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Pinewood VOC emissions protect from oxazolone-induced inflammation and dysbiosis in a mouse model of atopic dermatitis

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ABSTRACT

Pinewood, increasingly used in construction and interior fittings, emits high amounts of volatile organic compounds (VOCs), which tend to accumulate in indoor air. Whether indoor VOCs affect the development of atopic dermatitis (AD) is a matter of debate. We aimed to evaluate the effects of pinewood VOCs on the development of AD-like inflammatory phenotype and linked microbiome alterations, both hallmarks of AD. An oxazolone-induced mouse model of AD was exposed to three different VOC concentrations emitted by pinewood plates throughout the experiment. The disease course and associated immunological and microbiological changes were evaluated. To validate and translate our results to humans, human keratinocytes were exposed to a synthetic pinewood VOCs mixture in an AD environment. Pinewood emitted mainly terpenes, which at a total concentration of 5 mg/m^3 significantly improved oxazolone-induced key AD parameters, such as serum total IgE, transepidermal water loss, barrier gene alteration, inflammation, and dysbiosis. Notably, exposure to pinewood VOCs restored the loss of microbial richness and inhibit Staphylococci expansion characteristic of the oxazolone-induced mouse AD model. Most beneficial effects of pinewood VOCs were dose-dependent. In fact, lower (<3 mg/m^3) or higher (>10 mg/m^3) pinewood VOC levels maintained only limited beneficial effects, such as preserving the microbiome richness or impeding Staphylococci expansion, respectively. In the human *in-vitro* model, exposure of keratinocytes grown in an AD environment to a pinewood VOCs mixture reduced the release of inflammatory markers. In conclusion, our results indicate that airborne phytochemicals emitted from pinewood have beneficial effects on an AD-like phenotype and associated dysbiosis. These investigations highlight the effects of terpenes as environmental compounds in the prevention and/or control of atopic skin disease.

Abbreviations: AD, Atopic dermatitis; ALI, Air liquid interface; EtOH, Ethanol; GC–MS, Gas chromatography mass spectrometry; LDA, Linear discriminant analysis; OTUs, Operational taxonomic units; PCoA, Principal coordinate analysis; TCS, T cell supernatant; TEWL, Transepidermal water loss; TSLP, Thymic stromal lymphopoietin; VOC, Volatile organic compound.

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1. Introduction

Wood, especially soft wood like pine, is a popular natural material which is increasingly used in construction, for interior work, as floor covering or for furniture (Mantau et al., 2013; StBA, 2023; Destatis, 2024). Pinewood releases considerable amounts of volatile organic compounds (VOCs), consisting mainly of monoterpenes, which are especially high in newly-built houses or following renovation, then diminish over a period of 6–8 months (Englund, 1999; Ghirardo et al., 2010; Back et al., 2000; Wallner et al., 2015; Fürhapper et al., 2020). As modern lifestyle is linked primarily to an indoor environment (Brasche and Bischof, 2005), the effect of exposures to various indoor emissions on human health becomes increasingly important, especially because of potential accumulation of airborne chemicals due to recent energy-efficient airtight construction methods, often combined with insufficient ventilation (Overton, 2013; Deng et al., 2012).

Atopic dermatitis (AD) is a common, inflammatory skin disease primarily diagnosed in 15 – 20 % of children, besides 3 – 5 % of adults (Kay et al., 1994; Bieber, 2008). The clinical picture of AD depends on the stage of the disease but is mainly characterized by pruritus, skin dryness and skin lesions, exerting a profound impact on the quality of life of patients and their families (Eyerich et al., 2015; Kiebert et al., 2002). The pathogenesis is a complex interaction between genetic, immunological and environmental factors (Bieber, 2008; Kantor and Silverberg, 2017; Ahn, 2014), leading to a Th2-dominated immune response accompanied by an impaired skin barrier and a shift in the microbial skin composition (Gittler et al., 2012; Kaesler et al., 2014).

Data on the effect of indoor VOCs on the course of AD are scanty. On the one hand, epidemiological studies hint to increased risk of developing AD in children exposed to enhanced indoor total VOCs (Herbarth et al., 2006; Lee et al., 2012; Kwon et al., 2015). Furthermore, a clinical research study demonstrated that a mixture of VOCs increased the susceptibility of AD skin to allergen exposure (Huss-Marp et al., 2006). On the other hand, VOCs like α-pinene are known for their anti-inflammatory properties and topical application of essential oil extracted from a cypress native to the Asiatic continent have even displayed therapeutic effects in a model of AD (Rufino et al., 2014; Kim et al., 2015; Yang et al., 2015). The German Federal Environmental Agency, following a survey of 479 households, defined indoor total VOC levels below 1 mg/m^3 as good air quality, between 3 and 10 mg/m^3 as critical and above 10 mg/m^3 as unacceptable, without including health risk assessments (Krause et al., 1991; Umweltbundesamt, 2007). While the median total VOC levels measured in German households falls within the good air quality range (about 0.3 mg/m^3) (Umweltbundesamt, 2008); in new (or newly renovated) buildings, including timber houses, total VOC concentrations can exceed 3 mg/m^3 and the terpene α-pinene alone can reach values of 1 mg/m^3 or more (Wallner et al., 2015; Fürhapper et al., 2020; Brown et al., 1994; Krol et al., 2014). Considering the fact that children, through their closer contact with VOC sources, e.g., by crawling or playing on wood floors, could be exposed to even higher levels of VOCs, we designed a study to evaluate the effects of pinewood VOC emissions on AD development focusing on 5 mg/m^3 VOC concentration (within the range defined as critical in[28]). In addition, a lower concentration range (<3 mg/m^3), which corresponds to the one generally measurable in households and a higher concentration range (>10 mg/m^3), which might be realistic in occupational settings (e.g. in sawmills) (Edman et al., 2003), were evaluated. For this purpose we used an established murine model of AD (Amar et al., 2022) and exposed it directly to pinewood emitting in the aforementioned three concentrations. In addition, to assess if the results obtained with the murine AD model can be translated to human, we developed a human *in vitro* AD model combined to exposures to pinewood-specific VOCs.

2. Materials and methods

2.1. Preparation of pinewood samples with defined emission rates of VOCs

Solid kiln-dried pine timber boards (*Pinus sylvestris* L., mainly heartwood) were obtained from a German sawmill. To provide comparable material, the samples were composed from different boards. Each sample consisted of 10 pieces of wood of 14.5 cm (length) × 2.8 cm (width). The thickness differed from 1 to 2.5 cm, depending on the intended emission level in the cages. Total VOC (TVOC) levels chosen for our investigation covered 3 ranges of concentrations: <3 mg/m^3, 5 mg/m^3 and >10 mg/m^3 (Fig. 1, Supplementary Fig. 1), based on the hygienic assessment of indoor air pollution by the German Federal Environment Agency (Umweltbundesamt, 2007). The area specific emission rates (in μg m^{-2}h^{-1}) of the samples were first determined using the emission test chamber method according to DIN EN ISO 16000-9:2008 (DIN EN ISO 16000-9:2008-04, 2008), using stainless steel emission chambers (1 m^3) with defined climatic conditions (23 °C ± 2 °C, relative humidity of 50 % ± 5 %; air velocity of 0.1 to 0.3 m s^{-1}). To achieve the intended VOC concentration within cages, either the emission rates or the emitting surface area of the wooden samples were adjusted by storing them in the emission chamber or by partially covering them with self-adhesive, non-emitting aluminum foil, respectively. The wooden samples with intended VOC concentrations were wrapped in aluminum foil and stored at −20 °C until start of experiments.

2.2. Mice

Six weeks old female C57BL/6J mice were purchased from Charles River (Sulzfeld, Germany). Animals were housed in ventilated cages (VentiRack; Biozone, Margate, UK) under controlled humidity (60 %), temperature (22 ± 2 °C) with a 12 h light–dark cycle and were fed with conventional mouse diet (Altromin, Lage, Germany) and water *ad libitum*. To avoid cage effects, animals obtained from the same breeding compartment were distributed randomly to experimental groups for each experiment (3 mice/cage). Mouse experiments were conducted according to the European Convention for Animal Care and Use of Laboratory Animals and were approved by local ethics committee and government authorities (Approval number: ROB-55.2-2532.VET_02-16-198).

2.3. Exposure of mice to pinewood emissions and VOC analysis

Pinewood boards were placed at the bottom of the cages and covered with a metal frame to avoid mice gnawing their edges. The boards were covered with vermiculite (Moosmann GmbH & Co. KG, Ravensburg, Germany), which replaced conventional wood chips bedding to prevent any confounding emissions (bedding pre-tests performed in our laboratory, data not shown). Since the emission rate of wood samples decreases over time (Englund, 1999), pinewood boards were replaced weekly to guarantee the TVOC concentrations within the expected range throughout the experiments. To best mimic a real exposure scenario, mouse cages were changed every 12 h from a ventilated condition in ventilated cages ("window open" during nighttime) to a non-ventilated condition, characterized by normal air exchange without active ventilation ("window closed" during daytime) (Hernandez et al., 2020). Animals not exposed to pinewood emissions were housed with vermiculite bedding only and were subjected to similar ventilation cycles as for the pinewood-exposed animals. On day 1 and 7 of each exposure week cage air was sampled for VOC analysis. Measurements were performed using sorbent tubes filled with Tenax® TA (200 mg, 35/60 mesh) and spiked with 200 ng toluene-d_8 as internal standard. The tubes were connected to the cages and a sampling pump (Analyt-MTC Messtechnik GmbH, Müllheim, Germany) working at constant flow rate of 100 ml min^{-1}. Depending on the expected VOC concentration, the sampling

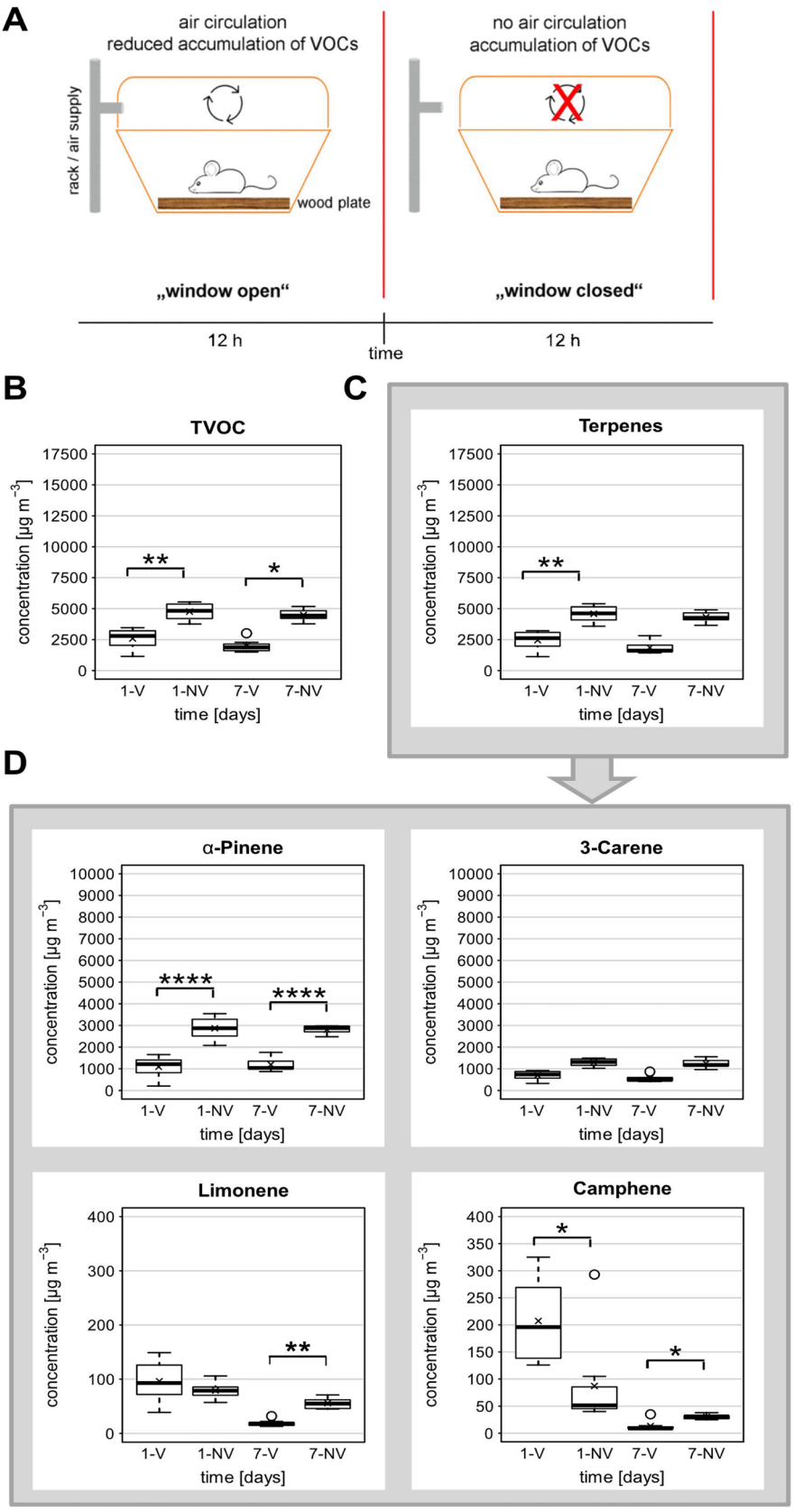

Fig. 1. Characterization of pinewood emissions at a concentration of 5 mg/m^3 in mouse cages. A) Representative scheme of daily exposure to pinewood emissions. Every 12 h, the air supply of the cages was changed from ventilated (V) to non-ventilated (NV), simulating open or closed windows, respectively. **B)** Levels of total volatile organic compounds (TVOC) and **C)** Terpenes. **D)** Individual VOCs in mouse cages during a representative experimental week, at day 1 and day 7. Air samples were analyzed with gas chromatography mass spectrometry (GC–MS). Boxplots depict minimum, 25th percentile, median, 75th percentile, and maximum.

Day 1, n = 8; day 7, n = 7 (V) and n = 5 (NV). *p < 0.05, ** p < 0.01, ***p < 0.001, ****p < 0.0001. Statistical analysis was performed by two-way ANOVA with Bonferroni post-hoc-test.

volume ranged between 0.1 and 1.0 L. The determination of VOC was performed according to DIN ISO 16000-6:2012 (DIN ISO 16000-6:2012-11, 2012). Thermal desorption (TD) analysis was performed by using the Ultra Series 2 50:50 (auto-sampling unit) and the Unity Series 2 (thermodesorption unit), (Markes International Ltd, Bridgend, UK). Identification and quantification of individual chemical compounds were performed by gas chromatography mass spectrometry (GC–MS) (model 7890A, 5975C; Agilent Technologies Inc., Santa Clara, USA). For separation a VF1701ms capillary column (CP9151, Agilent Technologies Inc.) was used with helium as carrier gas. The GC temperature program includes the following steps: 32 °C hold for 3 min, increase by 6 °C min^{-1} to 90 °C, 90 °C hold for 4 min, increase by 8 °C min^{-1} to 200 °C, increase by 12 °C min^{-1} to 240 °C, 240 °C hold for 2 min. The MS was operated in scan mode with 5 scans s^{-1} in the mass range of 22 and 300 u. Data were processed using the software MSD ChemStation E.02.00.493 (Agilent Technologies Inc.). For most of the VOCs the compound-specific response factors were determined with a seven-point calibration in the range from 1 to 1000 ng with reference compounds. Substances without reference compounds were quantified using the response factor of compounds with similar chemical structure or the internal standard. The VOCs deriving from pinewood emissions, mice, bedding, diet or excrements were analyzed. TVOCs, defined as the sum of all VOCs listed in Supplementary Table 1, corresponds to ≥95 % of the sum of all detected VOCs in mouse cages.

2.4. Induction and monitoring of an oxazolone-induced murine model of atopic dermatitis

To investigate the effects of exposure to pinewood VOCs on AD, a well-characterized AD model was induced on mouse ears (Amar et al., 2022). At the beginning of experiments (day 0) mice were sensitized once on each ear with 20 µl of a 0.8 % (w/v) oxazolone (4 Ethoxymethylene-2-phenyl-2-oxazolin-5-one, Sigma Aldrich, St. Louis, USA) solution solved in 100 % EtOH (Merck, Darmstadt, Germany). For challenge (start at day 7), 20 µl of a 0.4 % (w/v) oxazolone solution in EtOH was applied three times a week on each ear until the end of experiments (development of AD, day 16–18). Control groups received the same volume of EtOH. At every treatment point, body weight, transepidermal water loss [TEWL, Aquaflux AF200 system (Biox System Ltd, London, UK)] and ear thickness [via a caliper (Kroeplin; Schlüchtern, Germany)] were assessed. At the beginning and end of experiments also skin pH and total serum IgE were measured using a skin pH meter (Courage + Khazaka electronic GmbH; Cologne, Germany) and a sandwich ELISA (BD, New Jersey, USA), respectively (Amar et al., 2022).

2.5. Histological and immunohistochemical analysis

Skin tissue from mouse ears was fixed in 4 % paraformaldehyde at room temperature for 48 h, processed and embedded in paraffin. Tissue sections (4 µm) were stained with hematoxylin & eosin following a standard protocol. The sizes of epidermis and dermis were measured using the LASX software (Leica Application Suite X V. 3.4.2, Leica, Wetzlar, Germany) on a Leica DM4 B light microscope, as previously described (Amar et al., 2022). Immunohistochemical staining (IHC) was performed on 2 µm sections using an automated staining system (Leica). Briefly, after dehydration, the slides were pre-treated with epitope retrieval solution (ER)1 (for IBA1 and CD8) or ER2 (for CD4), incubated with the respective primary antibody (IBA1: Wako 019-1974; CD8: clone 309, Sino Biological 50389-R309; CD4: clone GHH4, Dianova DIA-404) and detected using the Bond™ Polymer Refine Detection Kit (Leica Biosystems). All primary antibodies were detected with a secondary anti-rabbit antibody provided in the kit. Only for the primary rat antibody (CD4), a secondary rabbit-anti-rat (Vector laboratories) was added. After counterstaining with haematoxylin, the slides were digitalized with an automated scanning system (Leica AT2, Wetzlar, Germany). Histopathological analysis of skin samples, including counting of positive cells and grading of inflammatory cell infiltration was performed by an experienced comparative pathologist using an Aperio Imagescope (version 12.4.0.7018) according to Watanabe et al. (Watanabe et al., 2009).

2.6. Real time-PCR analysis

For RT-PCR analysis, ear tissue was snap frozen in liquid nitrogen and disrupted using a Tissue Lyser LT (Qiagen GmbH, Hilden, Germany). RNA was extracted using the RNeasy Mini Kit (Qiagen) according to supplier's instructions and directly reverse-transcribed (RevertAid H Minus First Strand cDNA Synthesis Kit, Thermo Fisher Scientific). Quantitative real-time PCR was performed using SYBR Green PCR Kit Master Mix (Qiagen) and the LightCycler®480 System (Roche, Basel, Switzerland) according to the manufacturer's protocol. Primer sequences are listed in Supplementary Table 2. The expression levels were normalized to the house-keeping genes β-actin or GAPDH and to an unstimulated control and the relative changes were represented as $2^{-\Delta\Delta CT}$ ($\Delta\Delta CT = \Delta CT - \Delta CTControl$) (Marzaioli et al., 2014).

2.7. Exposure of a human in vitro model of atopic dermatitis to pinewood emissions

Primary human epidermal keratinocytes were isolated by suction blister from healthy donors. The study was approved by the local ethical committee of the Klinikum rechts der Isar, Technical University of Munich, Project number 5590/12 and 44/16 S. Cells were cultured in DermalLife basal medium (LifeLine Cell Technology; Carlsbad, USA) supplemented with DermalLife K LifeFactor Kit (Lifeline Cell Technology) in a T75 flask at 37 °C, 5 % CO_2 until 70 % confluency, then transferred to collagen pre-coated (1 % in PBS, collagen type I, Sigma-Aldrich, Taufkirchen, Germany) polycarbonate inserts (Merck Millipore, Burlington, MA, USA) with a density of 0.42×10^6 cells/transwell in 500 µl supplemented DermaLife basal medium plus 1.5 mM $CaCl_2$ (Carl Roth GmBH, Karlsruhe, Germany) to initiate the 3D culture. Each insert was cultured in a 6 well plate with 1.5 mM $CaCl_2$-containing medium. After 24 h, the medium in the transwell was removed to place the model in air–liquid interface while the medium in the lower chamber was further supplemented with 1.5 mM $CaCl_2$ and 50 µg/ml Vitamin C (Sigma). Prior to a 12 h-long stimulation with AD derived T cell supernatant (diluted 1:2), the 3D models were starved for 12 h in DermalLife Basal Medium without supplements. AD derived T cell supernatant was generated as explained in (Lauffer et al., 2018). In brief, AD full skin biopsies (n = 3) were cultivated in RPMI 1640 medium supplemented with 5 % human serum, 0.1 mM NEAA, 2 mM L-Glutamine, 1 mM sodium pyruvate and 100 U/ml penicillin/streptomycin (all Gibco, Paisley, UK) in presence of 60 U/ml IL-2 (Novartis Pharma, Nürnberg, Germany) at 37 °C and 5 % CO_2. Fresh medium containing 60 U/ml IL-2 was replaced three times a week until lesional T cells emigrated from the biopsy. T cells were expanded by α-CD3 and α-CD28 (BD Bioscience, San Jose, CA, USA) stimulation (each 0.75 µg/ml, α-CD3 pre-coated on plate in PBS, α-CD28 soluble) and finally stimulated for 72 h with α-CD3 and α-CD28 as described above. Cell- free supernatant was obtained, characterized by ELISA for IL-4, IL-13 (both BD Biosciences), IL-17, IL-22, IFNγ, TNF (R&D, Minneapolis, MN, USA) and mixed in equimolar ratio. Before exposure of the skin equivalents to pinewood emissions, the T cell supernatant was removed and replaced with DermalLife basal medium with supplements excluding hydrocortisone. Non stimulated cells were

handled the same way, omitting only T cell supernatants. Exposure to VOCs occurred in a VITROCELL® 12/6 CF *in vitro* exposure system for the direct exposure of cell cultures to airborne substances (Vitrocell Systems, Waldkirch, Germany), conducted at the Air-Liquid Interface (ALI) (Gminski et al., 2010). The exposure system was encased in a climate chamber (ESPEC PR-4ST, Japan) to ensure physiological conditions (37 °C) and ultimately to prevent the formation of VOC-condensation within the exposure system components. Test atmospheres were prepared using PVDF coated 3 L-gas-sampling bags (ALTEF®, Restek Corp., USA) spiked up to a total mass concentration of 5 mg VOCs/L with liquid reference substances, according to the mass proportions of the most representative pinewood VOCs displayed in Supplementary Table 3 (α-pinene, 3-carene, limonene and hexanal; Merck GmbH, Germany). Spiked gas sampling bags were allowed to equilibrate and volatilize the liquid test compounds for 60 min at normal pressure and at 37 °C. For exposure, a vacuum driven continuous mass flow was set at 3 mL/min, conducting the synthetic test atmosphere directly from the sampling bag to the epithelial cells at the ALI for 2 h. Control exposures occurred with clean air. The test concentration of 5 mg/L and the exposure duration of 2 h were chosen based on preliminary dose-finding experiments with epithelial cells exposed at the ALI, eliciting an effect without inducing toxicity (Gminski et al., 2010). Following a post-incubation period of 22 h under ALI conditions (37 °C, 5 % CO_2, Fig. 3C), the basolateral cell culture supernatant was harvested and stored at −80 °C until analysis.

2.8. Measurement of inflammatory mediators

For cytokine analysis in *in vivo* experiments, skin tissue from mouse ears was pre-cut in lysis buffer (Thermo Fisher Scientific, Waltham, MA, USA), sonicated and centrifuged at 12,000 g for 20 min at 4 °C. Supernatants were analyzed using a mouse Th Cytokine Panel (13-plex) LEGENDplex™ (BioLegend®, San Diego, CA, USA) according to manufacturer's recommendations. For *in vitro* experiments, cell culture supernatants were analyzed using the human inflammatory panel 1 (13-plex) LEGENDplex™ (BioLegend®). Concentration of all analytes was determined based on a known standard curve using data analysis software LEGENDPlex™ version v8.0. For 8-isoprostane determination, an 8-isoprostane ELISA kit (Cayman Chemical, Ann Arbor, MI, USA) was used as previously described (Alessandrini et al., 2009). Briefly, after mincing, sonicating and centrifuging ear samples, the level of 8-isoprostane was measured at 405 nm using the software Gen 5 3.08 (Agilent Technologies Inc.). Results from *in vivo* experiments were normalized to sample's protein content measured by BCA assay (Pierce Biotechnology, Thermo Fisher Scientific).

2.9. Microbiome sampling and 16S rRNA gene processing

Skin swabs collected from mouse ears were snap frozen in liquid nitrogen and stored at −80 °C until further processing. Microbial DNA was extracted as described previously (Amar et al., 2022; Lueders et al., 2004). The DNA samples were amplified for their V1-V2 hypervariable regions of the 16S rRNA gene using MiSeq Illumina universal primers (Illumina, San Diego, USA): 27f: 5′-AGAGTTTGATCMTGGC-3′, and 357r: 5′-CTGCTGCCTYCCGTA-3′. Purified amplicons at 10 ng/sample were indexed using Illumina adaptors from the Nextera XT Index Kit v2 Set B: adapter 1 (N7xx) and adapter 2 (S5xx) in a final volume of 25 μl. The Neb Next High-Fidelity 2X Mastermix (New England Biolabs) was used for both PCR amplification and indexing steps. A composite pool was prepared by combining 4 nM of purified DNA samples to ensure equal representations of barcoded libraries (Klindworth et al., 2013). The final pool was sequenced on an Illumina Miseq Platform using the MiSeq Reagent Kit v3 (600 Cycle). The control samples consisting of untreated processed swabs did not produce measurable amplicons and were therefore added to the sequencing pool in equal volumes.

2.10. Statistical analysis

Graphical representations and statistics were done by Prism version 7.0 (GraphPad Software, La Jolla, CA). Analysis of bodyweight, TEWL, ear thickness, serum IgE and skin pH was performed by two-way analysis of variance (ANOVA) with post-hoc Bonferroni test. The rest of the data was analyzed by one-way ANOVA with post-hoc Bonferroni test. Data was expressed as mean ± SD. For the microbiome analysis, the obtained amplicon reads were analysed following the UPARSE method (Edgar, 2013) as implemented in the online IMNGS platform (Lagkouvardos et al., 2016). Briefly, primers and barcode sequences were removed from each read, in addition of depleting short sequences (<200 bp), low-quality and chimeric reads. Downstream analysis including diversity, taxonomy binning, serial group comparison and correlations were performed on R program (R version 4.0.2) using the Rhea pipeline (Lagkouvardos et al., 2017). These scripts rely on the following R packages: ade4, GUniFrac, phangorn, Hmisc, corrplot, plotrix, PerformanceAnalytics, reshape, ggplot2, gridExtra, grid, ggrepel, gtable and their dependencies. A prevalence cut-off of 0.25 % and abundance cut-off of 0.5 were used and the data were normalized using the total sum scaling method. For β-diversity estimation, a principal coordinate analysis (PCoA) was performed to visualize the distance matrices in a space of two dimensions. A PERMANOVA test (vegan:: adonis) was achieved to determine if the separation between groups was significant, as a whole and in pairs. The non-parametric Kruskal Wallis and Mann Whitney tests were respectively used for multiple pairwise groups comparisons. Multiple test corrections were performed with the Benjamini and Hochberg procedure and corrected $p < 0.05$ were considered as significant. Linear discriminant effect size analysis (LEFSe) (Segata et al., 2011) was performed to search for a linear combination of variables (OTUs) that best separate the groups. It employs Kruskal-Wallis rank sum test to detect OTUs with significant differential abundances between groups, followed by a linear discriminant analysis (LDA) to estimate the effect size of each differentially abundant OTU.

3. Results

3.1 Characterization of pinewood VOCs in mouse cages

As typical for pine heartwood emissions (Englund, 1999); the VOC composition in the cages revealed 94–98 % of monoterpenes (Fig. 1, Supplementary Fig. 1). Notably, the daily non-ventilated condition mostly led to significant VOC accumulation in mouse cages compared to ventilated (Fig. 1, Supplementary Fig. 1). For pinewood emitting 5 mg/m^3 TVOCs (Fig. 1) we detected median TVOCs between 2.8 mg/m^3 (day 1) and 1.9 mg/m^3 (day 7) during ventilation and from 4.9 mg/m^3 (day 1) and 4.4 mg/m^3 (day 7) with no ventilation. In the lower concentration scenario (<3 mg/m^3) TVOCs varied between 0.6 mg/m^3 (day 1 and 7) during ventilation and 2.1 mg/m^3 (day 1 and 7) with no ventilation (Supplementary Fig. 1B). At day 1 these concentrations were significantly lower compared to TVOCs emitted by pinewood 5 mg/m^3 (Fig. 1B). In the higher concentration scenario (>10 mg/m^3), median TVOC values varied between 4.0 mg/m^3 (day 1) and 4.4 mg/m^3 (day 7) during ventilation and between 12.1 mg/m^3 (day 1) and 13.4 mg/m^3 (day 7) with no ventilation (Supplementary Fig. 1C), in both cases significantly higher compared to TVOCs emitted by pinewood 5 mg/m^3 (Fig. 1B). TVOCs were dominated by terpenes (Fig. 1C), particularly α-pinene and 3-carene. For pinewood 5 mg/m^3, the median concentration by no ventilation of α-pinene was 2.9 mg/m^3 at day 1 and 7 and of 3-carene 1.3 mg/m^3 at day 1 and 1.2 mg/m^3 at day 7 (Fig. 1D). For pinewood <3 mg/m^3, the median concentration of α-pinene was 1.3 mg/m^3 and of 3-carene 0.4 mg/m^3, both at day 1 and day 7 by no ventilation (Supplementary Fig. 1B, lower panel). In the non-ventilated condition both substances were lower compared to pinewood 5 mg/m^3 (Fig. 1D) at day 1 and α-pinene also at day 7. For pinewood >10 mg/m^3

by no ventilation the median concentration of α-pinene was 4.9 mg/m^3 at day 1 and 5.9 mg/m^3 at day 7 and of 3-carene 5.8 mg/m^3 at day 1 and 7 (Supplementary Fig. 1C, lower panel). Both compounds were significantly higher compared to pinewood 5 mg/m^3 (Fig. 1D) in absence of ventilation, and 3-carene also by ventilation. In addition to α-pinene and 3-carene, several other monoterpenes were detected in cage air, with camphene and limonene being the main ones. Small, but significant variations of these secondary terpenes were found between all pinewood samples (Fig. 1, Supplementary Fig. 1). Saturated aldehydes (mainly hexanal) and organic acids (mainly acetic and propionic acids) were detected at very low concentrations (≤0.1 mg/m^3) in all pinewood samples (data not shown).

3.2. Pinewood VOC emissions at 5 mg/m^3 dampen the oxazolone-induced AD-like inflammation

To disentangle the effects of pinewood VOCs on AD development, we first exposed an established murine model of oxazolone-induced AD (Amar et al., 2022) to a "critical" concentration of pinewood VOCs (Umweltbundesamt, 2007), namely to 5 mg/m^3 (Fig. 2A). As expected, application of oxazolone on mouse ears led to a typical increase of TEWL and ear thickness compared to non-sensitized EtOH controls (Fig. 2B, C), without affecting body weight (Supplementary Fig. 2B) (Amar et al., 2022). Surprisingly, exposure of oxazolone-treated animals to pinewood emissions significantly decreased both TEWL and ear swelling compared to oxazolone alone (Fig. 2B, C). Also, levels of serum total IgE (Fig. 2D) were significantly reduced in oxazolone-treated animals exposed to pinewood VOCs. Contrarily, pinewood VOCs did not affect skin pH (Fig. 2E). Histopathological assessment of oxazolone-treated mouse skin revealed a strong inflammatory cell infiltration (Fig. 2F, arrows) rich of mononuclear cells, granulocytes and lymphocytes (scores in Fig. 2H, Supplementary Fig. 3A and Supplementary Fig. 4) and epidermal hypertrophy (Fig. 2F, arrowheads and scores in Supplementary Fig. 3), in addition to parakeratosis, erosion, slight ulceration and edema (scores in Fig. 2H and Supplementary Fig. 3A). Noteworthy, additional exposure to pinewood VOCs significantly decreased inflammatory skin infiltration, in particular of granulocytes, CD4$^+$ T cells and mononuclear cells (Fig. 2F, H). Contrarily, CD8$^+$ cells were minimally affected (Supplementary Fig. 3B). Pinewood VOCs significantly reduced both epidermal and dermal thickness (Fig. 2G) as well as parakeratosis and slightly also epidermal hypertrophy, erosion and ulceration scores, whereas edema scores were minimally affected (Fig. 2H, Supplementary Fig. 3A). Overall, exposure to pinewood VOCs had no effect on EtOH control skin in any parameter analyzed (Fig. 2F-H, Supplementary Fig. 3A and 4). Analysis of skin barrier genes expression revealed a significant upregulation of *involucrin* induced by oxazolone, brought back to control levels by exposure to pinewood VOCs (Fig. 2I, top). Contrarily, *filaggrin 2* was not altered by oxazolone application, but its expression level was slightly increased in response to pinewood VOCs (Fig. 2I, bottom). Given the important role of oxidative stress in the development of AD (Galiniak et al., 2022) and the fact that terpenes are known antioxidants (Porres-Martinez et al., 2015; Roberto et al., 2010), we measured the level of 8-isoprostane, a known indicator of oxidative stress (Alessandrini et al., 2009), in ear skin lysates in our model. The level of 8-isoprostane was highly variable within the oxazolone group and was characterized by a slight increase only in some specimen, which was absent following exposure to pinewood VOCs (Fig. 2J). Lastly, the expression of thymic stromal lymphopoietin (*TSLP*), a candidate protein involved in AD development (Indra, 2013), was upregulated upon oxazolone application and noticeably restored by pinewood VOCs (Fig. 2K). Also, other inflammatory cytokines like IL-1β, TNF-α, IL-6 and IL-4 (Fig. 3A) and chemokines like KC/GRO, CCL2, CCL3 and CXCL2 (Fig. 3B) were significantly increased in ear skin samples following oxazolone treatment. Exposure to pinewood VOCs significantly decreased the levels of IL-1β, TNF-α and KC/GRO, slightly decreased IL-6 and CCL2, but had no effect on IL-4, CCL3 and CXCL2 (Fig. 3A, B). Again, none of these parameters were affected by pinewood exposures in EtOH controls.

To study whether the observed beneficial effects were dose-dependent, we exposed the AD model depicted in Fig. 2A to lower (<3 mg/m^3) and to higher (>10 mg/m^3) pinewood VOC concentrations. Neither of the tested VOCs had an impact on body weight (Supplementary Fig. 2A, C). While lower pinewood VOC levels (<3 mg/m^3) displayed no improvement of the oxazolone-induced increased TEWL and ear swelling (Supplementary Fig. 5A), exposure to higher pinewood VOC levels (>10 mg/m^3) led to a slight, but significant increase of both parameters only in the last time-point of evaluation (Supplementary Fig. 5B). Nevertheless, histological analysis of skin samples did not reveal variations in epidermal hypertrophy, inflammatory infiltration or in measurements of epidermal and dermal thickness (Supplementary Fig. 5C-E) following exposure to each of the two VOC doses. Overall, no significant variations in serum total IgE or skin tissue cytokines were detected in mice exposed to lower or higher pinewood emissions (Supplementary Fig. 5F-G). Furthermore, whilst no effect on either skin pH or 8-isoprostane was measured in skin homogenates following lower pinewood VOCs exposure (data not shown), higher pinewood VOCs induced only a slight, but not significant, increase of 8-isoprostane levels in both sensitized and non-sensitized animals, and no effects on skin pH (Supplementary Fig. 5H-I). Also *involucrin* expression was not altered by lower VOCs concentrations, whereas it was reduced in sensitized animals following higher VOCs exposure (Supplementary Fig. 6A,B, top panels). Similarly to pinewood 5 mg/m^3, also lower and higher pinewood VOCs had no effect on *filaggrin 2* (Supplementary Fig. 6A,B, lower panels). Other than slight effects on 8-isoprostane, again, either pinewood VOC concentration had an impact on EtOH controls. Taken together, pinewood VOC concentrations of 5 mg/m^3 efficiently attenuated the oxazolone-induced AD-like inflammation and restored the skin barrier function, while at lower levels (<3 mg/m^3) had no effects and at higher levels (>10 mg/m^3) showed limited pro-inflammatory effects on the AD phenotype.

3.3. Pinewood emissions exhibit anti-inflammatory properties in a human AD model

To translate our findings to human, we developed a human AD model using primary keratinocytes stimulated with T cell supernatant (TCS) from lesional skin of AD patients, and subsequently exposed it to a mixture of TVOCs, simulating native pinewood emissions, up to a total mass concentration of 5 mg/L (Fig. 3C, Supplementary Table 2). Stimulation of keratinocytes with TCS led to a slight increase of IL-1β, IL-6 and CCL2 compared to respective controls. Interestingly, and in line with our *in vivo* observations, exposure to pinewood VOCs significantly reduced CCL2 release both in non-stimulated and in stimulated keratinocytes, the last reaching control levels. In addition, exposure to pinewood VOCs decreased slightly IL-1β and near-to-significantly ($p = 0.06$) IL-6 release from stimulated keratinocytes (Fig. 3D). These data confirm the anti-inflammatory effects of pinewood emissions on a human *in vitro* AD model.

3.4. Pinewood VOC emissions prevent the oxazolone-induced skin microbiome dysbiosis

Given the importance of cutaneous bacterial dysbiosis in AD (Eyerich et al., 2018; Kobayashi et al., 2015; Mempel et al., 2003), we explored the effects of pinewood emissions on the skin microbiome in the mouse AD model. The PCoA, a distance-based approach for group comparison, showed that all treatments were able to shift the microbiome composition compared to untreated controls (Fig. 4A). Notably, exposure to 5 mg/m^3 pinewood VOCs induced significant changes in the skin microbiome of oxazolone-treated mice (Fig. 4B). In line with our previous observations, the oxazolone treatment led to a significant decrease in α-diversity (Amar et al., 2022). Interestingly, microbial diversity was

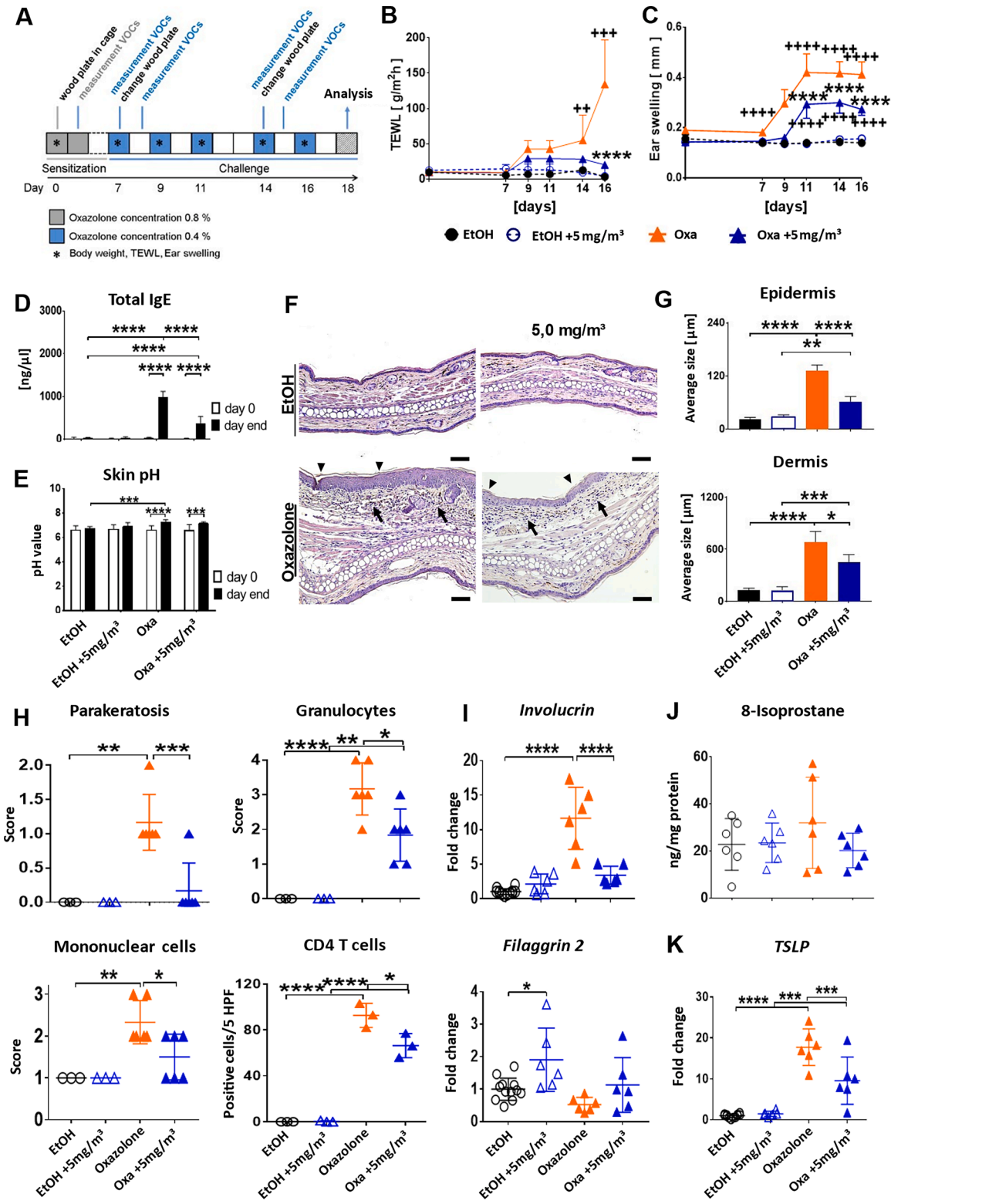

Fig. 2. Pinewood emissions at a concentration of 5 mg/m^3 dampen the oxazolone-induced AD-like inflammation A) Experimental scheme: an AD-like skin phenotype was induced by oxazolone application on mouse ears and mice were directly exposed to pinewood emissions. **B)** Measurement of transepidermal water loss (TEWL) and **C)** ear swelling. **D)** Total serum IgE levels and **E)** skin pH at the beginning (day 0) and end (day end) of experiment. **F)** Representative sections of mouse ears stained with haematoxylin and eosin (H&E). Arrows, inflammatory cell infiltrate; arrowheads, parakeratosis. Scale bar = 50 µm. **G)** Measurements of epidermis and dermis average sizes. **H)** Histological (left) and immunohistochemical (right) analysis of ear skin. **I)** Relative expression of key skin barrier genes. **J)** 8-isoprostane levels in ear lysates. **K)** Relative expression of thymic stromal lymphopoietin (*TSLP*) in mouse ears. One representative experiment out of two. Each data point represents an individual mouse. Data are expressed as mean ± SD. n = 6/group; n = 10 (Fig. 2I, EtOH). TEWL and ear swelling: $^{+}p < 0.05$; $^{++}p < 0.01$; $^{+++}p < 0.001$; $^{++++}p < 0.0001$ vs. EtOH; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ vs. Oxazolone; further graphs: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$. Statistical analyses were performed by two-way ANOVA (B-E) and one-way ANOVA (G-K) with Bonferroni post-hoc-test.

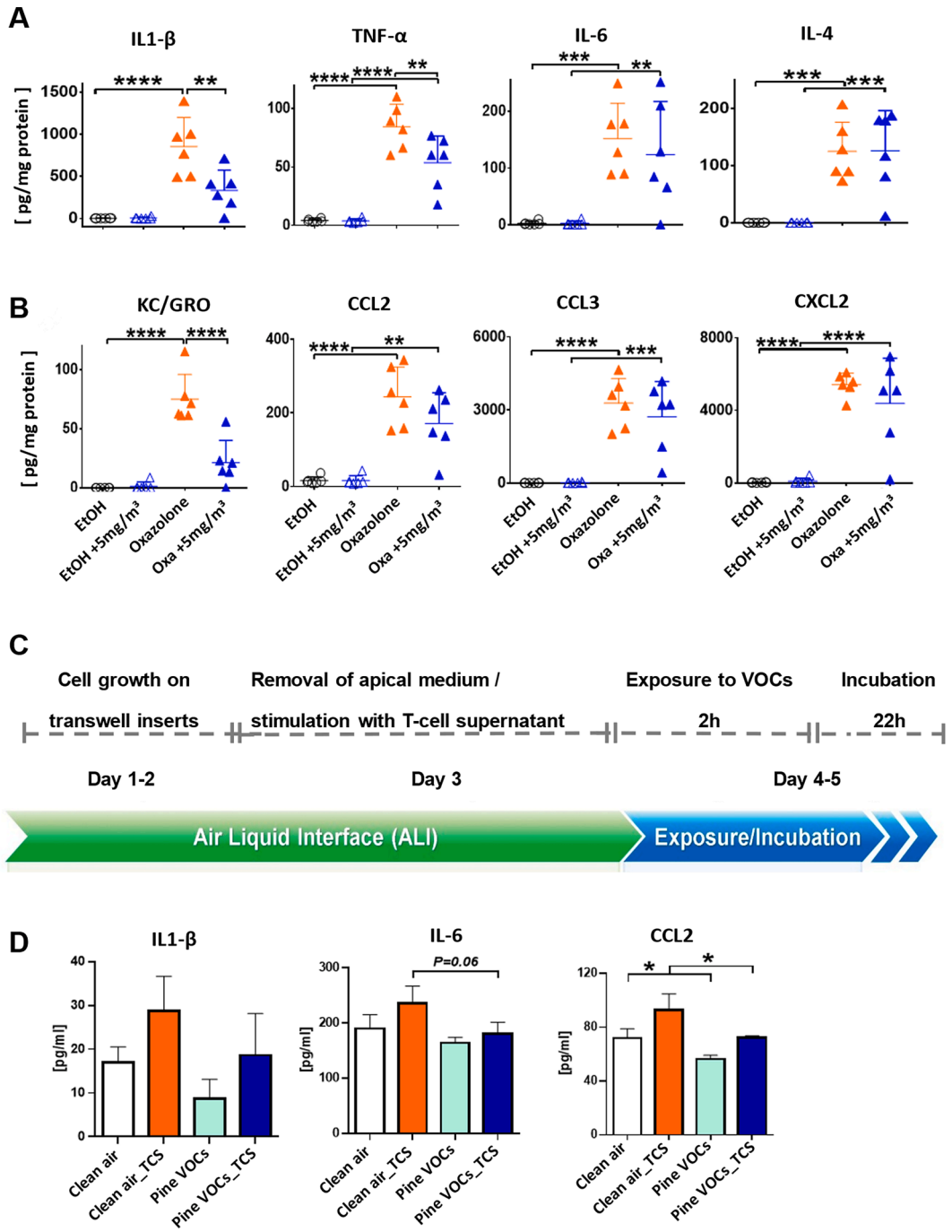

Fig. 3. Pinewood VOCs exhibit anti-inflammatory properties *in vivo* and *in vitro* in a human keratinocytes exposure system. Levels of key inflammatory **A)** cytokines and **B)** chemokines detected in mouse ear lysates following exposure to 5 mg/m^3 TVOCs concentrations. **C)** *In vitro* experimental setup to assess the effects of a 2 h exposure to a synthetic pinewood VOCs mixture on human keratinocytes stimulated or not with T-cell supernatant (TCS). **D)** Concentrations of key inflammatory mediators released in cell culture supernatants 22 h after incubation with VOCs. Each data point represents an individual mouse. Data are expressed as mean ± SD. A, B, n = 6/group; D, n = 3/group, where each data point represents the mean of two replicates of exposures. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001. Statistical analysis was performed using a one-way ANOVA with Bonferroni post-hoc-test.

recovered after exposure to pinewood VOC emissions (Fig. 4C). While the *Streptococcus* and *Cutibacterium* members did not display major changes following the different treatments, EtOH treatment alone was able to cause a clear drop of *Thiolapillus HQ191085* and *Novosphingobium* commensals with increased abundances of *Xanthomonas*. Markedly, the proportions of the latter group were restored to control levels by exposure to pinewood VOCs (Fig. 4D). Focusing on the AD-induced microbiome changes, the observed loss of microbial diversity upon oxazolone treatment was paralleled by an expansion of Staphylococci (Fig. 4D), both being characteristic AD features (Kong et al., 2012). In line with this, the LEFSe showed a significant association between the *Staphylococcus* group (LDA score = 5.7, p ≤ 0.05) and oxazolone treatment whereas an additional exposure to pinewood VOCs exhibited an association with *Jeotgalicoccus* (LDA score = 6, p ≤ 0.05) (Fig. 4E). The expansion of Staphylococci and *S. xylosus* in particular seems to take advantage of the loss of microbial diversity seen following oxazolone application, in agreement with our previous findings (Amar et al., 2022). Interestingly, the colonizing *S. xylosus*, displayed a drastic

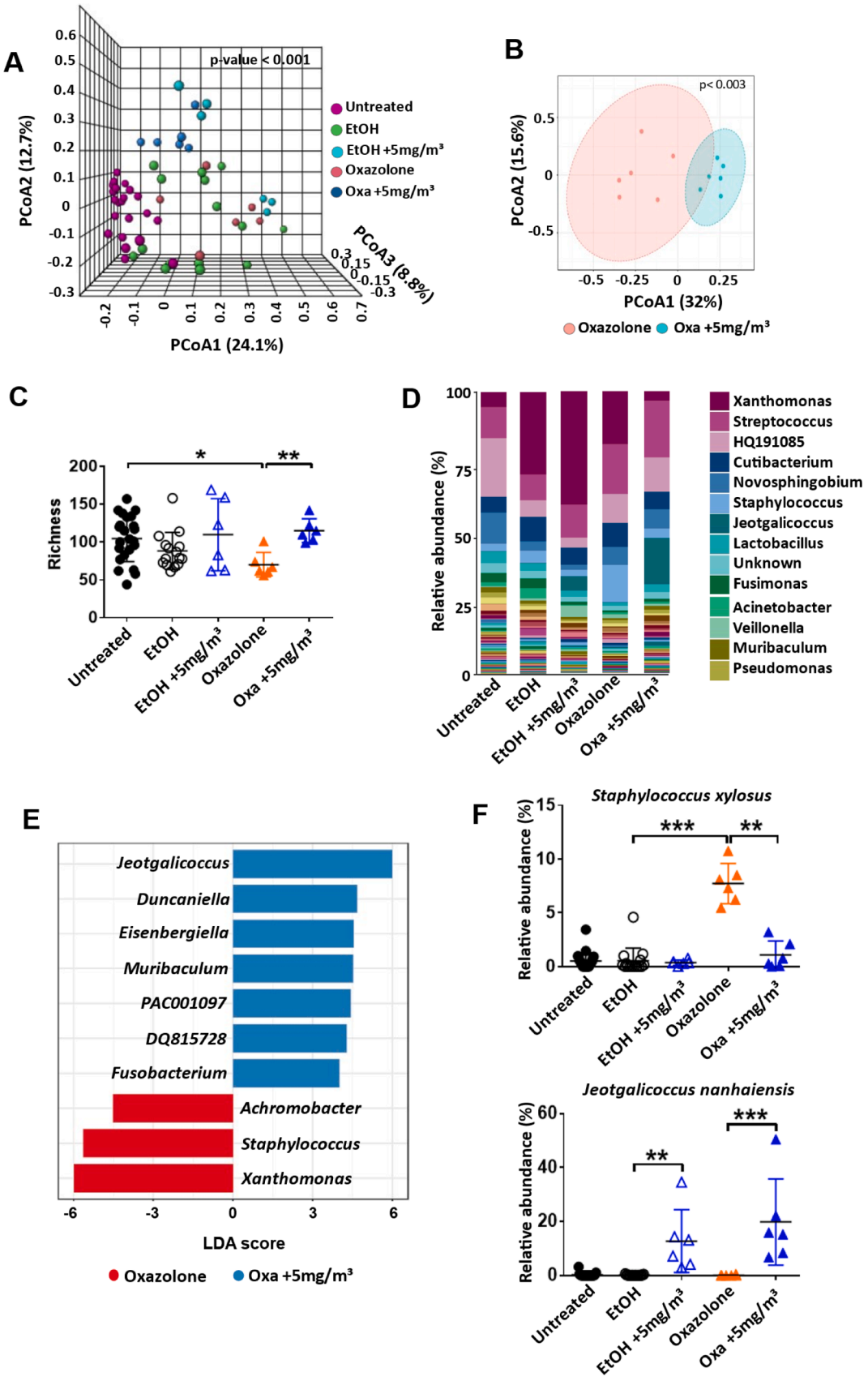

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Fig. 4. Pinewood VOCs at a concentration of 5 mg/m^3 correct the oxazolone-induced AD-like dysbiosis. A) PCoA of β-diversity analysis reveals clear microbiome changes in the experimental groups compared to untreated controls. **B)** PCoA plot of pairwise group comparison between oxazolone treated animals and those treated with oxazolone and pinewood emissions. The Bray Curtis index has been used to calculate similarity between samples depicted in the PCoA plots and the Permutational MANOVA (PERMANOVA) to test for statistical significance based on the distance matrix. **C)** Microbial richness (number of different OTUs /sample) estimated in treated and control animals. **D)** Taxonomic bar charts displaying the mean relative abundance of key genera in control and treated animals. **E)** LEFSe plot displaying genera associated with oxazolone treatment or the combination of oxazolone with pinewood VOCs at a concentration of 5 mg/m^3. **F)** Relative abundance of key taxa with major shifts among the oxazolone-treated animals and those additionally exposed to pinewood emissions. Data are expressed as mean ± SD. A-F: untreated, n = 23; EtOH, n = 15; all other groups, n = 6/group. Each data point represents a swab sample. Statistical significance was calculated using Kruskal Wallis and Wilcoxon Mann Whitney tests, respectively for multiple and pairwise groups comparisons. Multiple test corrections were performed with the Benjamini and Hochberg procedure to adjust p-values (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

decrease of relative abundance upon pinewood VOCs exposure (Fig. 4F, top). On the contrary, *J. nanhaiensis*, increased significantly both in EtOH control and oxazolone-treated mice following pinewood exposure (Fig. 4F, bottom).

After demonstrating the beneficial effects of 5 mg/m^3 TVOCs concentrations on the AD-like skin microbiome, we evaluated whether <3 mg/m^3 or >10 mg/m^3 pinewood TVOC concentrations would have similar effects. All treatments were able to shift the microbiome composition compared to untreated controls (Fig. 5A). Similarly to pinewood 5 mg/m^3, also concentrations of pinewood VOCs <3 mg/m^3 and >10 mg/m^3 induced significant changes of the microbiome compared to oxazolone (Fig. 5B). Comparably to pinewood 5 mg/m^3, lower concentrations of VOCs restored the microbial richness back to control levels (Fig. 5C), but were unable, contrarily to pinewood 5 mg/m^3, to reduce the relative abundance of Staphylococci in general (Fig. 5D, Supplementary Fig. 7A, B) and of *S. xylosus* in particular (Fig. 5F). On the other hand, higher concentrations of pinewood VOCs had only minor effects on α-diversity (Fig. 5C), but, similarly to pinewood 5 mg/m^3, induced a significant decrease of Staphylococci in general and of *S. xylosus* in particular (Fig. 5D, F, Supplementary Fig. 7A, B). Also, the EtOH-induced increase in *Xanthomonas* proportions was restored to control levels by both lower and higher pinewood VOC levels (Fig. 5D). Analogously to the results relative to pinewood 5 mg/m^3, the LEFSe analysis showed an association between exposure to lower or higher concentrations of pinewood VOCs and the *Jeotgalicoccus* group (Fig. 5E). Of note, the proportions of *J. nanhaiensis* increased upon exposure to pinewood volatiles in EtOH controls starting from 5 mg/m^3 up to >10 mg/m^3 and in sensitized animals following all pinewood VOCs concentrations (Fig. 5F, Supplementary Fig. 7B).

Taken together, these results indicate that pinewood volatiles at concentrations of 5 mg/m^3 are able to restore the oxazolone-induced skin dysbiosis. Lower or higher pinewood VOCs concentrations display individual beneficial effects for oxazolone-induced skin microbiome alterations, either restoring the loss of diversity or limiting Staphylococci colonization.

4. Discussion

The present findings demonstrate that exposure to pinewood VOCs at 5 mg/m^3 strikingly dampens the oxazolone-induced AD-like phenotype and associated dysbiosis. The oxazolone-induced AD-like phenotype was characterized by increased serum total IgE, skin barrier disruption and inflammatory cell infiltrate. The restoration of the skin barrier defect by pinewood VOCs was reflected by the significant decrease of TEWL and partial restoration of skin barrier genes. Also skin inflammation, characterized by increased ear thickness, inflammatory cell infiltration, in particular of granulocytes, macrophages and CD4$^+$ T cells, and by augmented key inflammatory markers like Th2 cytokines, TSLP, IL-1β and TNF-α, mirroring human AD (Danso et al., 2014), was dampened by VOC exposure. Likewise KC-GRO, involved in neutrophil recruitment (Sawant et al., 2021), was significantly reduced following pinewood VOCs exposure, corroborating the decreased granulocyte infiltration detected in the histological analysis (Fig. 2H, Supplementary Fig. 4). This is in line with previous investigations evaluating the anti-inflammatory or wound-healing effects of single terpenes or plant extracts in skin pathologic conditions, including AD (Yang et al., 2015; Amagai et al., 2017; Salas-Oropeza et al., 2021). Surprisingly, IL-4 release was not mitigated by pinewood VOCs, although treatment with pinewood oil fractions has recently shown to inhibit IL-4 and IL-13 release *in vitro* (Yang et al., 2021). Likely, in our experimental setting with real pinewood exposures, terpenes concentrations did not reach the minimal threshold level necessary for hampering IL-4 release. The same argument likely applies also to 8-isoprostane measurements, where pinewood VOCs displayed only slight effects in sensitized skin, supported also by recent findings on the low antioxidant potential of several monoterpenes in epithelial cells (Muilu-Makela et al., 2022). The data from the human *in vitro* system indicate that pinewood VOCs slightly dampen pro-inflammatory mediators by stimulated keratinocytes. The decreased secretion of inflammatory cytokines and in particular of CCL2, a key mediator in the development of inflammatory skin diseases (Vestergaard et al., 2004), is consistent with our *in vivo* data (Fig. 3B), and likely contributes to the reduction of tissue macrophages which we also observed by IHC (Fig. 2H and Supplementary Fig. 4).

Whilst lower (<3 mg/m^3) pinewood VOC concentrations had almost no effect in our model, higher (>10 mg/m^3) concentrations evoked late but significant alterations in TEWL and ear swelling and a slight increase in 8-isoprostane in both sensitized and non-sensitized mice, suggesting that extremely high pinewood VOC concentrations might impact negatively AD. In this respect, whereas α-pinene, limonene and 3-carene lack sensitization potential (Kawakami et al., 2020; Api et al., 2018), their oxidized form might drive allergic reactions (Karlberg and Lepoittevin, 2021). These results may be relevant for specific occupational settings (Edman et al., 2003).

A balanced skin microbiome, together with cutaneous epithelial and immune cells act as a functional unit of the barrier defense (Sanford and Gallo, 2013). In diseases such as AD a breakdown of this balance is commonly observed, where pathogens, particularly *Staphylococcus aureus*, often display a strong colonization (Park et al., 2013). We have previously shown that *in vivo* application of oxazolone triggers a mixed Th1-Th2 immune response associated with microbiome alterations sharing similarities with human AD dysbiosis (Amar et al., 2022). Notably, the application of the vehicle (EtOH) alone, due to its disinfectant and degreasing properties, modulates the cutaneous microbiota. Nevertheless, the key features of this AD-like model including a drop of microbial diversity and expansion of *Staphylococci* were only observed upon oxazolone application, indicating that EtOH is not the main driver of the observed microbiome changes (Amar et al., 2022). We also observed that in the absence of *S. aureus* from animal facilities, other members of the Staphylococci group like *S. xylosus* expands upon oxazolone treatment (Amar et al., 2022). Of note, similarly to *S. aureus*, *S. xylosus* has also been detected in moderate to severe forms of AD (Gonzalez, 2020; Higaki et al., 1999; Al-Saimary et al., 2013) and was largely documented to colonise mouse skin under inflammatory conditions (Kim et al., 2017). Interestingly, exposure to 5 mg/m^3 pinewood concentrations impeded the oxazolone-induced dysbiosis, keeping the microbial diversity to homeostatic levels and limiting the expansion of *S. xylosus*. The mechanism underlying the microbiome restoration could be the combination of both anti-inflammatory effects of pinewood volatiles with their direct antimicrobial action (Muilu-Makela et al., 2022). It has been shown indeed, that pinewood emissions are able to inhibit

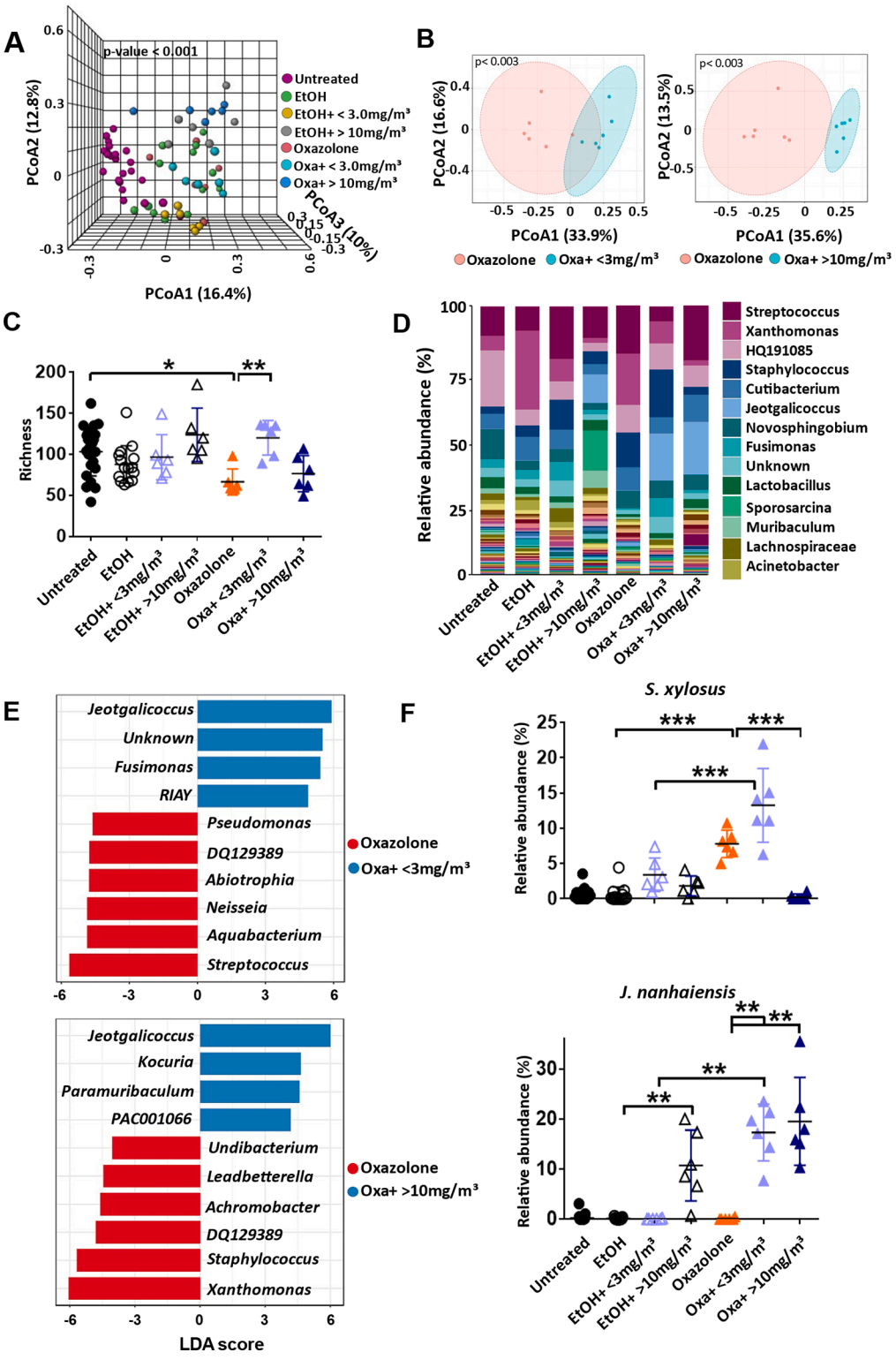

Fig. 5. Impact of lower and higher pinewood VOC concentrations on oxazolone-induced skin dysbiosis. A) PCoA plot of β-diversity analysis displaying microbiome shifts in the experimental groups compared to untreated controls. **B)** Pairwise group comparison of mice treated with oxazolone with those treated with oxazolone and exposed to <3 mg/m^3 or >10 mg/m^3 pinewood VOC concentrations. The Bray Curtis index was used to calculate similarity between samples and PERMANOVA to test for statistical significance. **C)** α-diversity expressed as microbial richness in control and treated animals. **D)** Taxonomy analysis of key abundant genera. **E)** LEFSe plot displaying genera associated with oxazolone treatment in comparison to combination of oxazolone with pinewood VOCs at low and high doses. **F)** Relative abundance of key taxa with major shifts among controls and treated animals. Data are expressed as mean ± SD. A-F: untreated, n = 23; EtOH, n = 15; all other groups, n = 6/group. Each data point represents a swab sample. Statistical significance was calculated using Kruskal Wallis and Wilcoxon Mann Whitney tests, respectively for multiple and pairwise groups comparisons. Multiple test corrections were performed with the Benjamini and Hochberg procedure to adjust p-values (*p < 0.05, **p < 0.01, ***p < 0.001).

the growth of a wide range of pathogens including *S. aureus*, *E. coli*, *S. pneumoniae* and *S. typhimurium* (Vainio-Kaila et al., 2017). Antimicrobial effects were also reported for single terpenes as α-pinene, 3-carene and limonene (Muilu-Makela et al., 2022). In addition, we show that exposure to pinewood volatiles *in vivo* possess a strong inhibitory potential against *Xanthomonas*, even if recent *in vitro* investigations demonstrated the inability of 1–2 % pinewood extracts to impede the growth of different *Xanthomonas* strains (Mačionienė et al., 2021). Exposure to pinewood VOCs led also to an increase of the *Jeotgalicoccus* member *J. nanhaiensis* on both healthy and diseased skin. The *Jeotgalicoccus* species, although ubiquitous and sharing many similarities with *Staphylococcus*, are still poorly studied (Schwaiger et al., 2010). Likely, the inhibitory effect of pinewood VOCs towards *S. xylosus* favors an ecological niche which allows the expansion of *J. nanhaiensis,* which may be beneficial since *Jeotgalicoccus* sp. have been shown to inversely correlate with atopic diseases (Ege et al., 2012).

Having assessed the effects of the TVOC mix limits our conclusions regarding which of the individual components could be responsible for the observed anti-inflammatory or antimicrobial effects. However, the aim of this study was to investigate the effect of pinewood VOCs on the development of AD using a real case scenario, simulating the indoor exposure to pinewood-specific total volatiles mix. The question of which terpene emitted from pinewood is mainly responsible for the beneficial effects observed should be matter of future investigations.

5. Conclusion

This study reveals for the first time the impact of exposures to pinewood as a source of VOCs on a murine model of AD. We demonstrated that pinewood volatiles at a concentration of <3 mg/m^3 had no effect on the mouse AD phenotype, but were able to restore the oxazolone-induced loss of microbiome diversity. Pinewood VOCs at 5 mg/m^3 demonstrated anti-inflammatory properties, hampered the development of an AD-like phenotype and restored the associated dysbiosis. The beneficial effects of pinewood VOCs were confirmed in a human *in-vitro* AD model. Higher pinewood VOCs concentrations of >10 mg/m^3, while displaying isolated pro-inflammatory effects on the AD phenotype, maintained beneficial effects for oxazolone-induced skin microbiome alterations by limiting Staphylococci colonization. This work identifies relevant taxa as biomarkers for the effects of terpenes on AD and calls for clinical studies to exploit the adjuvant therapeutic potential of these environmental compounds for the prevention and/or control of AD.

Ethics statement

Mouse *in vivo* experiments were conducted according to the European Convention for Animal Care and Use of Laboratory Animals and were approved by local ethics committee and government authorities (Approval n. ROB-55.2-2532.VET_02-16-198). *In vitro* experiments were performed following the approval n. 5590/12 and 44/16 S, Technical University of Munich.

Author contribution

Mouse experimental design: SH and FA. Analysis of VOCs: KB. Conduction and analysis of *in vivo* experiments: ES, SM, BS and FA. Conduction of *in vitro* experiments: SM and MG-K. Histology, gene and protein expression analysis: ES, KS, IMH, BS and FA. Immunohistochemical stainings and analysis: KS. Microbiome sample processing: SG. Microbiome data analysis: YA. Experimental data analysis: ES, YA and FA. Supervision: RG, BE, AG, JEvB, MO, CBS-W, SE and FA. Writing original draft: ES, YA, KB, MG-K, AG and FA. Review&Editing: All Authors. Funding acquisition: RG, TB, SH, MO, CBS-W, SE and FA.

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CRediT authorship contribution statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2024.109035.

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