Microbial Degradation of 2-Methylisoborneol in Forest Soil**

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Microorganisms use a complex array of chemical compounds to interact with their surroundings. They produce and process different molecules in response to changes in the environment or in their metabolism. One of the most well-known volatile organic compounds produced by microorganisms is the C11-terpenoid 2-methylisoborneol (2-MIB), which has received attention because of the off-flavor it confers to fresh and reservoir water as well as to cultured fish. Cleaning water supplies of the off-flavor 2-MIB has been of interest for the scientific community for years, with the use of techniques that are either expensive, e.g., activated carbon, or create toxic byproducts, e.g., ozonation. In the present study, soil samples from nature were collected from a forest and the volatile organic compounds produced by microbes were extracted and analyzed with focus on non-canonical terpenoid structures. HS-SPME-GC/MS analysis of soil samples revealed 1-methylcamphene (1-MC), 2-methylenebornane (2-MB) and 2-MIB as C11-terpenoids. Due to the high 1-MC/2-MIB ratio compared to previous reports, it was hypothesized that microbial degradation of 2-MIB was in place. Addition of synthetic 2-MIB to biologically active soil revealed complete degradation of the pollutant to 2-MB, 1-MC and 2-methyl-2-bornene (2-M2B). The results suggest the potential of using respective natural microorganisms for biodegradation of 2-MIB, with applications in water treatment, fishery and soil ecology.

Keywords: terpenoids, gas chromatography, mass spectrometry, environmental chemistry.

Introduction

To communicate with their surroundings, microorganisms use complex blends of chemical compounds. Changes in the environment or in their metabolism elicit specific responses, which involve the generation and processing of various molecules. In some cases, the specific response involves the emission of volatile organic compounds (VOCs), which in turn are perceived by other individuals establishing intra- and inter-specific communication patterns. One of the most well-known secondary metabolites produced by microorganisms is the C11-terpene 2-methylisoborneol (2-MIB), which is also a VOC and has received attention because its presence in water causes an unpleasant flavor perception, which is a problem for water supply management. 2-MIB has a smell described as muddy or camphor-like, and the threshold for odor and flavor effect is very low (<5 ng/L).[1] Even though it is a component of the characteristic blend of camembert and brie cheeses,[2] the combination of low detection threshold and unpleasant odor of this compound categorizes it as a contaminant of drinking water, wine and fishery products.[3–5]

Since the threshold for its perception by the human nose is very low, small amounts are enough for turning the product that contains it, unfit for human consumption. For that reason, the cleaning water supplies in order to get rid of the off-flavor 2-MIB, has been a topic of interest for the scientific community for years. 2-MIB does not decompose naturally, thus several attempts were made to remove or degrade it,

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using diversified methods ranging from UV light to degradation by specific enzymes.\[^{6-8}\] Activated carbon can successfully remove it, but the adsorbent gets quickly saturated from natural organic matter present in the water and needs to be changed constantly, leading to high demand for the material (reviewed recently by Mustapha and colleagues).\[^{9}\] On the other hand, ozonation is an option for chemical degradation, but has the disadvantage of generating many unwanted toxic byproducts. Biodegradation using bacterial strains or enzymes has been discussed as a possible solution to the problem, a technique that has the convenience of reduced by-product generation and no use of additional chemicals.\[^{10}\]

2-MIB is a non-canonical terpene produced by action of a methyltransferase, which methylates the C10 precursor geranyl pyrophosphate (GPP), and a terpene synthase that uses the formed C11 intermediate to form a bicyclic terpene with 11 carbon atoms. The biosynthetic pathway of 2-MIB was elucidated by different research groups\[^{11,12}\] and since then, further studies by us and others have uncovered the existence of additional non-canonical terpenes which require the activity of prenyl pyrophosphate MTases in their biosynthesis.\[^{13-15}\] The production of 2-methylisoborneol was demonstrated for a myriad of different organisms, mostly actinobacteria, and cyanobacteria, but also fungi.\[^{16-18}\] Even though 2-MIB has a clear olfactory effect in humans, its role in the microbial community is still under investigation. Recently, 2-MIB was shown to be responsible for insect attraction, evidencing its ecological role as elicitor of behavioral responses.\[^{19,20}\]

Terpenes in general seem to be important chemical cues for microorganisms, and their production and detection constitute a way to communicate with each other and their surroundings.\[^{21}\] The identification of terpenes that mediate these microbial interactions could be the key for understanding their ecological roles. For that, more information is needed on the quality and quantity of volatile terpenes that are present in natural ecosystems. Aiming at contributing to this niche of microbial ecology, soil samples from nature were collected from a forest, and the volatile organic compounds produced by microbes were extracted and analyzed. The goal was to qualitatively analyze the volatiles produced by the microbial community, with focus on non-canonical terpenes. Additionally, the biodegradation of 2-methylisoborneol by soil microorganisms was demonstrated.

### Results and Discussion

The soil for analysis was collected in seven different spots within the forest. Volatiles were extracted under laboratory conditions and analyzed with GC/MS. Mean peak area was calculated from results of peak integration of chromatograms obtained from the seven different spots. Analysis of the volatiles emitted by soil samples revealed the presence of 2-MIB, 2-methylenebornane (2-MB) and an unexpectedly large amount of 1-methylcamphene (1-MC), in comparison to previous studies. As shown in Table 1, 1-MC peaks corresponded to $17 \pm 6\%$ of the total chromatogram, 2-MIB peaks amounted to $21 \pm 9\%$ of the total, and 2-MB was responsible for the largest percentage of peak area, with $62 \pm 5\%$.

Microorganisms which synthesize 2-MIB and 2-MB were already shown to release also some C11 terpene side products.\[^{18,22,23}\] \textit{In vitro} investigations of respective C11 terpene synthases confirmed the production of these side products, which includes 1-MC.\[^{11,12,24}\] In all cases, the concentrations of 1-MC were relatively low, way behind the main products 2-MIB and 2-MB, and at a maximum of $3\%$ of total peak area. The only case in which the concentration of 1-MC was increased in relation to the main products was when the 2-MB synthase from \textit{P. fluorescens} was engineered to have its active site modified.\[^{25}\]

Since the relative amount of 1-MC in comparison to the other two C11 terpenes detected was way higher

### Table 1. Relative peak areas of C11-terpenes detected in fresh soil samples.

| Compound name              | Retention Index | Identification method* | Mean peak area % |
|----------------------------|----------------|------------------------|------------------|
|                            | DBWAXetr       | VB-5                   |                  |
| 1-Methylcamphene           | 1072           | 982                    | MS, RI, STD      | $17 \pm 6$ |
| 2-Methylenebornane         | 1108           | 1015                   | MS, RI, STD      | $62 \pm 5$ |
| 2-Methylisoborneol         | 1608           | 1183                   | MS, RI, STD      | $21 \pm 9$ |

*Identification based on comparison of measured MS spectra with NIST database (MS), comparison of retention indices with published data (RI), and confirmation of MS spectra and retention indices on both with authentic standards (STD).
than in earlier reports, we hypothesized that 2-MIB degradation might be occurring. Dehydration of 2-MIB by terpene-degrading bacteria was described for *Pseudomonas* sp. and *Sphingomonas* sp.\(^{[26]}\) To test our hypothesis, in an attempt to observe the biodegradation of the compound, 2-MIB was added to a sample of soil (from a mixture of the seven different areas), and the resulting volatiles were extracted and analyzed. The soil samples used for 2-MIB addition underwent two days of storage at room temperature in unsealed flasks. This step allowed the volatiles present in the fresh soil to escape, so that no background terpenes would disturb the subsequent analysis. The absence of terpenes in the soil samples was confirmed by headspace-GC/MS analysis, prior to the addition of 2-MIB. To exclude the possibility of spontaneous dehydration, the same amount of 2-MIB was also added to a sample of sterile soil (autoclaved). As shown in *Figure 1A*, in the soil sample that contained microbes, 2-MIB was completely degraded forming 2-MB, 1-MC and 2-methyl-2-bornene (2-M2B). When 2-MIB was added to sterile soil in turn, a large peak for 2-MIB is seen, with only traces of 2-MB and 1-MC (*Figure 1B*).

The chemical dehydration of 2-MIB, conducted with the use of thionyl chloride in pyridine, was shown to yield 2-M2B, 1-MC and 2-MB in the ratio 12:20:68.\(^{[27]}\) In the same study, treating 2-MIB with sulfuric acid was shown to dehydrate 2-MIB almost completely to a mixture of 2-MB and 1-MC in 1:1 ratio. It was suggested then that these conditions would resemble the natural water environment with mild acid catalysis. However, what we show here is the dehydration of 2-MIB to 2-MB and 1-MC dependent on the activity of microorganisms. When the integrated peaks were quantified, the difference between the two treatments

![Figure 1. Headspace-SPME-GC/MS analysis of natural soil sample (A) and sterile soil sample (B) after addition of 2.5 μg of 2-MIB.](image)
in 2-MIB dehydration became evident (Table 2). The sum of the area of all integrated peaks was considered 100%, and the fractions of each compound were compared regarding their relative peak area in percentage to the total peak area in the chromatogram. Microbial activity was responsible for degrading 2-MIB, forming mainly 2-MB (59.4% of total peak area) and 1-MC (37.3% of total peak area), with a small trace of 2-M2B (3.4% of total peak area). When microbes were excluded by sterilization, addition of 1-MIB generated a peak corresponding to the largest percentage of the total peak area with 86.8%, and small amounts of 1-MC (4.5% peak area) and 2-MB (8.6% peak area). They can be attributed to spontaneous dehydration occurring in the stock solution or impurities of the standard compound used for the experiment (not shown).

The results shown here promote the concept of 2-MIB removal with the use of naturally occurring soil microorganisms. Even though the microbial community was not further analyzed regarding its species or quantity, the results show a clear effect of microbial activity on the chemical profile of C11-terpene volatiles in soil. Since 2-MIB is a common off-flavor agent causing problems in water for human consumption and fish farms, the technique is an interesting alternative to the complex ecological functions of this volatile. The C11-terpene is not only an off-flavor for the human nose and palate. Recent research started to unveil the actual roles that this molecular signal can play as a semiochemical. When produced by Streptomyces species that live in the soil, 2-MIB was shown to attract Collembola, which in turn promote dispersion of spores that adhere and hitchhike on the arthropod.19 Analysis of Streptomyces gene expression also showed that 2-MIB production is linked to sporulation, evidencing a developmental role of 2-MIB in this specific case.

Another evidence of the ecological importance of 2-MIB was provided by Huang and colleagues.30 While investigating the soil chemistry effects on ant distribution, the group analyzed preferences of an ant species, Solenopsis invicta regarding its nesting habits. They could show that the ant species could detect the presence of 2-MIB in the soil and positively choose the direction of the air that contained the C11-terpene. The presence of 2-MIB in soil indicates the presence of actinomycetes, which in turn inhibit the growth of fungi which are harmful for the ants nesting. They also demonstrated that the number of actinobacteria colonies had a negative correlation to entomopathogenic fungi, which means that the presence of actinobacteria deterred the growth of the fungi. Since the fertilized queens are very susceptible to infection by these fungi, as well as other pathogens, the selection

| Compound            | Natural soil peak area % | Sterile soil peak area % |
|---------------------|--------------------------|--------------------------|
| 2-Methyl-2-bornene  | 3.4                      | 0.0                      |
| 1-Methylcamphene    | 37.3                     | 4.5                      |
| 2-Methylenebornane  | 59.4                     | 8.6                      |
| 2-Methylisoborneol  | 0.0                      | 86.8                     |

The removal of off-flavors by biodegradation was suggested a while ago by Elhadi and colleagues.29 Through ozonation followed by biological filtration, they were able to remove 47% to 100% of 2-MIB in the water, and showed the potential of biofiltration as an additional step for removal of off-flavors.

Besides the clear application of biofiltration on water cleanup from 2-MIB for human consume, microbial degradation adds another aspect to the complex ecological functions of this volatile. The C11-terpene is not only an off-flavor for the human nose and palate. Recent research started to unveil the actual roles that this molecular signal can play as a semiochemical. When produced by Streptomyces species that live in the soil, 2-MIB was shown to attract Collembola, which in turn promote dispersion of spores that adhere and hitchhike on the arthropod.19 Analysis of Streptomyces gene expression also showed that 2-MIB production is linked to sporulation, evidencing a developmental role of 2-MIB in this specific case.

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of the right nesting place has a direct effect on the survival of the queen and consequently the offspring. Therefore, there is an ecological correlation between mating of this ant species and presence of actinomycetes, evidenced by the release of chemical cues such as 2-MIB. Ultimately, the distribution of this ant species may be associated with the presence of actinobacteria in the soil.

More research is needed on the influence that soil microorganisms (and their chemicals production and release) have on the distribution of other organisms, including but not limited to arthropods. In the above-mentioned study with Solenopsis, it is suggested that the findings could be used for strategies to hinder invasion by ants, by for example managing the soil microbiota in a way to increase the population of beneficial actinomycetes which do not attract unwanted insects. In our study we showed a rapid degradation of 2-MIB by soil microbiota with formation of further C11 compounds such as 1-MC, whose presence may as well have unknown consequences for the microbial community. The microbial degradation of 2-MIB therefore offers a solution useful not only for control of the unpleasant smell generated by this compound, but also with potentially interesting applications for insect or pathogen control.

Conclusions

In this study, we analyzed soil samples regarding their non-canonical terpene profile. The finding of 1-methylcamphene in high amounts indicated the possibility of degradation of the commonly found 2-methylisoborneol. Further experiments revealed microbial degradation of 2-methylisoborneol, which is a compound of interest in water management due to its off-flavor properties and is a compound with newly discovered ecological functions. The findings indicate the possibility of using soil microorganisms for 2-methylisoborneol sustainable degradation.

Experimental Section

Chemicals and Materials

Standard solution of 2-methylisoborneol at 10 mg mL⁻¹ in methanol was obtained from Sigma-Aldrich (Germany). Pure standard compounds 2-methyl-2-bornene, 2-methylenebornane and 1-methylcamphene were synthesized by Enamine (Latvia). Standard compounds were diluted in ethanol or methanol (0.25 mg mL⁻¹) for analysis and for application in soil samples. Cycloheximide and streptomycin were purchased from Roth (Germany).

Collection of Soil

Soil samples were collected in September 2020 from the forest area in Rüdesheim, Hessen, Germany (initial collection point coordinates 50.0292520, 7.9097550), which is composed mainly of beech, oak and pine trees. For sample diversification, soil material was collected from seven different locations within one kilometer of distance from the initial collection point. One kilogram of soil was collected from each location. The soil material was collected with a shovel, from up to 10 cm under the surface, and put in separate plastic bags for transportation. Once in the laboratory, soil samples were sieved (0.4 cm garden sieve) separately and transferred to flasks for analysis.

Analysis of Soil Volatiles

The seven locations were analyzed separately regarding their volatile content. The amount of soil analyzed, as well as pre-treatment and extraction temperatures were achieved through preliminary experiments to determine optimal conditions. Soil material was inserted in 10 mL GC vials with magnetic screw caps. The amount of material was 2 g and allowed at least 80% of the vial to be comprised of headspace for analysis. Vials were submitted to pre-treatment with 10 min of heating at 95°C. An SPME fiber (100 μM PDMS, PalSystems) was used to extract the volatiles in the headspace for 30 min at 95°C.

For 2-MIB degradation experiments, 2.5 μg of standard compound was added to a vial containing 2 g of a mixture of soil from the seven different areas, and then submitted to 60 min incubation at room temperature. The quantity of standard compound applied to the soil samples was selected after a series of preliminary experiments (not shown), which indicated the optimal concentration to be detected under these experimental conditions. Six replicates for this experiment were performed. An SPME fiber (100 μM PDMS, PalSystems) was used to extract the volatiles in the headspace for 30 min at 95°C.

After extraction, the SPME fiber was introduced in the injector port of a GC/MS QP2010 SE (Shimadzu) and sampled for 1 min at 250°C in splitless mode. GC oven temperature program was optimized by us for
the measurement of C11-terpenes, and consisted of 40°C for 1.5 min, raised at 7°C/min to 150°C, 10°C/ min to 250°C, and 13°C/min to 300°C. At the MS, interface was set to 310°C and ion source to 270°C. The apolar column used was VB5 with restriction (Agilent), of 42 m length and 0.25 um thickness. Polar column was DBWAXetr (Agilent) of 30 m length and 0.25 thickness. The resulting volatiles were compared regarding their mass spectra (MS) and retention indexes (RI) against GC/MS data obtained from measuring synthetic reference compounds under the same conditions.

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Ethics Approval

No approval of research ethics committees was required to accomplish the goals of this study because experimental work was conducted with unregulated microbial species.

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Conflict of Interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

Author Contribution Statement

All authors contributed to the study conception and design. Sample collection was performed by Laura Drummond and Christian von Wallbrunn. Material preparation, data collection and analysis were performed by Laura Drummond. The first draft of the manuscript was written by Laura Drummond and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

References

[1] P. Ömür-Özbek, J. C. Little, A. M. Dietrich, ‘Ability of humans to smell geosmin, 2-MIB and nonadienal in indoor air when using contaminated drinking water’, Water Sci. Technol. 2007, 55, 249–256.
[2] C. Karahadian, D. B. Josephson, R. C. Lindsay, ‘Volatile Compounds from Pencillium sp. Contributing Musty-Earthly Notes to Brie and Camembert Cheese Flavors’, J. Agric. Food Chem. 1985, 33, 339–343.
[3] Z. Wang, I. H. Suffet, Al-Samarrai, ‘Sensory and chemical analysis methods for earthy and musty odors in drinking water caused by geosmin and 2-methylisoborneol’, Water Sci. Technol. Water Supply 2006, 6, 147–155.
[4] P. Piriou, R. Devesa, M. De Lalande, K. Gucina, ‘European reassessment of MIB and geosmin perception in drinking water’, J. Water Supply Res. Technol. – AQUA 2009, 58, 532–538.
[5] [6] R. M. Callejón, C. Ubeda, R. Ríos-Reina, M. L. Morales, A. M. Troncoso, ‘Recent developments in the analysis of musty odor compounds in water and wine: A review’, J. Chromatogr. A 2016, 1428, 72–85.
[6] D. Bruce, P. Westerhoff, A. Brawley-Chsworth, ‘Removal of 2-methylisoborneol and geosmin in surface water treatment plants in Arizona’, J. Water Supply Res. Technol. – AQUA 2002, 51, 183–197.
[7] F. Jüttnert, S. B. Watson, ‘Biochemical and ecological control of geosmin and 2-methylisoborneol in source waters’, Appl. Environ. Microbiol. 2007, 73, 4395–4406.
[8] A. Peter, U. Von Gunten, ‘Oxidation kinetics of selected taste and odor compounds during ozonation of drinking water’, Environ. Sci. Technol. 2007, 41, 626–631.
[9] S. Mustapha, J. O. Tijani, M. Ndaminito, A. S. Abdulkareem, D. T. Shuaib, A. K. Mohammed, ‘A Critical Review on Geosmin and 2-Methylisoborneol in Water: Sources, Effects, Detection, and Removal Techniques’, Springer International Publishing, 2021.
[10] X. Shao, K. Du, ‘Biodegradation of 2-methylisoborneol by enzyme separated from Pseudomonas mandelli’, Water Sci. Technol. Water Supply 2020, 20, 2096–2105.
[11] W. K. W. Chou, H. Ikeda, D. E. Cane, ‘Cloning and characterization of Pfl_1841, a 2-methylenebornane synthase in Pseudomonas fluorescens PFO-1’, Tetrahedron 2011, 67, 6627–6632.
[12] N. L. Brock, S. R. Ravella, S. Schulz, J. S. Dicketschat, ‘A detailed view of 2-methylisoborneol biosynthesis’, Angew. Chem. Int. Ed. 2013, 52, 2100–2104; Angew. Chem. 2013, 125, 2154–2158.
[13] L. Drummond, M. J. Kschowak, J. Breitenbach, H. Wolff, Y. M. Shi, J. Schrader, H. B. Bode, G. Sandmann, M. Buchhaupt, ‘Expanding the Isoprenoid Building Block
Repetoire with an IPP Methyltransferase from Streptomyces monomycini’, *ACS Synth. Biol.* 2019, 8, 1303–1313.

[14] V. Radhika, N. Ueda, Y. Tsuboi, M. Kojima, J. Kikuchi, T. Kudo, H. Sakakibara, ‘Methylated Cytokinins from the Phytopathogen Rhodococcus fascians Mimic Plant Hormone Activity’, *Plant Physiol.* 2015, 169, 1118–1126.

[15] S. Von Reuss, D. Domik, M. C. Lemfack, N. Magnus, M. Kai, T. Weise, B. Piechulla, ‘Sodorifen Biosynthesis in the Rhizobacterium Serratia plymuthica Involves Methylation and Cyclization of MEP-Derived Farnesyl Pyrophosphate by a SAM-Dependent C-Methyltransferase’, *J. Am. Chem. Soc.* 2018, 140, 11855–11862.

[16] C. Klausen, M. H. Nicolaisen, B. W. Strobel, F. Warnecke, J. L. Nielsen, N. O. G. Jørgensen, ‘Abundance of actinobacteria and production of geosmin and 2-methylisoborneol in Danish streams and fish ponds’, *FEMS Microbiol. Ecol.* 2005, 52, 265–278.

[17] S. Giglio, W. K. W. Chou, H. Ikeda, D. E. Cane, P. T. Monis, ‘Biosynthesis of 2-methylisoborneol in cyanobacteria’, *Environ. Sci. Technol.* 2011, 45, 992–998.

[18] D. R. Fravel, W. J. Connick, C. C. Grimm, S. W. Lloyd, ‘Volatile compounds emitted by sclerotia of Sclerotinia minor, Sclerotinia sclerotiorum, and Sclerotium rolfsii’, *J. Agric. Food Chem.* 2002, 50, 3761–3764.

[19] P. G. Becher, V. Verschut, M. J. Bibb, M. J. Bush, B. P. Molnár, E. Barane, M. M. Al-Bassam, G. Chandra, L. Song, G. L. Chalis, M. J. Buttner, K. Flärdh, ‘Developmentally regulated volatiles geosmin and 2-methylisoborneol attract a soil arthropod to Streptomyces bacteria promoting spore dispersal’, *Nat. Microbiol.* 2020, 5, 821–829.

[20] Q. Huang, C. A. Roessner, R. Croteau, A. I. Scott, ‘Engineering Escherichia coli for the synthesis of taxadiene, a key intermediate in the biosynthesis of taxol’, *Bioorg. Med. Chem.* 2001, 9, 2237–2242.

[21] R. Schmidt, V. De Jager, D. Zühlke, C. Wolff, J. Bernhardt, K. Cankar, J. Beekwilder, W. Van Ijcken, F. Sleutels, W. De Boer, K. Riedel, P. Garbeva, ‘Fungal volatile compounds induce production of the secondary metabolite Sodorifen in Serratia plymuthica PRI-2 C’, *Sci. Rep.* 2017, 7, 1–14.

[22] M. J. Kschowak, H. Wortmann, J. S. Dickschat, J. Schrader, M. Buchhaupt, ‘Heterologous expression of 2-methylisoborneol/2-methylisoborneol biosynthesis genes in Escherichia coli yields novel C11-terpenes’, *PLoS One* 2018, 13, e0196082.

[23] J. S. Dickschat, T. Nawrath, V. Thiel, B. Kunze, R. Müller, S. Schulz, ‘Biosynthesis of the off-flavor 2-methylisoborneol by the myxobacterium Nanocystis exedens’, *Angew. Chem. Int. Ed.* 2007, 46, 8287–8290; *Angew. Chem.* 2007, 119, 8436–8439.

[24] W. K. W. Chou, C. A. Gould, D. E. Cane, ‘Incubation of 2-methylisoborneol synthase with the intermediate analog 2-methylneryl diphosphate’, *J. Antibiot.* 2017, 70, 625–631.

[25] M. J. Kschowak, F. Maier, H. Wortmann, M. Buchhaupt, ‘Analyzing and Engineering the Product Selectivity of a 2-Methylenoborneane Synthase’, *ACS Synth. Biol.* 2020, 9, 981–986.

[26] R. W. Eaton, ‘Dehydration of the off-flavor chemical 2-methylisoborneol by the R-limonene-degrading bacteria Pseudomonas sp. strain 19-rlim and Sphingomonas sp. strain BIR2-rlima’, *Biodegradation* 2012, 23, 253–261.

[27] R. Schumann, P. Pendleton, ‘Dehydration products of 2-methylisoborneol’, *Water Res.* 1997, 31, 1243–1246.

[28] S. Sommer, L. Lang M., L. Drummond, M. A. Fraatz, H. Zorn, ‘Odor description of non-canonical terpenoids’, unpublished n.d.

[29] S. L. N. Elhadi, P. M. Huck, R. M. Slawson, ‘Removal of geosmin and 2-methylisoborneol by biological filtration’, *Water Sci. Technol.* 2004, 49, 273–280.

[30] H. Huang, L. Ren, H. Li, A. Schmidt, J. Gershenzon, Y. Lu, D. Cheng, ‘The nesting preference of an invasive ant is associated with the cues produced by actinobacteria in soil’, *PLoS Pathog.* 2020, 16, 1–21.

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