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Structure and function of the global topsoil microbiome

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Supplementary results and discussion

Environmental associations of the global topsoil microbiome

To determine the main predictors of taxonomic diversity based on our global metabarcoding soil data, we applied generalized least squares (GLS) regression models. The best-fit models revealed that soil pH predominantly explains the variation in taxonomic richness (r^2=0.633, $p<0.001$) as well as phylogenetic diversity (r^2=0.677, $p<0.001$) of bacteria. Bacterial phylogenetic diversity and richness displayed a quadratic relationship with soil pH, peaking at values 5-6, which is consistent with several smaller scale studies[1,2]. Soil pH was also the best predictor for the relative abundance of most phyla, as revealed by the random forest machine learning approach (Extended Data Fig. 4). However, the relative abundance and diversity of particular phyla such as Firmicutes and Thermotogae were mostly affected by climatic variables including temperature (Extended Data Fig. 1b, Extended Data Fig. 4). Compared to other bacterial phyla and the general trend of bacterial latitudinal diversity, Firmicutes, Chlamydiae, Thermotogae and WD272 and Cyanobacteria were clustered together in a cluster analysis based on their correlations to latitude and environmental variables (Extended Data Fig. 1b). The deviation of a few phyla from the general LDG trends can be explained by responses to edaphic and climate factors weakly related to latitude (Extended Data Fig. 1b) or contrasting effects at lower taxonomic levels. Thus, environmental filtering may be stronger at the level of phylum.

While several large scale studies have also reported soil pH as the main predictor of microbial taxonomic diversity[1-3], others have found temperature[4] and soil nutrient stoichiometry[5] as the main predictors of bacterial diversity from regional to continental scales. These conflicting results could reflect limited ranges of climate and soil pH covered in previous smaller scale studies or no one single variable explaining the bulk of diversity across different microbial taxa. Temperature was a negligible factor for the overall taxonomic diversity (Supplementary Table 3). Similarly to their functional gene diversity, the metabolic potential of bacteria (inferred from the relative abundance of genes belonging to metabolic OGs functions), respectively, showed a relationship with latitude (r^2=0.234, $p<10^{-11}$; r^2=0.10, $p<10^{-4}$, respectively) and a weak relationship with temperature ($p>0.05$; Supplementary Table 14). From these, we infer that the metabolic theory of ecology[6,7] may not be largely applicable to the global distribution of soil bacteria soil microbes and substrate availability,

local environment and biotic interactions may overrule each other's effect on the global distribution of soil bacteria.

Due to high microbial genomic plasticity[8,9] and functional redundancy in microbial communities[10,11], distributions of microbial taxa may not directly reflect distributions of microbial functions. The lack of a perfect fit between taxonomic and functional diversity may stem from expansion and reduction in gene families and horizontal gene transfer[12] and decoupling mechanisms of taxonomic and functional gene assemblies[8]. Thus, the joint effects of climate and soil factors on functional gene composition compared to the dominant effect of soil pH on taxonomic composition may explain the lack of perfect fit between taxonomic and functional gene composition on a global scale. Functional diversity of soil bacteria was positively correlated to taxonomic diversity, for all levels from OTUs to classes ($r=0.704\pm0.063$, mean±SD). Similarly, the richness of bacterial Operational Taxonomic Units (OTU) and functional Orthologous groups (OG) were strongly correlated ($r=0.504$, $p<0.001$). Unlike bacteria, functional and taxonomic richness of fungi were weakly correlated ($p>0.05$). Fungal functional richness was most strongly affected by C/N ratio ($r^2=0.357$, $p<0.001$) and less affected by pH ($r^2=0.103$, $p<0.001$). We used structural equation modelling (SEM, see Methods) to predict the response of functional diversity to environmental factors that may affect functional diversity directly and indirectly via the structuring of taxonomic diversity and the alteration of other parameters (see Methods). In bacteria, mean annual precipitation (MAP) was the key variable correlated to functional diversity, greatly exceeding the effect of pH (Extended Data Figs. 3g,h, 5c,g; Supplementary Table 3,5). Moreover, the pH effect on functional diversity was mostly mediated by altered taxonomic diversity (Supplementary Table 5). In fungi, functional diversity was negatively associated with soil pH, with a weak positive association to MAP (Extended Data Figs. 3g,h,5d,h; Supplementary Table 3,5). These results suggest that opposing responses to MAP and soil pH may partly explain the observed inverse pattern of functional diversity in bacteria and fungi (Fig. 1). Using SEM modelling, we also tested whether the observed correlations between bacterial diversity and environmental variables stem from microbial effect on or response to their environmental conditions. We thus tested the hypothesis that MAP could affect soil pH though its effect on bacterial diversity as a major component of topsoil microbial diversity. Comparing model fits suggested that soil pH is more likely to affect rather than to be affected by bacterial diversity in our global dataset (Model: MAP> soil

pH> Bacterial OG & OTU diversity; AIC: 26.00; RMSEA: 0.000, P=0.959, compared to Model: MAP> Bacterial OG & OTU diversity> soil pH; AIC: 27.23; RMSEA: 0.053, P=0.367)

Taxonomic and functional differences between biomes

Overall, bacteria were the most abundant microbial group in topsoil, especially bacteria from the phyla Proteobacteria (34.3% of all sequences), Actinobacteria (15.5%), Cyanobacteria (10.9%), Acidobacteria (10.0%), Planctomycetes (6.90%), Verrucomicrobia (4.0%) and Bacteroidetes (2.8%), followed by the fungal phyla Basidiomycota (2.6%) and Ascomycota (2.4%) (Supplementary Table 17). There were significant differences in the phylogenetic composition of bacteria among biomes (PERMANOVA: pseudo-F=7.292, r^2=0.321, p=0.001; Extended Data Fig. 2f,5i; Supplementary Table 14). Across all metagenomes, in genes for which OGs could be assigned, the most abundant broad function category (COG one-letter codes, as available in eggNOG), were *replication, recombination and repair* (8%) and *carbohydrate transport and metabolism* (7%) (Supplementary Table 17). Similar to taxonomic groups, functional gene composition (PERMANOVA; bacteria: pseudo-F=2.162, r^2=0.108, p=0.001; Extended Data Fig. 5k) and the relative abundances of functional categories were significantly discriminated across biomes (pseudo-F=5.495, r^2=0.235, p=0.001; Extended Data Fig. 2h,i; Supplementary Table 14). Because they describe mostly housekeeping gene roles, OG functional categories are known to show little variance between samples[9]; however, when small differences are significant across biomes, it indicates that certain gene functions have an effect on or respond to the environment and are thus ecologically important (this was tested using a non-parametric test that only compares ranks).

Correlation of Antibiotic resistance and fungal relative abundance

As carriers of ARGs, it is expected that the abundance of bacteria is somewhat correlated with that of ARGs. For example, (alpha) Proteobacteria, whose genomes are known to be enriched for ARGs, were a major bacterial phylum positively correlated with ARG relative abundance. However, this correlation was weaker than the positive correlation between ARG and fungal abundance, a correlation which is strong despite fungal genomes not being expected to carry genes closely matching models for bacterial ARGs, each with very strict cutoffs that should not allow for cross kingdom orthologous matches). We have also used SEM modelling to test possible scenarios of direct/indirect associations to put these correlations in environmental context and found that in all models fungal rather than bacterial abundance appears to be a better determinant of ARG abundance. In addition, we tested this by including fungal and bacterial abundance instead of B/F in the SEM

model (Fig. 3c). We found the model including fungal abundance has significantly better fit (RMSEA=0.065, PCLOSE=0.00; AIC=29.79) compared to the models including bacterial abundance (RMSEA=0.378, PCLOSE=0.278; AIC=55.80) or both fungal and bacterial abundances (RMSEA=0.456, PCLOSE=0.278; AIC=118.10).

While we used a specific bacterial ARG database, to examine the rate of false positive discovery of ARGs as a result of increasing fungal genomes in samples enriched for fungi, we characterized ARG profiles in simulated metagenomes based on samples that included only fungal genomes (n=15) from diverse fungal groups including those with high antibiotic production potential (including *Oidiodendron* and *Aspergillus*) using our methods (Supplementary Table 10). This analysis revealed a very low AR potential (3E-07±6E-07 read fraction) compared to the AR potential of a simulated community composed solely of bacteria (9E-04±3E-04 read fraction). We also re-analyzed our data using the CARD database, despite its limitations (see Methods), and our main results remained qualitatively unchanged (namely ARG abundance correlated to fungal (r=0.275, p=0.0001) rather than to bacterial abundance (r=-0.145, P=0.048), and fungal phyla/classes (particularly Ascomycota, Leotiomycetes) strongly correlated to ARG abundance (see Supplementary Table 18).

Correlation of Antibiotic resistance to functional genes

To further understand genomic mechanisms behind changes in antibiotic resistance (AR) potential in topsoil, we sought to identify gene functional pathways associated with antibiotic resistance genes (ARG) abundance. We expected that as a result of more intense fungal-bacterial competition in samples with high numbers of ARGs, there should exist a greater abundance of functions related to cell damage and nutrient stress responses. While we found a significant correlation between ARG relative abundance and specific antibiotic biosynthesis KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways (r=0.200, p=0.006), ARG relative abundance was more strongly correlated with the relative abundance of reactive oxygen species (ROS) protection factors such as peroxidases ($r=0.477$, $p<10^{-11}$) as well as the SEED categories of virulence ($r= 0.556$, $p < 10^{-15}$) and stress response ($r=0.283$, $p<10^{-5}$). Closer examination showed that peroxidases that were significantly correlated with ARGs mainly consisted of catalases, which contribute to the stress response by removing hydrogen peroxide (H_2O_2). The induction of ROS is known to be a mechanism through

which antibiotics can kill bacteria[13]. Fungi can also generate excessive ROS to compete with bacteria[14] or with each other[15], particularly under nitrogen limitation, which in turn promotes AR in the microbial community. There was no significant correlation between the relative abundance of fungal "Secondary metabolites biosynthesis, transport and catabolism" and soil C/N and ARG relative abundance. However, a detailed re-analysis at higher resolution level (subcategories) within this functional category revealed that the relative abundance of OGs related to the biosynthesis of polyketide which is involved in production of antibiotics strongly correlates with C/N ratio ($r=0.536$, $p<10^{-8}$) and ARG abundance ($r=0.415$, $p<10^{-8}$) (Supplementary Table **4**). In addition, analysis of fungal specific biosynthetic gene clusters (BGCs) with known antibiotic production reports revealed that the abundance of these clusters correlate significantly to C/N and ARG abundance in the global ($r=0.456$, $p<10^{-10}$; $r=0.346$, $p<10^{-4}$, respectively) and in the external validation dataset ($r=0.420$, $p<10^{-6}$; $r=0.381$, $p<10^{-5}$, respectively). These data together with evidence of fungi-bacteria antagonism in nature suggest that resistance may, at least partially, have arisen as a response to antibiotics as a means of competition, rather than as e.g. signalling agents, which has also been proposed[16]. Future experiments to test these hypotheses will improve our understanding of the forces shaping the distribution of ARGs and the conditions that select for resistant strains over sensitive ones.

Analysis of antibiotic resistomes from additional environments

To test if the positive relationship between ARGs and fungi observed in the topsoils sampled herein and oceans extends to other habitats, we analysed 233 additional soil metagenomes from non-forested natural (grassland and desert) and human-impacted soils, including pasture, lawn and agricultural soils available from MGRAST[17]. Due to the heterogeneity of the available sequencing data, a general approach was adopted to quality filter the samples. Sequences shorter than 90 bp length and those longer than 1000 bp (e.g. uploaded assemblies) were rejected to accommodate the data for our direct mapping approach. Thus, 3,350,904,460 (52.45%) of 6,388,725,921 processed reads from 214 samples passed our stringent quality control.

As the relative abundance of ARGs was to a large part explained by Bacteria to Fungi (B/F) ratio in two very diverse ecosystems (soil and ocean), we also analysed this in the additional soil sample sets obtained from MGRAST. For deserts, grasslands, pasture and lawn, fungi are also key associated

taxa of ARG relative abundance (Supplementary Table 8), and there was a significant correlation between ARG relative abundance and B/F abundance ratio ($r=-0.327$, $p<0.001$). However, in agricultural soils, the relative abundance of ARGs was unrelated to B/F abundance ratio ($r=-0.02$, $p=0.824$).

To compare patterns of ARG diversity in soils and oceans to human gut, human skin, we re-analysed the metagenomics data sets of the MetaHit project[18] (Supplementary Table **1**). MetaHit sequences were obtained from European Bioinformatics Institute Sequence Read Archive under accession ERP004605. After quality filtering, 4,891,268,695 out of 6,100,278,254 reads were retained from the MetaHit. Inclusion of data from MetaHit was limited to the 68 samples from Denmark that were sequenced on a HiSeq 2000 sequencer to maintain consistency with the Tara Oceans samples. We also included 310 skin samples from American patients (263 samples from ref[19]. and 57 skin samples from Human Microbiome Project[20] (HMP) sequenced on Illumina HiSeq 2000 (obtained from ftp://public-ftp.hmpdacc.org). These samples originated from 18 body sites and are thus assumed to represent the human skin microbiome; for this dataset 8,374,233,488 of 11,443,026,496 reads were retained after quality filtering (see Methods). The quality-filtered reads from all datasets were mapped to the corresponding databases using Diamond[21], with the exception that no merging of read pairs was attempted, because the chances of finding overlapping reads were too low (with a read length of 100bp and insert size of 300 bp (Tara Oceans) or 350 bp (MetaHit). Sequences for SSU and LSU were extracted from these metagenomics datasets for constructing taxon by sample matrices, as described in the miTag approach. ARG abundance matrices were also obtained from the Tara Oceans and MetaHit projects based on the published gene catalogues annotated using similar approach as in the current study.

In contrast to what we observed in the soil and ocean samples, we found no negative association between ARG relative abundance and B/F abundance ratio in the human samples (gut: $r=0.087$, $p=0.501$; skin: $r=0.384$, $p<0.001$). In addition, fungal relative abundance was not positively correlated with ARG relative abundance (gut: $r=0.038$, $p=0.753$; skin: $r=-0.269$, $p=0.036$) or diversity (gut: $r=-0.033$, $p=0.785$; skin: $r=0.078$, $p=0.169$) in these samples. However, similar to what we observed in the soil and ocean samples, sparse partial least squares (SPLS) regression analysis revealed that certain fungal phyla are among the phyla that were most strongly associated

with bacterial ARG relative abundance (Cryptomycota: r=0.263, p<0.001, q<0.001), ARG diversity (Chytridiomycota: r=0.232, p<0.001, q<0.001; Ascomycota: r=0.449 p<0.001, q<0.001) and ARG richness (Chytridiomycota: r=0.299, p<0.001, q<0.001; Ascomycota: r=0.366, p<0.001, q<0.001) in human skin. In human gut microbiome samples, ARG relative abundance showed no significant correlation with any of bacterial or fungal phyla. However, ARG diversity and richness were most strongly and significantly correlated with Bacteroidetes (r=0.392, p<0.001, q=0.027) and Chytridiomycota (r=0.499, p<0.001, q<0.001), respectively. At the class level, fungi were also among the main associated taxa with ARG diversity in the human gut and skin samples (Supplementary Table 8). These data suggest that, although the human ARG abundance patterns may be confounded by life style factors, such as antibiotics treatment as reported earlier[22], certain fungal taxa appear to be associated with a greater variety of ARG families and thus higher ARG diversity and richness in the human associated microbiomes.

Investigating antibiotic producing classes in fungal genomes

One of our questions was whether the association between the relative abundance of fungi and ARGs could result from the potential of fungi to produce antibiotics. Unfortunately, the relatively sparse existing knowledge about antibiotic biosynthetic gene clusters (BGCs) in fungi is not available in systematically structured resources; in addition, prediction of BGCs relies on gene order/genomic proximity, which makes it difficult to investigate their presence in environmental metagenome samples. Therefore, we inspected the presence of antibiotic production related gene systems in 259 published fungal genomes (Supplementary Table 12) representing different clades retrieved from public whole genome databases using antiSMASH[23] (version 4.0) to classify putative secondary metabolites. The major groups with strong correlations to ARG abundance were filamentous Ascomycota, including Leotiomycetes, Eurotiomycetes, Dothideomycetes and Sordariomycetes (Supplementary Table 8). These fungi were significantly enriched (chi-squared=89.40, $p<10^{-15}$, Kruskal-Wallis test) in BGCs known to produce antibiotics (2.20±1.77 BGCs) compared to other fungal genomes (0.33±1.64 BGCs), suggesting their higher potential to produce antibiotics. Independent of taxonomy, the number of antibiotic producing BGCs in each fungal genome correlated significantly with how strong the phyla or classes they belong to correlated to ARGs in our global soil dataset (r=0.47, $p<10^{-13}$; r=0.181, p=0.026, respectively). In particular, *Oidiodendron* (several OTUs of which strongly correlated with ARG abundance in our data; Supplementary Table 6) exhibited a high number of putative secondary metabolites and antibiotic producing BGCs

(Supplementary Table 12). Interestingly, BGCs were completely absent in the genome of the phyla Glomeromycota and Cryptomycota that showed a very weak correlation with ARGs (Supplementary Table 12). These results support our assumption that fungal antibiotic production may affect ARG abundance in topsoil.

Compared to topsoil, the relative abundance Ascomycota was negatively correlated with ARG relative abundance in the ocean. Lack of positive correlation between ARG and Ascomycota in the ocean may be explained by their different environmental associations as well as composition and function of these fungi in the ocean and topsoil (Supplementary Table **9**). The relative abundance of both Ascomycota and Basidiomycota negatively correlated with mean annual temperature (MAT; $r=-0.47$, $p<0.001$; $r=-0.29$, $p<0.01$, respectively) that was positively correlated with ARG abundance in the ocean (Fig. 2). In the ocean, Oomycota had a 5 times higher relative abundance than e.g. Ascomycota and therefore seem to be the major eukaryotic group. Oomycota are known to have strong antagonistic relationship with bacteria and are significantly negatively correlated with Ascomycota in our dataset ($r=-0.300$, $p<0.001$). Using SEM modelling and after accounting for the effect of either MAT or the correlation of Oomycota and ARG relative abundance, there was no correlation between the abundance of ARGs and Ascomycota. Despite a strong correlation between ARG relative abundance and oomycetes in the ocean, to the best of our knowledge there are no convincing records whether or not oomycetes produce antibiotics, although oomycetes are also known to have a strong antagonistic relationship with bacteria in several studies, which can suppress bacterial growth and fitness[24-27]. To investigate this further based on genomic analysis, we downloaded all oomycetes genomes available in FungiDB (n=20, http://fungidb.org) and used antiSMASH to predict 283 BGCs (14.15/genome on average). While the vast majority of discovered BGCs (51%) were classified as putative, 4% were classified as NRPS, 6% as Terpene and 9% as CF_saccharide (Supplementary Table 19). These classes are among the biosynthetic gene clusters that can potentially provide the biosynthetic pathways to produce known antibiotics[28]. Thus, we cannot exclude the possibility that the strong association of oomycetes and ARG relative abundance in oceans could stem from oomycetes directly producing antibiotics. Alternatively, oomycetes may stimulate other organisms to produce antibiotics, as antibiotic substances production can increase bacterial fitness[29], to compete with fast-growing oomycetes. Oomycetes may also stimulate other groups such as fungi[30] and algae[31,32] to produce antibiotic compounds.

Taken together, our results indicate that the relative size and diversity of environmental resistomes are determined by antagonistic interactions with eukaryotic microbes, especially various fungal classes, of which some produce antibiotics[33-37] , and fungi-like oomycetes. Regardless of whether they produce them by themselves or stimulate bacteria to produce antibiotics, these eukaryotes may be promising candidates for novel antibiotic discovery in environmental samples. The metagenomics analysis outlined here is also applicable to other habitats for identifying ARG-associated organisms.

Supplementary References

1 Fierer, N. & Jackson, R. B. The diversity and biogeography of soil bacterial communities. *Proc. Natl. Acad. Sci. USA* **103**, 626-631 (2006).

2 Chu, H. *et al.* Soil bacterial diversity in the Arctic is not fundamentally different from that found in other biomes. *Environ. Microbiol.* **12**, 2998-3006 (2010).

3 Lauber, C. L., Hamady, M., Knight, R. & Fierer, N. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Appl. Environ. Microbiol.* **75**, 5111-5120 (2009).

4 Zhou, J. *et al.* Temperature mediates continental-scale diversity of microbes in forest soils. *Nat. Commun.* **7**, 12083 (2016).

5 Delgado-Baquerizo, M. *et al.* It is elemental: soil nutrient stoichiometry drives bacterial diversity. *Environ. Microbiol.* **19**, 1176-1188 (2017).

6 Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M. & West, G. B. Toward a metabolic theory of ecology. *Ecology* **85**, 1771-1789 (2004).

7 Price, C. A. *et al.* Testing the metabolic theory of ecology. *Ecol. Lett.* **15**, 1465-1474 (2012).

8 Medini, D., Donati, C., Tettelin, H., Masignani, V. & Rappuoli, R. The microbial pan-genome. *Curr. Opin. Genet. Dev.* **15**, 589-594 (2005).

9 Stukenbrock, E. H. Evolution, selection and isolation: a genomic view of speciation in fungal plant pathogens. *New Phytol.* **199**, 895-907 (2013).

10 Allison, S. D. & Martiny, J. B. Resistance, resilience, and redundancy in microbial communities. *Proceedings of the National Academy of Sciences* **105**, 11512-11519 (2008).

11 Louca, S., Parfrey, L. W. & Doebeli, M. Decoupling function and taxonomy in the global ocean microbiome. *Science* **353**, 1272-1277 (2016).

12 Dinsdale, E. A. *et al.* Functional metagenomic profiling of nine biomes. *Nature* **452**, 629 (2008).

13 Dong, T. G. *et al.* Generation of reactive oxygen species by lethal attacks from competing microbes. *Proceedings of the National Academy of Sciences* **112**, 2181-2186 (2015).

14 Tornberg, K. & Olsson, S. Detection of hydroxyl radicals produced by wood-decomposing fungi. *FEMS Microbiol. Ecol.* **40**, 13-20 (2002).

15 Gessler, N., Aver'Yanov, A. & Belozerskaya, T. Reactive oxygen species in regulation of fungal development. *Biochemistry (Moscow)* **72**, 1091-1109 (2007).

16 Aminov, R. I. The role of antibiotics and antibiotic resistance in nature. *Environ. Microbiol.* **11**, 2970-2988 (2009).

17 Meyer, F. *et al.* The metagenomics RAST server – a public resource for the automatic phylogenetic and functional analysis of metagenomes. *BMC Bioinformatics* **9**, 386 (2008).

18 Forslund, K. *et al.* Disentangling the effects of type 2 diabetes and metformin on the human gut microbiota. *Nature* **528**, 262 (2015).
19 Oh, J. *et al.* Biogeography and individuality shape function in the human skin metagenome. *Nature* **514**, 59-64 (2014).
20 Consortium, H. M. P. Structure, function and diversity of the healthy human microbiome. *Nature* **486**, 207-214 (2012).
21 Buchfink, B., Xie, C. & Huson, D. H. Fast and sensitive protein alignment using DIAMOND. *Nat. Methods* **12**, 59-60 (2015).
22 Forslund, K., Sunagawa, S., Coelho, L. P. & Bork, P. Metagenomic insights into the human gut resistome and the forces that shape it. *Bioessays* **36**, 316-329 (2014).
23 Blin, K. *et al.* antiSMASH 2.0—a versatile platform for genome mining of secondary metabolite producers. *Nucleic Acids Res.* **41**, W204-W212 (2013).
24 Fedi, S. *et al.* Evidence for signaling between the phytopathogenic fungus Pythium ultimum and Pseudomonas fluorescens F113: P. ultimum represses the expression of genes in P. fluorescens F113, resulting in altered ecological fitness. *Appl. Environ. Microbiol.* **63**, 4261-4266 (1997).
25 Newell, S. Y. & Fell, J. W. Competition among mangrove oomycotes, and between oomycotes and other microbes. *Aquat. Microb. Ecol.* **12**, 21-28 (1997).
26 Mille-Lindblom, C., Fischer, H. & J Tranvik, L. Antagonism between bacteria and fungi: substrate competition and a possible tradeoff between fungal growth and tolerance towards bacteria. *Oikos* **113**, 233-242 (2006).
27 Mille-Lindblom, C. & Tranvik, L. J. Antagonism between bacteria and fungi on decomposing aquatic plant litter. *Microb. Ecol.* **45**, 173-182 (2003).
28 Tracanna, V., de Jong, A., Medema, M. H. & Kuipers, O. P. Mining prokaryotes for antimicrobial compounds: from diversity to function. *FEMS Microbiol. Rev.* **41**, 417-429 (2017).
29 Vining, L. C. Functions of secondary metabolites. *Annual Reviews in Microbiology* **44**, 395-427 (1990).
30 Takahashi, K. *et al.* Cladomarine, a new anti-saprolegniasis compound isolated from the deep-sea fungus, Penicillium coralligerum YK-247. *J. Antibiot.* **70**, 911 (2017).
31 Gómez-Gómez, L. & Boller, T. Flagellin perception: a paradigm for innate immunity. *Trends Plant Sci.* **7**, 251-256 (2002).
32 Nürnberger, T. & Brunner, F. Innate immunity in plants and animals: emerging parallels between the recognition of general elicitors and pathogen-associated molecular patterns. *Curr. Opin. Plant Biol.* **5**, 318-324 (2002).
33 Berdy, J. Bioactive microbial metabolites. *J. Antibiot.* **58**, 1 (2005).
34 Fleming, A. On the antibacterial action of cultures of a penicillium, with special reference to their use in the isolation of B. influenzae. *Br. J. Exp. Pathol.* **10**, 226 (1929).
35 Bérdy, J. Thoughts and facts about antibiotics: where we are now and where we are heading. *J. Antibiot.* **65**, 385-395 (2012).
36 Andersen, N. R. & Rasmussen, P. The constitution of clerocidin a new antibiotic isolated from Oidiodendron truncatum. *Tetrahedron Lett.* **25**, 465-468 (1984).
37 Li, L., Li, D., Luan, Y., Gu, Q. & Zhu, T. Cytotoxic metabolites from the antarctic psychrophilic fungus Oidiodendron truncatum. *J. Nat. Prod.* **75**, 920-927 (2012).