollination seasons (July–October)

Table 1 Overview of mugwort and ragweed DNA quantification success in air (positive samples/analyzed samples)

Sampling period	Particle size fraction	Mugwort (*Artemisia vulgaris*)	Ragweed (*Ambrosia artemisiifolia*)
2006	Coarse	16/16	12/12
	Fine	10/16	12/12
2007	Coarse	16/16	12/12
	Fine	8/16	12/12
2008	Coarse	16/16	12/12
	Fine	6/16	12/12
2009	Coarse	14/19	14/14
	Fine	7/19	14/14
2010	Coarse	20/22	17/17
	Fine	12/22	17/17
2006–2010	Coarse	82/89	67/67
	Fine	43/89	67/67

and their non-flowering seasons in spring (May) and winter (December) were chosen.

2.2 Primer design, testing and DNA extraction

The highly variable internal transcribed spacer (ITS) region of the multi-copy ribosomal DNA (rDNA) was chosen for analysis. Both forward primers are located within the ITS 1 region, whereas both reverse primers are located in the ITS 2 region (Figure ESM1 and Table 2). Primers were designed using Primer 3.0 with the forward primers in the ITS 1 region and the reverse primers in the ITS 2 regions (Rozen and Skaletsky 2003). They were tested using pDraw 32 (http://www.acaclone.com/) and through database comparison. The sensitivity was furthermore verified by PCR on dilution series down to ten DNA copies.

DNA was extracted with a commercial soil DNA extraction kit (LysingMatrixE, Fast DNA Spin Kit for Soil, MP Biomedicals) with slight alterations to the supplier's instructions (See Müller-Germann et al. 2015). For testing purposes, DNA was initially extracted from 50 mg of ragweed (*Ambrosia artemisiifolia*) and mugwort (*Artemisia vulgaris*) leaf material. For the qPCR measurements, DNA was extracted from air filter aliquots (1/8).

For verification, the test DNA extracts were amplified and cloned using the exact methods described in Müller-Germann et al. 2015. The products were sequenced at the DNA Core Facility of the Max Planck Genome Centre in Cologne using a ABI Prism Sequencer (Applied Biosystems, Darmstadt). The obtained sequences were submitted to the NCBI database and received the following accession numbers: KM487595-KM487598 (*Ambrosia artemisiifolia*) and KM487604-KM487611 (*Artemisia sp.*).

Quantitative real-time PCR (qPCR) was conducted to measure the amount of mugwort and ragweed DNA sequences. The Real-Time PCR MiniOpticon™ System for Real-Time PCR Detection (Biorad) was used for measurements using the Opticon Monitor™ Software (Version 3.1). Experimental setup and programming of the qPCR runs followed the supplier's instructions using the iScript TM One-Step RT-PCR Kit (Biorad) with the primer-specific annealing temperatures (Table 2).

2.3 Standard curves and preparation

The absolute quantification used in this study is based upon comparative measurements with a well-defined standard to determine the absolute amount of the target sequence. To prepare the standard dilutions, PCR products were cloned into *E.coli* Top 10 cells as described above. After 12 h of incubation from the grown colonies on the one hand, colony PCRs were performed and sequenced to validate the correct insertion and species specificity of the PCR product. On the other hand, these colonies were used to inoculate liquid media and grow pre-cultures. The selected colonies were transferred in 2 ml LB broth with 2 µl ampicillin (100 mg ml^{-1}) and incubated for 15 h at 300 rpm and 37 °C. Subsequently, PCRs of the pre-cultures were performed, to check whether the vector contained the correct plant DNA fragment using the following restriction fragment length polymorphism (2 µl PCR product 5 units *Taq*I (Fermentas)). After gel electrophoresis restriction, fragment patterns were compared to theoretical restriction fragments calculated by pDraw 32 (http://acaclone.com/).

After verification, 100 µl of the pre-culture was transferred to 45 ml liquid LB medium containing 45 µl ampicillin (100 mg ml^{-1}), which was incubated 15 h at 300 rpm and 37 °C. For the *E.coli* plasmid preparation of DNA, the GenElute™ Plasmid Miniprep Kit (Sigma-Aldrich) was used following the supplier's instructions.

The concentration of the plasmid DNA was measured with Bio-Rad SmartSpec 3000 UV/Vis

Table 2 Used primer pairs with their specific annealing temperatures

Primer	Sequence 5′-3′	Tm (°C)
Primer pair *Ambrosia artemisiifolia*		
Ambrosia artemisiifolia 62 for	CGG GGA TCG AAG CTT ATG T	55
Ambrosia artemisiifolia 640rev	GAA GCA TCA TCG CAA GAC AA	55
Primer pair *Artemisia vulgaris*		
Artemisia vulgaris 115 for	CTT TTG GAC CTC TTG TGA ATG CG	62
Artemisia vulgaris 460 rev	ATG TTC CCT TTG CGG AGA AAT	62

The location of the primer is visualized in Figure ESM1, Tm = Annealing temperature

spectrophotometer using distilled water (dH_2O) as a control. DNA extracts were diluted 1:10, and measurements were repeated at least four times for each plasmid preparation. These plasmid preparations were photometrically analyzed, and the ratio of A260/A280 was determined. For each plant, the plasmid preparation with the best ratio between 1.8 and 2.0 was chosen and used for the actual standard dilutions.

To calculate the plasmid copy number ($CopyNo_{Plasmid}$) per µl, we used Eq. 1 using the measured plasmid preparation concentration ($C_{Plasmid}$, g μl^{-1}) as well as the known length ($L_{Plasmid}$) and weight ($W_{Plasmid}$, $gmol^{-1}$) of an insert-containing plasmid (Lee et al. (2006) and Whelan et al. (2003)). See Table 3 for symbol explanation:

$$\begin{aligned} &\mathrm{CopyNo_{Plasmid}}(\mu l^{-1}) \\ &= \frac{N_A(\mathrm{mol}^{-1}) \times C_{\mathrm{Plasmid}}(\mathrm{g}\ \mu l^{-1})}{L_{\mathrm{Plasmid}} \times W_{\mathrm{Plasmid}}(\mathrm{g\ mol}^{-1})} \\ &= \frac{6.02 \times 10^{23}(\mathrm{mol}^{-1}) \times C_{\mathrm{Plasmid}}(\mathrm{g}\ \mu l^{-1})}{L_{\mathrm{Plasmid}} \times 660\ (\mathrm{g\ mol}^{-1})} \end{aligned} \quad (1)$$

For the standard curve, each preparation was then diluted in eight steps from 10^8 to 10^1 copies μl^{-1}.

In each qPCR run, the standard was measured three times and each sample two times. The derived copy number of the PCR product was normalized by the sampled air volume. Therefore, the discussed copy numbers in this study refer to the copies per m^3 of sampled air.

2.4 Data analysis

The quality of a qPCR can be assessed by the PCR efficiency (E), which indicates how much template was amplified per cycle (Bustin et al. 2009; Pfaffl 2004). Quantitative PCR results with PCR efficiencies less than 80% were not used for further analysis. For each double replicate, the average initial quantity of the template (DNA copies: N_x) and the number of DNA copies per m^3 of sampled air (C_x) were calculated: for coarse V_c and C_c, for fine V_f and C_f, and for the total particle size V_{tot} and C_{tot} (see Table 3 for equation parameter explanation, and calculations are given in Eqs. 2 and 3).

$$\begin{aligned} C_c &= (N_c - C_f \cdot V_c)\,{}^1\!/_{V_{tot}} \\ &= \left(N_c - N_f \cdot {}^{V_c}\!/_{V_f}\right) \cdot {}^1\!/_{V_{tot}} \end{aligned} \quad (2)$$

$$C_f = \left(N_f + N_f \cdot {}^{V_c}\!/_{V_f}\right) \cdot {}^1\!/_{V_{tot}} \quad (3)$$

The DNA copies for coarse and fine particle filters were calculated separately, as 10% of the fine particles are sampled on the coarse particle filters due to the air flow of the virtual impactor, which is corrected in Eq. 2. However, the fine particle samples are essentially free from coarse particle contamination (Solomon et al. 1983). For further details, see also Müller-Germann et al. 2015.

Table 3 Equation parameters

Parameter	Quantity
$CopyNo_{Plasmid}$	Copy number of the standard plasmid per µl extract
$C_{Plasmid}$	Concentration of the plasmid (including PCR product (g μl^{-1})
C_t	DNA concentration total (number of copies per cubic meter of air; m^{-3})
C_c	DNA concentration coarse (number of copies per cubic meter of air; m^{-3})
C_f	DNA concentration fine (number of copies per cubic meter of air; m^{-3})
$l_{Plasmid}$	Length of plasmid DNA and included PCR product in base pairs (bp)
$m_{Plasmid}$	Weight of plasmid and included PCR product in g mol^{-1}
N_A	Avogadro constant (mol^{-1})
N_t	Number of DNA copies (cp), total
N_c	Number of DNA copies (cp), coarse
N_f	Number of DNA copies (cp), fine
V_c	Sampled air for coarse flow (m^3)
V_f	Sampled air for fine flow (m^3)
V_t	Total air flow (m^3)

2.5 Quality control

To monitor contaminations during amplification and DNA extraction, in each qPCR run one negative control was included and extraction blanks were extracted along with sampled filters and analyzed in the qPCR. In Table ESM2, all analyzed blank samples are listed. To detect possible contaminations from the sampler and sample handling, blank samples were taken at regular intervals (~4 weeks). The blank filters were treated identically to the regular filters, but the pump was either not turned on at all ("mounting blanks") or only for 5 s ("start-up blank") as described in Fröhlich-Nowoisky et al. (2009). For both, mugwort and ragweed, 34 mounting and start-up blanks, respectively, were analyzed. During each extraction process, at least one blank filter was extracted in parallel and amplified in the qPCR run. For each qPCR run, a negative control was included to ensure a contamination-free amplification process and setup. For mugwort and ragweed, in total 20 extraction blanks were tested each.

No extraction blank, qPCR negative control, or sampling blank contained any DNA for ragweed, whereas for mugwort on one mounting and one start-up blank mugwort DNA was detected, however, both times only in one of the two replicates. Both blank samples were from the plant-specific main pollen season, which makes it more likely that minor amounts of mugwort may have settled on the filter while mounting it in the filter sampler (MZ 49a from 2006-08-02 and MZ 201a from 2008-08-07).

2.6 Meteorological data

For the correlation analysis, the meteorological data for temperature, relative humidity, precipitation, and wind speed were provided by the Landesamt für Umwelt, Wasserwirtschaft und Gewerbeaufsicht Rhineland-Palatinate Zentrale Immessionsmessnetz (ZIMEN), which was gathered at their station in Mainz-Mombach, Germany. The dataset consists of half-hour values for all the observed sampling periods.

2.7 Correlation analysis

Using the software environment for statistical computing and graphics, R (R Development Core Team 2011), individual sampling period averages, standard deviations, maximum and minimum values were calculated for temperature (°C), relative humidity (%), and wind speed (m s^{-1}). For the precipitation, the sum of precipitation (mm), the duration of precipitation (h, with a half-hour resolution), and the average precipitation strength (mm h^{-1}) were assessed for each sampling period. Single factor linear regression analysis was performed between meteorological factors and the corresponding copies per m^3 for mugwort and ragweed [total suspended particles (TSP), coarse, and fine particle samples] for (a) the entire dataset, (b) for the entire dataset disregarding the values after each annual maximum copies per m^3, (c) for each year individually, and (d) for the individual years up to the maximum copies per m^3.

2.8 Back-trajectory calculation

Back trajectories were calculated with Hysplit 4 (Draxler and Rolph 2003) for all of the sampling periods analyzed in this study using the Global Data Assimilation System (GDAS) meteorological datasets from the National Centers for Environmental Prediction (NCEP). The gridded meteorological datasets have a 1° horizontal resolution, divided into 23 vertical pressure levels. The maximum modeling height was set at 10,000 m above ground level. Trajectories were calculated backwards in 30 min intervals from the sampling location (49.99°N, 8.23°E). To assess a species-specific trajectory length, we followed Landolt-Börnstein (1988), which estimates the residence time of a particle based on its aerodynamic diameter. The aerodynamic diameter of a particle is defined as the diameter of a sphere with a density of 1 g cm^{-3} and the same aerodynamic properties as the particle and is primarily dependent on the particle shape and density. For reasons of simplicity, we used the lower bound of actual diameter, as both species have more or less spherical shape with width to length ratios of over 0.9 and furthermore the cell density of pollen is known to be variable, dependent on pollen age and humidity levels (http://www.pollenwarndienst.at/DE/de/allergie-infos/fuer-aerobiologen/pollenatlas.html). For ragweed, Harrington and Metger (1963) report densities for fresh pollen that ranged from 1.28 to 1.05 g cm^{-3} at 100 and less than 52% relative humidity, respectively, and a density of 0.84 g cm^{-3} for dried pollen at 52% relative humidity. This resulted in back-trajectory

run times of 27 h for mugwort (20 µm) and 32 h for ragweed (18 µm) during the flowering period. Outside the flowering season, the run times were arbitrarily set to 72 h to account for potential long-range transport from southern Europe and the transport of damaged or aged pollen of smaller aerodynamic diameters.

3 Results and discussion

3.1 Seasonal variation and comparison of the pollination periods

During the 5-year sampling period from 2006 to 2010 in Mainz, mugwort as well as ragweed DNA was successfully quantified from air filters on which a mixture of urban and rural continental boundary layer air masses was collected. In total, 89 filter pairs, each consisting of a coarse and a fine particulate matter filter, were analyzed for mugwort and 67 filter pairs for ragweed. Details are shown in Table 1 listing the number of analyzed filters for each year next to the number of filters with quantifiable DNA concentrations. In Fig. 1, we show the seasonal variations in atmospheric concentrations in the coarse (1a) and fine (1b) size fractions separately, whereas in Fig. 2, we compare plant-specific pollination periods and show both results for absolute concentrations (2a) and a comparison of the relative proportions (2b). It is important to remember, when viewing Fig. 2, that the pollination periods of both plants differ, so we are comparing time periods that only overlap by half a month.

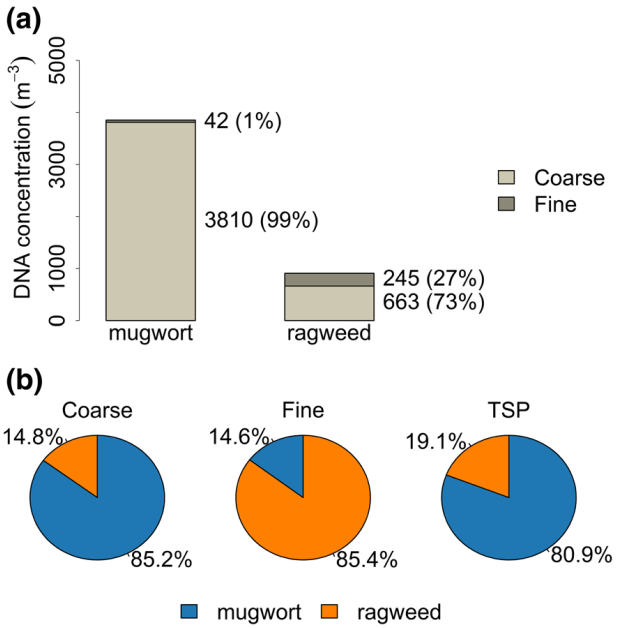

Fig. 2 Comparison of the ragweed and mugwort DNA concentrations within their characteristic pollination periods (ragweed: mid-August to end of September, mugwort: mid-July to end of August): **a** The number of DNA copies per cubic meter of air in the coarse and fine fraction averaged over all analyzed air samples (2006–2010) that lay within the characteristic pollination periods, **b** The relative proportions of the ragweed and mugwort copies per cubic meter of air shown for the coarse and fine fractions as well as TSP (coarse + fine) calculated using the values displayed in Fig. 2a

As intact mugwort pollen is spherical and 20–26 µm in diameter while ragweed pollen has a size of 18–22 µm (Table ESM1), their resulting aerodynamic diameters will lie well above the 3-µm sampler size separation between the coarse and fine fractions. Therefore, DNA from intact pollen grains

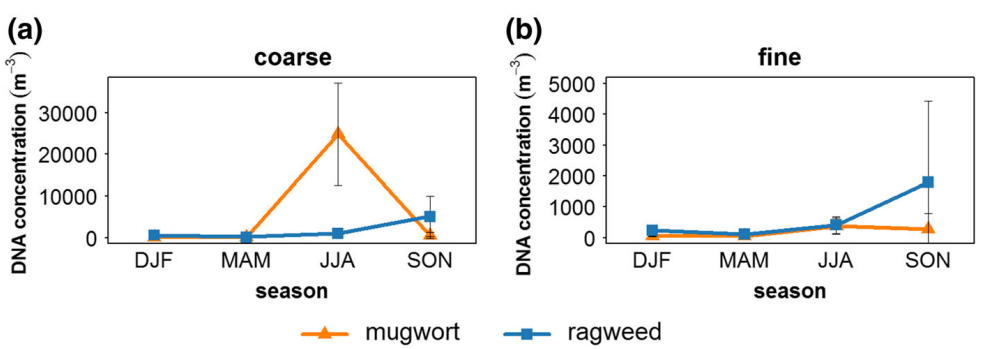

Fig. 1 Sum of the number of DNA copies per cubic meter of air for each meteorological season averaged over the 5 years of sampling (2006–2010): Results are shown for ragweed and mugwort within the **a** coarse particle samples and the **b** fine particulate matter samples. DJF (winter: December, January, February); MAM (spring: March, April, May); JJA (summer: June, July, August); SON (fall: September, October, November). The *error bars* correspond to the standard deviation between the years

will only be found in the coarse particle fraction along with plant fragments with aerodynamic diameters above the cutoff. Unsurprisingly, our data indicate that the process of pollen release is the largest contributor to the atmospheric DNA content. As can be seen in Fig. 1a, both mugwort and ragweed show their by far highest coarse fraction concentrations within their characteristic pollination periods. Mugwort is seen to peak in summer, while ragweed peaks in fall (pollination periods: mugwort: mid-July to August, ragweed: mid-August to September, see Figure ESM1 for more detail). Nonetheless, it is also possible that coarse fraction plant tissue is also released along with pollen, meaning that the DNA concentrations need not exclusively stem from pollen.

As presented in Fig. 2a, b (TSP) the mugwort concentrations within its pollination period are in total roughly four to five times as high as those for ragweed. Furthermore, when comparing the years individually the mugwort concentration in 2010 even reached the 20-fold concentration of ragweed (Figure ESM2). These observations are consistent with mugwort being far more abundant in Germany. For both plants, the proportion of DNA detected in the coarse fraction was much higher than in the fine fraction, varying between 99% for mugwort and 73% for ragweed for the coarse particles (Fig. 2a) and this again is consistent with pollen grains falling in the coarse size fraction.

However, as listed in Table 1, 92% of the coarse particle filters contained mugwort DNA, while 100% contained ragweed DNA. As the primers were equally sensitive and amplified still from 10 copies DNA, as tested in dilution series, a technical reason for this finding is less likely. For further details also see Table ESM3, where the exact copy numbers for all analyzed filter samples are provided. These findings seem to contradict the fact that mugwort is far more abundant in Germany than ragweed and thus could be expected to be found more frequently. However, for mugwort, 32 filter pairs, i.e., only 35% of the total analyzed filter pairs, lay within the specific mugwort pollination period and for ragweed 35 filter pairs and therefore over 50% lay within its characteristic pollination time. Within the mugwort-specific pollination period, from mid-July to August, 100% of the coarse filters contained quantifiable DNA. Nonetheless, quite surprisingly DNA from the far less abundant ragweed was found on all analyzed samples, regardless of size fraction or time of year, albeit at sometimes very low concentrations. This may be explained by long-range transport from southern Europe which is discussed in detail below.

In Fig. 1b the seasonal fine fraction concentrations are provided. When compared to the coarse fraction concentrations, seen in 1a, the seasonal progression of the inhalable fine fraction ragweed mirrors the coarse fraction concentrations at lower concentrations. The fine fraction mugwort concentration does show a minor peak in summer, coinciding with the mugwort pollination period; however, it is far less pronounced in comparison with ragweed.

Particles, collected in the fine particulate matter fraction, will consist of fragmented plant and pollen material. For ragweed, a frequent production of subpollen particles (SPPs) with a size range 0.5–4.5 µm has been observed (Bacsi et al. 2006). This SPP production is likely based on fragmentation processes, as it is known that ragweed pollen can burst under high humidity, as well as during heavy rainfall and thunderstorms, and release SPPs in great numbers (Huffman et al. 2013; Pummer et al. 2013; Steiner et al. 2015). The pollen nucleus DNA contained in some SPPs, due to their small aerodynamic diameters, is likely to be sampled predominantly in the fine particle fraction. In hydration tests with ragweed pollen, at least 35% burst within 90 min (Bacsi et al. 2006). For mugwort to our knowledge, no similar experiments have been conducted. It should also be noted that DNA from fragmented tissue material may be degraded by exposure to atmospheric photooxidants and thus might even be underestimated (Després et al. 2007).

While only 46% of the fine filter samples had quantifiable mugwort DNA, all analyzed fine filters contained ragweed DNA (Table 1). Even within its pollination period, mugwort was only found on 53% of the fine filters despite being far more abundant in the local surrounding than ragweed, which was detected in all fine particle filters. Furthermore, during the pollination period 27% of the total ragweed DNA concentration was found in the inhalable fine particulate fraction (Fig. 2a). Comparing the two species, the proportions of the highly allergenic ragweed to the native mugwort are reversed in respect to the coarse fraction with ragweed constituting 85.4% in the fine fraction (Fig. 2b). These results, therefore, firstly imply that mugwort pollen grains are far more stable than ragweed pollen grains. Secondly and more

importantly, along with the continual presence of ragweed in both size fractions these observations may be important factors explaining the high allergic potential of ragweed and the high sensitization rates observed among populations exposed to ragweed, as SPPs can reach and accumulate in the alveoli of the lungs and therefore might be promoting and enhancing allergic reactions (Mücke and Lemmen 2008).

3.2 Annual variation and the influence of meteorology

As illustrated by the large error bars seen in Fig. 1a, large variation in measured DNA concentrations was observed between the analyzed years. When comparing the DNA concentration patterns between the individual years in more detail (Figure ESM2), it becomes evident that the highest detected mugwort TSP DNA concentration for single filter samples during its pollination period varies between ~4000 copies per m^3 in 2006 and the fivefold amount of ~19,000 copies per m^3 in 2009. For ragweed, there is a similar picture. Between the different years, the TSP DNA concentration maxima during the pollination period ranged between ~6500 copies per m^3 (2008) and ~500 copies per m^3 (2010). This supports the findings of Straka (1975) that yearly pollen concentrations can vary enormously in strength. The local pollen amount is on the one hand influenced by the number, health, and size of the producing plants and on the other hand by the general growing conditions, i.e., climate and habitat characteristics (Fumanal et al. 2007). Although the main pollination season was generally more pronounced in the coarse particle fraction for both weeds, in 2008 and 2009 the fine particle filter samples display high DNA concentrations for ragweed within the pollination time, resulting in several high values from the middle of September until the middle of October 2008 with a maximum of ~2700 copies per m^3.

Due to the continuous spread of ragweed in Europe and especially within Germany, one could expect an increase in ragweed presence (Alberternst et al. 2006; Dahl et al. 1999; Jäger 2000; Mandrioli et al. 1998; Prank et al. 2013). However, within the Mainz air filter samples, this spread is not reflected by an increase in the DNA concentration over the 5-year measurement period. With the increasing public awareness of the spread of ragweed, a greater amount of ragweed plants was detected over the last decade. In Germany, it is not compulsory to remove ragweed plants. Therefore, combative measures are locally confined and sporadic. Nonetheless, these measures might also add to the variability and counteract a potential increase in ragweed DNA amount in the observation period from 2006 to 2010. As another explanation for the annual DNA variation observed for both weeds, one could discuss possible competitive behavior between the species, influences by meteorological conditions or long-distance transport playing a substantial role.

Figure 3 shows an almost inverse behavior of mugwort and ragweed throughout the 5 years. This could point to a possible competitive behavior between both plants for certain habitats as both weeds prefer similar growing conditions (Barney and Di Tommaso 2003; Bassett and Crompton 1975; see Table ESM1). However, the direct observation of the plant behavior makes competition effects less likely. Ragweed prefers heavily disturbed areas like agricultural fields, while mugwort avoids these disturbed areas. And even if competition takes place for a short period, it may have no effect on the long-term occupation of that habitat.

The onset and duration of characteristic pollination seasons also vary depending on different physical influences like seasonal climate, and meteorological factors (Dahl et al. 2013). Especially meteorological factors like precipitation, relative humidity, temperature, and wind direction as well as speed are known to have a strong influence on the life cycles of mugwort and ragweed, their successful pollen grain production,

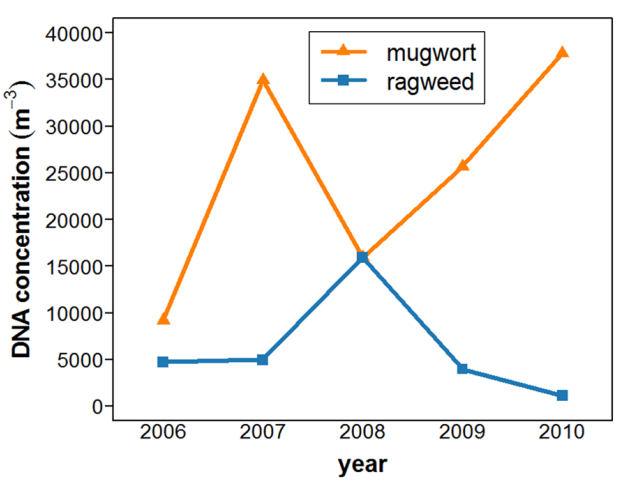

Fig. 3 Comparison of the mugwort and ragweed sums of TSP DNA copies per cubic meter of air for all years of sampling

release and distribution (Kaminski et al. 2010; Kasprzyk 2006; Peternel et al. 2008; Puc 2006). As shown in Figure ESM3, the linear regression analysis of the meteorological data with mugwort or ragweed DNA concentration in the coarse fraction produced no significant results true for all years. This is likely due to a too simplistic approach in the linear regression analysis. Firstly, it would require an immediate effect of a meteorological factor on the atmospheric concentrations. A temporal lag in the influence is probable. Nonetheless, the DNA concentrations of individual years do show high absolute Pearson values to differing meteorological factors. Therefore, we have presented the results, but make no attempt at in-depth interpretation. Preliminary results of a timeline analysis of the meteorological factors in the time period leading up to the pollination peak suggest that the temperature profile plays a key role in the onset of pollination of both species. Furthermore, there is evidence that implies that precipitation may play a role in the DNA concentration within the fine fraction. These observations are, however, preliminary and will be subject of future analysis.

3.3 Wind back trajectories and long-range transport

For ragweed the concentrations in all samples taken in November and December reached approximately 5–42%, (27–1085 copies per m^3) of maximum air mass sample concentrations of the same years. In each year, the analyzed November and December samples lay well after the first temperature drop below 0 °C at the sampling location (earliest freezing temperatures on 15th of October in 2009 and latest on November 23, 2008); this should rule out the pollen release in the sampling region as a source of the DNA concentrations (Buttenschøn et al. 2009). The presence may also be due to emission related to the degradation process; however, in the same samples mugwort only reaches 0–1.8% (0–309 copies per m^3) of its annual maximum concentration. Again, the far higher local mugwort abundancy would lead to higher concentrations if degradation was the main contributor.

As locally—emitted pollen and degrading plant matter seem unlikely as a predominant source for the high wintertime concentrations of ragweed, long-range transport from southern Europe may be a viable explanation. Pollen grains in the size range around 20 µm can be transported up to 1000 km into areas where they are not native, and for ragweed long-distance transport events have been reported in the past (Buttenschøn et al. 2009; Kaminski et al. 2010; Mandrioli et al. 1998; Piotrowska and Weryszko-Chmielewska 2006; Rousseau 2003; Smith et al. 2008; Stach et al. 2007; Zink et al. 2012). In Germany the pollen has been shown to originate from distant areas such as Slovakia, Hungary, Northern Italy, or Southern France (Smith et al. 2008; Zink et al. 2012). Other studies even report pollen reaching the UK and Denmark from southern France and Hungary (de Weger et al. 2016; Sommer et al. 2015). Furthermore, Grewling et al. (2016) were also able to show that the main allergen Amb a 1 maintained its immunoreactivity after a hypothesized long-range transport in both a size range above and below 10 µm aerodynamic diameter.

In Fig. 4, we show the calculated back trajectories for two samples with the highest wintertime ragweed concentrations (MZ-76 from 2006 (blue) and MZ-229 from 2008 (green) with TSP concentrations 1085 and 896 copies per m^3, respectively) along with the sample with the lowest measured wintertime concentration (MZ-376 from 2010 (red) with a TSP concentration of 27 copies per m^3). As can be seen, the back trajectories for MZ-76 seen in blue predominately stem from a southwesterly region in respect to our sampling location, from mid and southern France including

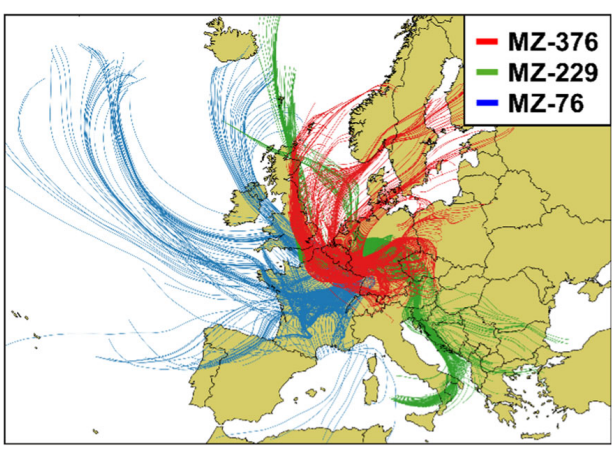

Fig. 4 Back trajectories calculated for fall–winter periods for mugwort (**a**) and ragweed (**b**): **a** 2009 of filter sample MZ 297 (2009-11-03 to 2009-11-10; *red colored*); 2010 of filter sample MZ 376 (2010-11-23 to 2010-11-30; *green colored*); **b** 2006 of filter sample MZ 76 (2006-12-07 to 2006-12-14; *blue colored*); 2008 of filter sample MZ 229 (2008-12-11 to 2008-12-18; *green colored*); 2010 of filter sample MZ 376 (2010-11-23 to 2010-11-30; *red colored*). Trajectories were calculated using the program Hysplit

the Rhône valley known for its extensive ragweed population (Thibaudon et al. 2014). Furthermore, data obtained from the RNSA (Réseau National de Surveillance Aérobiologique) for Avignon, a potential emission source, show concentrations of 0.53–1.06 grains m^{-3} 1 week prior to our sampling, which would account for the duration of the transport process from southern France. The back trajectories calculated for MZ-229, seen in green, mainly stem from a south-easterly direction, passing over the eastern Adriatic coast and to some extent the Pannonian Plain both known for their very extensive ragweed stock (Buttenschøn et al. 2009; Skjøth et al. 2010). In contrast, the back trajectories calculated for MZ-376 seen in red, with a low concentration of ragweed DNA, mainly stem from a north to northwesterly direction, with the most southern trajectories stemming from the Alps. These results strengthen the possibility of long-range transport from southern Europe as an explanation for the high wintertime concentrations.

If long-range transport of ragweed does play a significant role for the wintertime concentrations, reaching up to 42% of the annual maximum, it should also have a significant influence during the pollination season. Makra et al. (2006) report that ragweed alone makes up 47.3% of the annually measured pollen in Szeged in Hungary. Their measurements encompassed 24 taxa, including mugwort, which was shown to have an annual pollen concentration peak roughly of an order of magnitude smaller than that of ragweed. Next to possible differences in pollen stability leading to SPP production, high emission rates from southern Europe may also be a feasible alternative explanation for the high fine fraction concentrations, which could be caused by rupturing of the pollen during transport at high altitude. Large differences in emission rates between mugwort and ragweed would furthermore explain why mugwort is not as prominent in the fine fraction, as far less pollen is subjected to the environmental stresses of long-range transport.

4 Conclusions

Within this study, we successfully quantified DNA of the allergenic weeds mugwort and ragweed over a 5-year period in Mainz, Germany. For both weeds, we could demonstrate that their DNA concentrations are higher within the coarse particle fraction, containing particles with aerodynamic diameters larger than $\sim$3 µm. This is most probably due to the plants' pollen falling in this fraction, with actual diameters of $\sim$20 µm. Furthermore, there was a large variation between the DNA abundancy observed each year, which may be primarily explained by differing meteorological conditions. We could also demonstrate that, within the coarse fraction, more mugwort was present than ragweed, which coincides with mugwort being far more abundant within the local flora than ragweed.

Interestingly, the situation was reversed in the inhalable fine particle fraction ($<\sim$3 µm), with more ragweed DNA present than mugwort DNA. An explanation for this finding might be that ragweed pollen is less stable and has a higher tendency to burst under humid conditions, thereby producing SPPs, which are smaller in size and thus accumulate on the fine particle filter. We also observe high ragweed DNA concentrations outside the pollination season. Back-trajectory analysis pointed to long-distance transport from southern Europe, where the pollen emissions of ragweed are reported to far outweigh those of mugwort. This may be an alternative explanation of the high fine fraction concentrations, in general, as the pollen may be rupturing during long-range transport.

The high fine fraction concentrations may also be a factor contributing to the allergenic potential of ragweed and high sensitization rates seen among the population as small particles will spread further, heightening exposure, and furthermore penetrate deeper in the respiratory system. The influence of long-distance transport implies that not only the local vegetation must be taken into account when discussing possible health risks but also the pollen and pollen fragments reaching Germany from surrounding countries.

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Supplementary Material

Allergenic Asteraceae in air particulate matter: DNA-based analysis of mugwort and ragweed

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Supplementary Online Material

Meteorological influences

Interestingly, some years seem to show in total more significant correlations than others, e.g., for fine particles in ragweed in the year 2006 the temperature seems to correlate negatively with the DNA concentration, while wind speed criteria correlate positively. In the coarse particle fraction these correlations are not supported. For mugwort, however, the year 2008 sticks out. Here in the coarse particle fraction criteria describing the influence of relative humidity and precipitation, correlate either positively or negatively. Observations by other authors, who found e.g. influence by wind speed on the pollen dispersal, cannot be supported strongly with the presented data (Dahl et al. 2013). On the other hand, the findings do support the results of studies dealing with the comparison of meteorological data and mugwort and ragweed pollen concentration, which also found no continuous trends for the correlation (Kaminski et al. 2010; Puc 2006) . However, meteorological parameters should still influence mugwort and ragweed life cycles. It might be that these correlations can only be discovered using a smaller time scale. Furthermore, it is also possible that there is a temporal lag in the influence of meteorological parameters, which is not taken into account in our analysis.

Supplementary Figures

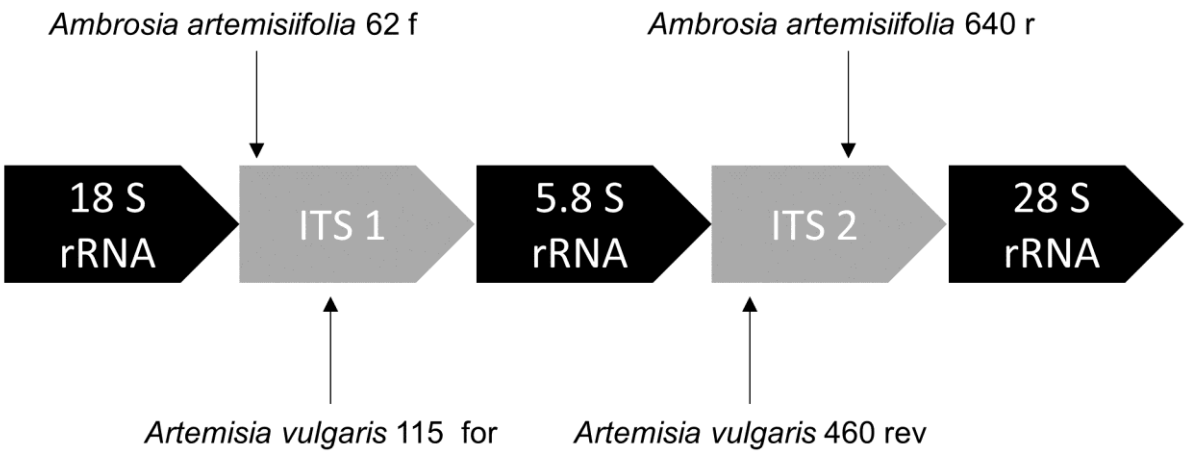
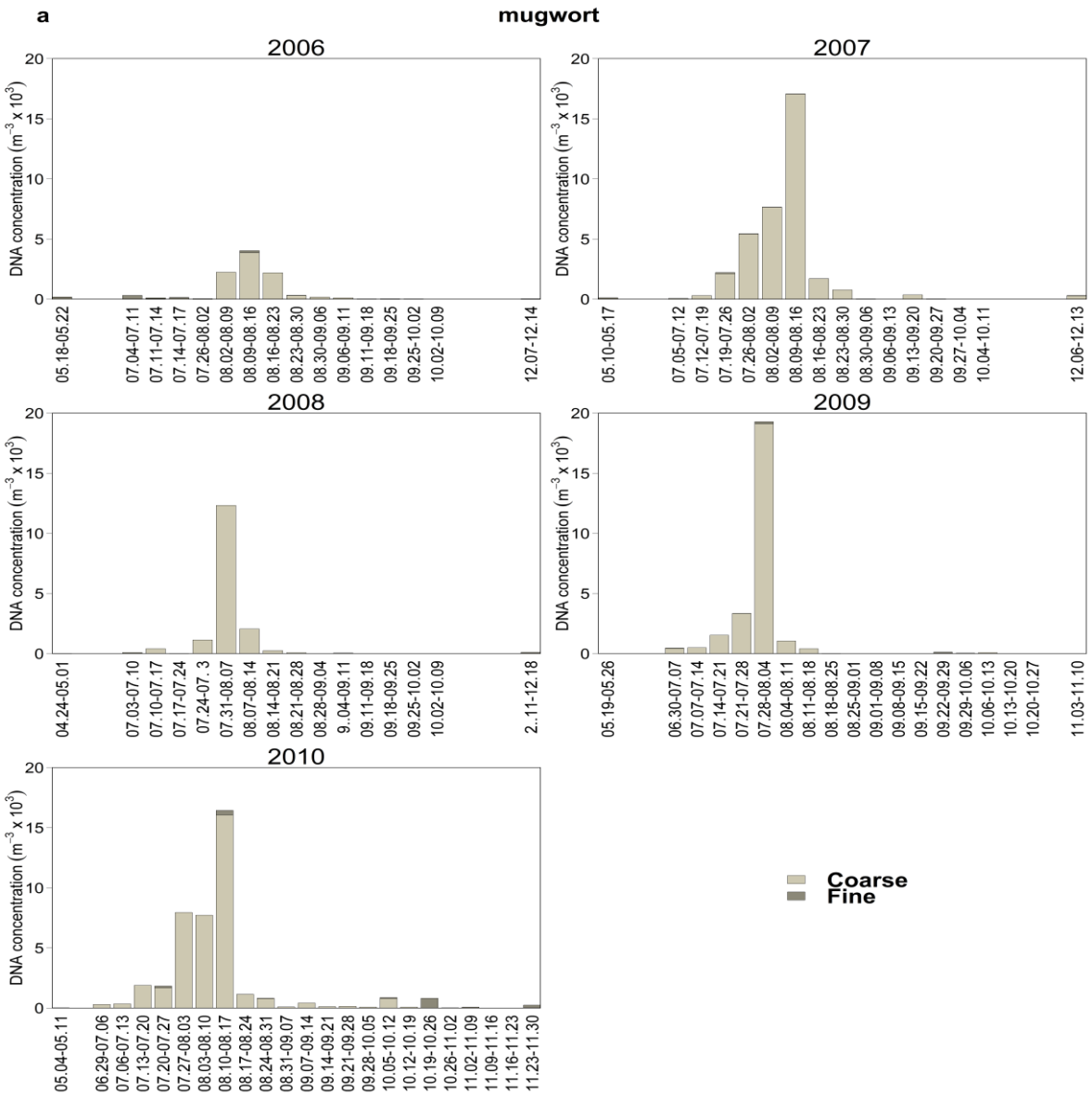


Fig. ESM1 Primer binding sites for mugwort and ragweed in the ITS region


a
mugwort
2006
2007
2008
2009
2010
DNA concentration (m-3 x 10^3)
Coarse
Fine

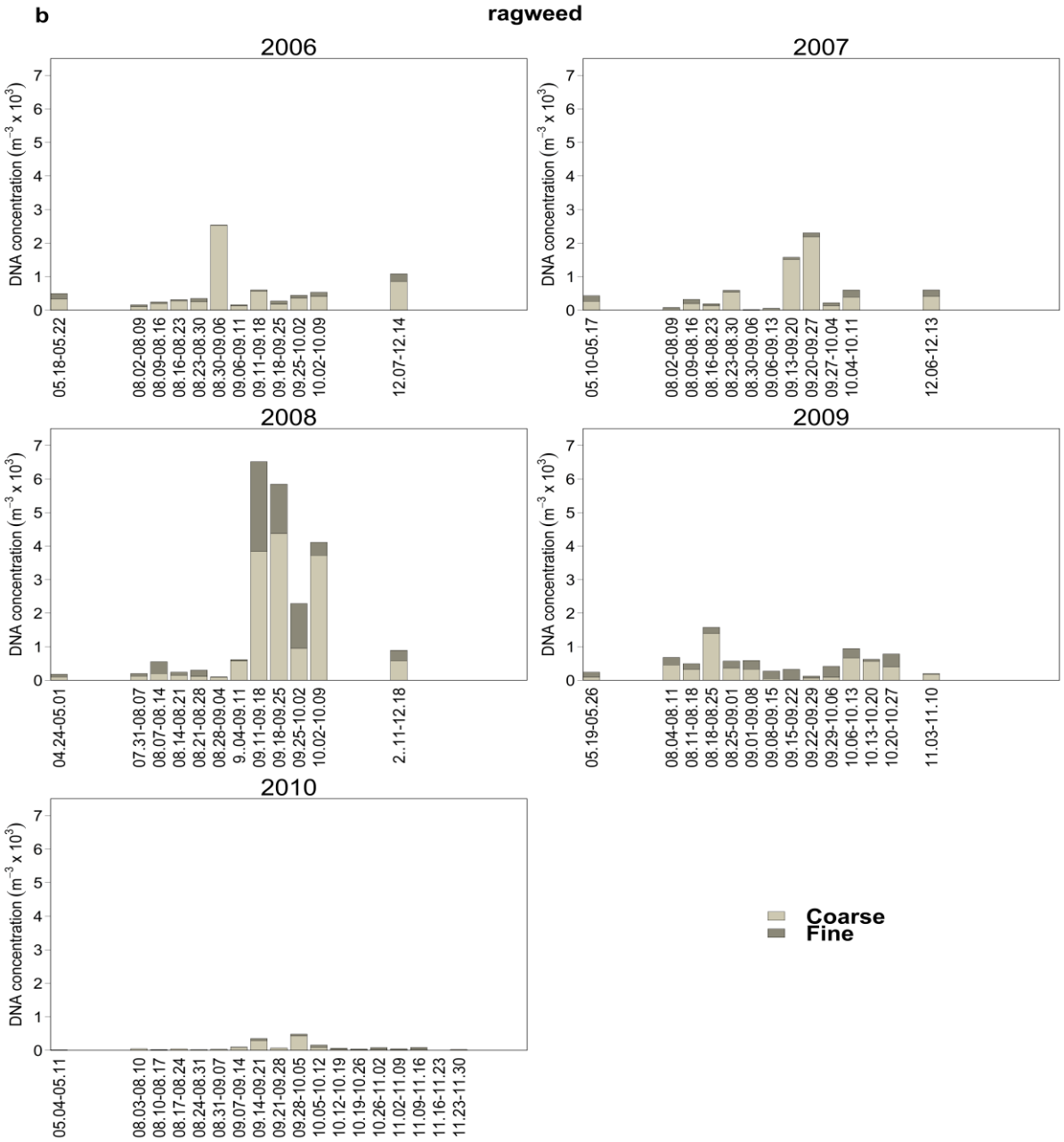


Fig. ESM2 Mugwort (a) and ragweed (b) DNA concentration in air from 2006 to 2010. DNA amount is given in copies per cubic meter of air: m^{-3}; m^{-3}x1000 for coarse and fine particle filters for each analyzed filter sample. The sum for the DNA concentration on coarse and fine particle filters gives the DNA concentration of the total air mass. Gaps in the x-axis represent non-analyzed time periods in late spring/early summer and late fall

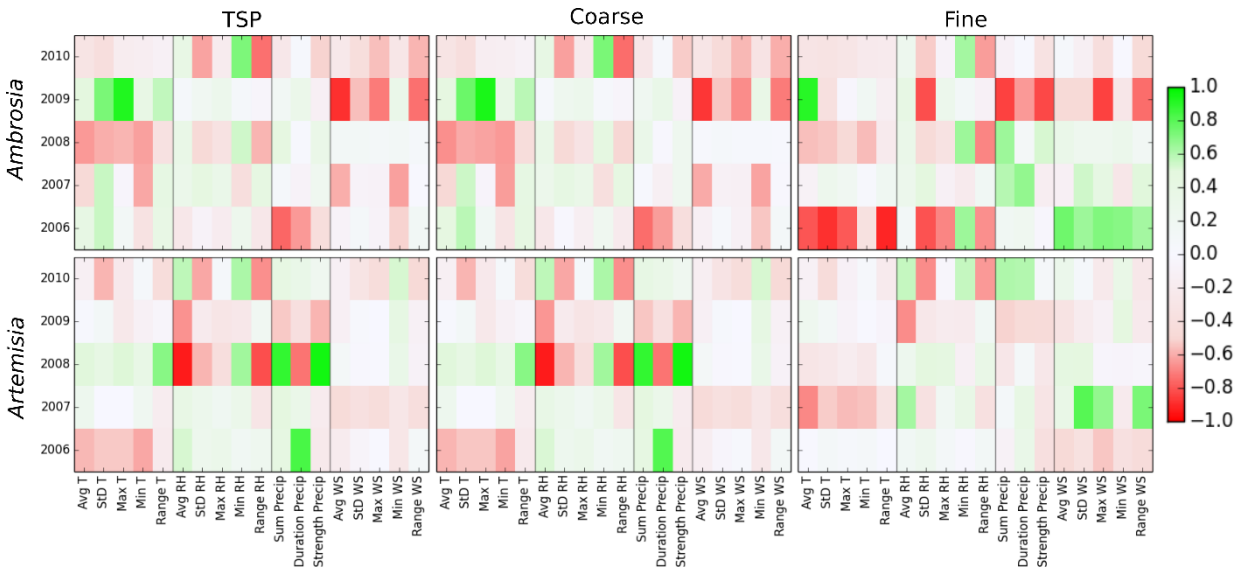


Fig. ESM3 Pearson heat map depicting the Pearson values (green +1 to red -1) between copy numbers of *Ambrosia* and *Artemisia* in the TSP, coarse and fine fractions and meteorological factors. For each year only the samples were factored into the analysis that lay before the peak coarse fraction copy numbers, which should correspond to the maximum pollination time point. Meteorological factors analysed were temperature (T in °C), relative humidity (RH in %), wind speed (WS), precipitation (Precip), the sum of precipitation duration as well as average precipitation strength

Supplementary Tables

Table ESM1 Comparison of mugwort and ragweed plant characteristics (n.k. = not known)

	Ragweed (*Ambrosia artemisiifolia*)		**Mugwort (*Artemisia vulgaris*)**	
Origin	North America and Canada	e.g., Gadermaier et al. (2004)	Europe	Barney and Di Tommaso (2003) and references therein
Habitat	Human disturbed open habitats, roadsides	Bassett and Crompton (1975)	Wastelands, roadsides	Holm et al. (1997)
Climate/soil conditions	Warm climate, grows in a wide range of soil types, prefers slightly acidic, sandy and muddy loam soils	Bassett and Crompton (1975), own observations	Wide temperature range, moist soils	Barney and Di Tommaso (2003)
European distribution pattern	Centre: Northern Italy, southern France, Carpathian Basin Spreading northwards	Dahl et al. (1999); Makra et al. (2004) Dahl et al. (1999)	Northern hemisphere and Mediterranean basin	Gadermaier et al. (2004)
Generation cycle	Annual	Peternel et al. (2005)	Rhizomatous perennial	Barney and Di Tommaso (2003)
Seed production	3000-62000 seeds depending on plant size	Dahl et al. (1999)	200 000 seeds per plant	Pawlowski et al. (1968)
Main pollination season	August-onset of frost depending on local climate some plants start to flower at the end of June	Buttenschøn et al. (2009); Dahl et al. (1999), own observations	End of July-end of August, can last longer in warmer regions	Gadermaier et al. (2004); Wopfner et al. (2005)
Pollen grain size	18- 22 µm	Gadermaier et al. (2004)	20 – 26 µm	Hayat et al. (2009)
Pollen production	10^9-2,5x10^9	Fumanal et al. (2007); Laaidi et al. (2003b)	3,19 x 10^9	Grewling et al. (2012); Piotrowska (2008)
Prevalence in atopic individuals	Europe: 35-80 % North America: 45 %	Bundesamt für Gesundheit (2005); Wopfner et al. (2005)	Europe: 14 % North America: n.k.	Spieksma et al. (1980)

Table ESM2 Start-up and mounting blank measurements (+ DNA detected on blank sample; - no DNA detected; n.a. not analyzed)

Description	Sampling Date	Sampling period (in days)	*Artemisia vulgaris*	*Ambrosia artemisiifolia*
MZ 48a	2006-08-02	Start-up blank	-	-
MZ 48b	2006-08-02	Start-up blank	-	-
MZ 49a	2006-08-02	Mounting blank	+	-
MZ 49b	2006-08-02	Mounting blank	-	-
MZ 56a	2006-09-11	Start-up blank	-	-
MZ 56b	2006-09-11	Start-up blank	-	-
MZ 58a	2006-09-11	Mounting blank	-	-
MZ 58b	2006-09-11	Mounting blank	-	-
MZ 132a	2007-09-06	Start-up blank	-	-
MZ 132b	2007-09-07	Start-up blank	-	-
MZ 133a	2007-09-07	Mounting blank	-	-
MZ 133b	2007-09-07	Mounting blank	-	-
MZ 140a	2007-10-11	Start-up blank	-	-
MZ 140b	2007-10-11	Start-up blank	-	-
MZ 141a	2007-10-11	Mounting blank	-	-
MZ 141b	2007-10-11	Mounting blank	-	-
MZ 201a	2008-08-07	Start-up blank	+	-
MZ 201b	2008-08-07	Start-up blank	-	-
MZ 202a	2008-08-07	Mounting blank	-	-
MZ 202b	2008-08-07	Mounting blank	-	-
MZ 207a	2008-09-04	Start-up blank	-	-
MZ 207b	2008-09-04	Start-up blank	-	-
MZ 208a	2008-09-04	Mounting blank	-	-
MZ 208b	2008-09-04	Mounting blank	-	-
MZ 213a	2008-10-02	Start-up blank	-	-

MZ 213b	2008-10-02	Start-up blank	-	-
MZ 214a	2008-10-02	Mounting blank	-	-
MZ 214b	2008-10-02	Mounting blank	-	-
MZ 271a	2009-07-14	Start-up blank	-	-
MZ 271b	2009-07-14	Start-up blank	-	-
MZ 272a	2009-07-14	Mounting blank	-	-
MZ 272b	2009-07-14	Mounting blank	-	-
MZ 277a	2009-08-11	Start-up blank	-	-
MZ 277b	2009-08-11	Start-up blank	-	-
MZ 278a	2009-08-11	Mounting blank	-	-
MZ 278b	2009-08-11	Mounting blank	-	-
MZ 281a	2009-09-01	Start-up blank	-	-
MZ 281b	2009-09-01	Start-up blank	-	-
MZ 282a	2009-09-01	Mounting blank	-	-
MZ 282b	2009-09-01	Mounting blank	-	-
MZ 290a	2009-10-13	Start-up blank	-	-
MZ 290b	2009-10-13	Start-up blank	-	-
MZ 291a	2009-10-13	Mounting blank	-	-
MZ 291b	2009-10-13	Mounting blank	-	-
MZ 346a	2010-07-06	Start-up blank	-	n.a.
MZ 346b	2010-07-06	Start-up blank	-	n.a.
MZ 347a	2010-07-06	Mounting blank	-	n.a.
MZ 347a	2010-07-06	Mounting blank	-	n.a.
MZ 353a	2010-08-10	Start-up blank	-	-
MZ 353b	2010-08-10	Start-up blank	-	-
MZ 354a	2010-08-10	Mounting blank	-	-
MZ 354b	2010-08-10	Mounting blank	-	-
MZ 358a	2010-08-31	Start-up blank	-	-
MZ 358b	2010-08-31	Start-up blank	-	-
MZ 359a	2010-08-31	Mounting blank	-	-

MZ 359b	2010-08-31	Mounting blank	-	-
MZ 365a	2010-10-05	Start-up blank	-	-
MZ 365b	2010-10-05	Start-up blank	-	-
MZ 366a	2010-10-05	Mounting blank	-	-
MZ 366b	2010-10-05	Mounting blank	-	-
Ex Blk	2008-08-06	Extraction blank	-	-
Ex Blk	2008-08-07	Extraction blank	-	-
Ex Blk	2008-08-19	Extraction blank	-	-
Ex Blk	2008-09-17	Extraction blank	-	-
Ex Blk	2008-11-12	Extraction blank	-	-
Ex Blk	2008-12-29	Extraction blank	-	-
Ex Blk	2009-11-03	Extraction blank	-	-
Ex Blk	2009-11-04	Extraction blank	-	-
Ex Blk	2009-11-12	Extraction blank	-	-
Ex Blk	2010-09-23	Extraction blank	-	-
Ex Blk	2010-09-23	Extraction blank	-	-
Ex Blk	2010-11-17	Extraction blank	-	-

Table ESM3 DNA copies per cubic meter of air; m^3 are given for mugwort (a) and for ragweed (b) in the sampling period from 2006-2010

a) **Mugwort**

Filter ID	Sampling period (2006)	Sampled air volume [m^3]	Coarse filters [m^{-3}]	Fine filters [m^{-3}]	Air masses [m^{-3}]
MZ 24	05-18 / 05-22	1703	95	99	194
MZ 41	07-04 / 07-11	3019	17	298	315
MZ 42	07-11 / 07-14	1315	34	86	120
MZ 43	07-14 / 07-17	1288	63	89	152
MZ 47	07-26 / 08-02	3032	8	39	47
MZ 50	08-02 / 08-09	2931	2247	0	2247
MZ 51	08-09 / 08-16	3015	3849	193	4042
MZ 52	08-16 / 08-23	3056	2197	0	2197
MZ 53	08-23 / 08-30	3054	306	21	327
MZ 54	08-30 / 09-06	3087	179	0	179
MZ 55	09-06 / 09-11	2139	121	0	121
MZ 59	09-11 / 09-18	2916	26	0	26
MZ 60	09-18 / 09-25	3073	32	0	32
MZ 61	09-25 / 10-02	3063	24	2	26
MZ 62	10-02 / 10-09	3033	12	1	13
MZ 76	12-07 / 12-14	3058	5	36	41

Filter ID	Sampling period (2007)	Sampled air volume [m^3]	Coarse filters [m^{-3}]	Fine filters [m^{-3}]	Air masses [m^{-3}]
MZ 108	05-10 / 05-17	3025	2	131	133
MZ 121	07-05 / 07-12	3034	58	9	67
MZ 122	07-12 / 07-19	3076	301	0	301
MZ 123	07-19 / 07-26	3001	2114	103	2217
MZ 126	07-26 / 08-02	3068	5404	38	5442
MZ 127	08-02 / 08-09	3064	7646	16	7662

MZ 128	08-09 / 08-16	3065	17056	22	17078
MZ 129	08-16 / 08-23	3038	1701	9	1710
MZ 130	08-23 / 08-30	3033	776	0	776
MZ 131	08-30 / 09-06	3025	25	0	25
MZ 134	09-06 / 09-13	3042	6	0	6
MZ 135	09-13 / 09-20	3043	364	0	364
MZ 136	09-20 / 09-27	3072	27	0	27
MZ 137	09-27 / 10-04	3046	15	0	15
MZ 138	10-04 / 10-11	3056	11	0	11
MZ 153	12-06 / 12-13	3052	276	33	309

Filter ID	**Sampling period (2008)**	**Sampled air volume [m^3]**	**Coarse filters [m^{-3}]**	**Fine filters [m^{-3}]**	**Air masses [m^{-3}]**
MZ 181	04-24 / 05-01	3046	4	23	27
MZ 194	07-03 / 07-10	3006	13	82	95
MZ 197	07-10 / 07-17	2995	406	3	409
MZ 198	07-17 / 07-24	3082	20	8	28
MZ 199	07-24 / 07-31	3039	1140	0	1140
MZ 200	07-31 / 08-07	3078	12333	0	12333
MZ 203	08-07 / 08-14	3010	2058	0	2058
MZ 204	08-14 / 08-21	3147	260	0	260
MZ 205	08-21 / 08-28	2969	85	0	85
MZ 206	08-28 / 09-04	3106	1	0	1
MZ 209	09-04 / 09-11	3069	1	51	52
MZ 210	09-11 / 09-18	1687	6	0	6
MZ 211	09-18 / 09-25	3050	8	0	8
MZ 212	09-25 / 10-02	3080	6	0	6
MZ 215	10-02 / 10-09	2994	16	0	16
MZ 229	12-11 / 12-18	3125	50	60	110

Filter ID	Sampling period (2009)	Sampled air volume [m^3]	Coarse filters [m^{-3}]	Fine filters [m^{-3}]	Air masses [m^{-3}]
MZ 261	05-19 / 05-26	3078	0	0	0
MZ 269	06-30 / 07-07	3171	418	48	466
MZ 270	07-07 / 07-14	3021	497	0	497
MZ 273	07-14 / 07-21	3065	1531	0	1531
MZ 274	07-21 / 07-28	2942	3339	18	3357
MZ 275	07-28 / 08-04	3119	19097	199	19296
MZ 276	08-04 / 08-11	3057	1055	0	1055
MZ 279	08-11 / 08-18	3016	406	0	406
MZ 280	08-18 / 08-25	3043	20	7	27
MZ 281	08-25 / 09-01	3056	4	0	4
MZ 284	09-01 / 09-08	3043	2	4	6
MZ 285	09-08 / 09-15	2986	0	0	0
MZ 286	09-15 / 09-22	3037	0	0	0
MZ 287	09-22 / 09-29	3030	44	84	128
MZ 288	09-29 / 10-06	3057	15	29	44
MZ 289	10-06 / 10-13	3015	97	0	97
MZ 292	10-13 / 10-20	2982	0	0	0
MZ 293	10-20 / 10-27	3045	0	0	0
MZ 297	11-03 / 11-10	3047	16	0	16

Filter ID	Sampling period (2010)	Sampled air volume [m^3]	Coarse filters [m^{-3}]	Fine filters [m^{-3}]	Air masses [m^{-3}]
MZ 335	05-04 / 05-11	3031	31	0	31
MZ 345	06-29 / 07-06	3006	302	0	302
MZ 348	07-06 / 07-13	3120	345	0	345
MZ 349	07-13 / 07-20	3052	1890	0	1890
MZ 350	07-20 / 07-27	3126	1678	154	1832

MZ 351	07-27 / 08-03	3013	7948	0	7948
MZ 352	08-03 / 08-10	3131	7721	0	7721
MZ 355	08-10 / 08-17	3038	16047	397	16444
MZ 356	08-17 / 08-24	3007	1136	9	1145
MZ 357	08-24 / 08-31	3022	792	32	824
MZ 360	08-31 / 09-07	3020	99	0	99
MZ 361	09-07 / 09-14	3142	420	0	420
MZ 362	09-14 / 09-21	3042	120	0	120
MZ 363	09-21 / 09-28	2931	154	1	155
MZ 364	09-28 / 10-05	3294	88	0	88
MZ 367	10-05 / 10-12	3129	773	105	878
MZ 368	10-12 / 10-19	2978	89	0	89
MZ 369	10-19 / 10-26	3050	9	809	818
MZ 370	10-26 / 11-02	2939	0	28	28
MZ 373	11-02 / 11-09	3047	22	62	84
MZ 374	11-09 / 11-16	3019	0	0	0
MZ 376	11-23 / 11-30	3083	55	194	249

b) **Ragweed**

Filter ID	Sampling period (2006)	Sampled air volume [m^3]	Coarse filters [m^{-3}]	Fine filters [m^{-3}]	Air masses [m^{-3}]
MZ 24	05-18 / 05-22	1703	332	165	497
MZ 50	08-02 / 08-09	2980	101	58	159
MZ 51	08-09 / 08-16	2990	196	51	247
MZ 52	08-16 / 08-23	3081	274	41	315
MZ 53	08-23 / 08-30	3104	249	101	350
MZ 54	08-30 / 09-06	3137	2529	12	2541
MZ 55	09-06 / 09-11	2157	126	35	161

MZ 59	09-11 / 09-18	2892	566	39	605
MZ 60	09-18 / 09-25	3033	176	100	276
MZ 61	09-25 / 10-02	3038	356	91	447
MZ 62	10-02 / 10-09	3008	408	126	534
MZ 76	12-07 / 12-14	3058	856	229	1085

Filter ID	**Sampling period (2007)**	**Sampled air volume [m^3]**	**Coarse filters [m^{-3}]**	**Fine filters [m^{-3}]**	**Air masses [m^{-3}]**
MZ 108	05-10 / 05-17	3075	268	163	431
MZ 127	08-02 / 08-09	3140	42	40	82
MZ 128	08-09 / 08-16	3115	195	128	323
MZ 129	08-16 / 08-23	3088	130	62	192
MZ 130	08-23 / 08-30	3033	537	56	593
MZ 131	08-30 / 09-06	3100	11	7	18
MZ 134	09-06 / 09-13	3092	42	15	57
MZ 135	09-13 / 09-20	3093	1513	63	1576
MZ 136	09-20 / 09-27	3123	2186	119	2305
MZ 137	09-27 / 10-04	3097	130	91	221
MZ 138	10-04 / 10-11	3056	391	211	602
MZ 153	12-06 / 12-13	3102	413	191	604

Filter ID	**Sampling period (2008)**	**Sampled air volume [m^3]**	**Coarse filters [m^{-3}]**	**Fine filters [m^{-3}]**	**Air masses [m^{-3}]**
MZ 181	04-24 / 05-01	2921	99	84	183
MZ 200	07-31 / 08-07	3027	126	76	202
MZ 203	08-07 / 08-14	2960	204	350	554
MZ 204	08-14 / 08-21	3044	150	93	243
MZ 205	08-21 / 08-28	2920	112	191	303
MZ 206	08-28 / 09-04	3055	93	11	104
MZ 209	09-04 / 09-11	3018	572	46	618

MZ 210	09-11 / 09-18	1659	3841	2678	6519
MZ 211	09-18 / 09-25	3025	4372	1476	5848
MZ 212	09-25 / 10-02	2980	952	1337	2289
MZ 215	10-02 / 10-09	2994	3727	386	4113
MZ 229	12-11 / 12-18	3074	582	314	896

Filter ID	Sampling period (2009)	Sampled air volume [m^3]	Coarse filters [m^{-3}]	Fine filters [m^{-3}]	Air masses [m^{-3}]
MZ 261	05-19 / 05-26	2664	92	150	242
MZ 276	08-04 / 08-11	2708	461	221	682
MZ 279	08-11 / 08-18	2729	327	166	493
MZ 280	08-18 / 08-25	2634	1397	181	1578
MZ 281	08-25 / 09-01	2712	363	211	574
MZ 284	09-01 / 09-08	2603	334	258	592
MZ 285	09-08 / 09-15	2583	54	220	274
MZ 286	09-15 / 09-22	2678	25	303	328
MZ 287	09-22 / 09-29	2721	61	63	124
MZ 288	09-29 / 10-06	2468	87	331	418
MZ 289	10-06 / 10-13	2630	668	278	946
MZ 292	10-13 / 10-20	2490	567	58	625
MZ 293	10-20 / 10-27	2560	401	383	784
MZ 297	11-03 / 11-10	2554	180	20	200

Filter ID	Sampling period (2010)	Sampled air volume [m^3]	Coarse filters [m^{-3}]	Fine filters [m^{-3}]	Air masses [m^{-3}]
MZ 335	05-04 / 05-11	2981	16	1	17
MZ 352	08-03 / 08-10	3080	48	1	49
MZ 355	08-10 / 08-17	2988	15	8	23
MZ 356	08-17 / 08-24	2958	45	1	46
MZ 357	08-24 / 08-31	2923	6	18	24

MZ 360	08-31 / 09-07	2921	32	3	35
MZ 361	09-07 / 09-14	3039	92	10	102
MZ 362	09-14 / 09-21	2992	293	63	356
MZ 363	09-21 / 09-28	2883	71	3	74
MZ 364	09-28 / 10-05	3240	431	54	485
MZ 367	10-05 / 10-12	3078	90	68	158
MZ 368	10-12 / 10-19	2930	30	38	68
MZ 369	10-19 / 10-26	3000	23	19	42
MZ 370	10-26 / 11-02	2770	27	56	83
MZ 373	11-02 / 11-09	2947	21	27	48
MZ 374	11-09 / 11-16	2970	28	61	89
MZ 376	11-23/ 11-30	3083	5	22	27

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Apendix C2:

Diversity, abundance, and seasonal dynamics of plant-pathogen Oomycetes in continental air

Lang-Yona, N., Pickersgill, D.A., Maurus, I., Teschner, D., Wehking, J., Thines, E., Pöschl, U., Després, V.R., & Fröhlich-Nowoisky J.

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Species richness, rRNA gene abundance, and seasonal dynamics of airborne plant-pathogenic Oomycetes

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Author contribution statement

J. F.-N., N.L.-Y., and D.A.P. wrote the paper. J.F.-N. U.P., V.R.D., N.L.-Y., D.A.P., D.T., and J.W. designed the research. J.F.-N. collected air samples and performed DNA extractions, cloning, and Sanger sequencing analysis. N.L.-Y. and I.M. performed qPCR analyses. D.A.P, D.T. and J.W. performed statistical correlations and analysis with meteorological data. J.F.-N, U.P., V.R.D., N.L.-Y, D.A.P., I.M., D.T., and J.W. discussed the results. All co-authors read and contributed to the manuscript.

Keywords

Airborne Oomycetes, Peronosporomycetes, plant pathogens, seasonal distribution, Sanger sequencing, qPCR analysis, Meteorological parameter

Abstract

Word count: 234

Oomycetes, also named Peronosporomycetes, are one of the most important and widespread groups of plant pathogens, leading to significant losses in the global agricultural productivity. They have been studied extensively in ground water, soil, and host plants, but their atmospheric transport vector is not well characterized. In this study, the occurrence of airborne Oomycetes was investigated by Sanger sequencing and quantitative PCR of coarse and fine aerosol particle samples (57 filter pairs) collected over a one-year period and full seasonal cycle in Mainz, Germany. In coarse particulate matter, we found 55 different hypothetical species (OTUs), of which 54 were plant pathogens and 29 belonged to the genus Peronospora (downy mildews). In fine particulate matter (< 3 µm), only one species of Hyaloperonospora was found in one sample. Principal coordinate analysis of the species composition revealed three community clusters with a dependence on ambient temperature. The abundance of Oomycetes rRNA genes was low in winter and enhanced during spring, summer, and fall, with a dominance of Phytophthora, reaching a maximum concentration of ~1.6 X 10^6 rRNA genes per cubic meter of sampled air in summer. The presence and high concentration of rRNA genes in air suggests that atmospheric transport, which can lead to secondary infection, may be more important than currently estimated. Thus, further investigations combining DNA sequencing and quantification of airborne Oomycetes may be useful for improved forecasting and management of related plant diseases.

Ethics statements

(Authors are required to state the ethical considerations of their study in the manuscript, including for cases where the study was exempt from ethical approval procedures)

Does the study presented in the manuscript involve human or animal subjects: No

Species richness, rRNA gene abundance, and seasonal dynamics of airborne plant-pathogenic Oomycetes

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Abstract

Oomycetes, also named Peronosporomycetes, are one of the most important and widespread groups of plant pathogens, leading to significant losses in the global agricultural productivity. They have been studied extensively in ground water, soil, and host plants, but their atmospheric transport vector is not well characterized. In this study, the occurrence of airborne Oomycetes was investigated by Sanger sequencing and quantitative PCR of coarse and fine aerosol particle samples (57 filter pairs) collected over a one-year period and full seasonal cycle in Mainz, Germany. In coarse particulate matter, we found 55 different hypothetical species (OTUs), of which 54 were plant pathogens and 29 belonged to the genus *Peronospora* (downy mildews). In fine particulate matter (< 3 µm), only one species of *Hyaloperonospora* was found in one sample. Principal coordinate analysis of the species composition revealed three community clusters with a dependence on ambient temperature. The abundance of Oomycetes rRNA genes was low in winter and enhanced during spring, summer, and fall, with a dominance of *Phytophthora*, reaching a maximum concentration of ~1.6×10^6 rRNA genes per cubic meter of sampled air in summer. The presence and high concentration of rRNA genes in air suggests that atmospheric transport, which can lead to secondary infection, may be more important than currently estimated. Thus, further investigations combining DNA sequencing and quantification of airborne Oomycetes may be useful for improved forecasting and management of related plant diseases.

1 Introduction

The dispersal of pathogenic microorganisms through the atmosphere has major implications for agriculture and public health. Some pathogens can travel over long distances, spreading diseases across and even between continents (Brown and Hovmoller, 2002;Burrows et al., 2009a;Burrows et al., 2009b;Womack et al., 2010;Després et al., 2012;Fisher et al., 2012;Fröhlich-Nowoisky et al., 2016).

Oomycetes are one of the most economically important and widespread group of plant pathogens. They are a diverse group of 'fungus-like` eukaryotic organisms distributed globally in diverse environments and spreading through water, seeds, soil, and air (Göker et al., 2007;Dick, 2013;Beakes et al., 2015). Historically, Oomycetes were classified as fungi due to their similarities in hyphal organization and in nutrition (osmotrophy). However, since their molecular analysis in recent years, they have been redefined as stramenopiles (or heterokonts), to which brown algae also belong (Baldauf et al., 2000;Latijnhouwers et al., 2003;Andersen, 2004;Spring and Thines, 2004;Garcia-Blazquez et al., 2008;Riisberg et al., 2009;Dick, 2013;Beakes et al., 2015).

Oomycetes mainly spread as zoospores, wall-less free-swimming cells, which are released from sporangia during wet conditions (such as water splash, ground or underground water) and at temperatures below 12°C (Walker and van West, 2007;Dick, 2013). They can generate survival structures (formed for over-wintering, hot summer temperatures, or drought survival), i.e., thick-walled chlamydospores (asexual) and oospores (sexual) that are temperature-resistant (Dick, 1995;Fay and Fry, 1997;Vercesi et al., 1999;Fry and Grünwald, 2010;Crone et al., 2013). Oospores and sporangia can be dispersed through the air or attached to soil and plant particles (Kakde et al., 2001;Docampo et al., 2011;Mallo et al., 2011;Delmas et al., 2014;Manzano et al., 2015). Moreover, the spores are known to stay viable in soil up to 10 years (Judelson, 2008;Spencer-Phillips and Jeger, 2012). Under optimal growth conditions, these spores can germinate and infect host plants.

More than 60% of known Oomycetes species are plant pathogens, such as species from the families Albuginaceae (white blister rusts), Peronosporaceae (downy mildews), and Pythiaceae (Aylor et al., 1982;Göker et al., 2007;Walker and van West, 2007;Garcia-Blazquez et al., 2008;Voglmayr, 2008;Thines and Kamoun, 2010;Beakes et al., 2015). An understanding of their diversity, dynamics, and spreading behavior in the atmosphere on local and larger scales is important to improve infection risk prediction and disease management strategies (West et al., 2008). Improving monitoring and forecast of infection risk would also be advantageous for both economic and environmental reasons: curative treatment of already infected plants usually is more expensive and more stressful for the environment than treatments which are applied before the disease actually infects the plants (Scholthof, 2006;Bebber and Gurr, 2015). However, for an improved disease forecasting and management a more precise knowledge of the time of arrival, diversity, and abundance of infectious spores is necessary. Moreover, understanding the dynamics of plant pathogens and influences thereupon is important in food security and climate change (Pautasso et al., 2012).

Because of the parasitic nature of many Oomycetes species they are often not detected with standard culture-based methods (Arcate et al., 2006;Spring and Thines, 2010). Therefore, spore traps and microscopy combined with meteorological data are used to forecast sporulation and infection risk (West et al., 2008;Delmas et al., 2014). The development and application of DNA-based detection and quantification methods for airborne Oomycetes could provide a faster monitoring and more accurate disease forecast (Lévesque, 2011;Judelson, 2012). Furthermore, DNA-based methods enable the detection of target organisms or genetic changes in pathogen populations by choice of primers,

which dependent on specificity allow the detection of all, some, or selected organisms in a sample (West et al., 2008).

Here, we combined DNA Sanger sequencing of the internal transcribed spacer (ITS) region with qPCR analysis of ribosomal RNA genes to investigate the species richness, rRNA gene abundance, and seasonal dynamics of airborne Oomycetes as well as their relationships with meteorological factors in continental air over a one-year period.

2 Material and Methods

2.1 Aerosol sampling

Aerosol samples (57 pairs of fine and coarse particle samples) were collected on glass fiber filters (Pall Corporation, Dreieich, Germany, Type A/E, 102 diameter) over one year in Mainz, Germany (March 2006 - April 2007) as described previously (Fröhlich-Nowoisky et al., 2009) and detailed in Table S1.

Briefly, a self-built high-volume-dichotomous sampler (Solomon et al. 1983), was operated with a rotary vane pump (Becker, Wuppertal, Germany, Type VT 4.25) at a total flow rate of ~0.3 $m^3 min^{-1}$, corresponding to a nominal cut-off diameter of ~3 µm. Thus, coarse particles with an aerodynamic diameter larger than ~3 µm were collected on one glass fiber filter (~0.03 $m^3 min^{-1}$), while the fine particles from the same air sample were collected on a second glass fiber filter (~0.27 $m^3 min^{-1}$). The sampling period was generally ~7 days, corresponding to a sampled air volume of 3000 m^3. A few samples were collected over shorter periods (volumes of ~400-2000 m^3). The sampling station was positioned on a mast about 5 m above the flat roof of the three-story high Max Planck Institute for Chemistry building located in the campus of the University of Mainz (49°59’31.36’’N, 8°14’15.22’’E). The sampled air masses represent a mix of urban and rural continental boundary layer air in central Europe. To ensure that all filters were DNA free prior to sampling, all glass fiber filters were baked overnight at 500°C prior to sampling and the loaded filters were packed in aluminum foil (baked at 500°C) and stored at -80°C until DNA extraction.

To detect possible contaminants from the sample handling or the sampler, blank samples were taken at 4-week intervals as previously described (Fröhlich-Nowoisky et al., 2009). Prebaked filters were mounted in the sampler in a similar manner for regular sampling, but the pump was either not activated (“mounting blanks”) or activated only for 5 s (“start-up blank”), respectively.

2.2 DNA extraction and amplification

Filter aliquots (about ⅛ – ¼ of the filter) were extracted with a soil DNA extraction kit (LysingMatrixE, FastDNASpin Kit for Soil, MP Biomedicals, Eschwege, Germany) according to the supplier’s instructions with the following modifications: After lysis the mixtures were centrifuged for 10 - 15 min, followed by an addition of 900 µL buffer (kit-supplied) and a second repeat of bead-beating and centrifugation step. Both supernatants were combined for the further extraction process. Finally, the DNA was dissolved in 100 µL elution buffer. Extraction kit blanks containing no filter and baked filter blanks were included as extraction blanks.

For each DNA extract up to two PCR reactions were performed with the primer pair ITS4Oo/ITS5 and nested primer pairs ITS4Oo/ITS1 or ITS4/ITS5 (White T J, 1990;Nikolcheva and Bärlocher, 2004). The 50 µL reaction mixture contained 1 - 2 µL template DNA, 0.33 µM of each primer (Sigma-Aldrich, Munich, Germany), 1× JumpStart™ PCR buffer (Sigma-Aldrich), 0.2 mM of each

dNTP (Sigma-Aldrich) and 2.5 units of JumpStart^{TM} REDTaq DNA polymerase (Sigma-Aldrich). A negative control containing no template DNA was included in all PCR runs.

The thermal cycling conditions (DNA Engine, Bio-Rad Laboratories, Munich, Germany) consisted of an initial 3 min denaturation at 94°C, followed by 35 cycles of 30 s denaturation at 94°C, and 60 s annealing at 49°C (for ITS4Oo/ITS5 and ITS4Oo/ITS1) or 30 s at 54°C (ITS4/ITS5), proceeded with 90 or 45 s, respectively of elongation at 72°C and 3 min of final extension at 72°C.

While PCR products were obtained for all coarse particle filter extracts, PCR amplicons were only detected on six fine particle filter extracts. No DNA could be amplified from any of the six mounting, six start-up, 12 extractions, and 36 PCR-blanks, indicating that no contamination occurred during sample handling and analysis in the laboratory.

Amplification products for sequencing were cloned using the TOPO TA Cloning® Kit (Thermo Fisher Scientific, Darmstadt, Germany) following the supplier's instructions. Colonies containing inserts were identified by blue-white selection and lysed in 20 µL H_2O for 10 min at 95°C. The inserts of 12-24 randomly picked colonies of each cloning reaction were amplified using 3 µL cell lysate in a 40 µL reaction. The PCR reaction mixture contained 1× PCR buffer (New England BioLabs, Frankfurt, Germany), 0.25 mM of each dNTP (New England BioLabs), 0.25 µM of each primer (Sigma-Aldrich) and 1.25 units of Taq DNA Polymerase (New England BioLabs). The PCR reactions were performed with the primer pair M13F-40 and M13R, and the thermal cycling conditions consisted of an initial 5 min denaturation at 94°C, followed by 40 cycles of 30 s denaturation at 94°C, 60 s annealing at 55°C, 60 s elongation at 72°C, and 15 min of final extension at 72°C. Up to 12 colony PCR products per original PCR product were sequenced.

The DNA sequences were determined with ABI Prism 377, 3100, and 3730 sequencers (Thermo Fisher Scientific) using BigDye-terminator v3.1 chemistry the Max Planck-Genome-centre Cologne, Germany (http://mpgc.mpipz.mpg.de/home/). The quality of all sequences was manually checked and the vector sequences were cut. Out of 499 sequenced clones 30 sequencing reactions failed.

For comparison with known sequences, database queries using the Basic Local Alignment Search Tool (BLAST) were performed via National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/). Each of the remaining 469 sequences was identified to the lowest taxonomic rank common to the top BLAST hits. Sixty sequences produced non-Oomycetes results and 6 sequences were assumed to be chimeric results of PCR recombination of the ITS1 and ITS2 regions and were excluded from further analysis. The Oomycetes DNA sequences were grouped into 55 OTUs (similarity scores ≥ 97%; Table S2). Fifty-two OTUs, obtained by direct PCR amplification, were used for the species richness analysis, whereas three (OTU 31, 32 and 55, Table S2) were excluded, as they were obtained by co-amplification of the 16S region (in an Acidiobacteria PCR with the primer pair Acid31/Eub518; Fierer et al. (2005)). For each filter, sequences that produced the same BLAST results were pairwise aligned using the BioEdit program (BioEdit Sequence Alignment Editor 7.2.5, http://www.mbio.ncsu.edu/bioedit/bioedit.html). Sequences with similarity scores ≥ 97% were clustered into an operational taxonomic unit (OTU) and can be seen as hypothetical species as the inner-species variability is lower than 3% (Robideau et al., 2011), with a mean intra-species variability of 0.5% and a mean inter species variability of 30%. The sequences from the obtained OTUs of the present study have been deposited in the GenBank database with the accession numbers MF095126 - MF095180, detailed in Table S3.

2.3 Quantitative PCR

The rRNA genes of four selected Oomycete taxa and total Oomycetes were quantified from coarse particle filter extracts using the CFX96 quantitative PCR (qPCR) instrument (Bio-Rad Laboratories). Selection was based on taxa identified by Sanger-sequencing. We used the SYBR green method for *Pythium*, Albuginaceae, and *Peronospora* and for the total Oomycetes (Lang-Yona et al., 2012;Müller-Germann et al., 2015), and the TaqMan probe method for the *Phytophthora* (Kox et al., 2007) as specified in Table 1. All qPCR reactions were performed in triplicates of 10 µL mixtures. The SYBR green reactions contained 5 µL SsoAdvanced universal SYBR green supermix (Bio-Rad Laboratories), 1 µL extracted DNA, 500 nM of each primer (500 nM, Sigma-Aldrich), and 3 µL sterile, filtered water (Sigma-Aldrich). TaqMan probe reactions contained 5 µL SsoAdvanced universal probes supermix (Bio-Rad Laboratories), 1 µL extracted DNA, 500 nM of each primer (500 nM, Sigma-Aldrich) 200 nM probe (200 nM, Eurofins Genomics, Ebersberg, Germany), and 2.6 µL sterile, filtered water (Sigma-Aldrich).

The thermal cycling conditions consisted of an initial 30 s or 2 min denaturation for SYBR green or TaqMan method respectively, enzyme activation at 98°C, followed by 40 cycles of 10 s denaturation at 98°C, and 25 s annealing for both reaction types, and extension at primer pair specific temperatures as detailed in Table 1. The examination of the melt peaks confirmed amplification of the single desired product.

The qPCR results are reported in gene copy number (GCN) per cubic meter of air. Oomycete genes were not detected in mounting, start-up, extraction, and qPCR blanks, verifying that no contamination occurred during sample handling and analysis.

2.4 gBlock DNA fragments and qPCR calibration

Calibration curves were derived using two self-designed gBlock DNA fragments (IDT, Iowa, USA). Fragment ‘gBlock A’ contains two binding sites and ‘gBlock B’ contains three binding sites for primer pairs detailed in Table 1 and illustrated Figure S1. Cross amplification of primer pairs was excluded using NCBI BLAST against Nucleotide collection (nr/nt) database. The designed amplicon size is based on the theoretical amplicon sizes of the selected taxa (Table 1).

Amplification efficiencies, tested on the gBlock fragments were higher than 90% in all qPCR assays, calculated from standard curves of 10-fold dilutions (10^9-10^1 rRNA gene copies). Limits of quantification, as calculated from the standard curves were 2.5 ± 0.6, 1.8 ± 1.1, 1.4 ± 0.6, 1.3 ± 0.5, and 1.0 ± 0.1 gene copies for total Oomycetes, Albuginaceae, *Phytophthora*, *Peronospora*, and *Pythium*, respectively (Forootan et al., 2017).

2.5 Meteorological data

Local meteorological data (temperature, relative humidity (RH), atmospheric pressure, wind speed, and precipitation) provided by the ZIMEN Luftmessnetz, Rheinland-Pfalz, Station Mainz-Mombach were provided in half hour values. The values were averaged for each sample period and are detailed in Table S1.

2.6 Statistical analysis

The Pearson correlation coefficient was calculated between the quantitative PCR values and meteorological factors, using OriginPro 9, to assess if there are potential significant linear correlations (p-value < 0.05).

Bray-Curtis (BC) dissimilarity in OTU composition was calculated for all aerosol filter pairs.

$$BC_{ij} = 1 - \frac{2C_{ij}}{S_i + S_j} \quad (1)$$

Here C_{ij} is the number of OTUs per filter pair, *i* and *j* have in common and *S* is the number of total OTUs (species richness) found on a filter pair *i* or *j*. A principle coordinate analysis (PCoA) was then performed on the resulting BC distances. Diversity clusters were identified using EM algorithm, using the R package mclust (vers.: 5.3) (Fraley and Raftery, 2002;Fraley et al., 2012).

3 Results and Discussion

The species richness and rRNA gene abundance of airborne Oomycetes were investigated over a one-year period and full seasonal cycle in Mainz, Germany, and correlated with meteorological factors to gain a better understanding of their seasonal dynamics in the atmosphere.

3.1 Species richness of airborne Oomycetes

Oomycetes were identified by PCR amplicon Sanger sequencing on all 57 coarse particle filters, but only on one fine particle filter (Table S2). This is consistent with previous observations of an enrichment of plant pathogenic fungi in the coarse particle fraction (Fröhlich-Nowoisky et al., 2009). A larger aerodynamic diameter is beneficial for a plant pathogen as a higher inertia will aid the impaction on a plants surface, thereby heightening the infection probability (Herries, 1961). Moreover, plant pathogens associated with aerosolized plant tissue fragments or soil particles will be concentrated in the coarse particle fraction (Després et al. (2012), and reference therein).

The 55 identified operational taxonomic units (OTUs) comprise up to 3.6-6.1% of the estimated 900-1500 existing Oomycetes species (Arcate et al., 2006;Walker and van West, 2007;Dotzler et al., 2008;Voglmayr, 2008;Sandle, 2014). Of all identified OTUs, 54 were identified as pathogens (Table S2). No information on the pathogenicity was found for the single OTU identified as *Pythium apiculatum.*

The detected OTUs were distributed over three families (Figure 1A). About 90% of the OTUs detected in coarse particle filters belonged to widespread obligate pathogenic family of Peronosporaceae to which all species of downy mildew belong. Albuginaceae (*Albugo* and *Wilsoniana*), to which many of white rusts belong, and Pythiaceae (*Pythium*), containing species known to cause root rot or damping off, represent 8% and 2% of the total identified OTUs, respectively. The Peronosporaceae OTUs were assigned to three (out of eight to seventeen) reported wind-dispersed genera (Riethmuller et al., 2002;Göker et al., 2004;Göker et al., 2007;Spencer-Phillips and Jeger, 2012) i.e., *Peronospora* (57%), *Hyaloperonospora* (29%), and *Pseudoperonospora* (4%). For just one OTU, discernment between *Peronospora* and *Hyaloperonospora* was not possible. From the three families described above, 24% of the OTUs were identified down to the species level (Table S2). The most abundant OTU, *Peronospora*

conglomerata, a pathogen for geraniums (Farr and Rossman, 2017), was found on 30% of the coarse particle filters of all seasons.

Although the genus *Phytophthora* was expected in the airborne fraction (Fall et al., 2015;Manzano et al., 2015), it was not detected by Sanger sequencing using the ITS primer pairs. However, it was the most abundant genus amongst the taxa quantified with qPCR. This is most probably due to a PCR selectivity of the general Oomycetes primers (albeit that the primers were previously reported to amplify *Phytophthora* spp.; Nikolcheva and Bärlocher (2004)). Further evidence for selectivity was a successful co-amplification of sequences that best matched *P. ramorum* (sequence similarity of 97%) using primers aiming at 16S rRNA of prokaryotes. This pathogen has a wide host range of 75 plant genera (e.g., oak, larch, rhododendron). *Phytophthora ramorum* was found in samples that were mostly taken in periods of higher humidity and rain (apart from MZ 15) during fall (Table S1). This corresponds to the spreading mechanism of *P. ramorum*, by airborne spores carried by wind-blown rain (Grunwald et al., 2008). The co-amplification in turn implies significant concentrations of airborne *P. ramorum* as it was the single oomycete identified amongst the abundant atmospheric bacterial population.

Seasonal dynamics of OTU composition of all analyzed samples is shown in Figure 1B. The BC dissimilarity index between two samples can vary between 1, indicating a completely different OTU composition, and 0, indicating an identical composition. Samples with half year differences, in general, show higher dissimilarities (values between 0.8 to 1) than samples in close temporal vicinity (along the diagonal) which show higher consistencies in OTU composition. This is not surprising in the seasonally changing climate of northern Europe as the plant pathogenic Oomycetes community structure will change with the annual vegetation cycle and will also change due to favorable meteorological conditions.

Samples taken at the late fall, winter, and spring show comparatively similar compositions. The summer samples, however, show a high sample-to-sample consistency (~0.5 and lower), and a nearly completely different OTU composition than samples from other seasons, apart from a transitional phase in the first half of the fall season. This again can be explained by the vegetative cycle of host plants. Different species of oomycetes are adapted to infect the different annual developmental stages of the hosts, such as leaf- or fruit-development (Latijnhouwers et al., 2003;Thines and Kamoun, 2010). The high consistency between winter 2007 and the two spring seasons could be due to the aerosolization of soil particles during winter which contain soil dwelling oomycetes species.

Principle coordinate analysis (PCoA) of the BC revealed three OTU composition clusters (Figure 2 A). However, the community clusters don't seem to be primarily defined by a distinct seasonality (Figure 1B), but rather through the average ambient temperature during sampling (distinguished by point color) as each cluster contains samples from at least three seasons (distinguished by point shapes). Other meteorological parameter, such as mean relative humidity, wind speed, sum and duration of precipitation, did not show correlations with OTU clusters. Therefore, the clusters were named "warm", "intermediate", and "cold". The mean temperatures for the different clusters are 9, 14 and 20°C (Figure 2B). Furthermore, the temperature distributions of the three clusters, analyzed by the Wilcoxon Rank test, were significantly different from another (p-value < 10^{-16}). The cold cluster had the lowest number of OTUs (22), which all fell in the two genera *Hyaloperonospora* and *Peronospora* (Figure 2C). The intermediate and warm clusters display higher species richness consisting of 35 and 28 OTUs, respectively. Taxonomically, both clusters are similar, with slight differences. The intermediate cluster contains the non-classified Peronosporaceae, while the warm cluster contains the only non-pathogenic OTU from the genus *Phytium*. A similar temperature

dependency has been previously shown to influence the distribution and seasonal abundancies of various *Halophytophthora* species in river water (Nakagiri, 2000). The changing atmospheric community structure can be explained by two possible scenarios: A rapid response of the phyllospheric Oomycetes community composition, shifting the abundance towards other species, with changing temperatures, or an influence of temperature on the emission or sporulation process, of different species. Sporangia are very sensitive to mild changes in temperature (Byrt and Grant, 1979;Suzaki et al., 1996), and zoospore formation has been shown to be induced by a drop in ambient temperature (Hardham and Hyde, 1997).

3.2 Ribosomal RNA gene abundance of airborne Oomycetes

To evaluate the abundance and seasonal variation of airborne Oomycetes in coarse particulate matter, rRNA genes of selected taxa and total Oomycetes were quantified using qPCR with the primer pairs detailed in Table 1. The concentration of rRNA genes for total Oomycetes ranges between ~1.4×10^4 up to ~5.1×10^6 GCN m^{-3} (Figure 3A and Table S4). Highest concentrations of rRNA genes were observed for *Phytophthora* (ranging between 4.3×10^3 to ~1.8×10^6 GCN m^{-3}), while Albuginaceae and *Peronospora* had lower values (ranging between 0 to ~3.3×10^4, and 0 to ~1.3×10^4 GCN m^{-3}, respectively). *Pythium* values were lowest (with maximum of ~33 GCN m^{-3}; MZ 11).

The seasonal averages of rRNA gene abundance are shown in Figure 3B. Total Oomycetes, *Phytophthora*, and *Peronospora* did not differ significantly during spring, summer, and fall, with highest levels observed during summer (~$1.6 \times 10^6 \pm 7.0 \times 10^5$, ~$4.9 \times 10^5 \pm 2.4 \times 10^5$, and ~$1.7 \times 10^3 \pm 9.6 \times 10^2$ GCN m^{-3}, respectively). This corresponds to the seasons with the highest host plant availability in Europe, and conditions most beneficial for growth (Ellenberg, 2009). The Albuginaceae exhibited highest values in fall (~$8.1 \times 10^3 \pm 5.0 \times 10^3$ GCN m^{-3}), while no significant seasonal variation and low rRNA gene abundances were observed for *Pythium*. All other taxa had lowest concentrations in winter, with ~$1.7 \times 10^5 \pm 6.2 \times 10^4$, ~ $6.0 \times 10^2 \pm 4.5 \times 10^2$, ~$7.3 \times 10^4 \pm 3.5 \times 10^4$, and ~$5.8 \pm 2.9$ GCN m^{-3} for total Oomycetes, Albuginaceae, *Phytophthora*, and *Peronospora*, respectively. Winter in central Europe is characterized by low vegetative yield (Ellenberg, 2009). Thus, a lower abundance of airborne plant pathogens can be expected. This finding is in concordance with previous studies of fungal spore abundance, reporting lower spore counts for lower outdoor temperatures (Tang (2009), and references therein).

Disease warning systems for plant pathogenic Oomycetes are based on temperature and leaf wetness, under the assumption that certain leaf moistures and temperatures will allow efficient infection of plants (Abraham et al., 1995;Madden et al., 2000;Gilles, 2004;Henderson et al., 2007;Reis, 2013). Our results show a medium to high positive correlation of total Oomycetes rRNA GCN m^{-3} with RH only during fall (See Table S5). No significant correlations were observed with temperature and precipitation. The inconsistency between the correlations found in our study and the disease forecasting system might be due to different factors, such as the 7-day sampling periods used in this study. Furthermore, while plant infection risk is correlated with RH and temperature (Palmieri et al., 2006;Li et al., 2014;Morales et al., 2018), atmospheric gene abundance of Oomycetes may not. The positive correlation with RH in fall could indicate preferential aerial transport under humid conditions, which is in agreement with previously reviewed dispersal of fungal and Oomycetes pathogens in tropical areas (Drenth and Guest, 2016).

As discussed above, temperatures might relate to preferential distribution of Oomycetes in a non-linear manner, i.e., specific temperatures trigger release of certain species (Jones, 2014). The differential influence of meteorological factors on oospore release is outlined in Fry et al. (2008).

Rain intensity and precipitation type (i.e., rain, drizzle, fog, etc.) could also affect the distribution of aerosolized Oomycetes. For example, prolonged rain will lead to wet deposition and therefore a washing of the atmosphere (Hemond and Fechner, 2015). However, short intensive rain may induce highly efficient release of oospores and Oomycetes fragments through a splash effect (Huffman et al., 2013), whereas a light drizzle could only have a minimal effect on aerosolization or deposition. Therefore, to estimate correlation with rain, further characterization of the precipitation type is required. Zoospores are free swimming in water films and can settle on surfaces and retract their flagella (Hardham and Hyde, 1997;Walker and van West, 2007). A subsequent secretion of a mucilaginous matrix affixes them to the surfaces, e.g., soil particles or leaf fragments which can then be aerosolized.

To further visualize common patterns in atmospheric presence, the rRNA gene abundance of the individual taxa was normalized by calculating the ratio to the highest taxon-specific value (Fig. 3C). Common periodical tendencies with similar sample-to-sample dynamics are observed amongst all taxa. This again is most probably an indication of a passive influence affecting the atmospheric presence of all taxa, such as meteorological factors influencing emission and deposition. Additionally, in Figure 3D the gene abundance of the different taxa in individual air samples display a linear correlation with the gene abundance of total Oomycetes. The linear tendency indicates near constant proportions of single taxa within the total airborne Oomycetes, which might be due to dispersal mechanisms (e.g., attached to soil particles or plant fragments (Sutton et al., 2006)), rather than species-related emissions (e.g., wind-dispersed sporangia etc.).

Our results demonstrate the presence of plant-pathogenic Oomycetes over a one-year period and full seasonal cycle in Mainz, Germany. Species composition analysis revealed occurrences of three plant-pathogenic families with seasonal dynamics and three community clusters with a dependence on ambient temperature. Higher concentrations of Oomycete rRNA genes in spring, summer, and fall, imply higher atmospheric transport rates in those seasons. The complementary input of the two methods combined in this study underlines the importance of parallel approaches in microbial ecology, where supportive analyses could help answering complex questions in this field. Further investigations and monitoring of airborne Oomycetes, combining high throughput DNA sequencing with quantitative approaches may be useful for improved forecasting and disease management.

4 Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

5 Author Contributions

J. F.-N., N.L.-Y., and D.A.P. wrote the paper. J.F.-N. U.P., V.R.D., N.L.-Y., D.A.P., D.T., and J.W. designed the research. J.F.-N. collected air samples and performed DNA extractions, cloning, and Sanger sequencing analysis. N.L.-Y. and I.M. performed qPCR analyses. D.A.P, D.T. and J.W. performed statistical correlations and analysis with meteorological data. J.F.-N, U.P., V.R.D., N.L.-Y, D.A.P., I.M., D.T., and J.W. discussed the results. All co-authors read and contributed to the manuscript.

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9 Tables

Table 1. Primers used for qPCR. The amplified region, primer names, sequences, length, and annealing temperatures of the different qPCR primer sets for analysis of the total Oomycetes, Albuginaceae, *Phytophthora*, *Pythium*, and *Peronospora***.**

Target	Amplified region	Primer name	Sequence	Amplicon length (bp)	Annealing temperature (°C)	Reference
Oomycetes	18S rRNA	AFP293-F AFP294-R	TTTCCGTAGGTGAACCTGCG GCGAGCCTAGACATCCAC	220-300	65	(Brouwer et al., 2003)
Albuginaceae	5.8S+18S rRNA	Albug-F Albug-R	GCTTCGGCTTGACACATTAG TCCGTCTCCTTGATGACCTT	93	62	(van Molken et al., 2014)
Phytophtora	18S rRNA	15Ph-F 279Ph-R All-Phy-P	TGCGGAAAGGATCATTACCACACC GCGAGCCTAGACATCCACTG FAM-TTGCTATCTAGTTAAAAGCA- TAMRA	248	60	(Kox et al., 2007)
Pythium	18S rRNA	Pyth664-F Pyth712-R	GCCCTTTCGGGTGTGTTACTAG CTGAATGGCAGAAGAACATCCTC	66	60	(Thomas et al., 2011)
Peronospora	5.8S+18S rRNA	Peron-F Peron-R	CACGTGAACCGTATCAACC GATAGGGCTTGCCCAGTAG	98	62	(Hukkanen et al., 2006)

10 Figure captions

Figure 1. Species richness and seasonal dynamics in composition of airborne Oomycetes. (A) Relative proportions of different genera (n.c. = not classified), and (B) heatmap of Bray-Curtis dissimilarity in OTU composition between all analyzed samples. The Bray Curtis index can vary between 0, for an identical OTU composition, and 1, for no common OTUs on the samples.

Figure 2. Temperature dependency of OTU composition. (A) Principle coordinate analysis of Bray-Curtis dissimilarities, revealing three temperature-dependent clusters with high, intermediate, and low average sampling temperatures. Point shape represents the sampling season, and color represents the average temperature for each aerosol filter pair. (B) Temperature distributions of the clusters (middle band: median, box: the 25^{th} to 75^{th} percentile, whiskers: 95% confidence interval. (C) Relative proportions of different genera within the three clusters (n.c. = not classified).

Figure 3. Oomycetes rRNA gene abundance retrieved from qPCR analysis. (A) The abundance (rRNA genes m^{-3} air) of selected taxa and total Oomycetes in coarse particle filter samples. Error bars represent standard deviation of triplicates. (B) Average seasonal rRNA gene abundance for selected taxa and total Oomycetes. Boxes limit 25 and 75% percentile, median presented as line, and mean values as point inside, connected in line. Error bars present 1% and 99% percentile. Outliers are shown (Student´s two sample *t*-test p-value < 0.01). (C) Gene abundance of selected taxa and total Oomycetes scaled to maximal taxon-specific values. (D) Gene abundance of selected taxa scaled to total Oomycetes gene abundance and correlated with gene abundance of the selected taxa. Color codes in all panels: total Oomycetes marked as black squares, Albuginaceae as green inverted triangle, *Phytophthora* as red circles, *Peronospora* as orange triangles, and *Pythium* as blue diamond.

Figure 1.TIF

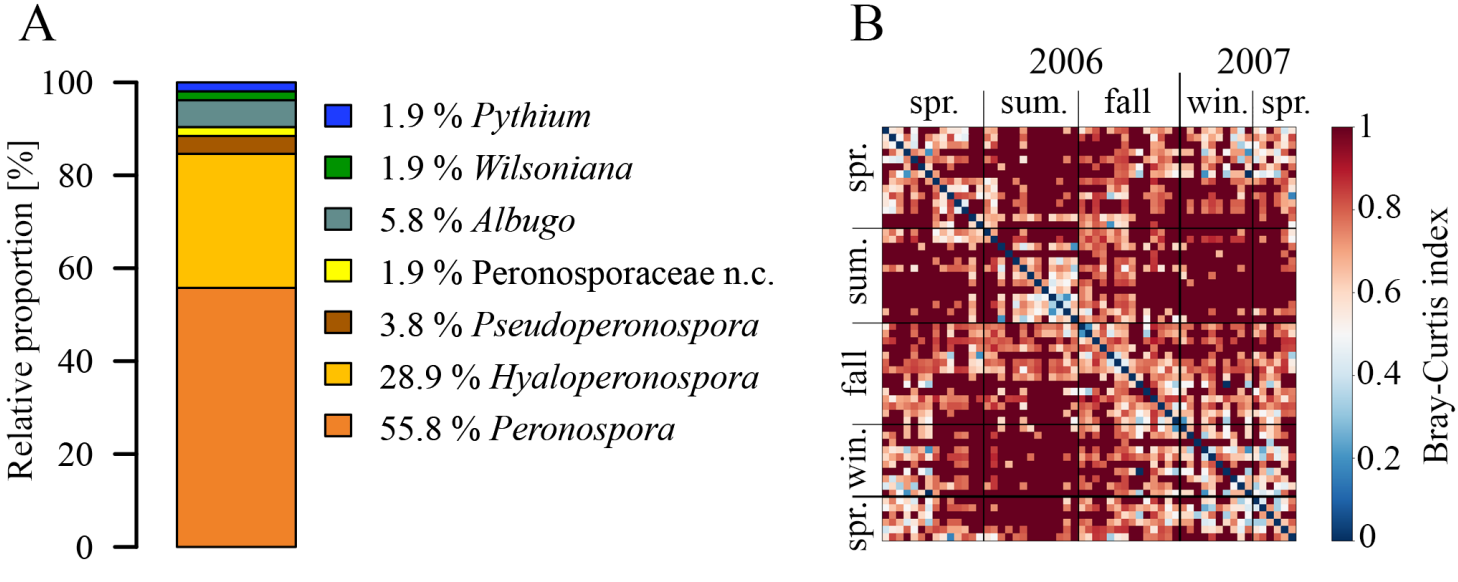


Figure 2.TIF

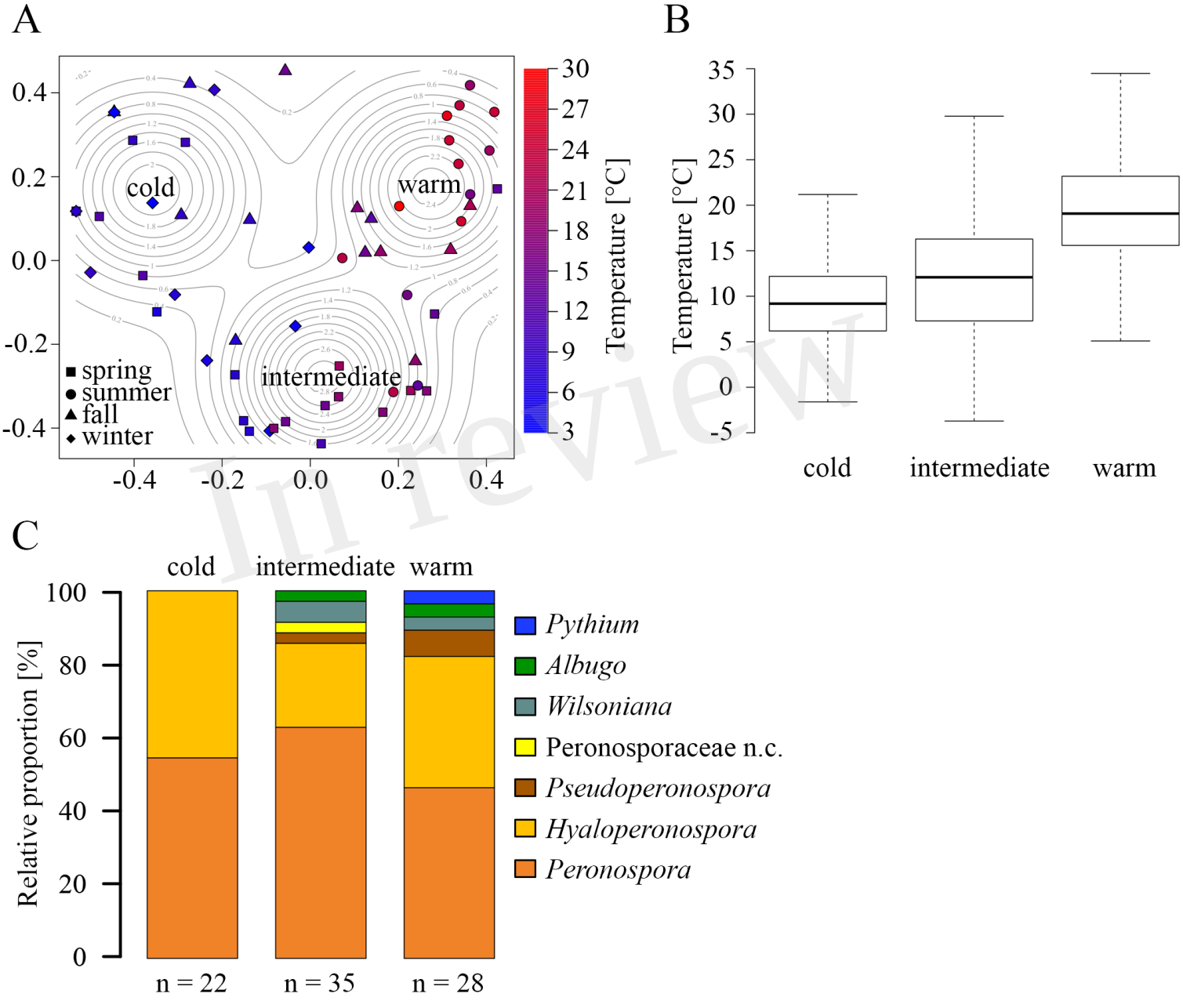
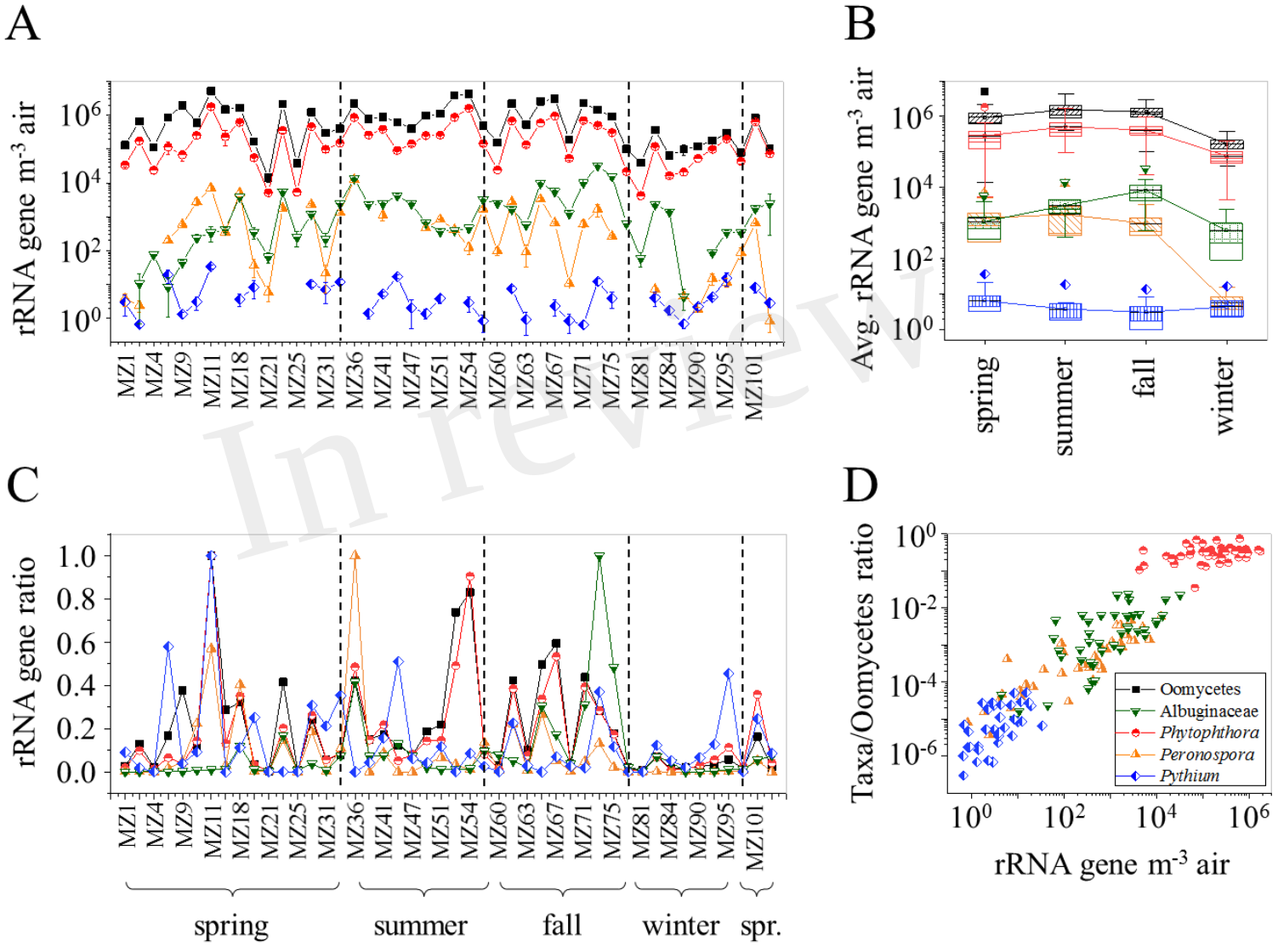


Figure 3.TIF



Supplementary Material

Species richness, gene abundance, and seasonal dynamics of plant-pathogen Oomycetes in continental air

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1 Supplementary Figures and Tables

1.1 Supplementary Figures

A

TTAACGAGTTTTCCCAGTCACGATTTTTGGATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCC

AFP293-F →

AGCTCCAATAGCGTATATTTAAGTTT**TTTCCGTAGGTGAACCTGCG**GAAGGATCATTACCACACCTAAAAAA

ACTTTCCACGTGAACCGTTTCAACCAAATATTTTGGGGGTCTTGTCTGGCGTATGGCTGCTGCTGTAAAAGGCG

GCGGCTGTTGCTGGGTGAGCCCTATCATGGCGAACGTTTGGGCTTCGGTCTGAACAAGTAGCTCTTTTTTAAAC

CATTACTTATTACTGATTATACTGTGGGGACGAAAGTCTCTGCTTTTAACTAGATAGCAACTTTCAGCA**GTGGA**

← AFP294-R

TGTCTAGGCTCGCTTTTTGCCCTTTCGGGTGTGTTACTAG → Pyth664-F

GATGTTTGAGACATTTTTTGTGAGGATGTTCTTC

← Pyth712-R

TGCCATTCAGTTTTTGTCATAGCTGTTTCCTGGCGGCCGC

B

15Ph -F →

TTAACGAGTTTTCCCAGTCACGATTTTTTGCGGAAAGGATCATTACCACACCTAATAATCTTTCCACTCAATCC

TACCAAGCCATTTCATTATGGGACTTGTACCACTATCATGGCGAATGTTTGACTTCGGTCCGGGCGAGTAGCTT

← All-Phy -P

TATTGTTTTAAACCCATTTCACAATTCTGATTATACTGTGGGGACAAAAGTCTCTGCTTTTAACTAGATAGCAA

← 279Ph -R

CTTTCAGCAGTGGATGTCTAGGCTCGCTTTTTCACGTGAACCGTATCAACC → Peron-F

AACATATATTGGGGGTTTGTTTG

← Peron-R

TGCGGTGGCTGCTGTGCATTTTGTGCTGGCTGGCTGCTACTGGGCAAGCCCTATC → Albug-F

TTTTGCTTCGGCTTGACAC

ATTAGCTCATGAATTGTATAGTTTGTTGTTCTTGTCAGAGTATACTTATGATAATCGAAAGGTCATCAAGGAGA

← Albug-R

CGGATTTTTGTCATAGCTGTTTCCTGGCGGCCGC

Supplementary Figure 1. Self-designed gBlock fragments for qPCR calibration. The two fragments contain multiple primer binding sites for amplification of (A) total Oomycetes (shaded bold) and the genus *Pythium* (blue) and (B) for the genera *Phytophthora* (red) and *Peronospora* (orange) as well as for the family Albuginaceae (green).

1.2 Supplementary Tables

Supplementary Table 2. Overview of air samples (aerosol filter pairs) and meteorological parameters. Sample running number, sampling period (date, season), meteorological data (average temperature, relative humidity, atmospheric pressure, wind speed, precipitation), and sampled air volume for the aerosol filter pairs**.**

Sample number	Sampling period			Meteorological data					Sampled air volume
	Start	Stop	Season	Temp. Avg [°C]	RH Avg [%]	Atm. pressure Avg [hPa]	Wind speed Avg [m s^{-1}]	Precipitation Avg [L m^{-2}]	[m^3]
MZ 1	24.03.2006	31.03.2006	Spring	11.19	81.70	1009.87	2.00	26.74	3008.88
MZ 2	31.03.2006	07.04.2006	Spring	8.07	72.10	1015.84	1.97	14.76	3079.44
MZ 4	07.04.2006	12.04.2006	Spring	8.67	55.29	1015.30	2.02	5.29	2217.60
MZ 6	15.04.2006	18.04.2006	Spring	11.95	77.68	1012.93	2.17	6.37	1276.56
MZ 9	20.04.2006	27.04.2006	Spring	14.54	67.04	1017.90	1.39	11.54	3024.00
MZ 10	27.04.2006	02.05.2006	Spring	10.13	69.84	1016.26	1.98	6.33	2109.60
MZ 11	02.05.2006	03.05.2006	Spring	15.52	61.91	1017.49	1.03	0.00	438.12
MZ 15	04.05.2006	09.05.2006	Spring	19.25	45.58	1018.91	2.22	0.00	2102.40
MZ 18	12.05.2006	15.05.2006	Spring	16.81	69.36	1019.25	1.72	7.69	1311.12
MZ 19	15.05.2006	16.05.2006	Spring	19.51	63.30	1016.49	1.31	0.10	430.56
MZ 21	17.05.2006	18.05.2006	Spring	17.75	74.16	1016.44	1.22	1.65	429.12
MZ 24	18.05.2006	22.05.2006	Spring	14.41	73.83	1008.61	2.36	15.31	1742.40
MZ 25	22.05.2006	23.05.2006	Spring	13.21	80.40	1010.62	2.20	5.39	435.96
MZ 26	23.05.2006	30.05.2006	Spring	13.57	75.09	1019.08	2.23	27.93	3109.68
MZ 31	01.06.2006	06.06.2006	Spring	13.15	65.74	1025.79	1.87	3.01	2223.00
MZ 33	08.06.2006	13.06.2006	Spring	21.28	55.67	1025.75	1.45	0.00	2188.80
MZ 35	14.06.2006	21.06.2006	Spring	22.48	65.58	1017.24	2.43	1.82	3011.40
MZ 36	21.06.2006	22.06.2006	Summer	21.91	53.55	1014.24	1.65	0.00	433.44
MZ 40	27.06.2006	04.07.2006	Summer	23.29	59.85	1021.53	1.25	0.00	3061.80
MZ 41	04.07.2006	11.07.2006	Summer	23.97	67.91	1019.30	1.27	13.91	3036.60
MZ 42	11.07.2006	14.07.2006	Summer	29.20	63.21	1022.90	1.38	3.01	1312.20
MZ 43	14.07.2006	17.07.2006	Summer	23.79	45.99	1027.78	1.90	0.00	1324.08
MZ 45	19.07.2006	21.07.2006	Summer	28.64	53.47	1020.21	1.35	0.00	881.28
MZ 47	26.07.2006	02.08.2006	Summer	23.08	71.54	1013.62	1.52	23.00	3036.60
MZ 50	02.08.2006	09.08.2006	Summer	19.28	68.88	1014.38	1.61	22.88	3051.72
MZ 51	09.08.2006	16.08.2006	Summer	15.62	80.16	1010.92	1.21	18.85	3054.24
MZ 52	16.08.2006	23.08.2006	Summer	18.26	76.38	1014.82	1.13	33.84	3104.64
MZ 53	23.08.2006	30.08.2006	Summer	15.57	82.12	1011.54	1.42	34.91	3122.28
MZ 54	30.08.2006	06.09.2006	Summer	19.20	73.00	1021.07	1.08	2.69	1557.36
MZ 59	11.09.2006	18.09.2006	Summer	21.10	71.74	1012.82	1.12	2.36	3034.08
MZ 60	18.09.2006	25.09.2006	Fall	17.97	76.60	1014.94	1.72	0.03	3024.00
MZ 61	25.09.2006	02.10.2006	Fall	16.92	82.31	1014.43	1.73	22.64	3049.20
MZ 62	02.10.2006	09.10.2006	Fall	12.77	85.29	1015.46	1.44	38.7	3081.96
MZ 63	09.10.2006	16.10.2006	Fall	14.39	83.43	1024.34	1.07	2.53	3087.00
MZ 66	16.10.2006	23.10.2006	Fall	13.07	84.21	1009.55	1.80	1.78	3013.92
MZ 67	23.10.2006	30.10.2006	Fall	14.65	81.88	1015.59	1.16	12.91	3039.12
MZ 69	02.11.2006	09.11.2006	Fall	7.79	81.64	1028.56	2.11	3.30	3036.60
MZ 70	09.11.2006	16.11.2006	Fall	8.88	84.09	1019.66	2.50	4.81	3064.32
MZ 71	16.11.2006	23.11.2006	Fall	8.93	84.29	1011.15	3.05	9.87	3071.88
MZ 74	23.11.2006	30.11.2006	Fall	9.16	91.67	1018.99	1.24	3.15	2827.44

MZ 75	30.11.2006	07.12.2006	Fall	8.80	81.66	1016.26	1.24	2.75	3013.92
MZ 77	14.12.2006	21.12.2006	Fall	4.01	80.43	1031.53	2.22	3.64	3021.48
MZ 81	28.12.2006	04.01.2007	Winter	5.36	81.42	1024.74	1.36	16.65	2993.76
MZ 82	04.01.2007	11.01.2007	Winter	9.51	78.31	1017.84	2.45	4.54	3061.80
MZ 83	11.01.2007	18.01.2007	Winter	7.59	79.01	1023.32	1.50	7.88	3066.84
MZ 84	18.01.2007	25.01.2007	Winter	5.38	65.08	1013.88	1.43	13.16	3109.68
MZ 88	01.02.2007	08.02.2007	Winter	4.89	82.79	1020.55	2.00	12.25	3059.28
MZ 89	08.02.2007	15.02.2007	Winter	6.56	81.34	1005.79	1.97	29.66	3056.76
MZ 90	15.02.2007	22.02.2007	Winter	4.99	81.43	1018.14	2.02	0.11	2991.24
MZ 93	22.02.2007	01.03.2007	Winter	8.41	78.49	1007.47	2.17	22.93	3054.24
MZ 95	08.03.2007	15.03.2007	Winter	8.00	69.86	1031.51	1.39	3.62	3024.00
MZ 96	15.03.2007	22.03.2007	Winter	6.81	71.54	1011.54	1.98	9.84	3104.64
MZ 97	22.03.2007	29.03.2007	Spring	9.04	63.73	1015.17	1.03	14.16	3039.12
MZ 101	05.04.2007	12.04.2007	Spring	12.41	61.24	1022.94	2.22	0.00	3056.76
MZ 102	12.04.2007	19.04.2007	Spring	17.38	54.49	1021.59	1.72	0.00	3099.60
MZ 103	19.04.2007	26.04.2007	Spring	16.51	55.78	1020.48	1.31	0.00	3054.24

Supplementary Table 2. Operational taxonomic units (OTUs) of identified Oomycetes. The OTU running number, frequency of occurrence (number of air samples in which the OTU was detected), best fitting NCBI accession numbers sorted by similarity score, taxonomic family, genus, and species name according to NCBI data base (if determined) and host plant families (numbers specify the corresponding host plant to the accession number of the pathogen). Species names are listed if available from NCBI sequences with similarity scores ≥ 97%.

OTU	Frequency of occurrence			NCBI accession numbers and similarity scores	Family, genus, species	Host plant family of best match sequences	References
	total	coarse	fine				
1	20	20		AY198279 (100%), AY198276 (97%)	Peronosporaceae, *Peronospora* sp.	Caryophyllaceae	(Voglmayr, 2003)
2	17	17		AY198300 (100%), AY198301 (99%), AY198298 (98-99%)	Peronosporaceae, *Peronospora* sp.	Rubiaceae	(Voglmayr, 2003;Göker et al., 2007)
3	17	17		AY919304 (98-99%), AY198246 (99%)	Peronosporaceae, *Peronospora conglomerata*	Geraniaceae	(Voglmayr, 2003;Göker et al., 2007)
4	16	16		AY198244 (99%), AY198243 (97%)	Peronosporaceae, *Peronospora* sp.	Veronicaceae	(Voglmayr, 2003;Göker et al., 2007)
5	15	15		AY198243 (99%), AY198241 (99%)	Peronosporaceae, *Peronospora* sp.	Veronicaceae	(Voglmayr, 2003)
6	14	14		AF528557 (99-100%), AF528556 (99%), FM863723 (99%)	Peronosporaceae, *Peronospora* sp.	Amaranthaceae	(Byford, 1967;Choi et al., 2008)
7	15	14	1	JF975613 (99%), JF975614 (99%), AY210985 (99%)	Peronosporaceae, *Hyaloperonospora*	Brassicaceae	(Voglmayr, 2003;Göker et al., 2007)
8	11	11		EU049225.1 (100%), EU049249.1 (99%)	Peronosporaceae, *Hyaloperonospora* sp.	Brassicaceae	(Voglmayr, 2003;Göker et al., 2007)
9	9	9		EF174902 (99%), EF174889 (98-100%)	Peronosporaceae, *Peronospora* sp.	Fabaceae	(Voglmayr, 2003)
10	7	7		AY929824 (97-98%)	Albuginaceae, *Wilsoniana amaranthi*	Amaranthaceae	(Mirzaee et al., 2013)
11	6	6		AY198240 (99-100%)	Peronosporaceae, *Peronospora violae*	Violaceae	(Voglmayr, 2003)
12	6	6		KF888604 (99%), KF888591 (99%)	Peronosporaceae, *Peronospora* sp.	Amaranthaceae	(Klosterman et al., 2016)
13	5	5		EU295529 (99%), AY695806 (99%)	Peronosporaceae, *Peronospora arborescens*	Papaveraceae	(Voglmayr, 2003;Göker et al., 2007)
14	5	5		KC494997 (96%), KC494998 (96%)	Peronosporaceae, *Hyaloperonospora* sp.	Brassicaceae	(Voglmayr et al., 2014)
15	4	4		AY531462 (100%), EU049260 (100%)	Peronosporaceae, *Hyaloperonospora* sp.	Brassicaceae	(Voglmayr, 2003;Göker et al., 2007)
16	4	4		EU049263 (99%), AY578093 (99%)	Peronosporaceae, *Hyaloperonospora*	Brassicaceae	(Voglmayr, 2003;Göker et al., 2007;Coates and Beynon, 2010)
17	4	4		DQ447120 (99%), EU049273 (99%)	Peronosporaceae, *Hyaloperonospora* sp.	Brassicaceae	(Voglmayr, 2003;Göker et al., 2007)
18	3	3		EU660054 (99%), EF126356 (99-100%)	Peronosporaceae, *Pseudoperonospora* sp.	Balsaminaceae, Cannabaceae, Cucurbitaceae	(Choi et al., 2005;Voglmayr et al., 2009)
19	3	3		AY531452 (100%), AY210987 (99%)	Peronosporaceae, *Hyaloperonospora parasitica*	Brassicaceae	(Voglmayr, 2003;Göker et al., 2007)
20	1	1		HQ643443 (99%)	Pythiaceae, *Pythium apiculatum*		
21	3	3		KM058096 (96%), AY198248 (90%)	Peronosporaceae *Peronospora* sp.	Veronicaceae	(Voglmayr, 2003;Göker et al., 2007)
22	2	2		AY531425 (100%), EU049276 (99%)	Peronosporaceae, *Hyaloperonospora* sp.	Brassicaceae	(Voglmayr et al., 2014)
23	2	2		GU583839 (100%), GU583838 (100%)	Peronosporaceae, *Hyaloperonospora erophilae*	Brassicaceae	(Voglmayr, 2003;Göker et al.,

						2007;Coates and Beynon, 2010)
24	2	2	EU049279 (99%), AY531452 (99%), AY198254 (99%)	Peronosporaceae, *Hyaloperonospora parasitica*	Brassicaceae	(Voglmayr, 2003;Göker et al., 2007)
25	3	3	EF174906 (99%), EF174949 (99%)	Peronosporaceae *Peronospora* sp.	Fabaceae	(Voglmayr, 2003;Mouchacca, 2005)
26	2	2	KM058101 (95%), KM058095 (94%)	Peronosporaceae *Peronospora* sp.	Ranunculaceae	(Riethmuller et al., 2002;Voglmayr, 2003;Voglmayr et al., 2014)
27	2	2	AY198280 (99%), KP271924 (98%)	Peronosporaceae, *Peronospora sp.*	1.Caryophyllaceae, 2.Polygonaceae	(Voglmayr, 2003;Petrželová et al., 2015)
28	2	2	EU049262 (99%)	Peronosporaceae, *Hyaloperonospora* sp.	Brassicaceae	(Voglmayr, 2003;Göker et al., 2007)
29	2	2	KC495031.1 (99%), EU049259.1 (99%)	Peronosporaceae, *Hyaloperonospora* sp.	Brassicaceae	(Voglmayr, 2003;Göker et al., 2007;Voglmayr et al., 2014)
30	2	2	KM058095 (97%), KM058101 (97%), KM058097 (97%)	Peronosporaceae, *Peronospora* sp.	Ranunculaceae	(Riethmuller et al., 2002;Voglmayr, 2003)
31	2	2	EU427470 (97%), DQ832718 (97%), DQ832717 (96%)	Pythiaceae *Phytophthora* sp.	Fagaceae, Ericaceae, Oleaceae, Theaceae, Adoxaceae; 3. Fabaceae	
32	2	2	EU427470 (96-97%), DQ832718 (96-97%), DQ832717 (96%)	Pythiaceae *Phytophthora* sp.		(Tyler et al., 2006;Grunwald et al., 2008)
33	1	1	KJ651417 (95%), HM636048 (94%), AY198307 (94%)	Peronosporaceae	Papaveraceae Urticaceae	(Voglmayr et al., 2014)
34	1	1	AY198293 (99%)	Peronosporaceae, *Peronospora valerianellae*	Valerianaceae	(Voglmayr, 2003)
35	2	2	AY198307.1 (99-100%), HM636049.1 (99-100%), AY608613.1 (97-98%)	Peronosporaceae, *Pseudoperonospora* sp.	1-3.Urticaceae 3.Cannabaceae	(Voglmayr, 2003;Choi et al., 2005;Göker et al., 2007)
36	1	1	AY211009 (99%), AY211010 (99%), EU049264 (99%)	Peronosporaceae, *Hyaloperonospora parasitica*	Brassicaceae	(Voglmayr, 2003;Göker et al., 2007)
37	1	1	AY210994.1 (98-99%), EU049210.1 (98-99%), AY198259.1 (98%)	Peronosporaceae, *Hyaloperonospora* sp.	Brassicaceae	(Voglmayr, 2003;Göker et al., 2007)
38	1	1	KM058095 (99%), KM058097 (99%), FJ384778 (98%)	Peronosporaceae, *Peronospora* sp.	Ranunculaceae	(Riethmuller et al., 2002)
39	1	1	AF241771 (100%)	Albuginaceae, *Albugo tragopogonis*	Asteraceae	(Long et al., 1975)
40	1	1	GQ390795 (99%), FJ394345 (99%)	Peronosporaceae, *Peronospora* sp.	Lamiaceae	(Thines et al., 2009;Henricot et al., 2010;Nagy and Horváth, 2011)
41	1	1	EU049207 (99%), EU049214 (99%)	Peronosporaceae, *Hyaloperonospora hesperidis*	Brassicaceae	(Göker et al., 2004;Voglmayr and Göker, 2011)
42	1	1	EF174893 (100%), EF174898 (99%)	Peronosporaceae, *Peronospora ervi*	Fabaceae	(Voglmayr, 2003)
43	1	1	AY198263 (99%), AY198264 (97%)	Peronosporaceae, *Peronospora* sp.	Boraginaceae	(Voglmayr, 2003;Göker et al., 2007)
44	1	1	KP271924 (97%), KM058096 (97%)	Peronosporaceae, *Peronospora* sp.	Polygonaceae	(Petrželová et al., 2015)
45	1	1	HM587262 (99%), FR825184 (99%)	Albuginaceae, *Albugo* sp.	Brassicaceae	(Kaur et al., 2011;Kemen et al., 2011)

46	1	1	AY198279 (95%), AY211016 (95%), AY198282 (94%)	Peronosporaceae, *Peronospora* sp.	1/3. Polygonaceae 2. Caryophylaceae	(Voglmayr, 2003;Petrželová et al., 2015)
47	1	1	AY198296 (99%), AY198295 (97%), AY198298 (95%)	Peronosporaceae, *Peronospora* sp.	1. Asteraceae 2. Lamiaceae 3. Rubiaceae	(Voglmayr, 2003)
48	1	1	DQ643921 (99%)	Albuginaceae, *Albugo portulacae*	Portulacaceae	(Choi et al., 2007)
49	1	1	HQ702191 88%), AY198247 (88%)	Peronosporaceae *Peronospora* sp.	Lamiaceae, Scrophulariaceae	(Voglmayr, 2003;Nagy and Horváth, 2011)
50	1	1	AY198276 (97%), AY198277 (97%)	Peronosporaceae *Peronospora* sp.	Caryophyllaceae	(Voglmayr, 2003)
51	1	1	AY198298 (94%), AY198296 (94%), AY198299 (94%)	Peronosporaceae *Peronospora* sp.	1/3. Rubiaceae 2. Asteraceae	(Voglmayr, 2003)
52	1	1	AY198246 (96%), EU295529 (94%)	Peronosporaceae *Peronospora* sp.	Geranicaceae, Papaveraceae	(Voglmayr, 2003;Göker et al., 2007)
53	1	1	AY198244 (95%), AY198243 (93%)	Peronosporaceae *Peronospora* sp.	Veronicaceae	(Voglmayr, 2003;Göker et al., 2007)
54	1	1	AY198243 (95%), AY198241 (94%)	Peronosporaceae *Peronospora* sp.	Veronicaceae	(Voglmayr, 2003;Göker et al., 2007)
55	1	1	EU427470 (97%), DQ832718 (97%), DQ832717 (96%)	Pythiaceae *Phytophthora* sp.	1/2. Fagaceae, Ericaceae, Oleaceae, Theaceae, Adoxaceae 3. Fabaceae	(Tyler et al., 2006;Grunwald et al., 2008)

Supplementary Table 3. NCBI accession numbers of most representative sequences of the identified OTUs.

OTU	NCBI accession number	OTU	NCBI accession number
1	MF095126	29	MF095154
2	MF095127	30	MF095155
3	MF095128	31	MF095156
4	MF095129	32	MF095157
5	MF095130	33	MF095158
6	MF095131	34	MF095159
7	MF095132	35	MF095160
8	MF095133	36	MF095161
9	MF095134	37	MF095162
10	MF095135	38	MF095163
11	MF095136	39	MF095164
12	MF095137	40	MF095165
13	MF095138	41	MF095166
14	MF095139	42	MF095167
15	MF095140	43	MF095168
16	MF095141	44	MF095169
17	MF095142	45	MF095170
18	MF095143	46	MF095171
19	MF095144	47	MF095172
20	MF095145	48	MF095173
21	MF095146	49	MF095174
22	MF095147	50	MF095175
23	MF095148	51	MF095176
24	MF095149	52	MF095177
25	MF095150	53	MF095178
26	MF095151	54	MF095179
27	MF095152	55	MF095180
28	MF095153		

Supplementary Table 4. Quantitative PCR results for rRNA genes (detailed in Table 1) of Oomycetes in coarse particulate matter. Filter sample running number, average rRNA gene abundance (gene copy number m^{-3}, n = 3 runs), and standard deviation (SD) for the total Oomycetes, Albuginaceae, *Phytophthora*, *Peronospora*, and *Pythium.*

Sample number	Season	Oomycetes		Albuginaceae		*Phytophthora*		*Peronospora*		*Pythium*	
		Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD
MZ 1	Spring	132647	8484	0.0	0.0	33623	3439	3.9	1.5	3.0	1.9
MZ 2	Spring	660248	21591	10.7	10.0	173014	2706	2.4	0.9	0.7	1.1
MZ 4	Spring	113338	15460	77.7	17.6	23865	5517	0.0	0.0	0.0	0.0
MZ 6	Spring	865499	50119	8.5	7.4	115448	47353	203.8	24.5	19.4	8.4
MZ 9	Spring	1938057	305849	44.7	8.1	67653	24690	596.1	48.0	1.3	2.3
MZ 10	Spring	606063	47769	237.3	61.9	252034	1393	2798.8	663.3	3.1	5.4
MZ 11	Spring	5144662	571473	336.7	162.5	1763896	94887	7147.6	1204.8	33.5	4.8
MZ 15	Spring	1476110	179703	427.2	42.8	232306	111720	353.2	25.2	0.0	0.0
MZ 18	Spring	1662387	140289	3917.0	732.2	619364	64675	5058.7	1107.4	3.7	6.5
MZ 19	Spring	168245	34162	349.2	126.4	55697	2645	36.3	20.2	8.4	14.5
MZ 21	Spring	14204	3734	65.7	23.8	5159	287	5.9	2.8	0.0	0.0
MZ 24	Spring	2139263	118944	5488.2	431.5	356575	55963	1824.6	121.1	0.0	0.0
MZ 25	Spring	38450	8292	248.8	120.1	5373	1272	0.0	0.0	0.0	0.0
MZ 26	Spring	1232300	68227	1198.3	154.7	461404	24784	2319.5	189.5	10.3	1.9
MZ 31	Spring	298467	19576	223.6	95.4	98818	5583	21.7	16.1	7.1	12.3
MZ 35	Spring	404940	12756	2450.4	86.3	151038	7119	1381.3	142.0	11.8	1.9
MZ 36	Summer	2177605	202571	13521.2	927.9	856443	51898	12541.0	2067.0	0.0	0.0
MZ 40	Summer	771889	65313	2333.8	346.7	260480	8349	0.0	0.0	1.4	2.4
MZ 41	Summer	898068	55655	2447.0	157.4	384782	32145	1095.7	328.7	5.2	0.6
MZ 45	Summer	624724	47700	4226.8	639.5	91053	5218	0.0	0.0	17.1	2.0
MZ 47	Summer	400480	39873	2437.5	223.6	144236	15927	0.0	0.0	2.1	3.6
MZ 50	Summer	963014	36186	657.3	54.8	251690	7361	466.2	78.0	1.4	2.5
MZ 51	Summer	1125035	61616	370.8	59.0	257359	20583	838.2	51.8	3.8	1.0
MZ 52	Summer	3795070	289972	399.0	84.8	866854	53189	421.4	68.0	0.0	0.0
MZ 54	Summer	4274819	386690	466.6	118.6	1595699	97904	125.0	49.0	2.9	1.4
MZ 59	Summer	488014	26877	3190.9	295.4	144514	18407	1687.7	220.8	0.8	1.4
MZ 60	Fall	157129	18839	2551.3	204.6	24311	4974	97.7	23.7	0.0	0.0
MZ 62	Fall	2173268	249307	1653.6	268.3	678461	34282	2785.7	391.4	7.6	1.6
MZ 63	Fall	526890	70651	580.9	109.2	134210	18534	90.3	56.7	0.9	1.6
MZ 66	Fall	2554584	317703	9871.4	766.9	596006	126244	3322.8	295.6	0.0	0.0
MZ 67	Fall	3063765	311458	5602.2	377.3	941664	8577	669.2	35.2	2.3	2.2
MZ 69	Fall	189725	5829	1246.2	177.8	52798	6570	11.0	0.0	0.9	1.5
MZ 71	Fall	2252326	343713	10124.8	148.2	693222	110423	615.7	150.2	0.7	1.2
MZ 74	Fall	1452678	112619	32519.3	1719.4	500398	35195	1659.2	661.9	12.4	2.8
MZ 75	Fall	920684	44942	15727.8	2814.3	306667	10006	269.4	22.4	3.9	2.0
MZ 77	Fall	100790	15436	637.0	137.6	21816	738	0.0	0.0	0.0	0.0
MZ 81	Winter	39722	1839	60.0	27.5	4270	1017	0.0	0.0	0.0	0.0
MZ 82	Winter	371992	36230	2298.2	174.5	119356	10380	7.3	1.8	4.1	1.8
MZ 84	Winter	64250	9658	1416.0	99.6	16360	1692	0.0	0.0	1.7	1.5
MZ 88	Winter	98477	32579	4.4	7.7	20999	249	4.6	0.5	0.7	1.2
MZ 90	Winter	121976	12513	0.0	0.0	53191	12016	1.9	3.3	2.2	0.5
MZ 93	Winter	176914	15957	87.2	6.0	98830	8827	15.1	5.6	4.3	0.3
MZ 95	Winter	292272	17911	349.7	33.2	199830	11312	11.6	3.5	15.2	6.4
MZ 97	Spring	79137	9529	337.7	34.9	43785	7617	88.0	33.8	0.0	0.0
MZ 101	Spring	837908	85860	1756.6	243.8	632423	60680	658.3	28.1	8.2	2.5
MZ 103	Spring	104578	15393	2453.5	2171.9	73155	2452	0.8	1.4	2.9	0.8

Supplementary Table 5. Correlation of Oomycetes rRNA gene abundance with temperature, relative humidity, and precipitation. Correlation coefficients for different taxa quantified by qPCR and seasons with temperature, relative humidity (RH), and precipitation. Correlations with winter samples are not presented due to the low number of qPCR-analyzed samples, low temperature range, lowest gene copy numbers, and insignificant correlations for this season.

	Temperature	RH	Precipitation
Albuginaceae			
Spring	0.332	-0.004	0.036
Summer	0.346	-0.723*	-0.500
Fall	-0.196	0.729*	-0.218
Phytophthora			
Spring	0.105	-0.148	-0.144
Summer	-0.361	0.139	-0.071
Fall	0.207	0.463	0.531
Peronospora			
Spring	0.169	-0.013	-0.046
Summer	0.005	-0.487	-0.331
Fall	0.195	0.534	0.472
Pythium			
Spring	0.209	0.003	-0.203
Summer	0.668*	-0.420	-0.308
Fall	0.195	0.534	0.472

*p-value < 0.05

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Apendix C3:

On the constraints and influences on the temporal and size fraction occurrences of airborne fungi

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(to be submitted)

On the constraints and influences on the temporal and size fraction occurrences of airborne fungi

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Keywords:

airborne fungi, primary bioaerosol, fungal spores, spore adaptations, plant pathogens, saprophytes

Abstract

Fungi play important roles in the environment, agriculture, and human health. Most fungal species spread by wind-driven dispersal of spores, determining their occurrence and distribution in different environments. The dynamics of airborne fungi and their dependence on lifestyle and environmental conditions, however, are not well characterized.

Here, we compare the airborne fungal composition in coarse and fine aerosol samples of continental boundary layer air, identified through isolated DNA sequences. This revealed that the composition of the airborne fungal community is temporally highly dynamic, however, not constant throughout the investigated 13-month period. We observed a high compositional identity of wintertime samples to spring and summer samples during the extremely warm winter 06/07, which may point to a very early initial spore dispersal due to warm conditions. Subsequent principal coordinates analysis revealed an ambient-temperature-dependent clustering of the samples and also revealed a separation of the coarse and fine fraction communities (cutoff 3 µm) The two communities displayed independent temporal dynamics as no discernable common patterns were identified between the coarse and fine filter samples.

To gain a deeper insight into the identified patterns, a subset containing the 25 most abundant species was formed and analyzed incorporating additional information on the fungi, including known nutritional and life-style preferences, fruiting form and spore dimensions. This allowed the identification of traits in the temporal and size fraction occurrences and correlations to meteorology, which were common amongst fungi with similar lifestyle preferences. We hypothesize the differences reflect lifestyle-dependent sporulation strategies which may facilitate and improve the assessment and forecasting of the abundance and spread of pathogenic fungi and related issues, such as crop protection, in view of land-use and climate change.

Introduction

Amongst the primary bioaerosol particles (PBAP) (Després et al. 2012) of the atmosphere, fungi are, in terms of number concentrations and impact, one of the most important groups. Typical number concentrations range from ~$10^3 - 10^4$ per cubic meter of air (Elbert et al. 2007; Fröhlich-Nowoisky et al. 2012), in the same orders of magnitude as bacteria, albeit with large variations depending on location, time of year, or even time of day. Fungal spores can show a large species-dependent variability in size, however, most species have spores in a size range of roughly 2 to 10 µm aerodynamic diameter (Elbert et al. 2007; Cao et al. 2014; Wang, Fang, and Lee 2008; Fröhlich-Nowoisky et al. 2009; Després et al. 2012; Huffman, Treutlein, and Pöschl 2010). As plant pathogens, fungi have a huge impact on agriculture and forestry (Strange and Scott 2005; Ostry and Juzwik 2008; Zabel and Morrell 2012) and, as pathogens and allergens, on human health (Horner et al. 1995). Their influence on atmospheric processes is also subject of ongoing discussions, as individual species have displayed ice-nucleating abilities far more efficient than non-biological aerosols and may influence cloud formation (Richard, Martin, and Pouleur 1996; Fröhlich-Nowoisky et al. 2015; Pöschl 2005; Fröhlich-Nowoisky et al. 2016).

Although wind-driven dispersal of spores is a passive process, through evolutionary adaptation to their lifestyles, fungi have heightened their sporulation success rate. These adaptations can be variations in numerous properties and will include aspects like the numbers of released spores, aerodynamic properties of spores, and the time of spore release, which we will cumulatively refer to as the "sporulation strategy". The evolutionary pressures leading to differing sporulation strategies will be factors such as the spatial distribution and abundancy of a nutrient source, for example, an obligate pathogenic fungus in comparison an unspecific generalist will have entirely different substrate constraints in the environment. In addition, the habitats to which fungal species are adapted should play a large role. On a global scale, the different seasonal climatic and vegetative cycles, will influence the presence of a nutrient sources, requiring a seasonal release of spores. On a local scale the habitats' microclimatic conditions, e.g., a sheltered forest atmosphere in comparison to open grasslands, will require differing strategies. Furthermore, meteorological factors are known to influence the fungal life cycle, from hyphal growth (van Laarhoven et al. 2015; Dowson, Boddy, and Rayner 1989; Donnelly and Boddy 1997) up to spore formation and liberation (Jones and Harrison 2004; Elbert et al. 2007), and will therefore ultimately also be major governing factors in the

sporulation strategy. Moreover, insight into the influence of meteorological factors on sporulation is of critical importance to understand and estimate the impacts of climate change, which is especially important for agriculture, due to the high abundance of fungal plant pathogens. The identification of occurrence and abundancy patterns and the main environmental factors influencing them are of key importance when it comes to developing forecast models or even large-scale global models, especially in view of the changing conditions in the Anthropocene.

As most studies dealing with temporal dynamics of the atmospheric microbiome focus on the taxonomic relations within the community as the foundation of analysis (Franzetti et al. 2011; Bowers et al. 2013, 2012; Yooseph et al. 2013; Fröhlich-Nowoisky et al. 2009, 2014, 2012), in this study, we aimed to focus more on the lifestyle-dependency of fungal atmospheric dynamics. To achieve this, we initially identify patterns in the temporal and size fraction occurrences and influence of meteorology in a dataset from a previous study by Fröhlich-Nowoisky et al., 2009, "High diversity of fungi in air particulate matter". The identified patterns are then explored in more detail by forming a subset of the 25 most abundant species and incorporating additional information, including nutritional and life-style preferences, fruiting form and spore dimensions. This allowed the identification of common patterns found amongst fungi adapted to similar lifestyles.

Material and methods

This study is based on the data obtained from a study by Fröhlich-Nowoisky et al., 2009. The following description is to be regarded as a short overview. For a detailed description of the applied laboratory methods please refer to the original publication.

Aerosol sampling

Sampling was conducted with a high-volume dichotomous sampler [self-built based on Solomon et al., "High-Volume Dichotomous Virtual Impactor for the Fractionation and Collection of Particles According to Aerodynamic Size" (Solomon, Moyers, and Fletcher 1983)] using sterilized glass fiber filters (PALL Corporation, Type A/A, 102-mm, sterilized at 500 °C for 12 h). The sampler was operated with a rotary vane pump (Becker VT.25) with a flow rate of 300 L min^{-1}, which corresponds to a cut-off of approx. 3 µm aerodynamic diameter, separating

the aerosols in the air sample onto separate coarse and fine particulate matter filters. The coarse particles (> 3 µm) were collected through a virtual impactor, in line with the inlet, with a flow rate 30 L min^{-1} while the fine particles (< 3 µm) were collected perpendicular to the inlet, with a flow rate of 270 L min^{-1}. Furthermore, it should be mentioned that an estimated 10 % of fine particulate matter, due to sampler design, is collected in the coarse fraction (Solomon, Moyers, and Fletcher 1983).

The sampling site was in Mainz (130 m a.s.l), Germany, on the roof of the old 3-storey Max Planck Institute for Chemistry building on the campus of the Johannes Gutenberg University (49°59´31.36´´ N, 8°14´15.22´´ E). The sampler was attached to a mast approximately 5 m in height above the roof top.

The surroundings in the direct vicinity of the sampling location are predominately urban to the north and east, whilst strongly dominated by agriculture to the south and west. The first small forests, approximately 2-5 km^2 in size, can be found approximately 3.5-5 km in distance to the north-west and south west. The first large forests, of over 100 km^2 can be found to the north and east at a range of 10-15 km.

The samples were collected over a 13-month period (March 2006-April 2007). The individual sampling durations were generally seven days, corresponding to an air volume of approximately 3000 m^3, with a few exceptions which had durations of 1-5 days (~400-2000 m^3). Altogether 42 sets of coarse and fine particulate filters were analyzed, which amount to a combined sampling time of ~37 weeks in the 13-month period. Information concerning the individual samples can be seen in table S1 in the supporting information of Fröhlich-Nowoisky et al. (Fröhlich-Nowoisky et al. 2009). To rule out contaminations during the sampling procedure, additional controls were performed. Blank samples comprised filters that were not used for sampling but only sterilized and extracted alongside the actual samples ("extraction blanks") to monitor the quality of the DNA extraction process. Two sets of filters were used to control the sampling procedure, where one filter set was mounted in the sampler and the pump turned on for 5 s ("start-up blanks") and the other filter sample set was equally mounted in the sampler but the pump was not turned on ("mounting blanks"). The sampling blanks were collected at regular intervals (~4 weeks) after a thorough cleaning of the sampler, and extraction blanks were included in each extraction process. None of the tested blanks contained detectable DNA.

DNA extraction and sequence analysis

Total DNA was extracted from filter aliquots (1/8–1/4) using a commercial soil extraction kit (LysingMatrixE, FastDNASpin Kit for Soil, MP Biomedicals). The extracted DNA was amplified via PCR with multiple primer pairs that target the ITS1-5.8S-ITS2 and 18S regions of the rRNA region (see Fröhlich-Nowoisky et al. (Fröhlich-Nowoisky et al. 2009). The amplification products were cloned using the TOPO TA Cloning Kit (Invitrogen) and ~12 to 24 colonies were randomly selected for further analysis, based on blue-white selection. A PCR was then performed on the selected colonies, using the vector specific primers M13F-40 and M13R (Sigma-Aldrich). To monitor possible contaminations, PCR blanks were included in all runs. The isolated cloned fragments were then further processed by a restriction fragment length polymorphism (RFLP) analysis to avoid unnecessary sequencing of identical sequences. The Max Planck-Genome-centre Cologne, Germany (http://mpgc.mpipz.mpg.de/home/) performed the sequencing of the selected amplification products. In total 1513 sequences were determined, of which 17 were removed due to chimeric results, resulting in 1496 sequences. The sequences were then grouped into 368 operational taxonomic units (OTUs) based on a 97 % sequence identity (OTU reference sequences accession numbers: FJ820489-FJ820856). Here, an OTU represents a hypothetical fungal species, as the intra-species variation of the ITS regions is usually below 3 % while the inter-species variation is on average 37 % (O'Brien et al. 2005; Schoch et al. 2012). The taxonomic affiliation of the OTUs was assessed using a BLAST search of the NCBI, whereby the OTUs were allocated to the lowest possible taxonomic level. The post-editing representative OTU sequences were on average ~630 base pairs in length. The relatively long sequences allowed Fröhlich-Nowoisky et al. to robustly identify the OTUs down to low taxonomic ranks (Approximately 30 % identified to species level, 25 % to genus level, remaining 45 % to family level or higher).

Dataset, subset, and additional information

Dependent on the performed analysis either the entire OTU dataset from Fröhlich-Nowoisky et al., 2009, as used or a subset of OTUs was used. The subset consisted OTUs found on at least 5 samples (roughly 10 % of the total samples) that were taxonomically identified to species- or genus-level. Furthermore, in the cases where multiple OTUs belonged to the genus and couldn't be differentiated on species-level, they were grouped indicated by *spp* (*species pluralis*) used in the used binomial name. The resulting OTU subset contained the most

abundant 28 of the original 368 OTUs which as 25 distinct taxa, which will be referred to as species due to the nature of the ITS region. A table listing the subset fungi along with the number of contained OTUs and the abundancy in the coarse and fine filter samples can be found in the supplementary table S1.

The low taxonomic ranks within the subset allowed additional information on the species or their genera to be gathered from literature (details in supplementary table S1):

The most prominent fruiting type was defined. This resulted in five categories. Mold-like (9 species), yeast-like (9 species) fungi, for primarily asexually reproducing species, and corticoid (7 species), bracket-forming (4 species), apothecium-forming (1 taxon) fungi for primarily sexually reproducing fungi.

It was differentiated whether the fungi are primarily ligninolytic (11 species), i.e. white-rot fungi or primarily non-ligninolytic fungi (14 species) dependent on other non-woody carbon sources.

It was assessed if the species are known saprophytes or are known at least to have saprophytic life-stages (19/25 species). Also, if the species are known to contain obligate or opportunistic pathogens (19/25 species; No differentiation was made between necrotrophic or biotrophic pathogens), or whether the species are known to be surface inhabitants or symbionts of plants (2/25 species).

Finally, spore dimensions were gathered. In cases where the species are known to produce both sexual and asexual spores, the dimensions of the asexual spores were used as these, to our knowledge, usually outweigh the sexual spore in terms of number concentrations.

Statistical analysis

For the foundation of presented analysis, a relational database was created with MySQL (MySQL Community Server Version 5.6.29). In a first step, all the entire fungal dataset along with associated information, such as meteorological and literature data, were evaluated and a database structure created to store all relevant information. The database allowed the storage of all data relevant for analysis, including sequence information along with an OTU grouping and hierarchical taxonomic tree structure using the nested set model (Celko 2004).

Furthermore, sampling information, including the sampling times, sampling volume, sampler type and sampler size fractioning, the location, was included.

Next to all data presented in Fröhlich-Nowoisky et al., 2009, and the additional information on the subset fungi, meteorological data from a weather station in Mainz-Mombach, provided by the ZIMEN Luft Messnetz of the Landesamt für Umwelt Wasserwirtschaft und Gewerbeaufsicht, were used. The meteorological data contain ½ h values for temperature, relative humidity, and precipitation and 1 h values for wind speed and direction for the entire 13-month sampling duration. For a unified taxonomic affiliation the higher taxonomic ranks of the species and genera were extracted from the catalogue of life (Roskov et al. 2015).

All calculations were performed either by direct database queries or using the script languages Python (Version 2.7; DB connector: MySQLdb module) or R Statistics (Version 3.1.0; DB connector: RMySQL Package).

A full list the formula described in the following, along with associated abbreviations, can be found in supplementary table S2.

To compare the OTU composition of the entire dataset between different air samples (β-diversity) the Bray Curtis Dissimilarity Index (*BC*) was, firstly, calculated between all TSP samples (total suspended particles: pooled OTUs from coarse and fine filter samples), and secondly, calculated between the samples subdivided into OTUs identified on coarse and fine fraction filter samples. A two-dimensional principle coordinate analysis (PCoA) was then performed on the resulting BC.

To compare the coarse and fine fractions occurrences of the subset fungi with their respective spore sizes a coarse-fine ratio ($\bar{S}$) was calculated:

$$\bar{S} = \frac{S_c - S_f}{S_{tot}} \qquad (1)$$

Here, S_{tot} is the total number of samples a taxon was found in, while S_c and S_f are the number of coarse or fine fraction occurrences, respectively. $\bar{S}$ can therefore vary between +1 and -1. A value of +1 indicates that a taxon was exclusively found in the coarse fraction, while -1 indicates occurrences exclusive to the fine fraction. A value of zero shows an even distribution between the size fractions. In Figure 4 the scale was replaced with fine (-1), even (0) and coarse (+1).

To compare $\bar{S}$ to the taxon-specific spore sizes, we calculated a shape-corrected theoretical aerodynamic diameter (d_a) for each subset taxon. To simplify calculations, non-spherical spores were treated as ellipsoids, which was true in most cases. Using the lengths and widths of the non-spherical spores the volumetric equivalent diameter (d_{vol}) was calculated (supplementary table S2).

The length to width ratio (q) allowed the calculation of the dynamic shape correction factor ($\varkappa$) assuming the spore polar axis is orientated horizontal to the airflow (Davis and Schweiger 2002). A horizontal orientation should be true for settling spores of homologous density in still air, as the horizontal orientation maximizes air resistance. Using d_{vol} and κ the theoretical aerodynamic diameter (d_a) of the elliptical spores could then be calculated:

$$d_a = \sqrt{\frac{\rho_{spore}}{\rho_0 \kappa}} d_{vol} \qquad (2)$$

With ρ_0 being the unit density (1 g cm^{-2}) and ρ_{spore} being the spore density: as the species and genus spore densities could not be found in literature and are known to vary between species, a density of 1 g cm^{-2} was uniformly used. Therefore, for spherical spores $d_a = d_{vol} = d$ and for non-spherical spores $d_a = (1/\kappa)^{1/2} d_{vol}$

Monthly relative frequency of species occurrences (RFO = Number of samples species was found on / the total number of analyzed samples in the month) were calculated for the subset. Samples on the border of two months were allocated to the month which was sampled longest.

To assess whether there are observable correlations between RFOs and meteorological parameters, the Spearman's Rank coefficients were calculated, along with p-values which describe the probability of random variables producing the observed correlation.

The meteorological data within the individual sampling periods were also pooled into months. The monthly sampling period averages, standard deviation, maximum and minimum values for temperature, humidity, and wind speed were calculated. For precipitation, the sum precipitation was calculated, along with an approximation of the duration of precipitation by summing every half hour with a precipitation larger than 0 mm and approximated precipitation strength (sum of precipitation / approximated duration).

Results and discussion

In the period from March 2006 to April 2007 in Mainz, Germany, 368 operational taxonomic units (OTU) isolated from the 42 analyzed air samples, comprising coarse and fine fraction particulate filters, were identified and discussed in Fröhlich-Nowoisky et al., 2009(Fröhlich-Nowoisky et al. 2009). Although the isolated DNA can potentially stem both from fungal spores and aerosolized hyphae. The hyphal fragments have, however, been found to be, at least, an order of magnitude lower in number concentration than spores ($\sim 10^2$ m^{-3})(Pady and Gregory 1963). The discussed sequences can therefore be assumed to stem primarily from spores, which is also strengthened by size fraction occurrences discussed below.

To gain an overview of the temporal dynamics of the atmospheric fungal community, Figure 1 shows the total suspended particles (TSP), i.e., combined coarse and fine fraction, inter-sample variability in composition of the entire dataset using the Bray-Curtis-Dissimilarity-Index. In general, air samples in close temporal vicinity (near the diagonal) display a higher similarity when compared to samples taken half a year later, demonstrating a clear seasonality in the composition of atmospheric fungal community. However, the short-term sample-to-sample variability in OTU composition is not constant throughout the 13-month period. On the one hand the plot regions A and C, corresponding to spring until late summer 2006 and winter 2006 to spring 2007, display long periods of relatively high sample-to-sample similarity (<0.75), with winter 2006 showing the highest consistency compared to the other seasons. On the other hand, the plot region B, corresponding to late summer and autumn of 2006, displays a low sample-to-sample consistency (predominately >0.75), which indicates a period of high diversity and dynamic variability in the atmospheric fungal composition. These findings coincide with the findings in Fröhlich-Nowoisky et al., 2009, which showed the absolute fungal species richness when grouped into meteorological seasons was highest in autumn and lowest in winter, while spring was slightly more diverse than summer.

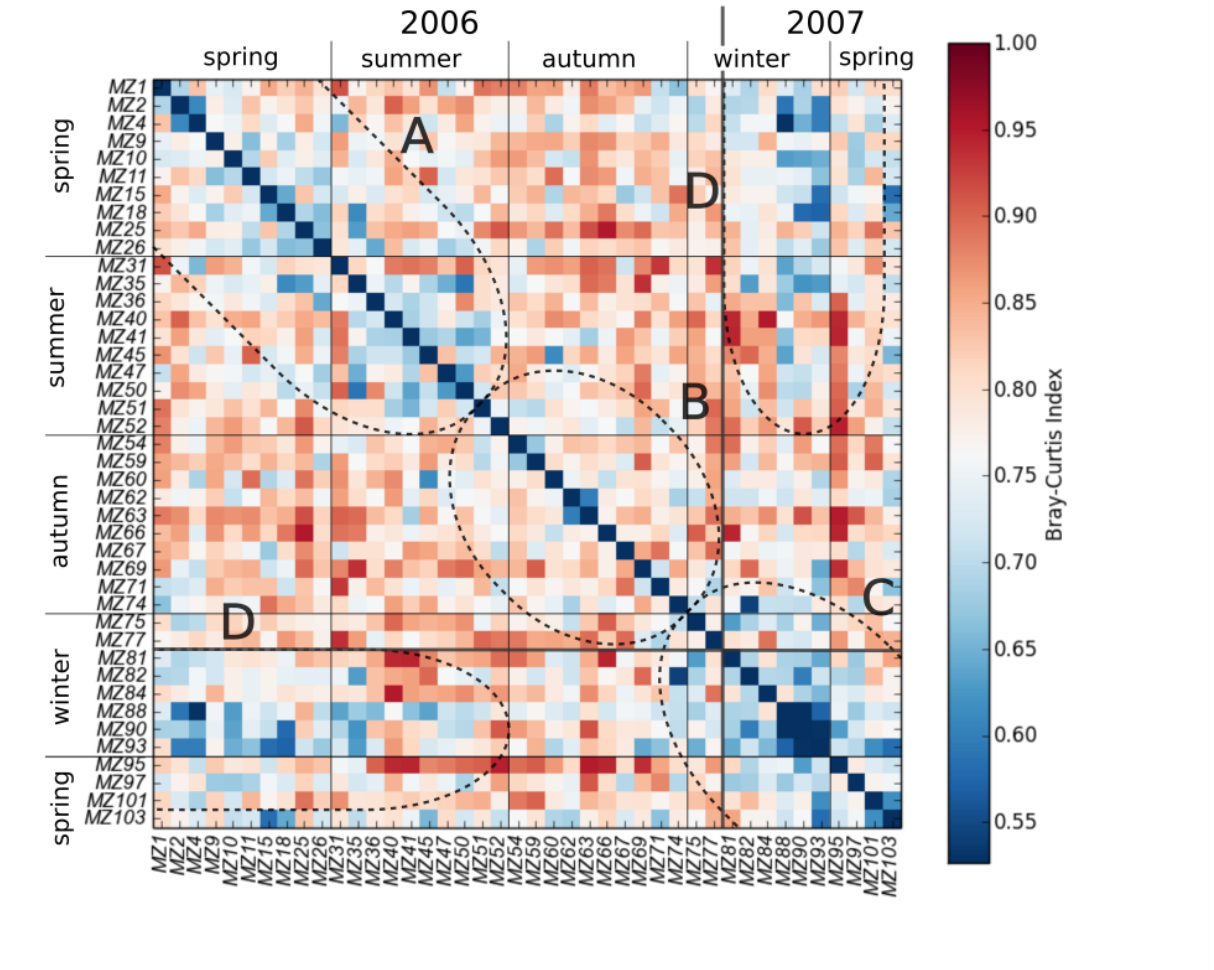

Figure 1: Inter-sample variability of OTU composition (β-diversity) for the entire dataset (TSP) using the Bray-Curtis Dissimilarity index. The consecutively numbered sample IDs of the sampling campaign are shown, which were also grouped into meteorological seasons. The Bray Curtis Index is the ratio of OTUs that differ between two samples, ranging from 1 being a completely different OTU composition to 0 being an identical composition. Note that the used color scale ranges from 1 to 0.52, as this was the lowest index calculated between two samples. A-D: Areas of interest referenced within the text.

Interestingly the end of winter 2006/07 (Figure 1 plot region D) displays high similarities to the samples taken in spring and even summer of 2006. The analyzed period in Germany was rather unique from a climatic perspective. According to the DWD (Deutscher Wetter Dienst), winter 2005/06 was the coldest in over a decade with an average temperature of -0.7°C while the winter of 2006/07 was, up to that time, the warmest in recorded history with an average temperature of +4.4°C (decadal winter time average 2001-10: +1.1°C). The unusually warm winter, is a potential explanation for the observed high similarities, which may have led to a much earlier sporulation onset and could be an indication of the far-reaching influence that a changing climate will have. The winter sample MZ 90 collected in mid-February with an

average temperature 4.99 °C even shows similarities to summer samples comparable to the sample-to-sample similarities within the summer season itself. An earlier onset of sporulation might benefit the spread and growth of fungal pathogens, prolong sporulation periods and allow for additional sporulation events for species with multiple generations per year. These in turn could have unforeseeable effects on, for example, agriculture, local flora and fauna, or the allergic sensitization and exposure towards fungal species (Boddy et al. 2014; Wolf et al. 2010; Gange et al. 2007; Fröhlich-Nowoisky et al. 2016).

To gain a deeper insight into the sample compositions and the hypothesized influence of temperature, in Figure 2a, a PCoA of the Bray-Curtis indices was performed. While the seasonal samples do, very roughly, cluster there is a clearer pattern observed when accounting for the average temperature during sampling. A diagonal increase in average temperature from the top-right to the bottom-left is seen, which also seems to account for the cases where there are larger distances between samples from the same season (temporally close samples). This is an indication that the composition of the airborne fungal community is to a certain extent influenced ambient temperature. This could be due to differing growth optima, an influence on sporulation, but also indirect influences such as a temperature influence on host vegetation. It should also be added that no patterns were observed when accounting for the other available meteorological parameters (seen in Supplementary Figure S1).

In figure 2b a PCoA was performed with separated coarse and fine filter sample OTU compositions. The coarse and fine samples show a clear vertical separation, albeit with some overlap. Furthermore, as shown by the lines connecting corresponding coarse and fine samples there are large differences and no discernable patterns observed between the fractions. Firstly, this indicates that there is a different composition in the coarse and fine fractions above and below the sampler cutoff of 3 µm, which is explainable by differing species-dependent spore sizes. Secondly, the size-dependent communities show little dependency on each other, i.e. clustering of coarse fraction samples does not necessarily lead a clustering of the corresponding fine fraction samples. This may be due to differing species-dependent sporulation strategies, e.g. species producing small spores may be aiming at different substrates than species that produce large spores, which could require different spore release patterns. Also, although it is conceivable that there is different influence of meteorological factors on coarse and fine fraction

spore populations, no clear patterning (as seen 2a) was discernable when accounting for any of the meteorological parameters (seen in Supplementary Figure S1) in the separate size fractions.

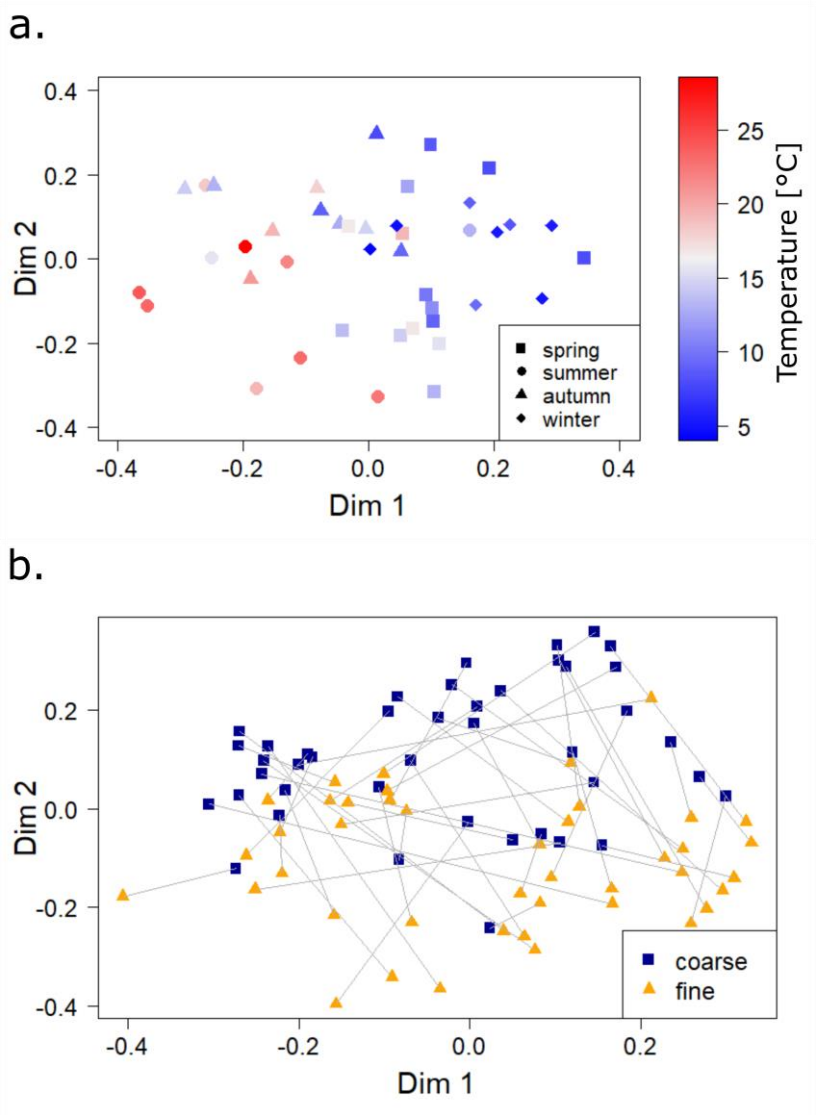

Figure 2: PCoA of Bray-Curtis Dissimilarities. a. PCoA of TSP samples (coarse + fine). The point colors correspond to the average temperature during sampling (color bar). The point shapes correspond to the season the sample was taken. b. PCoA of the separated coarse (blue) and fine (yellow) filter samples. The lines connect corresponding coarse and fine filter samples.

For a deeper insight into the observed seasonalities and the earlier sporulation observed in Figure 2, the RFO (relative frequency of occurrence), timelines of the subset fungi are shown in Figure 3. Boddy et al. (Boddy et al. 2014) investigated the influence of climate change on the development of the fruiting bodies of Basidiomycetes and reported an on average, 18-day earlier onset of springtime fruiting which was correlated with high winter temperatures (Boddy et al. 2014). Our results agree with their findings and point to a similar influence on

Ascomycetes (forming the group of non-ligninolytic fungi). April and May of 2006 display a high diversity with nearly all species showing an atmospheric presence within the two-month period. Furthermore, the occurrence shift is especially obvious amongst pathogens and potential pathogens. For example, the non-ligninolytic obligate pathogens (*Ascochyta* sp. to *Itersonilia perplexans*) display mid to high RFO in late spring (May 2006), which is observed again in mid to late winter (January and February 2007). Although, there is substantial research effort being invested into the influence of climate change on plant pathogens (Chakraborty 2013; Pautasso et al. 2012; West et al. 2012) due to the vast potential influences, there is further need to study and forecast the potentially diverse effects of a changing climate, such as rising temperatures or changing precipitation patterns, on the sporulation of fungi to develop strategies to limit harm to be expected.

In most cases the non-ligninolytic fungi, that is fungi involved primarily in the degradation or infection of non-woody herbaceous plant material, show a clear seasonality with one to two annual peaks that correspond to different stages in the annual life cycle of plants. The mold-like genera *Alternaria* and *Epicoccum* sp. to which many species of opportunistic plant pathogens belong, together with *Botryotinia fuckeliana* (anamorph *Botrytis cinereal)*, with a very large host range, display high RFO values throughout, from spring to late autumn, corresponding to the developing and fully developed vegetative stages of most plants in northern Europe. This reflects a generalist or unspecific sporulation strategy adoptable due to the unspecific nature of the pathogens. The more specialized obligate non-ligninolytic pathogens, *Ascochyta* sp. to *Itersonilia perplexans,* show more temporally confined atmospheric occurrences., in most cases early in the vegetative cycle of plants, aiming at the germinating and early plant developmental stages of plants. Additionally, in the case of *I. perplexans,* the cause of petal blight in its hosts (Gandy 1966), a second RFO peak is observed late in the year, presumably aiming at flowering host plants.

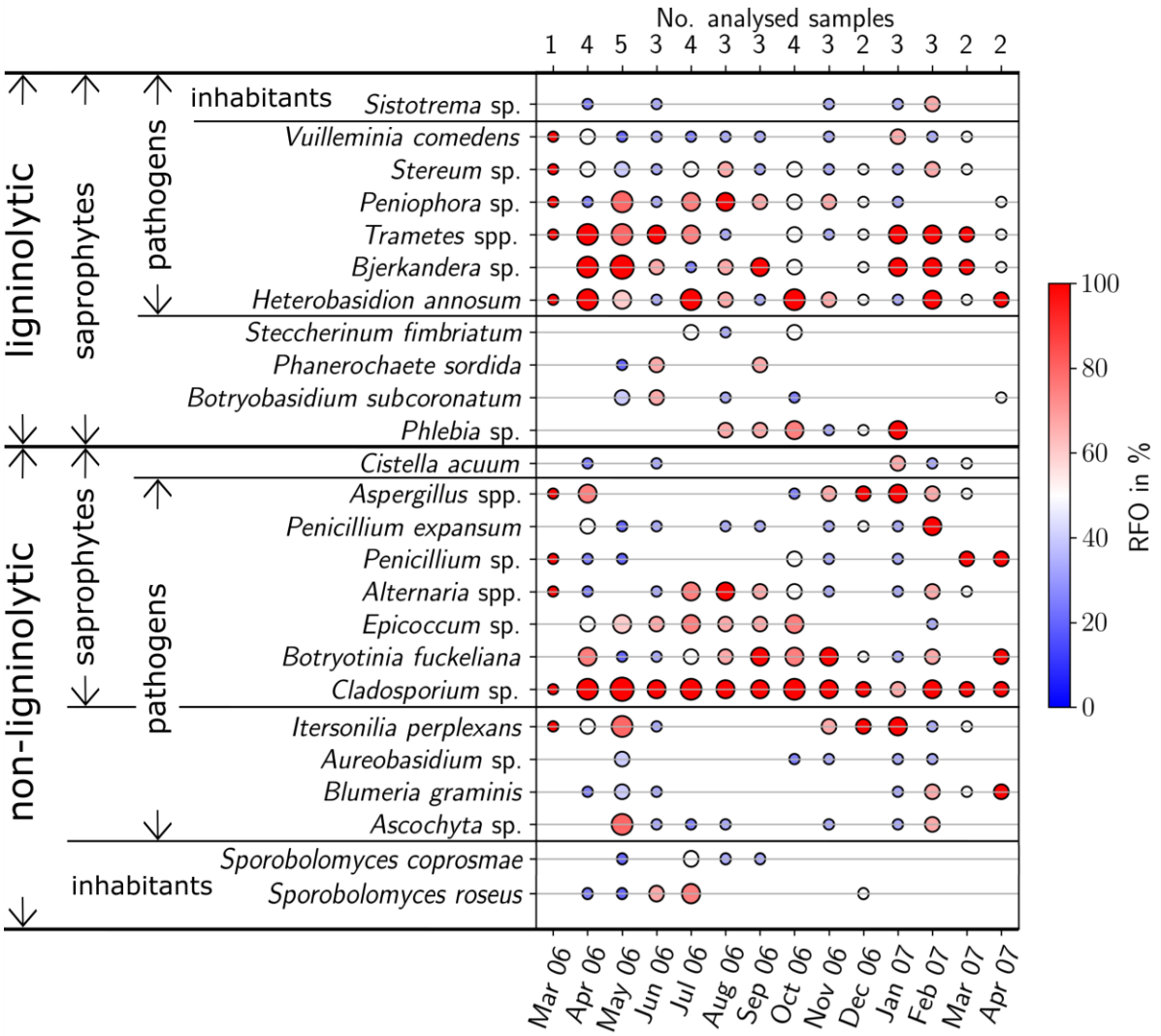

Figure 3: Temporal dynamics of the relative abundance of the subset fungi ordered according to the allocated lifestyle classification. The colors of the points correspond to the relative frequency of occurrence (RFO) for each species or genus and in a given month. The point sizes scale, both with the RFO-value and the number of analyzed samples for each month to additionally weight months with a higher number of analyzed samples (point size = RFO *no. analyzed samples/3).

Three genera of cosmopolitan molds, *Cladosporium Penicillium* and *Aspergillus* which are globally distributed and are mostly unspecific when it comes to habitats or substrates, are also observed. They are frequently amongst the most abundant atmospheric genera in studies (Fröhlich-Nowoisky et al. 2009, 2012; Larsen 1981; Mitakakis et al. 1997; Nayar and Jothish 2013; Shelton et al. 2002). *Cladosporium* sp. is the most abundant taxon in the study and is found in all but one sample, indicating a year-round high abundance. *Penicillium* sp. and *Penicillium expansum* were found with mostly mid to low RFO values spread throughout the

year, indicating a continuous atmospheric presence. Furthermore, there were an additional six *Penicillium* OTUs identified in the dataset that didn't meet the criteria of the subset. The continuous presence of the mold genera reflects the unspecific nature of their sporulation strategies, which can be adopted due to the ubiquity of potential substrate in the environment. *Aspergillus* spp. shows high spring but also winter RFO values, that might reflect the saprophytic nature of the isolated species, aiming at plant litter as a substrate.

In contrast to the non-ligninolytic fungi, the ligninolytic fungi show a distinct difference in occurrence patterns. These fungi to a large part will rely on wood degradation as one of their main energy sources. The saprophytic fungi, apart from *Phlebia* sp., mainly show sporadic occurrences and low RFO values, this might reflect the relatively large distance to larger forested areas (approx. 3.5 km) or even a shielding effect of the forest canopy reducing spore emissions into the free atmosphere. However, the fungi identified as ligninolytic pathogens or tree pathogens are amongst the most abundant identified within the study showing a year-round presence with frequently high RFO values. This may reflect the height of emission above the ground. Saprophytic fungi will mainly release their spores from fruiting bodies near the forest floor, while pathogenic fungi or fungi colonizing trees and dead attached branches will generally release their spores from a greater altitude into more turbulent airflow and therefore travel greater distances. It is also possible that tree pathogenic fungi produce significantly more spores over long periods of time compared to wood saprophytes. Many tree pathogens are known to form long-term fruiting bodies e.g., for *Bjerkandera adusta* which produces fruiting bodies year round (Kuo 2010). Such a sporulation strategy will heighten the infection probability in cases where, for example, a wounded or weakened host is needed, while saprophytes can generally rely on having dead wood in close vicinity to their emission source. So, like the cosmopolitan mold fungi, ligninolytic pathogens could be releasing vast quantities of spores with a less distinct seasonality not due to the ubiquity of substrate in the environment but rather due to its scarcity and therefore lower inoculation success rate.

To further investigate a possible influence of meteorological factors, as seen in Figure 2a. In Figure 4 the Spearman's rank correlation coefficients between the RFO values, seen in Figure 3, and the meteorological factors, seen supplementary Figure S1, are shown. The strongest correlations are observed with temperature and wind speed and only a few significant correlations are observed with relative humidity and precipitation, both known to be important

factors for short-term spore release (Jones and Harrison 2004) . However, they primarily have more short-term influence on sporulation and atmospheric residence, which may be masked by the grouping into months.

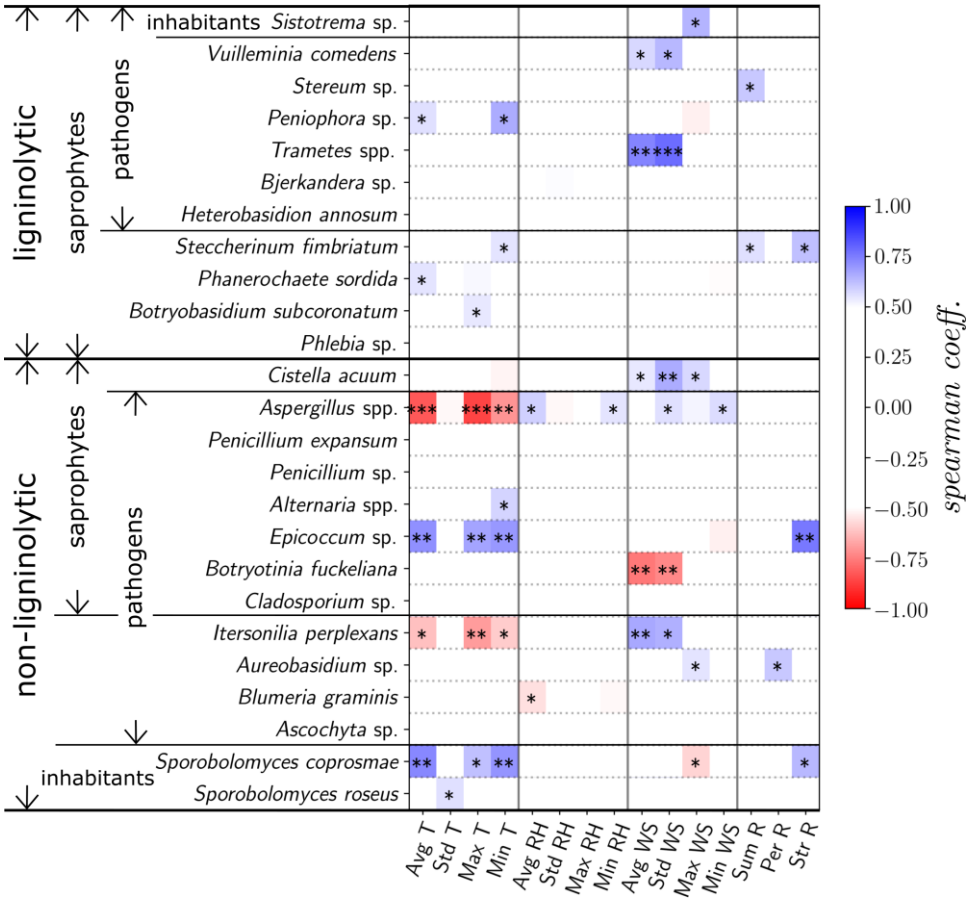

Figure 4: A Spearman's rank correlation matrix between the subset fungi RFOs in TSP with meteorological factors (shown in supplementary Figure S3). The values range from blue (+1) to red (-1), indicating a monotone increase or decrease, respectively, between the compared variables. The color scheme was chosen that begins coloration outside of threshold of -0.5 to 0.5. The p-value is the probability of a random dataset of the same size producing the same results ('*' < 0.05; '**'<0.01; '***' < 0.001)

The non-ligninolytic fungi show the strongest correlations. Temperature especially displays strong positive and negative correlations. Temperature is well known to be a main factor in germination hyphal growth and sporulation (Ayerst 1969; Tommerup 1983) which also makes sense for fungi with seasonal dispersal strategies, aimed at different stages in the vegetative cycle of plants. The ligninolytic fungi on the other hand show fewer sporadic correlations. The more significant correlations are observed with wind speed, seen for the species of *Trametes*. This might reflect the need for higher wind speeds for liberation from the forest canopy.

Aerodynamic properties of spores are also important as they are a determining factor in the potential atmospheric residence time and spore-deposition. In Figure 5 we show the calculated aerodynamic diameter for subset fungi against the ratio of the coarse to fine particle fraction occurrences (cut-off: 3 µm). Factors such as spore density, inhomogeneous density distribution and spore surface composition, will also affect a spore's aerodynamic properties. However, due to lack of information in literature, a uniform density of 1 g cm^{-3} was applied to all species. The few examples of densities mentioned by Gregory (Gregory 1961) range between 0.56 – 1.44 g cm^{-3}, which would translate to a reduction or increase of the aerodynamic diameters by ~25 % or ~20 %, respectively. It is mentioned that spores are typically slightly denser than water, in the range of 1.1-1.2 g cm^{-2}. Also the hydration of the spores has been observed to have a large effect on the aerodynamic size of spores (Reponen et al. 2001). Despite these uncertainties, an obvious increase in calculated diameters can be observed in Figure 5 over the range from fine to coarse. This is also an indication that the isolated DNA predominantly stemmed from spores rather than hyphal fragments. The yeast-like *Sporobolomyces coprosmae* (No. 19) seemingly contradicts the increasing trend, overweighing in the coarse fraction despite having relatively small spores. However, the larger vegetative cells of the plant surface inhabiting fungus (calculated aerodynamic diameter: 3.5 – 5.3 µm) may predominantly have been sampled as these can be aerosolized from the plant surfaces through splash-dispersal.

When considering the additional information, three groups can be identified (ellipses I-III). All the ligninolytic fungi, with exception of *Vuilleminia comedens*, lie within the central group III that are more or less evenly distributed between the size fractions. Non-ligninolytic fungi on the other hand display two distinct groups: Group I containing species predominant in the fine fraction to species evenly distributed between the size fractions, and Group II with species dominating in the coarse particle fraction. Group I exclusively contains the cosmopolitan mold fungi genera *Cladosporium, Penicillium* and *Aspergillus*, while Group II contains all plant pathogens and plant surface inhabiting fungi.

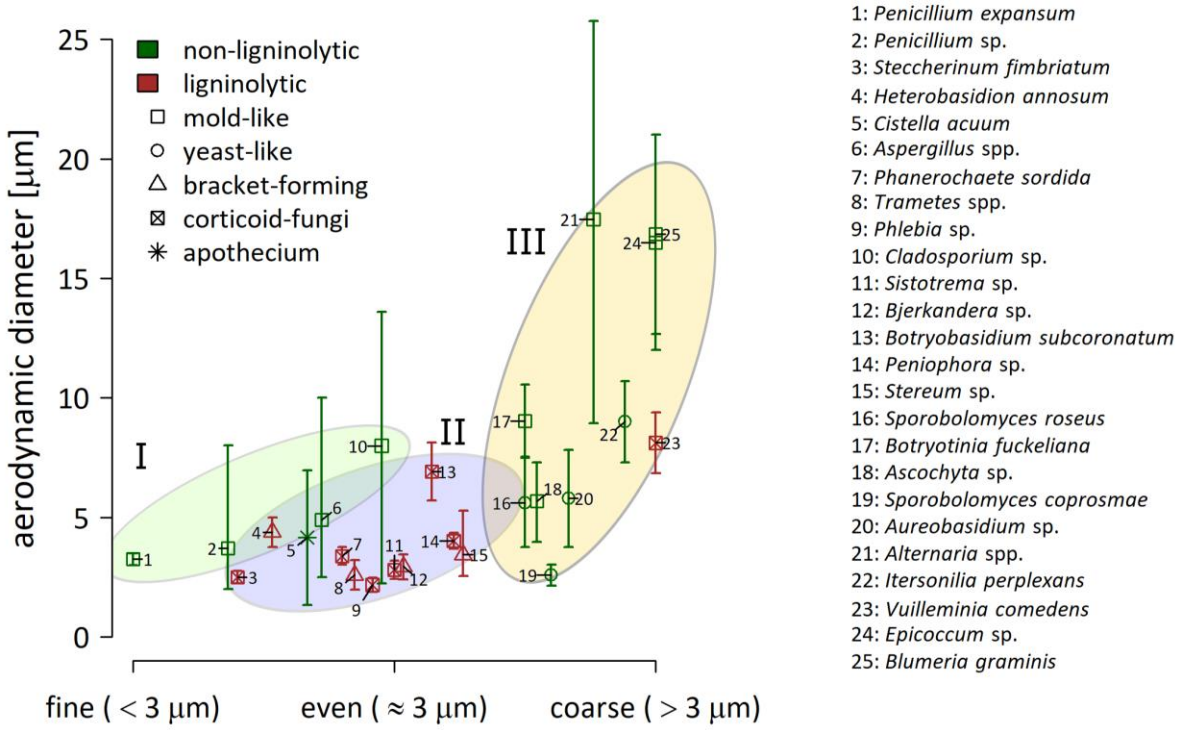

Figure 5: The estimated aerodynamic diameter against the size distribution for the subset fungi. The estimated aerodynamic diameter was calculated using spore dimensions found in literature (see supplementary table S2). The size distribution was calculated by subtracting the number of occurrences in the fine fraction from the occurrences in the coarse fraction and dividing the difference by the TSP occurrences. This shows which size fraction and to what extent the species were predominately found in. The color and point types correspond to the lifestyle categorization displayed in the legend. The shaded ellipses represent three identified clusters. I: hypothesized sedimentation adapted spores; II: hypothesized impaction adapted spores; III: hypothesized forest adapted spores.

The three groups could again reflect different distinct dispersal strategies dependent on the primary environment and preferred substrates of the fungi. In general, a large aerodynamic diameter and therefore higher inertia has the benefit of being able to overcome the laminar airflow close to a plant's surface. This will result in a higher impaction probability compared to small spores that will tend to follow the airflow around the plant's surface. This is reflected by the species within Group II, all of which are plant pathogens, live on plant surfaces or in the case of ligninolytic *Vuilleminia comedens* known to inoculate dying, still-attached branches (Boddy and Thompson 1983).

Group I is exclusively made up of the cosmopolitan mold fungi (*Cladosporium*, *Penicillium* and *Aspergillus*) which are either evenly distributed between the fractions or overweigh in the fine fraction, i.e., display an aerodynamic diameter around or lower than the 3 µm cut-off. These

genera are known to be very unspecific when it comes to substrates and environments. This may be reflected in the small spore sizes. The mold fungi can produce large quantities of small conidia and rely on sedimentation (e.g., on plant litter) for a successful inoculation. Furthermore, the asexual conidia are produced in chains. Dependent on conditions and the species, the mold fungi conidia are known to be liberated in a mixture of single spores and multi-spore chains (Gregory 1961). This leads to a broader aerodynamic size range and thereby does not rule out surface impaction. This may also be a factor playing a role in the even distribution of *Cladosporium* sp. between the fractions.

The ligninolytic fungi and, additionally, *Cistella acuum*, a degrader of pine needles, form Group III, with aerodynamic diameters around the sampler cut off of 3 µm. These fungi should all be concentrated in woody areas. Dead wood is not as ubiquitous as, for example, decaying plant litter, making a sufficient spread, key to a successful inoculation. Furthermore, many tree pathogens, such as *Heterobasidion annosum,* can only infect wounded hosts and then further spread across the root systems. Damaged hosts and tree stumps under normal conditions will be relatively scarce, which would make a longer distance spread of a large number of spores essential. Moreover, the small spores which cluster around 3 µm could be an adaptation to a forest environment. The forest canopy will act as a wind shield resulting in reduced wind speeds and turbulence. A small aerodynamic diameter will aid the spore spread in calmer conditions due to the longer atmospheric residence times in wind conditions that, dependent on forest type, can be reduced by a factor of four (Moon, Duff, and Tolhurst 2016).

In summary we were able to identify lifestyle-dependent patterns temporal and size fraction occurrences which to large part would have remained hidden by solely viewing the dataset from a taxonomic perspective. This approach opens up new possibilities for the analysis of the atmospheric microbiome. For instance, a similar classification scheme could be used as a simplification for larger-scale atmospheric and forecast modelling of bioaerosol emissions, making the source habitat emission estimates over time of parameters like number concentrations and physical characteristics of the different spore types a far less daunting task. We also presented evidence for a significant shift in early year sporulation due to the unusually warm winter of 2006/2007. This should be of concern for diverse fields, such as food security, agriculture and human health, which will be central challenges for the Anthropocene. Further

systematic studies are needed to assess and prepare for potentially far-reaching effects of climate change on the atmospheric microbiome.

Acknowledgements

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Supplementary material

Figure S1 Meteorological factors

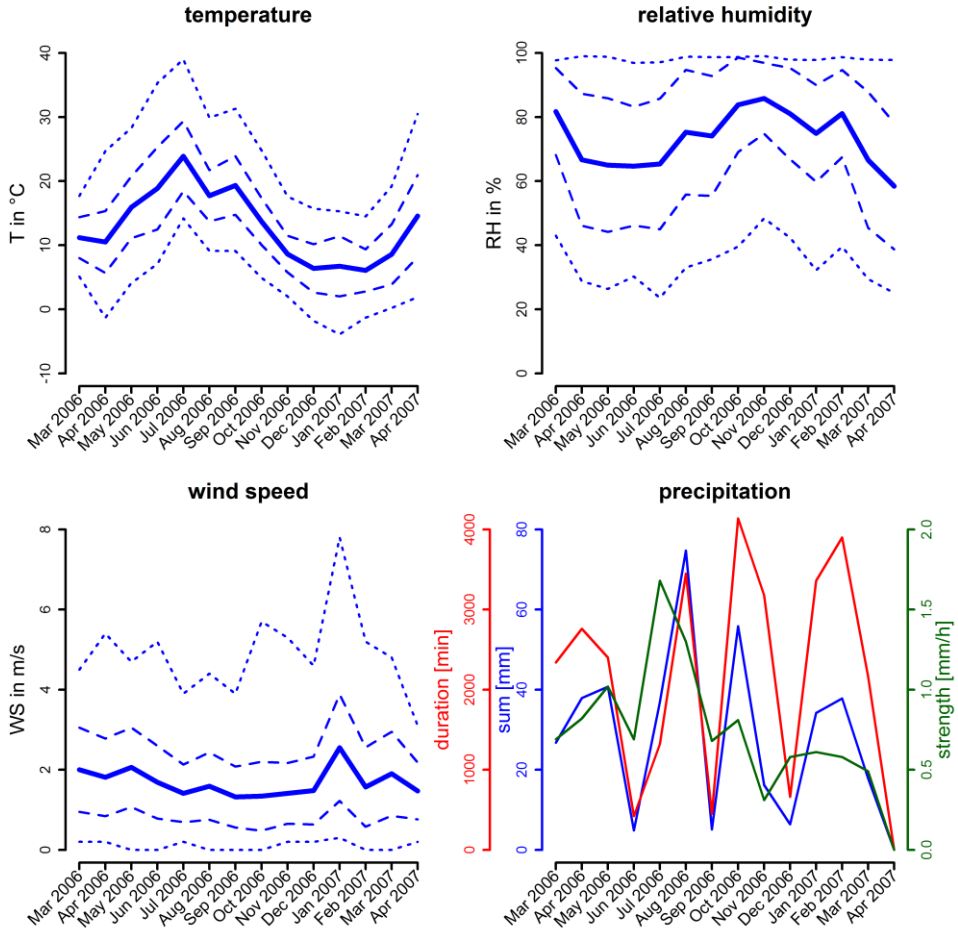

Figure S1: Overview of the meteorological factors used for the correlation analysis seen in figure S2. The values were calculated by grouping the values that lay within the sampling periods of the individual months. For temperature, relative humidity and wind speed the solid lines represent the averages, the dashed lines the standard deviation and the dotted lines the maxima and minima. For precipitation the line colors correspond to the three different y-axes.

Table S1 subset fungi

Table S1: Overview of the subset fungi. Dispersal is main means reproduction. The categories ligninolytic, saprophytic, pathogenic, inhabitant (symbionts or known non-pathogenic plant surface inhabitants) are marked with X if the trait is a significant lifestyle or nutritional type. The taxonomic ranks are from the Catalogue of Life database (Bisby et al. 2010). TSP, coarse and fine fractions are the numbers of samples (coarse and fine fractions combined), coarse and fine fraction occurrences. The OTUs refer to the IDs used in Fröhlich-Nowoisky et al. (Fröhlich-Nowoisky et al. 2009). In many cases taxon spore sizes and information were found in the Mycobank database (Robert et al. 2013) from which the citations were extracted.

Name	fruiting-form	ligninolytic	saprophytic	pathogenic	inhabitant	taxonomy	spore dimensions (l x w) in µm	OTU ID	TSP	coarse	fine	literature spore dimensions
Cladosporium sp.	mold-like		X	X		Davidiellaceae, Capnodiales, Dothideomycetes, Ascomycota	(3 - 35)x(2 - 10)	AMC1	41	35	37	(Al-Doory and Domson 1984)
Botryotinia fuckeliana	mold-like		X	X		Sclerotiniaceae, Helotiales, Leotiomycetes, Ascomycota	(9 - 12)x(7 - 10)	AMC2	24	20	8	(Gilman 1957)
Epicoccum sp.	mold-like		X	X		Pleosporaceae, Pleosporales, Dothideomycetes, Ascomycota	(12 - 21)	AMC3	18	18		(Schol-Schwarz 1959)
Alternaria spp.	mold-like		X	X		Pleosporaceae, Pleosporales, Dothideomycetes, Ascomycota	(18 - 83)x(7 - 18)	AMC5	12	11	1	(Al-Doory and Domson 1984)
								AMC10	9	8	2	

Penicillium sp.	mold-like	X	X	Trichocomaceae, Eurotiales, Eurotiomycetes, Ascomycota	(2 - 8)	AMC8	11	3	10	(Martinez, Calvo, and Ramirez 1982)
Penicillium expansum	mold-like	X	X	Trichocomaceae, Eurotiales, Eurotiomycetes, Ascomycota	(3 - 3.5)	AMC6	12		12	(De Hoog 2000)
Ascochyta sp.	mold-like		X	Not assigned, Pleosporales, Dothideomycetes, Ascomycota	(6 - 16)x(3.4 - 5.6)	AMC7	11	9	3	(Kovachevski 1936)
Blumeria graminis	mold-like		X	Erysiphaceae, Erysiphales, Leotiomycetes, Ascomycota	(25 - 38)x(10 - 17)	AMC9	10	10		(Sperr 1973)
Aureobasidium sp.	yeast-like		X	Dothioraceae, Dothideales, Dothideomycetes, Ascomycota	(7 - 13)x(3 - 6.5)	AMC12	6	5	1	(Kockova-Kratochvilova, Cernakova, and Slavikova 1980)
Cistella acuum	apothecium	X		Hyaloscyphaceae, Helotiales, Leotiomycetes, Ascomycota	(3 - 20)x(1 - 5)	AMC13	6	2	4	(Petersen and Læssøe, n.d.)
Aspergillus spp.	mold-like	X	X	Trichocomaceae, Eurotiales, Eurotiomycetes, Ascomycota	(2.5 - 10)	AMC4 AMC15	13 5	7	7 5	(Noble, Lidwell, and Kingston 1963)

Heterobasidion annosum	bracket	X	X	X	Bondarzewiaceae, Russulales, Agaricomycetes, Basidiomycota	(4.5 - 6.5)x(3.5 - 4.5)	BMC1	30	12	26	(Petersen and Læssøe, n.d.)
Bjerkandera sp.	bracket	X	X	X	Meruliaceae, Polyporales, Agaricomycetes, Basidiomycota	(4 - 5)x(2 - 3)	BMC2	29	22	21	(Shaw and Forest 1988)
Trametes spp.	bracket	X	X	X	Polyporaceae, Polyporales, Agaricomycetes, Basidiomycota	(4.5 - 6.5)x(1.5 - 2.5)	BMC3 BMC10	25 8	16 3	19 5	(Ryvarden and Johansen 1980)
Peniophora sp.	corticioid fungi	X	X	X	Peniophoraceae, Russulales, Agaricomycetes, Basidiomycota	(6.5 - 8)x(3 - 3.5)	BMC4	22	14	9	(Whelden 1936)
Stereum sp.	bracket	X	X	X	Stereaceae, Russulales, Agaricomycetes, Basidiomycota	(5 - 12)x(2 - 4)	BMC5	19	14	9	(Eriksson, Hjortstam, and Ryvarden 1978)
Itersonilia perplexans	yeast-like			X	Cystofilobasidiaceae, Cystofilobasidiales, Tremellomycetes, Basidiomycota	(12.5 - 17)x(6 - 9)	BMC6	17	16	1	(Ingold 1983)
Vuilleminia comedens	corticioid fungi	X	X	X	Corticiaceae, Corticiales, Agaricomycetes, Basidiomycota	(18 - 23)x(5 - 7)	BMC7	13	13		(Bernicchia and Gorjón 2010)
Phlebia sp.	corticioid fungi	X	X		Meruliaceae, Polyporales,	(3.5 - 4.5)x(1.5 - 2)	BMC8	12	6	7	(Bridge Cooke 1956)

						Agaricomycetes, Basidiomycota						
Sporobolomyces roseus	yeast-like				X	Not assigned, Sporidiobolales, Microbotryomycetes, Basidiomycota	(7 - 14)x(3 - 6)	BMC9	8	7	3	(Ramírez Gómez 1957)
Botryobasidium subcoronatum	corticioid fungi	X	X			Botryobasidiaceae, Cantharellales, Agaricomycetes, Basidiomycota	(8 - 12)x(5 - 7)	BMC11	7	5	4	(Donk 1931)
Sistotrema sp.	corticioid fungi	X	X	X	X	Hydnaceae, Cantharellales, Agaricomycetes, Basidiomycota	(2.7 - 3.7)x(2.3 - 3)	BMC12	6	3	3	(Münzenberger et al. 2012)
Sporobolomyces coprosmae	yeast-like				X	Not assigned, Sporidiobolales, Microbotryomycetes, Basidiomycota	(2.5 - 5)x(2 - 2.5)	BMC14	5	4	1	(Hamamoto and Nakase 1995)
Phanerochaete sordida	corticioid fungi	X	X			Phanerochaetaceae, Polyporales, Agaricomycetes, Basidiomycota	(5 - 7)x(2.5 - 3)	BMC15	5	2	3	(Eriksson, Hjortstam, and Ryvarden 1978)
Steccherinum fimbriatum	corticioid fungi	X	X			Meruliaceae, Polyporales, Agaricomycetes, Basidiomycota	(3 - 3.5)x(2 - 2.5)	BMC16	5	1	4	(Bernicchia and Gorjón 2010)

1

Table S2 Statistical abbreviations and formula

Symbol	Definition
BC_{ij}	Bray Curtis Dissimilarity Index between sample i and j
C_{ij}	common OTUs found on sample i and j
$S_{i/j}$	number of OTUs found on sample i or j
	$$BC_{ij} = 1 - \frac{2C_{ij}}{S_i + S_j}$$
$\overline{S}$	coarse-fine ratio
$S_{tot/c/f}$	number of occurrences tot: In total; c: in the coarse fraction; f: *in the fine fraction*
	$$\bar{S} = \frac{S_c - S_f}{S_{tot}}$$
d_{vol}	volumetric equivalent diameter
d_a	aerodynamic diameter
w, l	spore width and length
K	dynamic shape correction factor
q	spore length to width ratio
ϱ_0	unit density (1 g cm^{-3})
ϱ_{spore}	spore density
	$$d_{vol} = (w^2 \times l)^{\frac{1}{3}}$$ $$\kappa = \frac{8}{3}\frac{q^{-1/3}}{\left\{\frac{q}{(q^2-1)} + \frac{1}{\sqrt{q^2-1}}\left[1 - \frac{1}{2(q^2-1)}\right]\ln\left(\frac{q+\sqrt{q^2-1}}{q-\sqrt{q^2-1}}\right)\right\}}$$ $$d_a = \sqrt{\frac{\rho_{spore}}{\rho_0 \kappa}}\, d_{vol}$$
$RFO_{x,y}$	Relative Frequency of Occurrence for taxon x in time period y
$N_{x,y}$	Number of samples tested positive for taxon x in time period y
$N_{tot,y}$	Number of total samples taken in time period y
	$$RFO_{x,y} = \frac{N_{x,y}}{N_{tot,y}}$$

Apendix C4:

Diversity and seasonal dynamics of airborne archaea

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Diversity and seasonal dynamics of airborne archaea

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Abstract. Archaea are widespread and abundant in many terrestrial and aquatic environments, and are thus outside extreme environments, accounting for up to ∼10 % of the prokaryotes. Compared to bacteria and other microorganisms, however, very little is known about the abundance, diversity, and dispersal of archaea in the atmosphere. By means of DNA analysis and Sanger sequencing targeting the 16S rRNA (435 sequences) and *amoA* genes in samples of air particulate matter collected over 1 year at a continental sampling site in Germany, we obtained first insights into the seasonal dynamics of airborne archaea. The detected archaea were identified as *Thaumarchaeota* or *Euryarchaeota*, with soil *Thaumarchaeota* (group I.1b) being present in all samples. The normalized species richness of *Thaumarchaeota* correlated positively with relative humidity and negatively with temperature. This together with an increase in bare agricultural soil surfaces may explain the diversity peaks observed in fall and winter. The detected *Euryarchaeota* were mainly predicted methanogens with a low relative frequency of occurrence. A slight increase in their frequency during spring may be linked to fertilization processes in the surrounding agricultural fields. Comparison with samples from the Cape Verde islands (72 sequences) and from other coastal and continental sites indicates that the proportions of *Euryarchaeota* are enhanced in coastal air, which is consistent with their suggested abundance in marine surface waters. We conclude that air transport may play an important role in the dispersal of archaea, including assumed ammonia-oxidizing *Thaumarchaeota* and methanogens.

1 Introduction

Archaea have long been thought to occur only in restricted, extreme environments. However, since their formal recognition as an independent domain of life about two decades ago (Woese et al., 1990), they have been found in a wide variety of habitats from hydrothermal vents to aquatic and soil environments (Auguet et al., 2010; Bintrim et al., 1997; Boetius et al., 2000; Jurgens et al., 2000; Ochsenreiter et al., 2003; Schleper et al., 1997; Takai et al., 2001). Archaea are now known to be abundant, diverse, and widespread organisms that act as major players in the nitrogen and carbon cycle (Offre et al., 2013; Pester et al., 2012; Schleper et al., 2005).

Despite the recognition of the ubiquitous nature of archaea, their possible presence in the atmosphere is poorly investigated. While the atmosphere may not be a key habitat of archaea, it may act as the primary medium for the dispersal of microorganisms among the different ecosystems on the Earth's surface (Fröhlich-Nowoisky et al., 2012). For other

bioaerosols such as bacteria and fungi, studies have been conducted that describe the composition, diversity, abundance, seasonal, and even daily variation of these organisms in the atmosphere, and explore their distribution and biogeography (e.g., Després et al., 2012; Fröhlich-Nowoisky et al., 2012; and references therein). These matters are still unanswered for archaea.

The few published reports on airborne archaea are from anthropogenic environments such as compost piles or biosolids (e.g., Baertsch et al., 2007; Moletta et al., 2007; Thummes et al., 2007) and a handful of natural and urban environments (Bowers et al., 2013; Brodie et al., 2007; Cao et al., 2014; Cho and Hwang, 2011; Radosevich et al., 2002; Robertson et al., 2013; Smith et al., 2013; Yooseph et al., 2013). The extent of these studies remains very limited, and the number of airborne archaea sequences presented in the literature is small. Thus, the first insights into the presence of archaea in the atmosphere could draw only very preliminary conclusions about the composition from single sites and specific sampling times. In particular, nothing is known about which metabolic groups of archaea are present in the air.

Archaea have various types of metabolisms, some of which are involved in key biogeochemical processes like the production of methane, the assimilation of amino acids, or the oxidation of ammonia (Cavicchioli, 2011; Pester et al., 2011). Nitrification is a central process of the global nitrogen cycle that can be divided into two steps: the oxidation of ammonia to nitrite and the oxidation of nitrite to nitrate. Ammonia-oxidizing bacteria and archaea (AOB and AOA, respectively) use the enzyme ammonia monooxygenase (encoded by the *amoA* gene; Falkowski et al., 2008; Gruber and Galloway, 2008; Kowalchuk and Stephen, 2001) to perform the conversion to nitrite, which is the rate-limiting step. Since the discovery of the importance of the *amoA* gene, it has been widely used to explore the diversity and abundance of AOA, even though the presence of the gene does not necessarily prove the potential for ammonia oxidation (Pester et al., 2011). Thus, *amoA*-gene-based studies have allowed for a better understanding of the ecological and evolutionary factors shaping the community assembly in AOA and the factors that drive community distribution (Auguet et al., 2010; Cao et al., 2011; Cavicchioli, 2011).

The main purpose of this study was first to determine whether airborne archaea are present all year round, and whether they display seasonal dynamics. Furthermore, we wanted to test the hypothesis of whether the composition of airborne archaea depends on the type of ecosystem found below the air masses. For the first purpose, we gathered samples for 1 year in continental boundary layer air at one sampling site and identified archaeal communities by sequencing the 16S rRNA and the *amoA* gene. For the second objective, we compared the results of the continental air site with discrete sampling from other geographical regions, including both coastal and continental air, to understand the composition of airborne archaea under both a local and a more global perspective.

2 Material and methods

2.1 Aerosol sampling

As it is well established that decontamination of sampling devices is a necessary prerequisite for reliable molecular genetic analysis (Després et al., 2007), the quartz and glass fiber filters used were baked at high temperatures to eliminate possible biological traces. To secure a continuous quality assurance, we regularly cleaned the samplers with ethanol to prevent colony growth in the interiors and checked the air flow rates weekly. For some sampling sites (e.g., Germany), we separated the particles into coarse and fine mode, while for other sites or campaigns (e.g., North America) total suspended particulate matter was analyzed. As small variations occur between the different sampling sites within the sampling strategies, the specific details are given per site. With the main analyses focusing on the samples from Germany and Cape Verde only, the detailed sampling strategies for these sites are presented here; the information for the other sites is available in the Supplement. However, for an overall comparison, the main sampling information is listed in Table 1 and Supplement Table S1.

2.1.1 Germany

Over 1 year, 47 pairs of fine and coarse particle samples were collected in Mainz, Germany (March 2006–April 2007). A self-built high-volume dichotomous sampler (Solomon et al., 1983) was used, which was operated with a rotary vane pump (Becker VT 4.25) at a total flow rate of $\sim 0.3\,m^3\,min^{-1}$, corresponding to a nominal cutoff diameter of $\sim 3\,\mu m$. Thus, coarse particles with an aerodynamic diameter larger than $\sim 3\,\mu m$ were collected on one glass fiber filter ($\sim 0.03\,m^3\,min^{-1}$), while the fine particles from the same air sample were collected on a second glass fiber filter ($\sim 0.27\,m^3\,min^{-1}$). The sampling period was generally ~ 7 days, corresponding to a sampled air volume of $3000\,m^3$. A few samples were collected over shorter periods (volumes of ~ 400–$2000\,m^3$). The sampling station was positioned on a mast about 5 m above the flat roof of the three-story-high Max Planck Institute for Chemistry building (MPIC) on the campus of the University of Mainz (49°59′31.36″ N, 8°14′15.22″ E). The sampled air masses represent a mix of urban and rural continental boundary layer air in central Europe. All glass fiber filters were baked overnight at 500 °C prior to sampling, and the loaded filters were sealed in decontaminated aluminum foil and stored at −80 °C until analysis.

To detect possible contaminants from the sampler and sample handling, blank samples were taken at 4-week intervals. Prebaked filters were mounted in the sampler like for

Table 1. Overview and diversity parameters of aerosol filter samples and detected archaea. Sampling details of continental and coastal sites are given: number of air samples, aerosol size range (total suspended particles (TSP) and particulate matter (PM)), and obtained number of DNA sequences. Statistical parameters: species richness (S measured, S^* estimated), Shannon index (H'), Shannon evenness (E), and Simpson's index (D), not available (n.a.; see also Table S3). The measured species richness S in North America, China, and UK needs to be interpreted with caution as only few sequences were available.

	Continental		Coastal		
Sampling region	Germany	North America	Cape Verde	China	UK
Elevation	130 m	2370 m	69 m	21 m	0 m
Above ground	20 m	1 m	14 m	1.5 m	40 m
Latitude	49°59′31.36″ N	39°6′0″ N	14°55′33.96″ N	23°32′52.7994″ N	52°57′02″ N
Longitude	8°14′15.22″ E	105°5′30″ W	−23°29′40.92″ W	113°3′57.6″ E	1°07′19″ E
Samples	47, 47	20	26	14	12
Sampling duration	Mar 2006–Apr 2007	July 2011	Feb 2011–Mar 2011	Jul 2006	spring 2004
Filter type	glass	glass	quartz	quartz	glass
Average flow rate	0.3 m^3 min^{-1}	1 m^3 min^{-1}	1 m^3 min^{-1}	1 m^3 min^{-1}	1.120 m^3 min^{-1}
Size range	< 3 µm, > 3 µm	TSP	PM_{10}	TSP	< 4.49 µm
Sequences	435	20	72	7	11
S	57	1	25	1	4
S^*	137	n.a.	67	n.a.	n.a.
H'	3.32	n.a.	3.07	n.a.	n.a.
E	0.82	n.a.	0.95	n.a.	n.a.
D	0.05	n.a.	0.05	n.a.	n.a.

regular sampling, but the pump was either not turned on at all ("mounting blanks") or only for 5 s ("start-up blank").

2.1.2 Cape Verde

Filter sampling was part of the Cape Verde Dust project (CV-Dust). The sampling station was positioned ∼ 1500 m from the coast at 69 m elevation, latitude 14°55′33.96″ N, longitude 23°29′40.92″ W. For the 26 Cape Verde samples (Table 1 and Supplement Table S1), Whatman quartz filters were used and sampling was done between February and March 2011 with a PM_{10} high-volume sampler (Tisch PM10, TE-6001) placed 14 m above ground level and with a flow rate of 1 m^3 min^{-1}. Sampling periods ranged from less than 24 h to 3 days. Prior to sampling, filters were decontaminated by baking for 6 h at 500 °C, and after sampling they were stored in decontaminated bags and kept frozen at −80 °C until DNA extraction. The air at this site is primarily coastal with influence from continental air masses, which may carry particles from Saharan or Sahel dust events.

2.2 DNA extraction and amplification

Filter sample aliquots (about 1–25 % of the filter) were extracted with a commercial soil DNA extraction kit (Lysing Matrix E, FastDNA SPIN Kit for Soil, MP Biomedicals) according to the supplier's instructions with the following modifications (depending on the character of the filter): centrifugation (10–15 min) after the lysis, followed by addition of 900 µL buffer provided by the extraction kit and a second round of beating and centrifugation. The supernatants of both extraction steps were combined for the further extraction process. Finally, the DNA was dissolved in 100 µL elution buffer. Decontaminated filters were included during the extractions as extraction blanks. Extraction blanks without filters were prepared as well.

For the laboratory work, regular quality control and assurance measurements were taken. All polymerase chain reactions (PCRs) were set up with sterile equipment in a DNA-free environment. As it was difficult to amplify airborne archaea (see also Supplement), we used three primer pairs as well as nested primers to increase the amplification success. Thus, with the DNA extract from each of the filters, up to four PCRs were performed using normal and nested primer pairs targeting the 16S rRNA gene. The number of PCRs was dependent on the successful amplification. To detect even minimal amounts of archaeal DNA, an additional PCR and eventually nested PCRs were only performed if the first PCR failed. The 25–50 µL reaction mixture contained the template DNA (0.5–1 µL sample extract), 1 × PCR buffer, 0.2 mM each dNTP (Roth), 0.33 µM each primer (Sigma Aldrich), 2 mM $MgCl_2$, and 2.5 units of JumpStart REDTaq DNA polymerase. A negative control was included in all PCR runs.

The thermal profile of the 16S PCR was as follows: initial denaturing at 94 °C for 3 min, 34 cycles with 94 °C for 30 s, annealing at 55 °C for 1 min for primer pair A2F/958r (Baker and Cowan, 2004; Wani et al., 2006) or at 52 °C for 20 s for primer pair 109f/934r (Grosskopf et al., 1998), elongation at 72 °C for 1.5 min, and a final extension step

at 72 °C for 5 min. Nested PCRs were done with primer pair 109f/934r and primer pair 109f/927r (Baker and Cowan, 2004; Grosskopf et al., 1998), respectively. The thermal profile for both pairs was as follows: initial denaturing at 94 °C for 2 min, 29 cycles with 94 °C for 30 s, annealing at 52 °C for 1 min, elongation at 72 °C for 1 min, and a final extension at 72 °C for 10 min. A contamination occurred within one extraction blank in the samples of Cape Verde. The PCR product was cloned and sequenced and the sequences grouped into operational taxonomic units (OTUs). The OTUs occurring in this extraction blank PCR were completely removed from the analysis, and thus also from any other filter sample.

An *amoA* analysis was performed for 18 coarse filters from Mainz, Germany (Table S1). For the archaeal *amoA* gene, the primer pair amo19F–amo643R with the nested primer pair amo111F–amo643R was used (Leininger et al., 2006; Treusch et al., 2005). The PCR mix was as described above. The PCR consisted of an initial denaturation at 94 °C for 4 min, 9 cycles at 94 °C for 45 s, annealing at 55–65 °C for 1 min, elongation at 72 °C for 1 min and 19 cycles at 94 °C for 45 s, 55 °C for 1 min and 72 °C for 1 min, and a final extension step at 72 °C for 10 min.

Amplification products of each filter sample selected for sequencing were cloned using the TOPO TA Cloning Kit (Invitrogen) following the supplier's instructions. Colonies containing inserts were identified by blue–white selection and lysed in 20 µL water for 10 min at 95 °C. The cloning efficiency was between 5 and 200 colonies per cloned PCR product. The inserts of 12–24 randomly picked colonies were amplified for each PCR product using 1.5–3 µL lysate in a 25–40 µL reaction. If fewer colonies were available, all colonies were amplified and sequenced.

2.3 Sequence and phylogenetic analysis

DNA sequences were determined with ABI Prism 377, 3100, and 3730 sequencers (Applied Biosystems) using BigDye Terminator v3.1 chemistry at the Max Planck Genome Centre, Cologne. To assure quality, we studied the individual chromatograms for background noise and cut the vector sequences. Sequences were screened for possible chimera using the Bellerophon program (Huber et al., 2004). The alignment was conducted using the MUSCLE (multiple sequence comparison by log expectation) package (Edgar, 2004) and thereafter manually checked. The taxonomic identification was only based on sequence data. The sequences from each sampling site were clustered into OTUs, which represent artificial species. For the 16S rRNA sequence, the similarity of sequences within a species is thought to be at least 97 % (Stackebrandt and Goebel, 1994). As the PCR products can be inserted during the cloning process into the vector sequence in both directions and the sequencing was conducted from one direction, the reads sometimes cover different sections of the analyzed sequence or they only have a few base pairs of overlap. Thus, for the identification of OTUs, sequences were grouped together according to their sequence coverage to provide the longest possible length for an automatic analysis with the Mothur software package (Schloss et al., 2009). Sequence blocks were cut to the longest possible length to retain as many sequences as possible. For Germany this was ∼ 370 base pairs (bp), for North America ∼ 470, for China ∼ 600, and for the UK ∼ 760. Sequences that were too short for the chosen blocks were compared manually to the other sequences to determine whether they fitted into one of the automatically identified OTUs or formed a new one. OTUs and their reference sequences were determined with the Mothur package. For the sequences from Cape Verde, OTUs were created by using the USEARCH v5.2.3.2 package (Edgar, 2010) at the 97 % discrimination level and manual BLAST procedures.

For the phylogenetic identification of the OTUs, two approaches were used. Firstly, a broad taxonomic identification by comparing the sequences of interest with the sequences in well-known databases and, secondly, a more detailed analysis by calculating phylogenetic trees. The results from the comparison against databases were used to identify potentially closely related archaea strains. From these strains we then used type species to build a basic tree for the phylogenetic tree analysis. The basic tree sequences are listed in Table S2.

For these comparisons, with known sequences, both the Basic Local Alignment Search Tool (BLAST), via the website of the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/), and the SINA aligner (Pruesse et al., 2012), which includes several microbial databases, were used.

For the phylogenetic analysis, a basic tree was constructed that mainly included sequences from cultured, identified type species and a few from uncultured species (Table S2). For identification the representative sequences of each OTU were then included. For OTUs where the representative sequences did not overlap the area used for the tree calculation, the best match sequences were used as placeholders, but only when the similarity between the sequence and its best match was higher than 97 %. The model of evolution best suitable for the sequence sets was chosen using the "find best DNA/protein model" option in Mega5.2.2 (Tamura et al., 2011). Phylogenetic trees were constructed by means of the maximum likelihood algorithm using Tamura's three-parameter substitution model with gamma distributed rates for the analysis of the 16S sequences and 1000 replicates for the boot strap values. Additionally, we also tested the neighbor-joining algorithm, which agreed with maximum likelihood results.

The *amoA* gene DNA sequences were likewise aligned with MUSCLE. OTUs were formed when the sequence similarity scores were > 85 % according to Pester et al. (2012). Phylogenetic trees were built on the nucleotide level based on Tamura's three-parameter model as described above and on the amino acid level using the WAG+G+F model (Whelan and Goldman, 2001).

The sequences of the present study have been deposited in the GenBank database under the following accession numbers: for 16S rRNA genes KF683446–KF683516 (Cape Verde), KF558331–KF558337 (China), JQ249390–JQ249751 (Germany), JQ976019–JQ976037 (North America), KF558321–KF558330 (United Kingdom), and for the *amoA* gene sequences (KF824904–KF824908).

2.4 Quantitative PCR assays

All quantitative PCR (qPCR) reactions were performed on an iCycler thermocycler equipped with a MyiQ detection system (Bio-Rad, Munich, Germany) and the data were analyzed using the iQ3 Optical System software (Bio-Rad). As a standard for archaea, a pure culture of *Methanosarcina thermophila* was used (Lueders et al., 2004), and for the bacteria, chromosomal DNA of *Escherichia coli* K12 (Stubner, 2002) was used. Both standards were serially diluted and used for the construction of calibration curves in each reaction. Duplicates were analyzed for the standards, and triplicates for the filter extracts.

For the actual quantification process the following primer pairs were chosen: for archaea 364f (5′-CGG GG(CT) GCA (GC)CA GGC GCG AA-3′) and 934r (5′-GTG CTC CCC CGC CAA TTC CT-3′) and for bacteria 519f (5′-CAG C(AC)G CCG CGG TAA (AGCT) (AT)C-3′) and 907f/926r (5′-CCG TCA ATT CMT TTR AGT TT-3′), as commonly used (Burggraf et al., 1997; Grosskopf et al., 1998; Lane, 1991). The qPCR assays were based on SYBR Green (Ambion). Each reaction had a volume of 25 µL and the following mixture for archaea (conditions for bacteria in brackets): 12.5 µL SYBR Green JumpStart Taq ReadyMix, 1.5 (4) mM $MgCl_2$, 0.66 (0.5) µM of each primer, and 5 µL of the template DNA and 1 µM FITC. For the assay, the program used was as follows: 94 °C for one cycle for 6 (8) min, followed by 45 (50) cycles at 94 °C for 35 s (20 s), 66 °C (50 °C) for 30 s (20 s), 72 °C for 45 s (50 s), and 85.5 °C (75 °C) for 6 s for signal reading.

2.5 Statistical analysis

Although the number of the here analyzed Sanger sequences is low compared to modern next-generation sequencing techniques, we performed statistical analyses to get a first indication of species richness and possible correlations with meteorological conditions. However, species richness measurements were only performed for the sites with most sequences (Germany and Cape Verde). Correlation analyses with meteorological conditions were only performed for Germany, where most sequences were analyzed.

To assess whether there were significant correlations between the diversity of sampled archaea and the meteorological factors, the relative species richness per sample j ($S_{R,j}$), as well as the relative species richness per month k ($S_{R,k}$), was calculated (see Table S3). The statistical analysis was performed using the R package (R Development Core Team, 2005). Pearson's linear regression coefficients were calculated between the relative species richness and the sampling period or monthly averages for temperature, relative humidity, and wind speed, as well as for the sum of precipitation per sample, to assess the influence of the single factors on the sampled diversity.

To examine whether a combination of meteorological factors correlates best with the sampled diversity, the stepwise AIC method (Akaike information criterion) for variable selection (Yamashita et al., 2007) was conducted. A more detailed linear regression analysis (Neter et al., 1996) was then performed on the best results of the Pearson and stepwise AIC test. A more detailed description of the analysis can be found in the Supplement.

3 Results and discussion

3.1 Abundance and diversity of airborne archaea in continental boundary layer air

From the 47 air filters sampled at Mainz, Germany (Table S1), we retrieved a total of 435 archaeal 16S rRNA gene sequences that grouped into 57 OTUs. The aerosols at this site were sampled by means of size selection with a cut point at 3 µm. Archaea sequences could be amplified in all coarse samples (> 3 µm), but only in 21 % of the fine particle filter samples (< 3 µm). To better understand this phenomenon, we quantified copies (cp) of archaea and bacteria 16S rRNA genes in a subset of six air filter samples. We found the abundance of archaea in air to be only between ~ 1 and ~ 10 gene copies per cubic meter of air, while that of bacteria was found to be $\sim 10^4$ to $\sim 10^6$ in the same air samples (Table S4). These results are strikingly different from observations in soil and ocean surface water, where archaea represent about 10 % of the total prokaryotic abundance (Bates et al., 2011; Cao et al., 2012; DeLong, 1992; Karner et al., 2001; Kemnitz et al., 2007; Yin et al., 2013). One of the explanations might be that archaea could be more susceptible to UV light or other damage caused by, for example, components from the fine aerosol than bacteria. However, the fact that archaea were more numerous in coarse particle filters may indicate that archaea are often attached to soil dust particles as it also has been shown for bacteria (Jones and Harrison, 2004). The observation that archaea are more difficult to amplify than other airborne microbes has been stated in other publications as well (Bowers et al., 2009, 2013; Fierer et al., 2008; Woo et al., 2013; Woo, 2012).

This study gives first insights that airborne archaea were present all year long in air from the continental boundary layer. Bioaerosols in air have a lifetime of about a week (Burrows et al., 2009), implying that the aerosols sampled may have traveled several thousand kilometers. Therefore, this study's data represent not only a snapshot of the actual

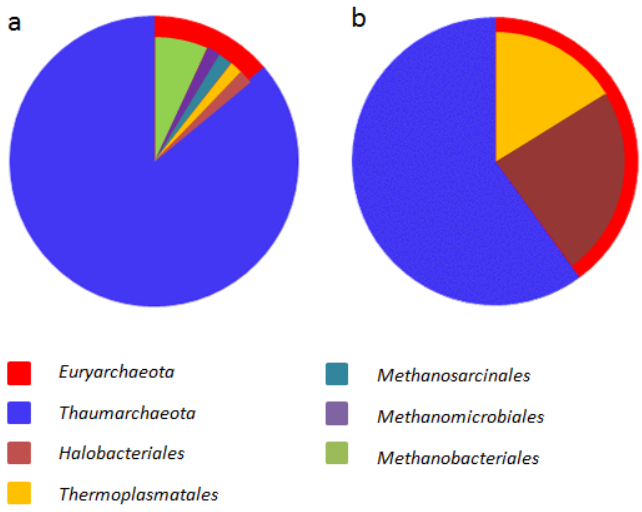

Figure 1. Species richness of airborne archaea. Relative proportions of different phyla (*Thaumarchaeota* and *Euryarchaeota*) and, within the *Euryarchaeota*, of different orders for continental air, Mainz **(a)**, and coastal air, Cape Verde **(b)**, respectively.

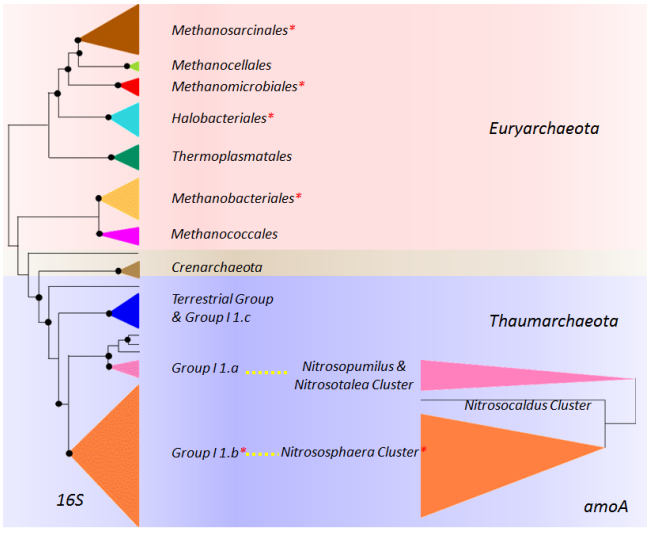

Figure 2. Phylogeny of *Euryarchaeota* and predicted ammonia-oxidizing *Thaumarchaeota*. Schematic illustration of a collapsed maximum likelihood tree based on the OTU sequences found in Mainz, Germany, in the 16S rRNA gene (left) and archaeal *amoA* gene (right), as well as basic tree sequences (Table S2). Black dots represent boot strap values > 80 %. Red stars indicate where OTUs from airborne archaea (Mainz, Germany) fall. The *amoA* cluster names are given according to Pester et al. (2012); the figure has been laid out as in Pester et al. (2011).

diversity but also include information about possible sources and distribution patterns.

All archaeal 16S rRNA gene OTUs at the German sampling site belonged either to *Euryarchaeota* (14 %) or *Thaumarchaeota* (86 %; see also Fig. 1a and Supplement Fig. S1, Table S5). *Thaumarchaeota* are widespread on Earth (Pester et al., 2011) and are detected in various environments such as freshwater, ocean, sediments, and hot springs, with an especially high abundance in soil (Bates et al., 2011; Brochier-Armanet et al., 2008, 2011; Leininger et al., 2006; Ochsenreiter et al., 2003; Schleper and Nicol, 2010). Based on phylogenetic analysis, all OTUs of the *Thaumarchaeota* were affiliated with the 16S rRNA sequences from the soil group I.1b (Fig. S1, Table S5). Group I.1b archaea were composed of a high number of related phylotypes (Fig. S1), similar to what is observed in soils (Auguet et al., 2010).

In addition to the 16S rRNA gene, the phylogenetic relationships of the *amoA* gene, which is used as a reliable marker to study the diversity of archaeal ammonia oxidizers, was also analyzed (Cao et al., 2011, 2013; Junier et al., 2010). The *amoA* gene was successfully detected on a subset of 18 coarse particulate matter samples, spanning all four seasons (Table S1). The 176 archaeal *amoA* sequences grouped into 5 OTUs. Thus, the diversity of archaeal *amoA* genes was lower than that of archaeal 16S rRNA genes. Strengthening the results from the analysis of archaeal 16S rRNA OTUs, all *amoA* OTUs belonged to the genus *Nitrososphaera*, affiliated with the group I.1b of soil archaea identified using the nucleotide (Fig. 2) and the amino acid sequences. Both findings support observations made for airborne bacteria showing that airborne microbial diversity is closely linked to soil diversity, and that soil and soil dust might be a primary source of airborne microorganisms (Brodie et al., 2007; Després et al., 2007, 2012; Fierer et al., 2008; Jones and Harrison, 2004; Lighthart and Shaffer, 1995; Prospero et al., 2005).

In comparison to *Thaumarchaeota*, the number of OTUs of *Euryarchaeota* was low. Euryarchaeotal 16S rRNA gene sequences were present in 32 % of the samples and attributed to five well-known orders. *Halobacteriales*, *Thermoplasmatales*, *Methanomicrobiales* and *Methanosarcinales* were represented by a single OTU each, while 50 % of the OTUs belonged to the *Methanobacteriales* (Figs. 1a and S1, Table S5). The presence of predicted methanogens may be surprising, as Ochsenreiter et al. (2003) suggested that oxic soils support only the *Thaumarchaeota* group I.1b. However, a recent study by Angel et al. (2012) proved that methanogenic *Euryarchaeota*, despite being obligate anaerobic, are present in oxic soils and regain their methanogenesis activity when deprived of oxygen. Other studies finding methanogens in soil attributed their presence to the effect of livestock introducing methanogens into the soil with their feces (Gattinger et al., 2007; Nicol et al., 2003; Radl et al., 2007). Similar to *Thaumarchaeota*, *Euryarchaeota* in air are likely to originate from aerosolized soil or other emission sources.

Thaumarchaeota had a normalized species richness (S_n, number of detected OTUs divided by number of investigated samples; Table S3) of approx. 2 to 3 throughout the year (Fig. 3a), with OTU *Arch1* being the most frequent

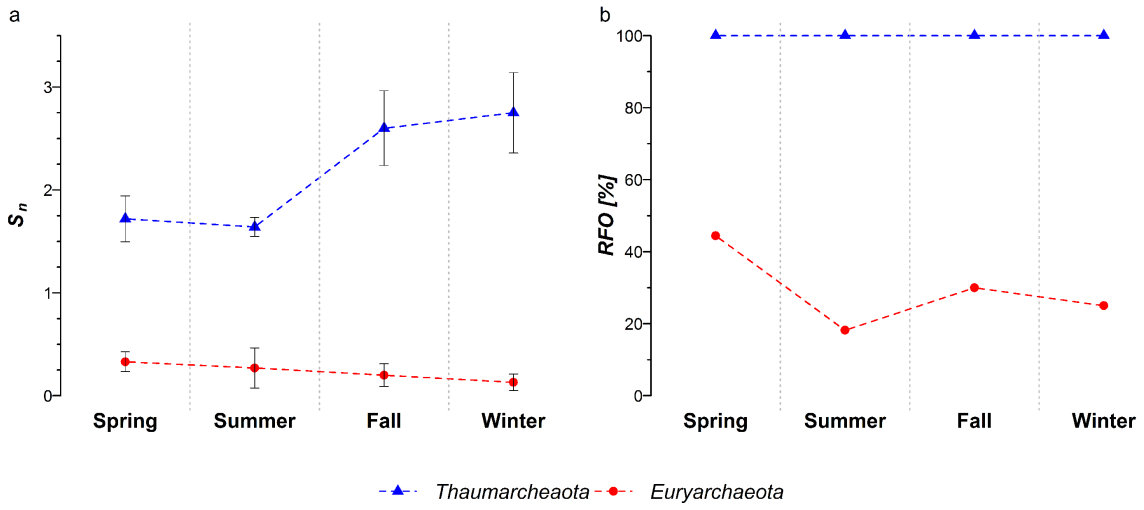

Figure 3. Overview of the species richness in Mainz. Seasonal variations in the species richness of *Euryarchaeota* and *Thaumarchaeota* in total suspended particles (TSP) normalized by the number of investigated air samples **(a)** and in the relative frequency of occurrence (RFO) of the phyla *Euryarchaeota* and *Thaumarchaeota* in Mainz, Germany **(b)**.

OTU present in 70 % of the samples. In contrast, the S_n of *Euryarchaeota* was always below 1, and the two most frequent OTUs (*Arch12* and *Arch13*) were detected in only 11 % of the investigated air samples. Based on the Chao-1 index (Table S3), we obtained an estimate of $\sim$ 137 OTUs for the actual species richness of archaea in the investigated air samples (see also Supplement for detailed discussion). This species richness is only about 50 % of that reported by Brodie et al. (2007) for two urban sites in North America. However, in urban air, the composition and abundance of aerosol microbes seems to be unique. Bacteria, for instance, are often released from strong point sources (Després et al., 2012; Fang et al., 2007; Shaffer and Lighthart, 1997), and this may also be true for archaea. While the number of investigated samples and DNA amplification products used in this study certainly underestimates the actual diversity of archaea in air, Fröhlich-Nowoisky et al. (2009) detected 368 fungal species in a slightly smaller but otherwise identical data set.

3.2 Seasonally and meteorologically derived dynamics of airborne archaea

Seasonal variation within the species richness of *Thaumarchaeota* shows highest diversity in the fall/winter period is illustrated in Fig. 3a. The sampling site in Germany is situated in an area with extensive agriculture. After the harvest of particular grains or rapeseed at the end of summer, fields either remain bare over fall and winter or are prepared, for example, by plowing and tilling of the upper 10–30 cm for seeding. Soil turnover might influence the number of *Thaumarchaeota* available for aerosolization, as the surface soil is enriched with *Thaumarchaeota*, and their frequency decreases with depth (Jia and Conrad, 2009; Leininger et al., 2006).

As meteorological conditions are also known to influence airborne communities, we compared the statistical variance of archaeal diversity in relation to average wind speed, temperature, relative humidity, and the sum of precipitation, since temperature and relative humidity have already been found to correlate with airborne fungal composition (Fröhlich-Nowoisky et al., 2009). On a short-term samplewise basis, we found that the relative species diversity of archaea correlates significantly with wind speed (negative correlation, p value $= 0.01$; Table S6, Fig. S2a). On a long-term monthly basis, multiple linear correlation analysis revealed that the normalized species richness (S_n; Fig. 4a) negatively correlated with both temperature and wind speed, while the relative species richness ($S_{R,k}$ Fig. 4b) showed a significant positive correlation with relative humidity and wind speed, although wind speed played a secondary role in both cases (p value $(S_n) = 0.02$ and $(S_{R,k}) = 0.01$). These findings coincide with our results that archaea diversity is highest in the fall/winter period, as temperature decreases during that time in the sampling regions. In fall and winter relative humidity also increases. At this point, however, no answer can be given regarding whether the meteorological factors are causing the diversity change, or whether the correlations are merely coincidental with the diversity and meteorology following similar annual cycle and other factors, e.g., increased aerosolization sources, cause the phenomenon. However, as illustrated in, for example, Fig. 4a between December 2006 and March 2007, the successive steep rise and fall of S_n is predicted rather accurately by the linear model, indicating that the correlation might not be coincidental.

Although S_n stayed constant for *Euryarchaeota* throughout the year, the relative frequency of occurrence (RFO) – that is, the proportion of samples in which these species

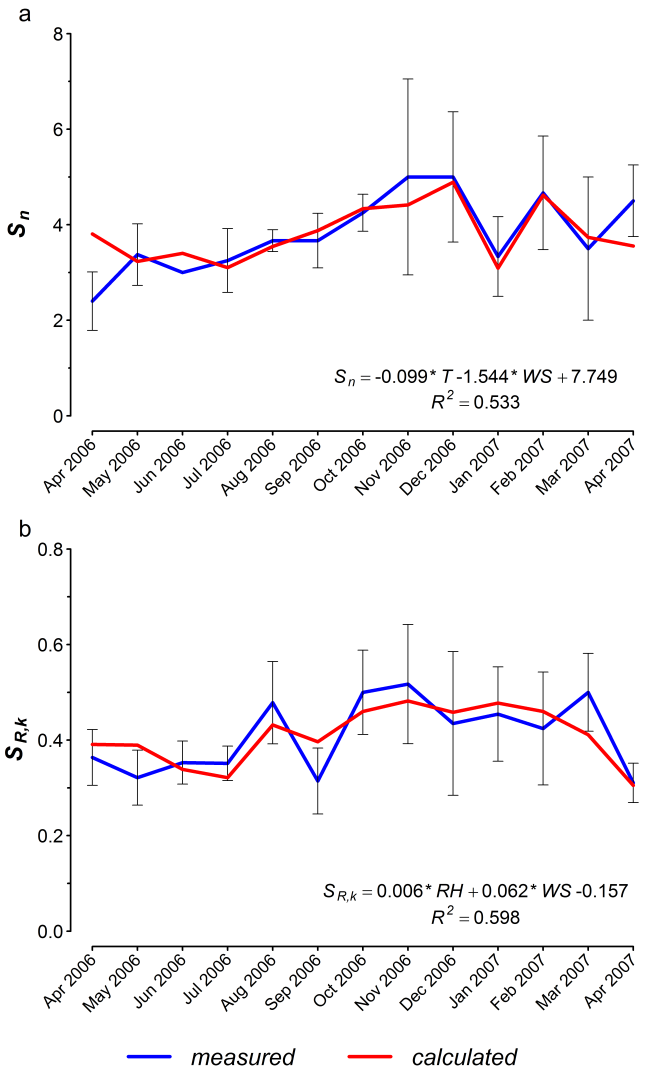

Figure 4. Time series depicting the most significant results from a multiple linear regression analysis between two diversity parameters – normalized species richness per month (S_n) and relative species diversity per month ($S_{R,k}$) – and meteorological factors. The observed S_n and $S_{R,k}$ curves (blue) were calculated with the equations given in Table S3. The modeled S_n and $S_{R,k}$ curves (red) were calculated using the monthly averages of the corresponding meteorological factors in the equations depicted in the plots. **(a)** Time series of S_n. Best variable subset: T and WS (p value = 0.011), with error bars being $SE_n(S_n)$. **(b)** Time series of $S_{R,k}$. Best variable subset: RH and WS (p value = 0.022), with error bars being $SE_n(S_{R,k})$. RH: average relative humidity [%]; WS: average wind speed [m s^{-1}]; T: average temperature [°C].

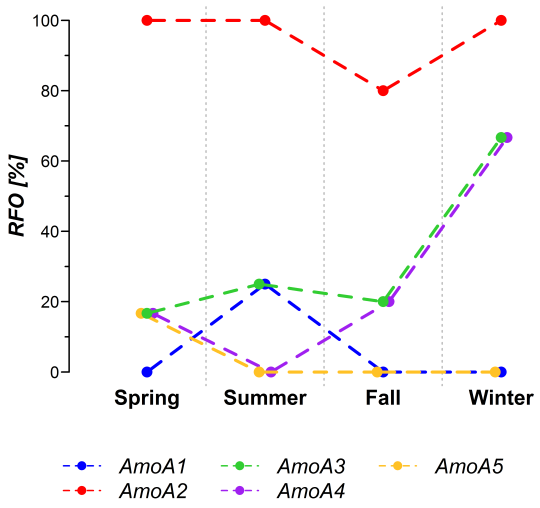

Figure 5. Seasonal variations in the relative frequency of occurrence (RFO) of the individual *amoA* OTUs detected in Mainz, Germany.

were detected – exhibited variations (Fig. 3b). In spring, *Euryarchaeota* were present in almost 50 % of the air samples, while over the rest of the year they were found in only 20–30 % of them. A constant source of *Euryarchaeota* could be the nearby river Rhine, as *Methanomicrobiales* and *Thermoplasmatales* can be found in freshwater (Auguet et al., 2010; Cao et al., 2013; Galand et al., 2006) and might thus be aerosolized. The increase in the RFO in spring may be linked to anthropogenic activities like fertilization of the soil. Although methanogens have been shown to be present in low numbers in aerated soils (Angel et al., 2012), the increase in the here-predicted methanogens in spring might be due to fields being fertilized with manure, and thus their methanogen composition may be affected (Gattinger et al., 2007; Nicol et al., 2003; Radl et al., 2007). All of the *Euryarchaeota* detected here are known to be common in livestock manure (St-Pierre and Wright, 2013).

3.3 Ammonia-oxidizing archaea in air

Within the air samples analyzed over a whole year, a total of five OTUs were discovered among the *amoA* gene sequences. As depicted in Fig. 5, two OTUs were present throughout the year, while the others were detected only sporadically. From comparison of the two most common OTUs, *AmoA2* showed a high relative frequency of occurrence, while the other was always below 60 %. Within our air filter samples we found that the 16S rRNA gene OTU *Arch1* and *amoA* OTU *AmoA2* occur on 94 % of the filter samples together, while no other OTUs showed such a high co-occurrence. This co-occurrence suggests that both genes may actually have been amplified from the same microorganisms. This observation confirms that the *Nitrososphaera amoA* cluster may be equivalent to the 16S rRNA cluster I.1b, as already indicated by other evidence (Bartossek et al., 2012; Pester et al., 2012).

The composition of *amoA* OTUs in the sampled air seems to be a combination of OTUs that are present throughout the year and others that occur only sporadically. OTUs that occur over the entire season might originate from a constant source like the river Rhine or sparsely vegetated soil, as it has been

Table 2. Summary of published airborne archaea sequence information. Sampling location, the most likely source of the air masses, the number of reported OTUs, the information as to which archaeal phyla they were attributed to, and the sequencing technique performed. * Another study by Bowers et al. (2013) analyzed bacteria and fungi in air collected at the Colorado Front Range over a 14 month period with the Illumina HiSeq technique. Within this study, archaea were amplified as well; however, no detailed information is provided.

Location	Air mass	OTUs	*Euryarchaeota* (%)	*Thaumarchaeota* (%)	Other (%)	Technique	Publication
North America (Salt Lake City, Utah)	Continental	3	0	100	0	Sanger sequencing	Radosevich et al. (2002)
Middle America (St Antonia & Austin, Texas)	Continental/urban	307	70	15	15	Microarray	Brodie et al. (2007)
Asia (East Korea)	Coastal	1	100	0	0	Sanger sequencing	Cho and Hwang (2011)
North America (Mt. Bachelor)	Transpacific plume	11	82	0	18	Microarray	Smith et al. (2013)
Asia (Beijing)	Maritime influence	–	0.8	–	–	Hiseq Illumina	Cao et al. (2014)
Europe (Mainz, Germany)	Continental	58	14	86	0	Sanger sequencing	This study
North America (Colorado)	Continental	1	0	100	0	Sanger sequencing	
Africa (Cape Verde)	Coastal	25	40	60	0	Sanger sequencing	
Europe (UK)	Coastal	4	100	0	0	Sanger sequencing	
Asia (China)	Coastal	1	100	0	0	Sanger sequencing	

shown that AOA are present in rivers, freshwater environments, and soils (Cao et al., 2013; Liu et al., 2011; Pouliot et al., 2009). In contrast, OTUs that occur only sporadically might originate from unique sources like localized spreading of compost.

The presence of different *amoA* OTUs in air could indicate that air might serve as a medium to distribute AOA globally. In this case, fertilization, which is known to decrease AOA frequency and diversity in comparison to AOB (He et al., 2012; Xu et al., 2012), might also decrease the spreading of AOA to some extent.

3.4 Comparison between locations

Initial analyses suggest that the composition of airborne microorganisms shows biogeographic patterns, especially between continental, coastal, and marine sites, but also across different land-use types, though only a few studies have considered or attempted to study this phenomenon by means of molecular or cultural methods (e.g., Bovallius et al., 1978; Bowers et al., 2011; Després et al., 2007; Fröhlich-Nowoisky et al., 2012; Shaffer and Lighthart, 1997; Womack et al., 2010).The comparison between sampling sites can in general be challenging when different sampling and analysis procedures are used. Although the local geographic and meteorological conditions need to be taken into account in a comparison between sites, the height at which the samples were taken for this study is negligible as the continental boundary layer air up to 1000 m is fairly well mixed and various atmospheric measurements can be compared. We thus compared archaea diversity detected in the continental air of Mainz with the archaea diversity we discovered in an additional subset of 26 air filter samples from coastal air in Cape Verde, which were collected in winter 2011. The approximately 6 m difference in sampling height is negligible. The air of Cape Verde represents typical coastal air and is influenced by a combination of marine and continental air masses. The 72 sequences detected in Cape Verde air were separated into 25 different OTUs. Both *Euryarchaeota* and *Thaumarchaeota* were present (Fig. 1b, Table S5).

While 86 % of the OTUs were *Thaumarchaeota* in Mainz, only 60 % could be affiliated with this archaeal group in Cape Verde. Out of the *Thaumarchaeota* OTUs detected in Cape Verde, 73 % were identical to OTUs detected in Mainz, and more than 50 % were discovered in several air samples. This might imply that the airborne *Thaumarchaeota* of Cape Verde disperse on a larger scale. However, the few OTUs abundant in Germany were discovered only at the Mainz site. This might indicate that OTUs that are abundant and present throughout the year at this site are dispersed locally and do not originate from far away.

Euryarchaeota in Cape Verde were not very diverse and only contained *Halobacteriales* and *Thermoplasmatales* (Fig. 1b). In Germany, in addition to one OTU of *Halobacteriales*, different methanogens were found. The lack of methanogens in Cape Verde and the dominance of a limited set of *Euryarchaeota* may be due to the relatively stronger influence of the marine environment compared to terrestrial ecosystems impacted by small-scale agriculture. Interestingly, we did not find *Euryarchaeota* from the marine group II, which are typical for ocean surface layers (Galand et al., 2009; Hugoni et al., 2013; Massana et al., 1997). Although the results for Cape Verde are based on a preliminary data set only, our results might point to general differences in the composition of airborne archaea in continental versus coastal air.

To follow up on the hypothesis that the composition of airborne archaea depends on the type of ecosystem found beneath the air masses, samples from other continental and coastal sites were investigated. We successfully amplified archaea from 1 of 20 random filter samples taken in Colorado (Huffman et al., 2013). The 20 sequences obtained from this filter all fell into one OTU which was identical to the 16S rRNA gene OTU *Arch31*, a *Thaumarchaeota*, also found in Mainz. In China and the United Kingdom we tried to amplify archaea 16S rRNA gene sequences from 14 and 12 filter samples, respectively, taken from coastal sites with influences from marine and continental air. We could only successfully amplify archaea 16S rRNA gene sequences from one filter sample at each site. All seven sequences from the

Chinese site belonged to one OTU, which was identical to *Arch16*, a predicted methanogen, observed in Mainz air. In the UK sample set, we amplified 11 sequences from a filter on which primarily air masses from the North Sea were sampled (Gysel et al., 2007). These sequences grouped into four OTUs, all of which belonged to the order *Thermoplasmatales* (*Euryarchaeota*). These results support the observations from Cape Verde that the diversity of *Euryarchaeota* in coastal samples might be higher and their composition different compared to continental samples.

We also compared our results with the scarce literature available for airborne archaea (Table 2). Continental airborne archaea from Salt Lake City (Radosevich et al., 2002) belonged to *Thaumarchaeota*, supporting the hypothesis that *Thaumarchaeota* prevail in continental air. Using microarray techniques targeting 16S rRNA genes, Brodie et al (2007) counted, in two cities in Texas, 30 % *Crenarchaeota* (under the assumption that these would be assigned to *Thaumarchaeota* in a reanalysis) and 70 % *Euryarchaeota*. However, the influence of marine air from the nearby Gulf of Mexico cannot be judged. To our knowledge, there are no sequences of airborne archaea from coastal sites available in the literature. However, Cho et al. (2011) found one single archaea OTU in marine air from the East Sea (Korea), which was identified as belonging to the *Halobacteriales* (*Euryarchaeota*). Using microarrays, Smith et al. (2013) found within the very few archaea detected in air influenced by marine air masses that 82 % of the OTUs belonged to the *Euryarchaeota*. As the number of available sequences and literature is limited, more analyses need to be done to confirm or reject the hypothesis about biogeography in airborne archaea.

4 Conclusions

With this study we gained first insights into the seasonal behavior of airborne archaea diversity, composition, and abundance in a continental, agriculture-dominated sampling site. The measured abundance of airborne archaea (1 to 10 16S rRNA gene copies per cubic meter of air) was much lower than the abundance of airborne bacteria (10^4–10^6 cp m^{-3}) and the ratio of archaea to bacteria was orders of magnitudes lower than in soil and marine environments. We show that the diversity of common soil *Thaumarchaeota* (I.1b), which are present throughout the year, correlates positively with relative humidity and negatively with temperature. This, together with the increased number of bare agricultural fields after harvest being available for aerosolization processes, may lead to the observed diversity peaks in fall and winter. In general, the diversity of the *amoA* gene was much lower than in the 16S rRNA gene. The OTU *AmoA1* correlates with *Arch1*, the most abundant OTU from the 16S region. This could point to associations of an *amoA* OTU with an archaeal 16S rRNA gene OTU. The few predicted methanogenic *Euryarchaeota* found show no seasonal trends in diversity but a slightly increased relative frequency of occurrence in spring, when fields are fertilized with manure. The comparison of this continental site with airborne archaea at a coastal site in Cape Verde indicates differences in community composition and that the diversity of *Euryarchaeota* might increase in coastal air. These results were supported by data from additional sampling sites in the United Kingdom, China, and North America, and also concurred with the very limited information available in the literature.

We conclude that air as a transport medium might play an important role in habitat and niche formation for archaea. Thus, the analysis of archaea diversity, abundance, and composition in air is important in order to better understand their behavior and evolutionary history in their primary ecosystems.

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Diversity and seasonal dynamics of airborne archaea

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Supplement Text

Aerosol sampling at additional sites

China

Samples of total suspended particles (TSP) were collected on quartz fiber filters with a high-volume filter sampler (Anderson Instruments, Smyrna, GA; 1.5 m above the ground, sample air flow 1 m^3 min^{-1}; sampling time ~24 h) during the Program of Regional Integrated Experiments of the Pearl River Delta Region (PRIDE-PRD) Campaign in July 2006 in Backgarden (21 m elevation, 23°54'80.56'' N, 113°06'63.89'' E, South China). Prior to sampling the 14 filters were decontaminated at 500 °C for at least 12 h. The samples were stored in a freezer at −80 °C until DNA extraction. The sampling site was situated in a rural farming environment ˜60 km northwest of the megacity Guangzhou on the edge of the highly populated PRD region. Due to the prevailing monsoon circulation at the time of sampling, the marine air masses came mainly from the south to southeast, making this site a rural receptor site for the regional pollution resulting from the outflow of the urban cluster around Guangzhou (Garland et al., 2008; Rose et al., 2010).

North America

The sampling site was located in a part of the Manitou Experimental Forest in a semi-arid, montane ponderosa pine zone in the Central Rocky Mountains 35 km northwest of Colorado Springs, CO and 15 km north of Woodland Park, CO (2370 m elevation, latitude 39°6'0" N, longitude 105°5'30" W). Total aerosol samples for DNA analysis were collected during the BEACHON-RoMBAS campaign in July 2011 onto 150 mm diameter glass fiber filters (Machery-Nagel, Type MN 85/90, 406015) using a free-standing high-volume sampler (Digitel DHA-80) operated at 1 m^3 min^{-1}, 1 m above the ground and located approximately 50 m from the sampling trailer. Filters were baked at 500 °C for 12 h prior to sampling. After sampling, filters were stored in decontaminated aluminum bags at -80 °C until DNA extraction. Twenty samples were tested for Archaea presence. The sampled air represents rural continental boundary layer air (Huffman et al., 2013).

United Kingdom

Samples on glass fiber filters (Graseby Andersen Hi-Vol six-stage impactor, sample air flow 1.120 m^3 min^{-1}, sampling time 21–35 h) were provided by the School of Earth, Atmospheric, and Environmental Sciences, University of Manchester, United Kingdom (UK). The 12 sam-

ples were collected as part of the Tropospheric ORganic CHemistry (TORCH) field campaign during spring 2004. Prior to use, the glass fiber filters were decontaminated by baking, and the loaded filters were transported frozen and stored in a freezer at −20 °C until DNA extraction. The TORCH2 campaign took place at the Weybourne Atmospheric Observatory (WAO, 52°57'02'' N, 1°07'19'' E), which is located on the North Norfolk coastline near Weybourne, UK. Norfolk is a sparsely populated rural region without large population centers or industrial areas. As detailed by Gysel et al. (2007) the air masses encountered at this station represent aged polluted outflow from London, the West Midlands or the European continent, or relatively clean air masses transported across the North Sea region by northerly winds.

Quantitative analysis of airborne Archaea

In contrast to other airborne microorganisms (e.g., Fungi, Fröhlich-Nowoisky et al., 2009 and Bacteria (data not shown)), which were studied from the identical air filter samples, the amplification of airborne Archaea 16S rRNA gene sequences was more challenging and PCR products were often only produced in a nested PCR or not at all. This was also true for air filter samples from China, the United Kingdom, and North America which were analyzed successfully for Fungi (Fröhlich-Nowoisky et al., 2012; Huffman et al., 2013) while Archaea could only be amplified for some of the samples (this study).

One explanation might concern the primer choice. Primers that are supposed to cover Archaea or subgroups might not have 100% identity across all Archaea lineages (Klindworth et al., 2013; Nicol and Prosser, 2011). In addition, Archaea taxonomy is continuously increasing due to the discovery of new species. However, the primers used for amplification in this study have been used successfully for soil samples (Angel et al., 2012; Grosskopf et al., 1998). To increase amplification success we also used a combination of 3 primer pairs as well as nested PCR as described in section 2.2. The primer pairs used amplify Archaea sequences from several taxonomic lineages, thus it can be assumed that they would equally amplify at least these lineages in air filter samples. Therefore, it is more likely that the Archaea lineages were either not present in the air or only in very limited amounts. To judge and compare Archaea to Bacteria abundance on the air filter samples we quantified Archaea and Bacteria by performing qPCR assays including a standard with a correlation coefficient of 0.98 in Bacteria and 0.99 in Archaea and PCR efficiencies of 102.6% in Bacteria and 91.5% in Archaea.

We compared the abundance of airborne Archaea and Bacteria with those in environments that are potential bioaerosol sources (see material and methods, Table S4). Although

only very rough estimates can be made as concentrations vary between regions, depth, temperatures, seasons etc., in general Archaea represent about ~10% of the total prokaryotes quantities in ocean surface water as well as in normal soil (Bates et al., 2011, Cao et al., 2012; DeLong, 1992; Karner et al., 2001; Kemnitz et al., 2007; Yin et al., 2013,).

In one kg of soil the copy numbers of Archaea 16S rRNA genes range, very roughly, between ~10^9 to 10^{11} while Bacteria have between ~10^{11} to 10^{12} copies (Table S4). *Thaumarchaeota AOA* often even prevail over *AOB* (Leininger et al., 2006, Pereira e Silva et al., 2012). For air, our results show that Bacteria were easily quantifiable and varied between 10^5 and 10^6 16S rRNA gene copies per m^3 of air, which is close to abundances measured for ocean surface water and in agreement with other studies (Bauer et al., 2002, Burrows et al., 2009, Harrison et al., 2005, DeLeon-Rodriguez et al., 2013). In contrast, Archaea 16S rRNA gene copies were often below the detection limit (< 10 copies), and varied approximately between 1 and 10 copies per m^3 air. Although the quantification is based on six filter samples only, Archaea are likely orders of magnitude lower than the Bacteria in the same air masses.

Supposing that soil is the primary source of airborne Archaea and Bacteria, the questions remains why Archaea are so difficult to amplify in air, or are not present in amplifiable quantities, while Bacteria are. It might be that Bacteria have important sources other than soils (e.g., plant surfaces), which Archaea might not have. Another possibility might be that Archaea are more susceptible to UV damage or other damaging compounds associated with fine particulate matter than Bacteria, or decompose faster after sampling. Thirdly, in contrast to Bacteria, Archaea might occur in larger particles and thus deposit faster from the air. However, the explanations can currently only be speculative and future studies are needed. The observation that airborne Archaea are low in number has also been made by Woo and colleagues (2013), and our observation that airborne Archaea are difficult to amplify in general is also consistent with the experience other researchers have made (Bowers et al., 2009, 2013; Fierer et al., 2008; Woo et al., 2013; Woo, 2012).

The difficulty to obtain DNA extracts that yielded enough archaeal DNA to be amplified, also limited the number of samples that could successfully be analyzed. We calculated rarefaction curves for the air filter samples from Mainz and Cape Verde which agree with our assumptions that although we exhausted laboratory possibilities the gained data can currently only serve as a first rough estimate of the actual species richness of Archaea in air.

Statistical analysis of airborne Archaea diversity with meteorological parameters

Meteorological conditions are also known to influence airborne communities and bioaerosol composition (Despres et al., 2012 and references therein), e.g., temperature and relative humidity have been found to correlate with airborne fungal composition (Fröhlich-Nowoisky et al., 2009). We thus compared the diversity of airborne Archaea (the normalized (S_n) and relative species richness ($S_{R,i,}$, see material and methods)) with meteorological parameters, such as averaged wind speed, temperature, relative humidity and the sum of precipitation for the individual sampling periods. To discriminate between short and long term influences, we performed linear correlation analyses on a sample to sample basis and also on a monthly basis.

In an initial step, the influence of single meteorological factors was assessed. The Pearson coefficients were calculated between the diversity parameters, $S_{R,j,}$ S_n and $S_{R,k}$, and the meteorological factors to preselect possible dependencies for further testing. The results of the averaged meteorological parameters can be seen in Table S6. Due to the nature of environmental samples and the limitations of the applied methods a high variance was expected within the diversity parameters. To account for this, relatively low Pearson coefficient boundaries, of +/- 0.3 for the sample to sample diversity parameter ($S_{R,i}$) and +/- 0.5 for the a monthly diversity parameters (S_n and $S_{R,k}$), were set as a threshold for further testing. The higher boundary for the monthly parameters was chosen as these are based on more data, thus reducing the expected intrinsic error.

Detailed single factor linear regression analyses were then performed on promising candidates. The results can be seen in Table S7 and Figures S2a, S3.

In a second step to assess the dependency of the diversity parameters on combined meteorological factors, multiple factor linear regression analyses were performed. To preselect the best combinations of factors, the best factor subsets were selected using the AIC method. Detailed linear regression analyses were then performed, the results of which can be seen in Table S7 and Figures S2b and 4 of the main text.

On a sample to sample basis, $S_{R,i}$ showed a significant negative correlation to average wind speed (p-value=0.012; Figure S2a). The incorporation of additional variables also revealed a negative correlation to wind speed with a positive correlation to relative humidity (p-value=0.015; Figure S2b).

While on the short term the influence of wind speed seems to play an important role for the observed diversity, on a monthly basis the Pearson's coefficients showed that relative humidity correlated best with diversity. The best single factor for both, S_n and $S_{R,k}$, was found to be relative humidity which in both cases was positively correlated (p-values=0.05 and 0.005; see Figure S3a and b). A negative correlation to temperature was also shown by $S_{R,k}$ (p-value=0.049; See Figure S3c). The multiple linear correlation analysis revealed that S_n negatively correlated with both average temperature and wind speed, while $S_{R,k}$ showed a significant positive correlation with average relative humidity and wind speed, though wind speed in both variable sets played a secondary role (p-value (S_n)=0.02, p-value ($S_{R,k}$)=0.01; Figure 4, main text).

The sampling site is situated in an area which has a seasonal cycle with lower temperatures and high relative humidity in the fall/winter period. Also, on average the wind speed increases due to the Westerlies prevailing at this time of the year. Thus, the observed positive correlation with relative humidity, as well as the negative correlation with temperature on a long term basis, coincides with our finding that the Archaea diversity increases in the fall/winter period. However, it is not possible at this point to determine whether the meteorological parameters influence the diversity of Archaea directly. For the soil environment it has been found that Archaea diversity reacts to changes in soil temperature and soil moisture (Rasche et al., 2011) thus meteorological parameters could indirectly due to their effect on soil conditions, influence the Archaea diversity. On the other hand the diversity could just be influenced by the changing accessibility of aerosolization sources, e.g., availability of bare, harvested fields. In this case the meteorological parameters would not cause changes in diversity, but only be characteristic for the season when the number of bare fields increases.

Supplement Figures S1-S3

Figure S1a

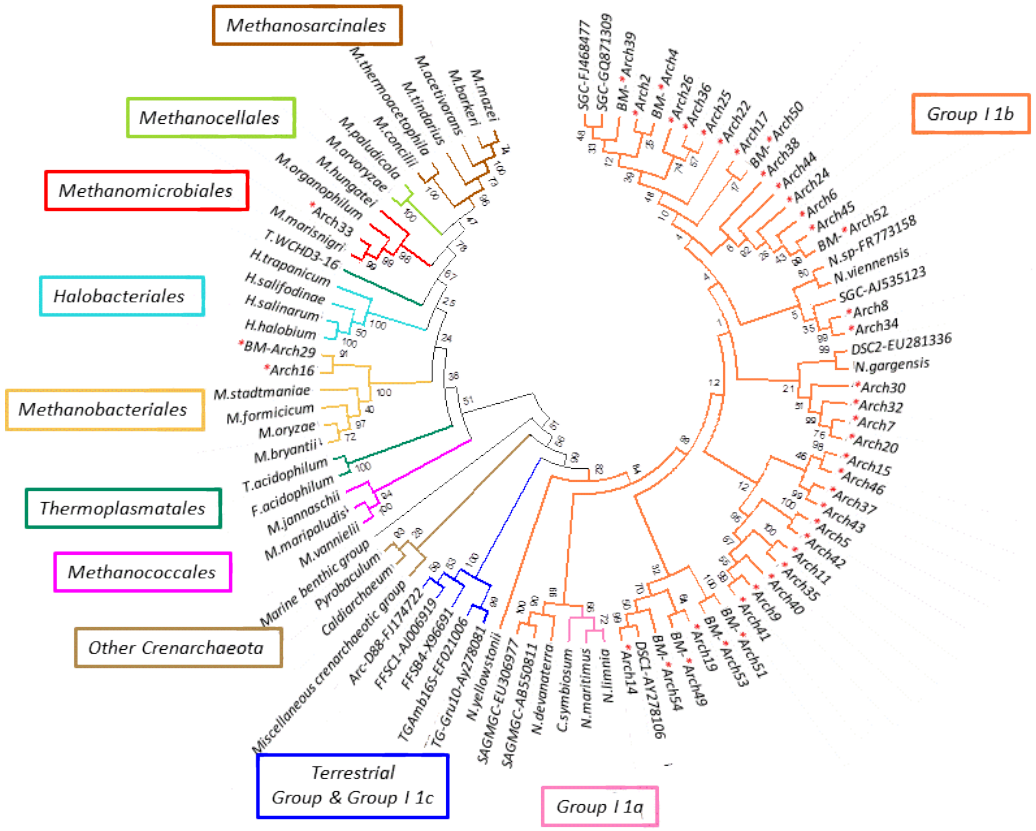

Figure S1b

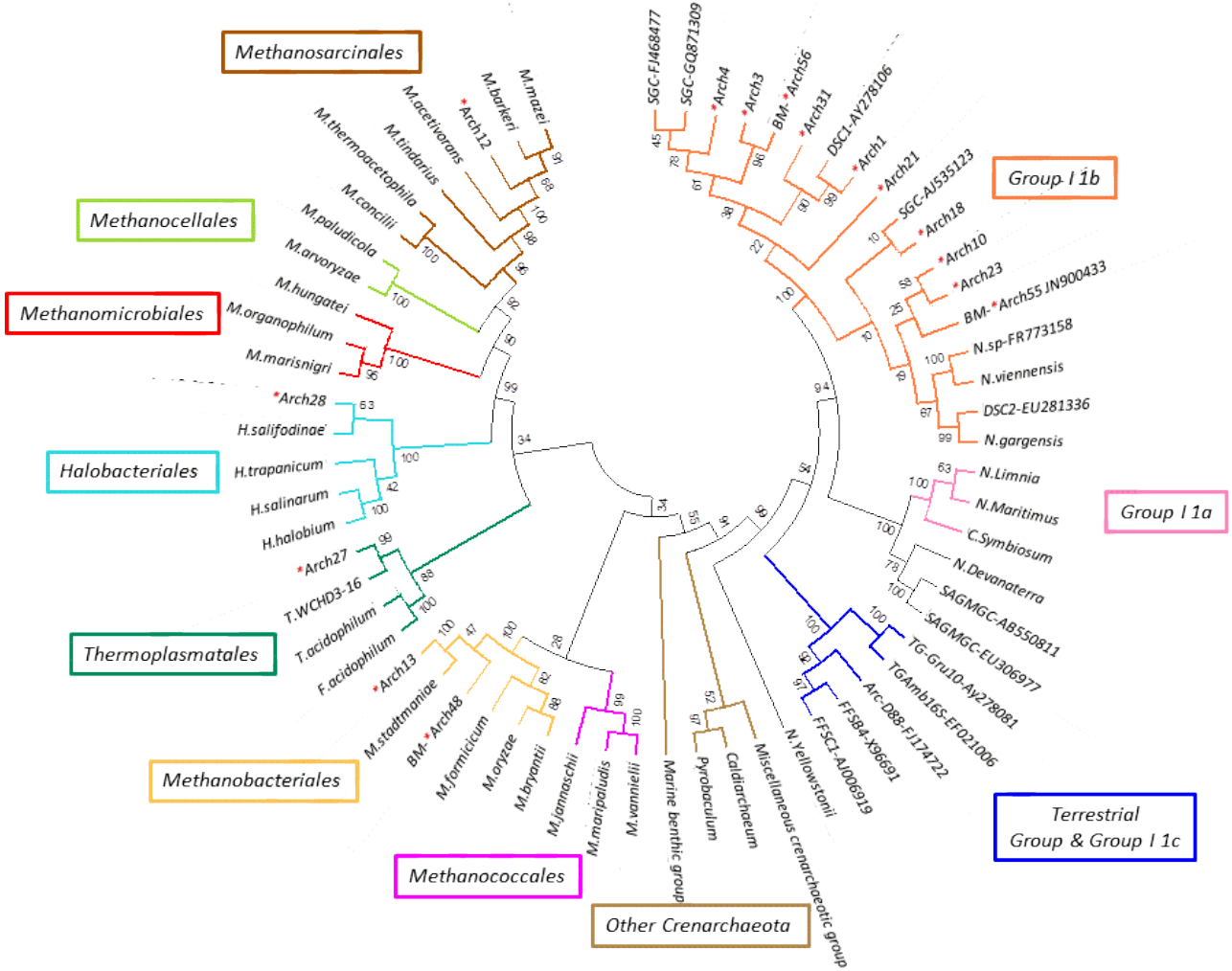
Methanosarcinales
Methanocellales
Methanomicrobiales
Halobacteriales
Thermoplasmatales
Methanobacteriales
Methanococcales
Other Crenarchaeota
Terrestrial Group & Group I 1c
Group I 1a
Group I 1b
M.mazei
M.barkeri
*Arch12
M.acetivorans
M.tindarius
M.thermoacetophila
M.concilii
M.paludicola
M.arvoryzae
M.hungatei
M.organophilum
M.marisnigri
*Arch28
H.salifodinae
H.trapanicum
H.salinarum
H.halobium
*Arch27
T.WCHD3-16
T.acidophilum
F.acidophilum
*Arch13
M.stadtmaniae
BM-*Arch48
M.formicicum
M.oryzae
M.bryantii
M.jannaschii
M.maripaludis
M.vannielii
Marine benthic group
Pyrobaculum
Caldiarchaeum
Miscellaneous crenarchaeotic group
N.yellowstonii
FFSC1-AJ006919
FFSB4-X96691
Arc-D88-FJ174722
TGAmb16S-EF021006
TG-Gru10-Ay279081
SAGMGC-EU306977
SAGMGC-AB550811
N.Devanaterra
C.Symbiosum
N.Maritimus
N.Limnia
N.gargensis
DSC2-EU281336
N.viennensis
N.sp-FR773158
BM-*Arch55 JN900433
*Arch23
*Arch10
*Arch18
SGC-AJ535123
*Arch21
*Arch1
DSC1-AY278106
BM-*Arch56
*Arch31
*Arch3
*Arch4
SGC-GQ871309
SGC-FJ468477

Figure S1: Phylogenetic Tree of airborne Archaea in Germany. A Maximum Likelihood Tree was calculated for the published and obtained sequences of this study representing known phylogenetic lineages (given here species names). They are also summarized in Table S2. Two different sequence parts of the 16S region were used, depending on the coverage by the OTUs of the airborne Archaea (a, b). Different orders are given in different colors. Red stars mark the presence of airborne Archaea of Mainz.

Figure S2a

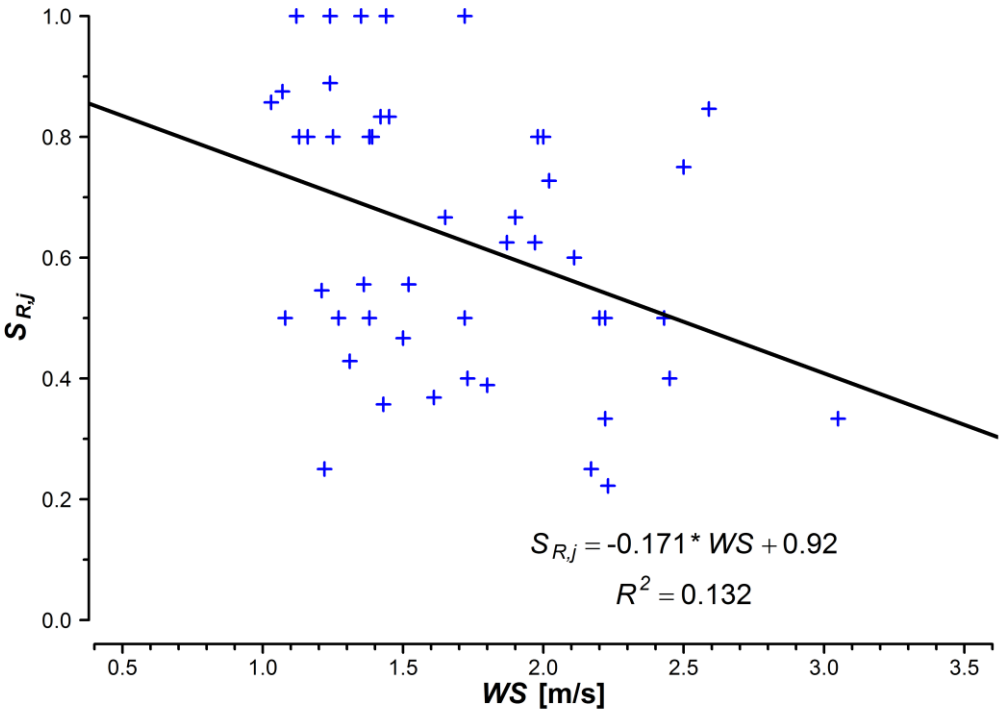

Figure S2b

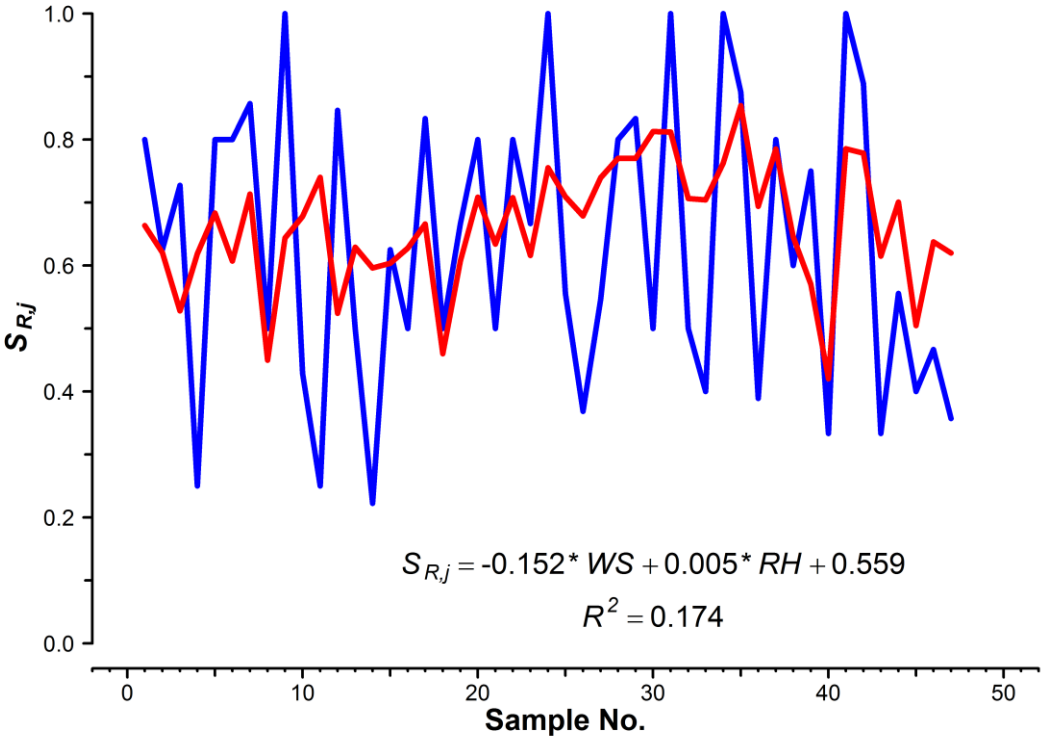

Figure S2: The most significant results of the linear regression analysis between the relative species diversity per sample ($S_{R,j}$) and meteorological factors. (a) Best single factor: $S_{R,j}$ as a function of wind speed (p-value = 0.012). (b) Best variable subset (*WS* and *RH*): The sample IDs given on the x-axis correspond to the sampling time given in Table S1. The values for the observed $S_{R,j}$ curve (blue) were calculated using the equation given in Table S3. The modeled $S_{R,j}$ curve (red) was estimated using the depicted equation that was obtained by regression analysis (p-value = 0.015) including the *RH* and *WS* values measured during each individual sampling period. *WS*: average wind Speed [m/s]; *RH*: average relative humidity [%].

Figure S3a

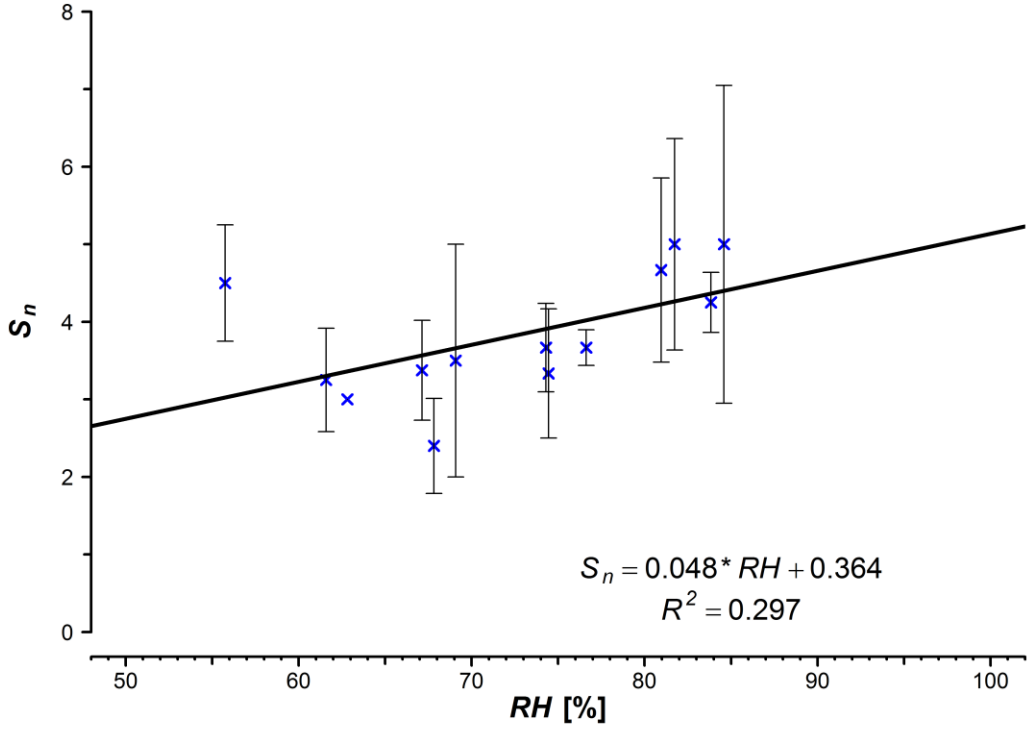

Figure S3b

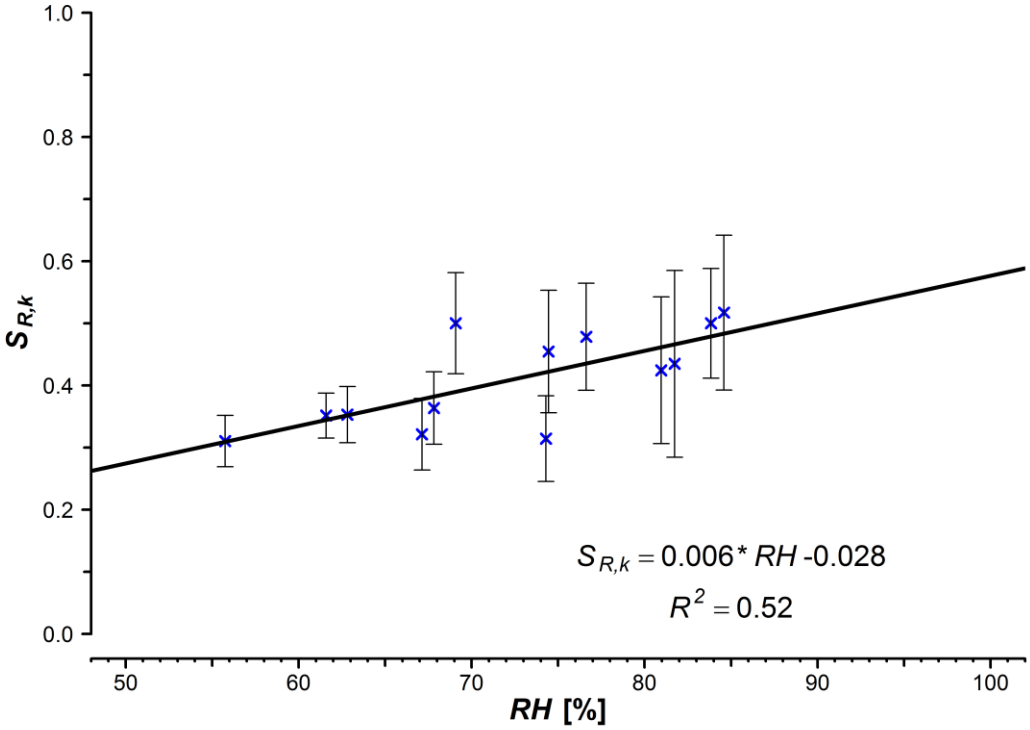

Figure S3c

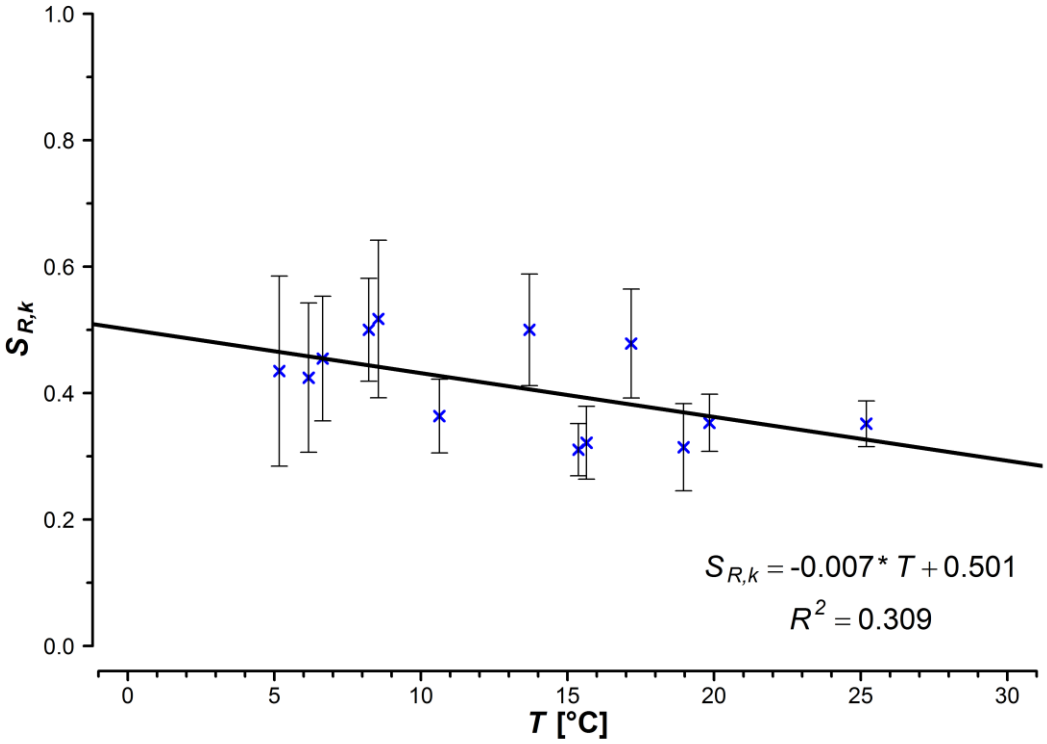

Figure S3: The most significant results of a single variable linear regression analysis between the two dependent variables, normalized species richness per month (S_n) and relative species diversity per month ($S_{R,k}$), and meteorological factors. The values for S_n and $S_{R,k}$ were calculated with the equations given in Table S3 (a) S_n as a function of *RH* (p-value = 0.055). Error bars: $SE_n(S_n)$ (b) The relative species diversity per month ($S_{R,k}$) as a function of *RH* (p-value = 0.005). Error bars: $SE_n(S_{R,k})$ (c) The relative Species per month ($S_{R,k}$) as a function of T (p-value = 0.048). Error bars: $SE_n(S_{R,k})$. *RH*: average relative humidity [%], *T*: average temperature [°C].

Supplement Tables S1-S7

Table S1. Overview of air samples (aerosol filters) positive for DNA analysis. Sample ID (location and running number), sampling period, sequence region (16S rRNA gene, *amoA*), number of sequences obtained. Abbreviation of sampling location: Cape Verde (CV), China (CH), Germany (MZ), North America (NA), United Kingdom (UK).

Sample ID	**Sampling period**	**Sequence region**	**Number of sequences (16S, *amoA*)**
MZ 1	24.03.2006 - 31.03.2006	16S	5
MZ 2	31.03.2006 - 07.04.2006	16S	8
MZ 4	07.04.2006 - 12.04.2006	16S, *amoA*	11, 12
MZ 6	15.04.2006 - 18.04.2006	16S	4
MZ 9	20.04.2006 - 27.04.2006	16S	5
MZ 10	27.04.2006 - 02.05.2006	16S	5
MZ 11	02.05.2006 - 03.05.2006	16S	7
MZ 15	04.05.2006 - 09.05.2006	16S, *amoA*	16, 12
MZ 18	12.05.2006 - 15.05.2006	16S	7
MZ 19	15.05.2006 - 16.05.2006	16S	7
MZ 21	17.05.2006 - 18.05.2006	16S	4
MZ 24	18.05.2006 - 22.05.2006	16S	13
MZ 25	22.05.2006 - 23.05.2006	16S	12
MZ 26	23.05.2006 - 30.05.2006	16S, *amoA*	18, 12
MZ 31	01.06.2006 - 06.06.2006	16S	8
MZ 33	08.06.2006 - 13.06.2006	16S	10
MZ 35	14.06.2006 - 21.06.2006	16S	6
MZ 36	21.06.2006 - 22.06.2006	16S, *amoA*	10, 4
MZ 40	27.06.2006 - 04.07.2006	16S, *amoA*	9, 12
MZ 41	04.07.2006 - 11.07.2006	16S	5
MZ 45	19.07.2006 - 21.07.2006	16S	18
MZ 47	26.07.2006 - 02.08.2006	16S	5
MZ 50	02.08.2006 - 09.08.2006	16S, *amoA*	9, 12
MZ 51	09.08.2006 - 16.08.2006	16S	5
MZ 52	16.08.2006 - 23.08.2006	16S, *amoA*	9, 3
MZ 54	30.08.2006 - 06.09.2006	16S, *amoA*	19, 12
MZ 59	11.09.2006 - 18.09.2006	16S	11

MZ 60	18.09.2006 - 25.09.2006	16S, *amoA*	5, 12
MZ 62	02.10.2006 - 09.10.2006	16S, *amoA*	6, 9
MZ 63	09.10.2006 - 16.10.2006	16S, *amoA*	12, 9
MZ 66	16.10.2006 - 23.10.2006	16S	4
MZ 67	23.10.2006 - 30.10.2006	16S, *amoA*	12, 12
MZ 69	02.11.2006 - 09.11.2006	16S	20
MZ 71	16.11.2006 - 23.11.2006	16S	1
MZ 74	23.11.2006 - 30.11.2006	16S	8
MZ 75	30.11.2006 - 07.12.2006	16S	18
MZ 77	14.12.2006 - 21.12.2006	16S	5
MZ 81	28.12.2006 - 04.01.2007	16S	5
MZ 82	04.01.2007 - 11.01.2007	16S	8
MZ 84	18.01.2007 - 25.01.2007	16S	9
MZ 88	01.02.2007 - 08.02.2007	16S, *amoA*	3, 7
MZ 90	15.02.2007 - 22.02.2007	16S, *amoA*	9, 9
MZ 93	22.02.2007 - 01.03.2007	16S, *amoA*	21, 11
MZ 95	08.03.2007 - 15.03.2007	16S, *amoA*	9, 4
MZ 97	22.03.2007 - 29.03.2007	16S	5
MZ 101	05.04.2007 - 12.04.2007	16S, *amoA*	15, 12
MZ 103	19.04.2007 – 26.04.2007	16S, *amoA*	14, 12
CV 1*	03.02.2011 - 04.02.2011	16S	10
CV 2	04.02.2011 - 05.02.2011	16S	9
CV 4	05.02.2011 – 06.02.2011	16S	10
CV 5	06.02.2011 - 07.02.2011	16S	11
CV 6	07.02.2011 - 08.02.2011	16S	4
CV 17	17.02.2011 - 19.02.2011	16S	6
CV 19	21.02.2011 - 23.02.2011	16S	5
CV 20	23.02.2011 - 24.02.2011	16S	9
CV 21	24.02.2011 - 27.02.2011	16S	4
CV 27	08.03.2011 - 11.03.2011	16S	4
NA 1*	24.07.2011 - 25.07.2011	16S	20
CH 4*	10.07.2006 - 11.07.2006	16S	7
UK 1*	06.05.2004 - 13.05.2004	16S	11

*For CV 26, NA 20, CH 14 and UK 12 air filter samples were tested in total (Table 1)

Table S2. Information on the sequences (16S rRNA gene) used for constructing phylogenetic trees. Accession numbers are given as well as the name of the species if available. Phylogenetic trees where these sequences have been used can be found in Figures 2 and S1.

	Acc Nr	Taxonomy
Euryarchaeota	**Methanosarcinales**	
	M59144	*Methanosarcina barkeri*
	AF028691	*Methanosarcina mazei*
	M59137	*Methanosarcina acetivorans*
	M59135	*Methanolobus concilii*
	M59141	*Methanosaeta thermoacetophila*
	Methanomicrobiales	
	M60880	*Methanospirillum hungatei*
	M59134	*Methanoculleus marisnigri*
	M59131	*Methanogenium organophilum*
	Methanobacteriales	
	M59124	*Methanobacterium bryantii*
	AF028690	*Methanobacterium oryzae*
	M36508	*Methanobacterium formicicum*
	AY196684	*Methanosphaera stadtmaniae*
	NR074235	*Methanobrevibacter smithii*
	NR044801	*Methanobrevibacter filiformis*
	Methanocellales	
	AB196288	*Methanocella paludicola*
	AM114193	*Methanocella arvoryzae*
	Thermoplasmatales	
	M38637	*Thermoplasma acidophilum*
	AJ224936	*Ferroplasma acidophilum*
	AF050618	*WCHD3-16, Thermoplasmatales*
	Methanococcales	
	M36507	*Methanococcus vannielii*
	M59126	*Methanocaldococcus jannaschii*
	U38484	*Methanococcus maripaludis*
	Halobacteriales	
	DQ256409	*Haladaptatus jilantaiense*
	EU887285	*Haladaptatus litoreus*
	DQ344973	*Haladaptatus paucihalophilus*
	DQ344974	*Haladaptatus paucihalophilus*
	GQ282623	*Halgranum amylolyticum*
	GQ282624	*Halgranum gelatinilyticum*
	EF645681	*Haloarcula argentinensis*
	EF645693	*Haloarcula marismortui*
	EF645688	*Haloarcula vallismortis*
	GQ282625	*Halobacteriaceae archaeon*
	AJ002949	*Halobacterium halobium*
	M38280	*Halobacterium halobium*
	U17364	*Halobacterium saccharovorum*
	AJ002947	*Halobacterium salinarium*
	AJ496185	*Halobacterium salinarum*
	U68538	*Halobacterium salinarum*
	AJ548827	*Halobacterium sp*
	D11106	*Halococcus morrhuae*
	AB004877	*Halococcus salifodinae*

Euryarchaeota	Z28387	*Halococcus salifodinae*
	AB037474	*Haloferax alexandrines*
	D14128	*Haloferax denitrificans*
	AJ420376	*Haloferax dombrowskii*
	AY458601	*Haloferax sulfurifontis*
	AY425724	*Haloferax volcanii*
	AF002984	*Halogeometricum borinquenss*
	EU887286	*Halogeometricum rufum*
	EU887283	*Halogranum rubrum*
	DQ417339	*Haloplanus natans*
	EU931578	*Haloplanus sp.*
	DQ987877	*Halorubrum luteum*
	AY149598	*Halorubrum tibetense*
	D14125	*Halorubrum trapanicum*
	EU931577	*Halosarcina limi*
	AB454051	*Salarchaeum japonicum*
	JN196516	*Salarchaeum sp.*
Thaumarchaeota	**Group I.1a**	
	U51469	*Cenarchaeum symbiosum*
	DQ085097	*Nitrosopumilus maritimus*
	AEGP01000029	*Cnitrosoarchaeum limnia*
	Group I.1b	
	EU281334	*Nitrososphaera gargensis*
	FR773157	*Nitrososphaera viennensis*
	FR773158	*Nitrososphaera sp.*
	Group I.1c	
	AJ006919	*FFSC1, Thaumarchaeota, I.1c*
	X96691	*FFSB4, Thaumarchaeota, I.1c*
	Group III	
	EU239960	*Nitrosocaldus yello*wstonii
	Soil Crenarchaeotic Group (SGC)	
	GQ871309	*W5P1-DO1, Thaumarchaeota, SGC*
	FJ468477	*F5, Thaumarchaeota, SCG*
	AJ535123	*Gitt-GR, Thaumarchaeota, SCG*
Thaumarchaeota	**Terrestrial Group (TG)**	
	EF021006	*Amb_16S, Thaumarchaeota, TG*
	AY278081	*GRU10, Thaumarchaeota, TG*
	FJ174722	*Arc-D88, Thaumarchaeota, TG*
	South African Gold Mine Group (SAGMGC)	
	AB550811	*NG-W-081028-3-5. Thaumarchaeota, SAGMGC*
	EU306977	*ArcB-cD06, Thaumarchaeota, SAGMGC*
	Dominant Soil Crenarchaeotic Group (DSC)	
	AY278106	*Thaumarchaeota, DSC1*
	EU281336	*Thaumarchaeota, DSSC2*
Crenarchaeota	**Crenarchaeota**	
	JN881579	*Caldiarchaeum*
	HQ214608	*Miscellaneous Crenarchaeotic Group*
	GQ267189	*Marine Benthic Group*
	JN227488	*Nitrosotalea devanaterra*
	Thermoproteales	
	NR040935	*Pyrobaculum*
	HF546082	*Sulfolobales*
	KC139249	*Desulfurcoccales*
	NR041774	*Acidilobales*
	EF552404	*Fervivicoccales*

Table S3. Definitions.

Symbol	Quantity/ Definition
D	Simpson's index (Hill et al., 2003), $D = \sum_{i=1}^{S} \frac{n_i(n_i-1)}{N(N-1)}$
E	Shannon evenness, $E=H'/\ln S$
H'	Shannon index, $H' = -\sum_{i=1}^{S}(P_i \ln P_i)$
m_j	Number of sequences analyzed for air sample j
m_k	Number of sequences analyzed for air samples in a month k
n_i	Frequency of occurrence of an individual species i (number of samples in which species i was detected)
N	Cumulative frequency of occurrence of investigated sample, $N = \sum_{i=1}^{S} n_i$
n	Number of investigated air samples
P_i	Relative proportion of an individual species i, $P_i = n_i/N$
S	Species richness measured (number of detected individual species)
S_j	Number of detected individual species in air sample j
S_k	Number of detected individual species in air samples of a month k
S^*	Species richness estimated with the Chao-1 approach (Chao, 1984; Hill et al., 2003), $S^*=S + a^2/(2b)$, a = number of species detected only once (singletons) b = number of species detected twice (doubletons)
S_n	Normalized species richness, $S_n = S/n$
$S_{R,j}$	Relative species diversity per sample, $S_{R,j} = S_j/m_j$
$S_{R,k}$	Relative species diversity per month, $S_{R,k} = S_k/m_k$
$SE_n(S_n)$	Normalized standard error of S_n: $= \frac{SE_{\bar{S}}}{\bar{S}} S_n$ $\bar{S}$ = average number of species per sample $SE_{\bar{S}}$ = standard error of $\bar{S}$
$SE_n(S_{R,k})$	Normalized standard error of $S_{R,k}$: $= \frac{SE_{\overline{S_{R,J}}}}{\overline{S_{R,J}}} S_{R,k}$ $\overline{S_{R,J}}$ = average relative species diversity per sample $SE_{\overline{S_{R,J}}}$ = standard error of $\overline{S_{R,J}}$

Table S4: Estimates of the abundance of Bacteria and Archaea in different environments. The number of copies (cp) of the 16S rRNA gene was quantified using qPCR.

Source	Archaea	Bacteria	Ratio A/B	Reference
Soil [cp kg^{-1}]	~10^{9} – 10^{11}	~10^{11} – 10^{12}	~10^{-2} – 10^{-1}	Cao et al., 2012; Kemnitz et al., 2007
Ocean surface water [cp L^{-1}]	~10^{6} – 10^{7}	~10^{8} – 10^{9}	~10^{-2}	Yin et al. 2013
Air [cp m^{-3}]	~1 – ~10^{1}	~10^{4} – 10^{6}	~10^{-4} – 10^{-6}	This study

Table S5. Operational taxonomic units (OTUs) attributed to Archaea 16S rRNA genes. Operational ID; frequency of occurrence (number of air samples and coarse or fine particle filters in which the OTU was detected (n.a.= not available); taxonomic family and genus name according to phylogenetic analysis based on the Maximum likelihood approach. Information is given for Germany, Cape Verde, United Kingdom, China, and North America.

OTU	**Frequency of occurrence**			**Family, genus**
Germany	**total**	**coarse**	**fine**	
Arch1	33	32	2	*Thaumarchaeota, former Group I 1b*
Arch2	26	26	0	*Thaumarchaeota, former Group I 1b*
Arch3	20	20	4	*Thaumarchaeota, former Group I 1b*
Arch4	17	16	2	*Thaumarchaeota, former Group I 1b*
Arch5	15	15	0	*Thaumarchaeota, former Group I 1b*
Arch6	13	13	1	*Thaumarchaeota, former Group I 1b*
Arch7	11	11	0	*Thaumarchaeota, former Group I 1b*
Arch8	9	9	0	*Thaumarchaeota, former Group I 1b*
Arch9	8	7	1	*Thaumarchaeota, former Group I 1b*
Arch10	8	7	1	*Thaumarchaeota, former Group I 1b*
Arch11	7	7	0	*Thaumarchaeota, former Group I 1b*
Arch12	5	4	1	*Methanosarcinaceae, Methanosarcina*
Arch13	5	4	1	*Methanobacteriaceae; Methanosphaera*
Arch14	5	5	0	*Thaumarchaeota, former Group I 1b*
Arch15	4	4	0	*Thaumarchaeota, former Group I 1b*
Arch16	3	2	1	*Methanobacteriaceae*
Arch17	3	3	0	*Thaumarchaeota, former Group I 1b*

Arch18	3	3	0	*Thaumarchaeota, former Group I 1b*
Arch19	3	3	0	*Thaumarchaeota, former Group I 1b*
Arch20	3	3	0	*Thaumarchaeota, former Group I 1b*
Arch21	2	2	0	*Thaumarchaeota, former Group I 1b*
Arch22	2	2	0	*Thaumarchaeota, former Group I 1b*
Arch23	2	2	0	*Thaumarchaeota, former Group I 1b*
Arch24	2	2	0	*Thaumarchaeota, former Group I 1b*
Arch25	2	2	0	*Thaumarchaeota, former Group I 1b*
Arch26	2	2	0	*Thaumarchaeota, former Group I 1b*
Arch27	1	1	0	*Thermoplasmatales*
Arch28	1	0	1	*Halobacteriaceae, Halococcus*
Arch29	1	1	0	*Methanobacteriaceae, Methanobrevibacter*
Arch30	1	1	0	*Thaumarchaeota, former Group I 1b*
Arch31	1	1	0	*Thaumarchaeota, former Group I 1b*
Arch32	1	1	0	*Thaumarchaeota, former Group I 1b*
Arch33	1	1	0	*Methanomicrobiaceae; Methanoculleus*
Arch34	1	1	0	*Thaumarchaeota, former Group I 1b*
Arch35	1	1	0	*Thaumarchaeota, former Group I 1b*
Arch36	1	1	0	*Thaumarchaeota, former Group I 1b*
Arch37	1	1	0	*Thaumarchaeota, former Group I 1b*
Arch38	1	1	0	*Thaumarchaeota, former Group I 1b*
Arch39	1	0	1	*Thaumarchaeota, former Group I 1b*

Arch40	1	1	0	*Thaumarchaeota, former Group I 1b*
Arch41	1	1	0	*Thaumarchaeota, former Group I 1b*
Arch42	1	1	0	*Thaumarchaeota, former Group I 1b*
Arch43	1	1	0	*Thaumarchaeota, former Group I 1b*
Arch44	1	1	0	*Thaumarchaeota, former Group I 1b*
Arch45	1	1	0	*Thaumarchaeota, former Group I 1b*
Arch46	1	1	0	*Thaumarchaeota, former Group I 1b*
Arch47	1	0	1	*Thaumarchaeota, former Group I 1b*
Arch48	1	1	0	*Methanobacteriaceae, Methanobrevibacter*
Arch49	1	1	0	*Thaumarchaeota, former Group I 1b*
Arch50	1	1	0	*Thaumarchaeota, former Group I 1b*
Arch51	1	1	0	*Thaumarchaeota, former Group I 1b*
Arch52	1	1	0	*Thaumarchaeota, former Group I 1b*
Arch53	1	1	0	*Thaumarchaeota, former Group I 1b*
Arch54	1	1	0	*Thaumarchaeota, former Group I 1b*
Arch55	1	1	0	*Thaumarchaeota, former Group I 1b*
Arch56	1	1	0	*Thaumarchaeota, former Group I 1b*
Arch57	1	1	0	*Thaumarchaeota, former Group I 1b*

OTU	**Frequency of occurrence**			**Family, genus**
Cape Verde				
	total	**coarse**	**fine**	
CV1	1	n.a.	n.a.	*Halobacteriaceae*

CV2	1	n.a.	n.a.	*Thaumarchaeota, former Group I 1b*
CV3	1	n.a.	n.a.	*Halobacteriaceae, Halococcus*
CV4	1	n.a.	n.a.	*Thaumarchaeota, former Group I 1b*
CV5	1	n.a.	n.a.	*Thaumarchaeota, former Group I 1b*
CV6	1	n.a.	n.a.	*Thaumarchaeota, former Group I 1b*
CV7	1	n.a.	n.a.	*Thermoplasmatales*
CV8	1	n.a.	n.a.	*Thaumarchaeota, former Group I 1b*
CV9	1	n.a.	n.a.	*Halobacteriaceae*
CV10	1	n.a.	n.a.	*Thaumarchaeota, former Group I 1b*
CV11	1	n.a.	n.a.	*Thaumarchaeota, former Group I 1b*
CV12	1	n.a.	n.a.	*Thermoplasmatales*
CV13	1	n.a.	n.a.	*Thermoplasmatales*
CV14	1	n.a.	n.a.	*Thaumarchaeota, former Group I 1b*
CV15	1	n.a.	n.a.	*Halobacteriaceae*
CV16	1	n.a.	n.a.	*Halobacteriaceae*
CV17	1	n.a.	n.a.	*Halobacteriaceae*
CV18	1	n.a.	n.a.	*Thaumarchaeota, former Group I 1b*
CV19	1	n.a.	n.a.	*Thaumarchaeota, former Group I 1b*
CV20	1	n.a.	n.a.	*Thaumarchaeota, former Group I 1b*
CV21	1	n.a.	n.a.	*Thaumarchaeota, former Group I 1b*
CV22	1	n.a.	n.a.	*Thermoplasmatales*
CV23	1	n.a.	n.a.	*Thaumarchaeota, former Group I 1b*

CV24	1	n.a.	n.a.	*Thaumarchaeota, former Group I 1b*
CV25	1	n.a.	n.a.	*Thaumarchaeota, former Group I 1b*
OTU	**Frequency of occurrence**			**Family, genus**
UK	**total**	**coarse**	**fine**	
UK1	1	n.a.	n.a.	*Thermoplasmatales*
UK2	1	n.a.	n.a.	*Thermoplasmatales*
UK3	1	n.a.	n.a.	*Thermoplasmatales*
UK4	1	n.a.	n.a.	*Thermoplasmatales*
OTU	**Frequency of occurrence**			**Family, genus**
China	**total**	**coarse**	**fine**	
Ch1	1	n.a.	n.a.	*Methanobacteriaceae*
OTU	**Frequency of occurrence**			**Family, genus**
North America	**total**	**coarse**	**fine**	
Col1	1	n.a.	n.a.	*Thaumarchaeota, former Group I 1b*

Table S6: Pearson coefficients results. Pearson coefficients between the diversity indices $S_{R,j}$, S_n and $S_{R,k}$, and the meteorological factors, average temperature (Temp), average relative humidity (RH), average wind speed (WS) and sum of precipitation (Precip). The asterix mark the cases selected for further testing (See Table S7 and Figures S2a, S3).

	Temp	**RH**	**WS**	**Precip**
$S_{R,j}$	-0.051	0.238	-0.357 *	0.087
S_n	-0.431	0.545 *	-0.302	-0.265
$S_{R,k}$	-0.556*	0.721 *	0.170	0.236

Table S7: Overview the most significant results of the linear regression analysis between the Archaea 16S rRNA gene sequence diversity and meteorological parameters. The "+" and "–" specify whether the diversity parameter has a positive or negative correlation to the meteorological factor (Temp = temperature, RH = relative humidity, WS = wind speed, Precip = precipitation, Sign = significance).

		Temp	RH	WS	Precip	R^2	p-value	Sign.
per sample	Best single variable							
	$S_{R,j}$ (Fig. S2a)			-		0.132	0.012	<0.05
	Best variable subset							
	$S_{R,j}$ (Fig. S2b)		+	-		0.174	0.015	<0.05
per month	Best single variable							
	S_n (Fig. S3a)		+			0.297	0.054	~0.05
	$S_{R,k}$ (Fig. S3b)		+			0.520	0.005	<0.01
	$S_{R,k}$ (Fig. S3c)	-				0.309	0.049	<0.05
	Best variable subset							
	S_n (Fig. 4a)	-		-		0.533	0.022	<0.05
	$S_{R,k}$ (Fig. 4b)		+	+		0.598	0.011	~ 0.01

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Apendix C5:

Community composition and seasonal changes of archaea in coarse and fine air particulate matter

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Community composition and seasonal changes of archaea in coarse and fine air particulate matter

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Abstract. Archaea are ubiquitous in terrestrial and marine environments and play an important role in biogeochemical cycles. Although air acts as the primary medium for their dispersal among different habitats, their diversity and abundance is not well characterized. The main reason for this lack of insight is that archaea are difficult to culture, seem to be low in number in the atmosphere, and have so far been difficult to detect even with molecular genetic approaches. However, to better understand the transport, residence time, and living conditions of microorganisms in the atmosphere as well as their effects on the atmosphere and vice versa, it is essential to study all groups of bioaerosols. Here we present an in-depth analysis of airborne archaea based on Illumina sequencing of 16S rRNA genes from atmospheric coarse and fine particulate matter samples and show seasonal dynamics and discuss anthropogenic influences on the diversity, composition, and abundance of airborne archaea.

The relative proportions of archaea to bacteria, the differences of the community composition in fine and coarse particulate matter, and the high abundance in coarse matter of one typical soil related family, the Nitrososphaeraceae, point to local phyllosphere and soil habitats as primary emission sources of airborne archaea.

We found comparable seasonal dynamics for the dominating Euryarchaeota classes and Crenarchaeota orders peaking in summer and fall. In contrast, the omnipresent Cenarchaeales and the Thermoplasmata occur only throughout summer and fall. We also gained novel insights into archaeal composition in fine particulate matter (< 3 µm), with Cenarchaeaceae, Nitrososphaeraceae, Methanosarcinales, Thermoplasmata, and the genus *Nitrosopumilus* as the dominating taxa.

The seasonal dynamics of methanogenic Euryarchaeota point to anthropogenic activities, such as fertilization of agricultural fields with biogas substrates or manure, as sources of airborne archaea. This study gains a deeper insight into the abundance and composition of archaea in the atmosphere, especially within the fine particle mode, which adds to a better understanding of the overall atmospheric microbiome.

1 Introduction

In addition to bacteria and eukaryotes, archaea constitute one of the three independent domains of life (Woese et al., 1990). In the beginning of archaeal research in the 1880s, primarily methanogenic archaea were discovered and cultivated, so the belief arose that archaea are exclusively extremophiles (Cavicchioli, 2011; Farlow, 1880; Schleper et al., 2005). However, during the last decades, cultivation- and culture-independent methods, such as DNA sequencing, have substantially improved the understanding of archaea and proved that they are also abundant in various environments such as marine or soil habitats, where they can represent more than 10 % of the microbial community (Buckley et al., 1998; Cao et al., 2012; Cavicchioli, 2011; Delong, 1998; Robertson et al., 2005; Yilmaz et al., 2016).

So far, diversity studies for archaea have mainly concentrated on the major habitats also known for bacteria such as marine and soil environments (Bintrim et al., 1997; Buckley et al., 1998; DeLong, 1992; Ochsenreiter et al., 2003). In the global marine environment the abundance of archaea is approximately 1×10^{28} archaeal compared to 3×10^{28} bacterial cells (Karner et al., 2001), with archaea accounting for 2–10 % in surface waters and for 20–40 % in deep ocean water (Massana et al., 1997).

The abundance and composition of archaea in soil vary between different soils types (Bates et al., 2011). All cultivated methanogens belong to the kingdom Euryarchaeota and are strictly dependent on anaerobic conditions with low redox potentials (Le Mer and Roger, 2010); thus, they are only present in small numbers in many soils. The fertilization with livestock manure adds anaerobically adapted organisms to the surface of agriculturally used soils. Thus, even in aerated soils, core anaerobic populations seem to survive albeit in low number (Angel et al., 2012). Another issue influencing the abundance and composition of archaea in soil is – as also observed in water columns – the depth (Karner et al., 2001). Analyses of soil depth profiles revealed changing diversity patterns with depth (Bundt et al., 2001; Pesaro and Widmer, 2002) in composition and number.

Next to the well-established major habitats, the atmosphere is another environment in which microorganisms can be detected; however, it remains unclear whether the atmosphere can be considered a natural habitat or whether it only represents a medium of dispersal for terrestrial and marine microorganisms and their spores (Bowers et al., 2009, 2011, 2012, 2013; Smith et al., 2013; Womack et al., 2010; Yooseph et al., 2013). For airborne bacteria and archaea the main known emission sources are surface waters and the surface layer of soils (Womack et al., 2010). Therefore, the different abundances and composition of archaea within water and soil columns are of special interest to understand possible emission sources for airborne archaea. For bacteria, which are abundant in air, the concentration of 16S rRNA gene copies quantified using qPCR in soil was 10^{11} to 10^{12} gene copies kg^{-1} and for archaea 10^{9} to 10^{11} gene copies kg^{-1} (Cao et al., 2012; Kemnitz et al., 2007). In ocean surface waters the concentration is lower but estimated to be 10^{8} to 10^{9} gene copies L^{-1} for bacteria and 10^{6} to 10^{7} gene copies L^{-1} for archaea (Kemnitz et al., 2007; Yin et al., 2013), whereas only 10^{4} to 10^{6} bacterial gene copies m^{-3} air have been detected (Cao et al., 2012; Fröhlich-Nowoisky et al., 2014; Kemnitz et al., 2007; Yin et al., 2013). Interestingly, in contrast to bacteria, it seems challenging to detect, amplify, and analyze archaea in air, as their concentration of 100 ppm is much lower than the abundance of bacteria (Cao et al., 2012; Fröhlich-Nowoisky et al., 2014). Until now, it remains unclear whether these observations are biased by technical obstacles or reflect the true abundances. The largest study on airborne archaea is to our knowledge by Fröhlich-Nowoisky et al. (2014) and is based on Sanger sequencing. However, in Fröhlich-Nowoisky et al. (2014) the number of sequences were low, the observations had little statistical support, and the analysis of the microbiome of aerosolized archaea was difficult. Therefore, we present an in-depth next-generation sequencing study of airborne archaea collected on coarse and fine particulate matter filters over 1 year in Mainz, Germany. We attempt to compare the composition, diversity, and abundance to the same characteristics as in other habitats, which also allows an inference about the primary emission sources of airborne archaea.

2 Material and methods

2.1 Aerosol sampling

As described in Fröhlich-Nowoisky et al. (2009), in total 24 pairs of air filter samples (i.e., 20 filter pairs of one fine and one coarse particle filter sample each, 2 pairs of start-up air filter blanks, and 2 pairs of mounting filter blanks) were analyzed within this dataset. The air filters were installed on an in-house-built high-volume dichotomous sampler (Solomon et al., 1983). The whole sampling campaign lasted 1 year in Mainz, Germany (March 2006–April 2007). The rotary vane pump (Becker VT 4.25) worked with a flow rate of $\sim 0.3\,m^3\,min^{-1}$. The particles were split according to their aerodynamic diameter by a virtual impactor. Particles with an aerodynamic diameter larger than the nominal cut-off of $\sim 3\,\mu m$ and, due to the sampling device, an additional 10 % of the fine particles were sampled in line with the inlet on one glass fiber filter (flow rate: $\sim 0.03\,m^3\,min^{-1}$) representing the coarse fraction. The fine particles were collected on a second glass fiber filter perpendicular to the inlet ($\sim 0.27\,m^3\,min^{-1}$) which was essentially free from coarse particles (Solomon et al., 1983). To get a representative dataset for the whole year, five random samples, consisting of a coarse and fine filter, were analyzed for each of the four seasons of the sampling campaign. The sampling period of a single filter pair was generally 7 days except for filter pairs MZ 11 (24 h), MZ 15 (5 days), and MZ 31 (5 days; Table S1 in the Supplement). The sampled air masses represent a mixture of urban and rural continental air, as the sampler was positioned on the roof of the Max Planck Institute for Chemistry on the campus of the University of Mainz (49°59′31.36″ N, 8°14′15.22″ E). To reduce the sampling of particles emitted from the ground, the sampling device was on a mast about 5 m above the flat roof of the three-story building.

2.2 Extraction, amplification, and sequencing

The DNA extraction and sequencing was part of the Earth Microbiome Project (EMP – http://www.earthmicrobiome.org/, last access: 4 November 2016) using the MoBio PowerMag Soil DNA Isolation kit and the Illumina GAIIx sequencer with the sequencing by synthesis technology. As shown before, this technology is suitable for analyzing mi-

crobial communities in soil, water, and human skin (Caporaso et al., 2011).

For the PCR amplifications the 515f/806r primer set (Fwd: GTGCCAGCMGCCGCGGTAA; Rev: GGACTACHVGGGTWTCTAA) described in Caporaso et al. (2011) proved to be suitable, as shown by Bates et al. (2011). It covers the conserved flanking regions ideal for amplifying bacteria and archaea over the V4 region of the 16S rRNA gene (Bowers et al., 2013; Huse et al., 2008; Muyzer et al., 1993). In addition, the primer pair is preferred for this amplification as it exhibits only few biases against individual bacterial taxa. As suggested in Caporaso et al. (2011) each DNA extract was amplified in triplicate. These triplicates were combined and purified using a 96-well PCR clean-up kit from MO BIO. The utilized PCR reaction was performed; amplicons were purified and sequenced using the GAIIx.

2.3 Grouping of sequences into OTUs and taxonomic identification

The sequences were analyzed using the Quantitative Insight Into Microbial Ecology (QIIME) toolkit (Caporaso et al., 2010). To assign sequences to operational taxonomic units (OTUs), we used QIIME's closed reference OTU picking script, which uses Uclust (Edgar, 2010) and the Greengenes reference database (gg_13_8_otus/rep_set/97_otus.fasta, last update 15 August 2013; McDonald et al., 2012) with 97 % similarity. For the actual identification process a corresponding taxonomy map provided by the Greengenes database was used. Sequences, which did not match to any Greengenes reference set OTU, were discarded for the downstream analysis.

2.4 Controls

Prior to the sampling procedure all filters were baked in sealed aluminum foil bags overnight at 500 °C. To best conserve the DNA of the collected bioaerosols, after the sampling procedure all filter samples were stored at −80 °C until analysis. To detect possible contaminants from the sampling device and the filter handling, blank filters were taken at 4-week intervals. Contamination-free, prebaked filter pairs were mounted on the sampler as for regular sampling, but the pump was not turned on at all (mounting blanks). In addition, small environmental samples were taken to collect air exclusively around and from the interior of the sampling device by turning the pump on for 5 s only (start-up filter blanks). A detailed list of all analyzed air and blank filter samples with their individual sampling details can be found in the Supplement in Tables S1 and S2.

The DNA of the blank filters was extracted and quantified in parallel to the actual filter samples. Often, the detected DNA concentrations on blanks can be too small to be quantified or to build usable sequencing libraries (Cao et al., 2014). However, as the start-up blanks were briefly exposed to environmental air, they also could contain DNA. Within this study we controlled the actual filter changing process by sequencing two mounting blanks, i.e., MZ 23 and MZ 73. Two sequences were obtained from the fine particle filter of MZ 23 and 408 archaeal sequences (371 sequences on the coarse particle blank filter and 37 sequences on the fine particle blank filter) were detected on MZ 73. On the coarse particle filter of MZ 23 no archaeal sequences were detected. Minimal DNA amounts are to be expected, as the mounting blanks were briefly exposed to the air during the mounting process. The sequences on the mounting blanks were assigned to five archaeal families (Cenarchaeaceae, Methanobacteriaceae, Methanoregulaceae, Methanosaetaceae, Methanomassiliicoccaceae). The handling of the sequences obtained with next-generation sequencing techniques, e.g., for amplicon sequencing of environmental air sample controls, is neither well established nor standardized. To ensure that all contaminants were removed comprehensively from the dataset, we decided to omit all identified families from the data if present in more than 1 % of all detected archaeal sequences of the mounting blanks.

The subsequently deleted families (from 404 sequences) Methanoregulaceae (8.5 %, 3 OTUs), Methanomassiliicoccaceae (17.6 %, 3 OTUs), and the largest family of the Methanobacteriaceae (72.4 %, 4 OTUs) all belonged to the Euryarchaeota (see also Table S2). In total 2341 sequences remained for the downstream analysis.

Likewise, two pairs of start-up air filter blanks were sequenced. But as they sampled the air for 5 s the obtained sequences were not treated like the mounting blanks. On these four filter samples 709 archaeal sequences were found, distributed with 328 sequences on MZ 22 (326 sequences on coarse, 2 sequences on fine) and 381 sequences on MZ 72 (3 sequences on coarse, 378 sequences on fine).

2.5 Statistical analysis

All data management and most of the analyses were performed using a MySQL database and R statistics if not stated otherwise (R-Team, 2011).

To characterize the biodiversity of the archaea community and thus to approximate the likely diversity, several statistical parameters were calculated: species richness estimators, rarefaction curves, and community diversity indices using the software tool EstimateS (Colwell et al., 2012).

2.6 Meteorological analysis

As a possible correlation between the abundance of taxonomic ranks in an air mass and meteorological parameters can be either following a monotone or specifically a linear relationship, in this study the Pearson product-moment correlation coefficient (r_K) testing for a linear regression and the Spearman's rank (r_R) for fine, coarse, and total suspended particles (TSPs) were used. The meteorological parameters

tested were wind speed in $m\,s^{-1}$ (average and maximum), temperature in °C (range and maximum), relative humidity in %, and the sum of precipitation in mm. The meteorological data were provided in hourly data for wind speed and half-hourly values for all other meteorological parameters by the ZIMEN Luftmessnetz of the Landesamt für Umwelt Wasserwirtschaft und Gewerbeaufsicht of Rhineland-Palatinate. All averages were calculated for the exact sampling periods (Table S1). The correlation analysis using the Pearson product-moment correlation coefficient (r_K) and Spearman's rank (r_R) were calculated for different taxonomic levels, i.e., kingdom, phylum, and class level. Only results with r_K or r_R over 0.5 or under −0.5 were interpreted. However, no significant correlations between the relative abundance and the meteorological factors were found.

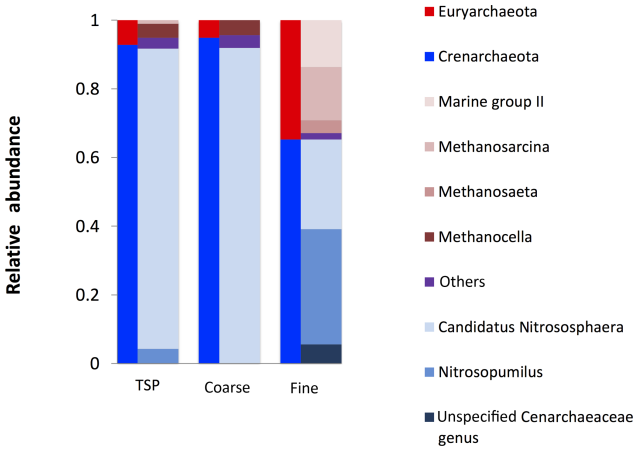

Figure 1. Archaeal community composition for total suspended (TSP), the same airstream split into coarse, and fine particulate matter on the level of phyla (red/blue) and genera (pastel colors).

3 Results and discussion

3.1 Overall diversity

To determine the archaeal diversity in air, 20 air filter pairs were sampled and analyzed for 1 year in Mainz, Germany. Each filter pair consists of one filter collecting particles with aerodynamic diameters smaller than 3 µm (fine particulate matter) and one collecting primarily coarse particles, which are larger than 3 µm. On 39 (97.5 %) of the 40 analyzed filters (20 air filter pairs) archaeal DNA could be detected. In total 2341 sequences could be assigned to archaea (Table 1). More archaeal sequences were detected on coarse particle filters (109 sequences on average per sample) than on fine particle filters (8 sequences on average per sample) for which the number of sequences ranged from 0 to 42. On all but one fine particle filter, MZ 81 sampled in December 2006, archaeal sequences were discovered. The highest number of sequences, i.e., 601, were detected on the coarse particle filter MZ 74 from November 2006. The 2341 archaeal sequences were assigned to 52 OTUs. Out of these OTUs, 17 OTUs were found in coarse as well as in fine particulate matter. As listed in Table 1 the coarse particle filters comprised 2180 sequences distributed among 41 OTUs, whereas only 161 sequences assigned to 28 OTUs were identified on the fine particle filters.

In total only 7 % of all archaeal sequences stem from fine particle filters, whereas 93 % stem from coarse particle filters. Specifically, on 75 % of the coarse particle filters 20 or more archaeal sequences were found, while on 70 % of the fine particle filters less than 6 archaeal sequences could be detected.

The community structures of both size fractions differ remarkably in composition (Fig. 1). In the fine fraction the genus *Nitrosopumilus* is the dominant taxon. This Thaumarchaeota genus shows a relative abundance of 33.5 % over all archaea sequences found on all samples in fine particulate matter. The cultivable *Nitrosopumilus maritimus* is a well-known representative of the genus *Nitrosopumilus*. These chemolithoautotrophic nitrifying archaea have primarily been sampled from marine sources. They form straight rods with a diameter of 0.17–0.22 µm and a length of 0.5–0.9 µm (Könneke et al., 2005) and are thus one of the smallest organism known today. With this size even long-distance transport from marine sources might be conceivable. The same can be said for species of marine group II. However, *N. maritimus* and species of marine group II have been found in soil samples (Leininger et al., 2006; Treusch et al., 2005) – in contrast to the coarse fraction, where only the genera *Methanocella* and the *Candidatus Nitrososphaera* were found with relative proportions of more than 3 %. Due to the much higher number of sequences isolated from the coarse particle fraction in comparison to the fine fraction, the TSP composition resembles that of the coarse particle fraction (Fig. 1).

Taking the relative distribution over the entire course of the year into account, on class level the Thaumarchaeota also dominate the fine particle fraction, except fort two fine filters sampled during fall where the Euryarchaeota even have a higher relative abundance than the Thaumarchaeota (93 and 92 %).

The Crenarchaeota, primarily represented by Thaumarchaeota (99 %), are the dominating phylum in the coarse particle mode. Next to Thaumarchaeota a single OTU of the miscellaneous Crenarchaeotal group (MCG; Kubo et al., 2012) representing seven sequences was found on a single coarse spring filter sample. No Euryarchaeota were observed on 65 % of the fine particle filters and 50 % of the coarse particle filters. A closer look at taxonomic assignments and the contribution of sequences to individual families reveals that most sequences within the coarse particle fraction belong to the Nitrososphaeraceae family. While this family is only present in 10 % of the fine particle filters it was identified on 75 %

Table 1. Number of sequences and indices estimating the archaeal diversity in Mainz for coarse and fine particle filter samples and total suspended particles (TSPs).

Size fraction	*n* (Samples)	Sq (Sequences)	Sq / *n*	OTU (operational taxonomic unit)	S_{Chao1} (Chao1)	*H* (Shannon)	*D* (Simpson)
Coarse	20	2180	109	41	64	3.09	0.83
Fine	20	161	8.1	28	41	3.65	0.88
TSP	20	2341	117.1	52	63	3.36	0.84
Fröhlich-Nowoisky et al. (2014)	47	435	9.3	57	137	3.32	0.82

of the coarse particle filters. In soil surveys the I.1.b group of Crenarchaeota has constantly been found (Ochsenreiter et al., 2003), with the Nitrososphaeraceae being one of the most abundant archaea families therein. Thus, the aerosolization of soil and soil dust as a primary source can be hypothesized for this family. Within this family the genus *Nitrososphaera* is an abundant taxon specifically in agricultural soils (Zhalnina et al., 2013). The landscape of the surrounding area of the sampling location is dominated by agricultural fields and the emitted soil particles are thus likely to contain the genus *Nitrososphaera*. Soil and soil dust are classically discussed as primary emission sources for airborne bacteria (Després et al., 2007, 2012; Fierer et al., 2008). Therefore, when attached to large soil particles these organisms should be mainly collected in the coarse particle fraction. To our knowledge, the only cultivated *Nitrososphaera* species, *Nitrososphaera viennensis*, has a much smaller diameter (irregular cocci with a diameter of 0.6–0.9 µm; Stieglmeier et al., 2014), which should be, if in single cell status, collected in the fine particle mode. The hypothesis that soil particles identified through Nitrososphaeraceae are mainly collected on coarse particles is also strengthened by the results of community analysis of the fine particle filters. The observed increase of the relative abundance of the Euryarchaeota could also be interpreted as the decline of Nitrososphaeraceae as soil particles are less frequent in the fine mode. On the phylum level the Nitrososphaeraceae family forms the main difference between the two size fractions.

The diversity estimator Chao1 (Table 1) and the rarefaction curves (Fig. 2) predict a relatively low diversity for archaea in Mainz air (S_{Chao1}; 64 and 41 for coarse and fine, respectively). On the other hand, the relative abundances of the OTUs and the diversity calculated by Shannon (*H*) or Simpson (*D*) (Table 1) are slightly higher for the fine particle fraction. This might be because of the small sequence number but is surely driven by the relative dominance of Nitrososphaeraceae sequences in the coarse particulate matter (Fig. 1).

Most results of this study are in agreement with the previous Sanger-sequencing-based study by Fröhlich-Nowoisky et al. (2014), which analyzed 47 air filter pairs including

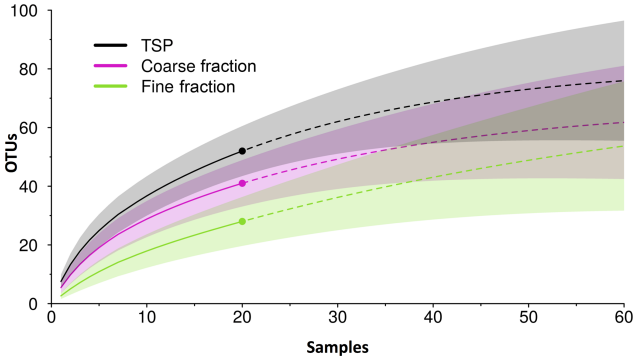

Figure 2. Rarefaction curve of species richness for total suspended (TSP), coarse, and fine particulate matter. TSP is a single airstream split into coarse and fine particulate matter. The solid curves represent the interpolated number of OTUs as a function against the number of samples. The dashed lines represent the extrapolations and the dots the sample size of this study. The colored areas represent the 95 % confidence intervals.

the 20 filter pairs we focussed on in this study. However, in Fröhlich-Nowoisky et al. (2014), only a limited number of clones were sequenced, resulting in a total of 435 sequences, as compared to 2341 sequences obtained from the current study (Table 1). Fröhlich-Nowoisky et al. (2014) concluded that archaea occur far more often in coarse particulate matter than in fine particulate matter as archaeal DNA could only be detected on 21 % of the fine particle filters, which is consistent with the results of this study. Another consistency compared to the study of Fröhlich-Nowoisky et al. (2014) is the high abundances for Group I.1.b on coarse particle samples. This can be explained by the higher relative abundance of Nitrososphaeraceae in the coarse particle fraction discovered in this study.

The main difference between the Sanger and the Illumina approach is the estimated species richness, with 137 species from Sanger estimating almost double the amount compared to the Illumina approach, which estimates 63 species. This can be caused by several issues: first, a possible lack of taxonomical depth caused by the shorter sequences compared to the Sanger approach and the usage of different primer pairs;

second, by the closed-reference-based taxonomic assignment and a possible lack of taxonomical depth in the used reference dataset; and, third and most likely, by the smaller number of sequences from more filter samples used in Fröhlich-Nowoisky et al. (2014).

As the used primers also amplified bacterial sequences, the following observation could be made. The ratio of archaea and bacteria suggests a very low proportion of airborne archaea in comparison to airborne bacteria (Fig. 3). In total, 0.07 % of the total reads could be assigned to archaea, while the rest (5.7×10^6 reads) consists of bacterial, mitochondrial, and plasmid DNA. After the sequences of mitochondria and plastids are eliminated, the ratio of archaea to bacteria increases only to 0.1 %, which is widely different from the ratios discovered in the soil and marine environment.

This extremely low ratio is an interesting phenomenon as in most possible emission sources the proportion of archaea is higher than in air.

Several studies, focusing on airborne bacteria and archaea, found that archaeal DNA in air is extremely low (Cao et al., 2014; Yooseph et al., 2013; Smith et al., 2013). Cao et al. (2014) found a proportion of 0.8 % of archaea when compared to bacteria in PM_{10} and $PM_{2.5}$ using Illumina HiSeq data (2014). Yooseph et al. (2013), who analyzed the urban prokaryotic metagenome of New York, on a multistep approach based on taxonomic classifications for their metagenomic reads and assigned to the different organism groups, found that 0.48 % of their sequences were archaeal, with roughly 80 % Euryarchaeota and 20 % Crenarchaeota and Thaumarchaeota. Both studies therefore agree with the 0.1 % archaeal sequences found in Mainz air.

Next to comparisons of species diversity and composition, the ratio of bacteria to archaea might be an indicator of the possible emission sources, as the aerosolization process is likely to equally affect all microorganisms from an emission source. We therefore compared the detected ratios with ratios of possible emission sources such as soils, surface water, and the phyllosphere reported in literature (Fig. 3).

We found that compared to soil the microbial habitat, which is often discussed as the primarily emission source, differs strongly from our and other air studies. Although archaeal abundance in aerated soils increases with depth (Kemnitz et al., 2007), the proportion known for surface soil is still much higher than the proportions in air. Thus, soil alone seems an unlikely emission source. Also in sea water their abundance increases with depth reaching up to 39 % (Karner et al., 2001). As Mainz is not close to oceans, emission from sea water seems unlikely as a primary source. The only larger emission surface from water might be the river Rhine which is very likely one of the primary sources in the study area.

In a review by Vorholt (2012) it is convincingly shown that the abundance of archaea in the phyllosphere is less than 1 % of the total microorganism load (Fig. 4), which is similar to the 0.1 % we found. With a total area of 10^9 km^2 of the upper and smaller leaf surface, the phyllosphere surface habitat is approximately twice the size of the land surface and is supposed to comprise up to 10^{26} cells worldwide (Vorholt, 2012); therefore, it could present a significant emission source (Woodward and Lomas, 2004) in the studied area. Thus, the phyllosphere might be the local primary emission source.

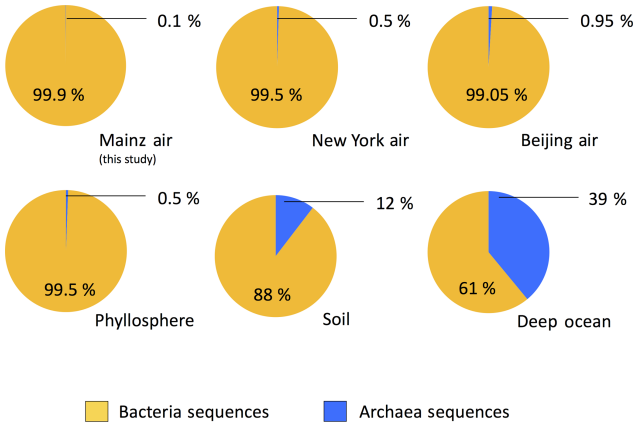

Figure 3. Relative proportions of archaeal (blue) and bacterial (yellow) sequences detected in environmental samples. Proportions for soil are based on Kemnitz et al. (2007), for the deep ocean on Karner et al. (2001), and for the phyllosphere on Delmotte et al. (2009) and Knief et al. (2012). The proportions of the Mainz air are based on this study. The data for the New York air are published in Yooseph et al. (2013) and the data of Beijing are based on Cao et al. (2014).

The situation might, however, differ for individual groups found in the air filter samples, such as the Nitrososphaera family. This family includes typical soil microorganisms, which would point to soil as a primary emission source. The presence of this family in the air might be, on the one hand, caused by the diversity of the phyllosphere. Especially for annual plants, the microorganism diversity of the phyllosphere is primarily driven by soil and the soil microbiome surrounding the sampling site (Knief et al., 2010). On the other hand, the explanation especially for the findings in the coarse fraction is that larger soil particles carry many typical soil archaea. Thus, based on the proportions of bacteria and archaea, the most likely interpretation is that the microbiome detected in the Mainz air primarily originates from the phyllosphere and is complemented by small soil particles, which add a large amount of typical soil archaea. Unfortunately, there is a lack of literature on archaea of the phyllosphere; thus, the identification of the emission source based on the composition cannot be answered for certain.

Based on the identified genera, however, the phyllosphere and the soil can both be the primary emission source. But as the microbiome of the soil drives the composition of the phyllosphere, comparing taxonomy alone will not lead to a final answer.

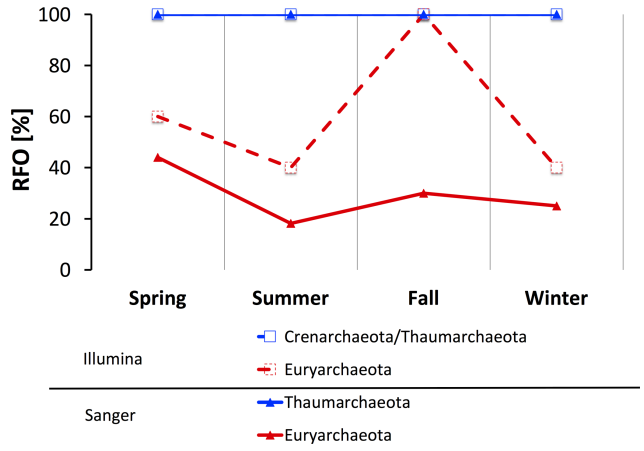

Figure 4. Seasonal variation in the relative frequency of occurrence (RFO) of airborne archaea on the phylum level. The relative frequency of occurrence – the proportion of samples in which these taxa were detected – is given for both phyla, i.e., Thaumarchaeota and Euryarchaeota. The graph based on Sanger sequencing represents the data published in Fröhlich-Nowoisky et al. (2014), whereas the remaining data comprises the results of this study.

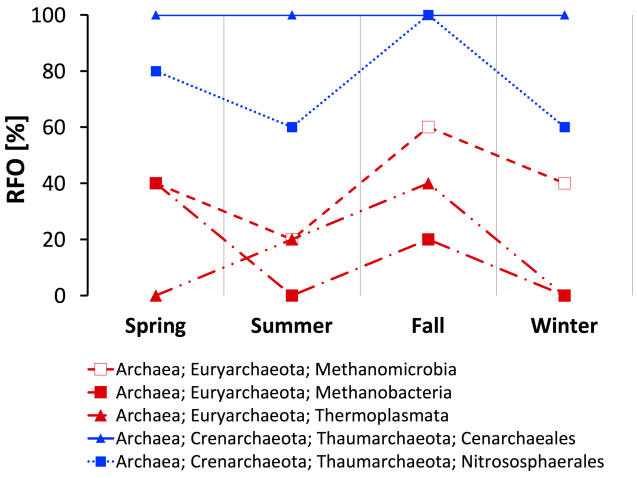

Figure 5. Seasonal variation in the relative frequency of occurrence of dominating Euryarchaeota classes and Crenarchaeota orders within this study.

3.2 Seasonal dynamics

To better understand the seasonal dynamics of archaea in the atmosphere the availability of emission sources over different seasons per year can be analyzed. As mentioned, from the 2341 archaeal sequences 168 could be assigned to Euryarchaeota. Based on their relative frequencies of occurrence (RFO), Thaumarchaeota are present all year, whereas Euryarchaeota are less abundant and their RFO values show seasonal peaks in spring and fall (Fig. 4).

Although the seasonal increasing or decreasing trends of the RFO values over the year are similar to Fröhlich-Nowoisky et al. (2014), overall they are higher.

Fröhlich-Nowoisky et al. (2014) suggested the nearby river Rhine as a potential permanent source for Methanomicrobiales and Thermoplasmatales as they are known to be present in river water throughout the year (Auguet et al., 2009; Cao et al., 2013). The RFO values of the orders shown in Fig. 5 present a slightly different picture: Methanomicrobia were observed in every season with RFO values around 40 %; thus, the Rhine could contribute continuously to the aerosolized Methanomicrobia. However, the Thermoplasmata group was exclusively found in summer and fall samples, arguing against an emission from an omnipresent source like the Rhine.

Alternatively to the Rhine, potential emission sources for several groups of Euryarchaeota – especially in agricultural areas as around Mainz – are biogas substrates and livestock fertilization methods (Fröhlich-Nowoisky et al., 2014). Figure 5 shows that Methanomicrobia and Methanobacteria both have their highest relative RFO during fall and another increase during the springs in 2006 and 2007. This supports the hypothesis, of livestock manure being a possible emission source, as both classes are commonly known to be present in the microbiome of livestock and the typical times for fertilization of fields with manure are in spring and fall (Nicol et al., 2003; Radl et al., 2007). As all methanogen groups, they have been reported in biogas reactors, too (Jaenicke et al., 2011). For the Thermoplasmata the peaks in summer and fall might be linked to the usage of biogas reactor substrates, which are also applied to agricultural fields as fertilizer. The differing RFO values of Thermoplasmata and other Euryarchaeota might be caused by their sensitivity to temperature and especially to pH, which only allows their survival in moderate to high temperatures and low pH environments.

The hypothesis that aerosolized archaea are linked to agricultural activities is also supported by the seasonal variation of the RFO of the order of the Nitrososphaerales within the Thaumarchaeota that is also present in the Euryarchaeota classes as discussed. Nitrososphaerales were found in agricultural soil samples close to the sampling area of our study (Ochsenreiter et al., 2003; Zhalnina et al., 2013) and thus can be considered a typical agricultural soil microorganism.

4 Conclusions

This study gains a deeper insight into the diversity of airborne archaea. The overall abundance of archaea in the atmosphere compared to bacteria is very low, which is comparable to the ratio found for the phyllosphere. We found the Nitrososphaeraceae family out of the I.1.b group of Crenarchaeota to be the major archaeal family in coarse particulate matter. The groups Cenarchaeaceae, Nitrososphaeraceae, Methanosarcinales, and Thermoplasmata as well as the genus *Nitrosopumilus* could be observed within the fine particulate matter.

The observed seasonal dynamics for the dominating Euryarchaeota classes and Crenarchaeota orders, which peak in summer and fall, might be a result of agriculture in the surrounding area. Therefore, anthropogenic activities like fertilization with livestock manure or substrates of biogas reactors might influence the diversity of airborne archaea as their occurrence is increased during the main fertilization seasons.

This combination of findings provides support for the conceptual premise that the occurrence of archaea in air might be driven by the microbiota of the phyllosphere but the influence of livestock manure gains an edge over the phyllosphere through the fertilization seasons. Additionally, groups emitted with soil as carrier particles seem to have a major influence on the community composition. For a further understanding of the dependencies of airborne microorganisms on their sources, future studies should additionally explore possible source habitats to gain as complete a picture as possible.

We conclude that the understanding of the seasonality, diversity, and composition of airborne archaea as one very small fraction within the bioaerosols is an important contribution to the understanding of the patterns driving the whole atmospheric microbiome.

Data availability. The post-library-split sequence dataset is available from the Edmond digital repository at: https://doi.org/10.17617/3.1l (Wehking et al., 2018).

The Supplement related to this article is available online at https://doi.org/10.5194/bg-15-4205-2018-supplement.

Author contributions. JW and VRD wrote the paper. VRD, UP, JF-N, DAP, JW, RMB, and DT designed the research. JF-N and RMB performed the sample collection and laboratory work. JW performed downstream analysis. JW and DAP performed correlations with meteorological data. All co-authors discussed the results, read and contributed to the manuscript.

Competing interests. The authors declare that they have no conflict of interest.

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Community composition and seasonal changes of archaea in coarse and fine air particulate matter

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Supplementary material.

Table S1: List of air filter samples analysed for Archaea presence. While the air masses collected on MZ 11, MZ 15, and MZ 31 were sampled over 1, 5, and 5 days, respectively, all other air filter samples were taken over a 7-day period corresponding to ~ 3000 m^3 of sampled air. Air masses analysed and sequenced with Illumina within this study are bold. For comparison the samples analysed and published in Fröhlich-Nowoisky et al (2014) were included, n.a. = not available as these air masses were not sequenced with Illumina but exclusively via Sanger sequencing.

Sample ID	Sampling period	Number of sequences 16S Sanger (coarse, fine)	Number of sequences 16S Illumina (coarse, fine)
MZ 1	24.03.2006 - 31.03.2006	5	n.a.
MZ 2	31.03.2006 - 07.04.2006	8	n.a.
MZ 4	07.04.2006 - 12.04.2006	11	n.a.
MZ 6	15.04.2006 - 18.04.2006	4	n.a.
MZ 9	20.04.2006 - 27.04.2006	5	n.a.
MZ 10	27.04.2006 - 02.05.2006	5	n.a.
MZ 11	**02.05.2006 - 03.05.2006**	**7**	**3 - 80**
MZ 15	**04.05.2006 - 09.05.2006**	**9 - 6**	**195 - 81**
MZ 18	12.05.2006 - 15.05.2006	7	n.a.
MZ 19	15.05.2006 - 16.05.2006	7	n.a.
MZ 21	17.05.2006 - 18.05.2006	4	n.a.
MZ23 blank	18.05.2006		0 - 2
MZ 24	18.05.2006 - 22.05.2006	13	n.a.

MZ 25	22.05.2006 - 23.05.2006	12	n.a.
MZ 26	**23.05.2006 - 30.05.2006**	**12 - 6**	**301 - 103**
MZ 31	**01.06.2006 - 06.06.2006**	**8**	**208 - 5**
MZ 33	08.06.2006 - 13.06.2006	10	n.a.
MZ 35	14.06.2006 - 21.06.2006	5 - 1	n.a.
MZ 36	21.06.2006 - 22.06.2006	10	n.a.
MZ 40	27.06.2006 - 04.07.2006	9	n.a.
MZ 41	**04.07.2006 - 11.07.2006**	**5**	**19 - 284**
MZ 45	19.07.2006 - 21.07.2006	14, 4	n.a.
MZ 47	**26.07.2006 - 02.08.2006**	**5**	**32 - 23**
MZ 50	**02.08.2006 - 09.08.2006**	**9**	**196 - 132**
MZ 51	09.08.2006 - 16.08.2006	5	n.a.
MZ 52	**16.08.2006 - 23.08.2006**	**9**	**234 - 2**
MZ 54	**30.08.2006 - 06.09.2006**	**4 - 15**	**183 - 574**
MZ 59	**11.09.2006 - 18.09.2006**	**11**	**597 - 3**
MZ 60	18.09.2006 - 25.09.2006	5	n.a.
MZ 62	**02.10.2006 - 09.10.2006**	**6**	**318 - 351**
MZ 63	09.10.2006 - 16.10.2006	12	n.a.
MZ 66	16.10.2006 - 23.10.2006	4	n.a.

MZ 67	23.10.2006 - 30.10.2006	12	29 - 321
MZ 69	02.11.2006 - 09.11.2006	20	n.a.
MZ 71	16.11.2006 - 23.11.2006	1	n.a.
MZ73 blank	23.11.2006		371 - 37
MZ 74	23.11.2006 - 30.11.2006	8	797 - 2
MZ 75	30.11.2006 - 07.12.2006	11 - 7	n.a.
MZ 77	14.12.2006 - 21.12.2006	5	n.a.
MZ 81	28.12.2006 - 04.01.2007	5	5 - 0
MZ 82	04.01.2007 - 11.01.2007	8	202 - 21
MZ 84	18.01.2007 - 25.01.2007	5 - 4	n.a.
MZ 88	01.02.2007 - 08.02.2007	3	93 - 2
MZ 90	15.02.2007 - 22.02.2007	9	122 - 485
MZ 93	22.02.2007 - 01.03.2007	15 - 6	597 - 2
MZ 95	08.03.2007 - 15.03.2007	9	n.a.
MZ 97	22.03.2007 - 29.03.2007	5	n.a.
MZ 101	05.04.2007 - 12.04.2007	11 - 4	36 - 4
MZ 103	19.04.2007 – 26.04.2007	10 - 4	109 - 5
	∑ (+contaminants)		4285 - 2507
	∑ (-contaminants)		2180 - 161

Table S2: Air filter blank samples analyzed for archaeal contamination. All families found on mounting blank filters comprise together 410 sequences: families were discarded from the data if present in more than 1% of all detected archaeal sequences on the mounting blanks, i.e., the Methanoregulaceae (8.54%), Methanomassiliicoccaceae (17.56%), and the Methanobacteriaceae (72.44%).

	MZ 23		MZ 73			
	coarse	fine	coarse	fine	∑	%
Cenarchaeaceae	0	0	1	1	2	0.49
Methanobacteriaceae	0	0	297	0	297	72.44
Methanoregulaceae	0	0	0	35	35	8.54
Methanosaetaceae	0	2	1	1	4	0.98
Methanomassiliicoccaceae	0	0	72	0	72	17.56