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Clogmia albipunctata Larvae Influence the Odour Dynamics of Rotting Organic Matter

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ABSTRACT

Environmental malodors, particularly from wastewater treatment plants, are a growing concern due to their impact on health and well-being. The drain fly, *Clogmia albipunctata* (Williston, 1893) (Diptera: Psychodidae, Psychodinae, Paramormiini), is commonly found in sewage treatment plants and household drains, where its larvae help unclog drains and consume biofilm and bacteria that cause odors. This study examined the effect of these larvae on malodor by adding them to containers with rotting nettle (*Urtica dioica* L.) and dewatered sewage sludge. Odor differences between control and treatment containers were assessed by measuring volatile organic compounds (VOCs) with a PTR–ToF mass spectrometer and hedonic tone ratings by nonprofessional people. Chemical analysis showed a significant increase in odorous compound concentrations and composition, as well as a higher total odor active value (SOAV), in sewage sludge with larvae, possibly caused by enhanced evaporation and decomposition. However, results for nettle sludge medium were inconclusive, possibly due to fewer larvae, but did show decreased concentrations of some key odorants due to larval activities. No significant results were yielded from the hedonic ratings, potentially because laypeople were used instead of a trained panel. This study provides a unique case of insects influencing odor dynamics in rotting organic matter and can potentially inform future research and strategies for managing environmental malodors in wastewater treatment processes.

1 | Introduction

Malodors from industrial sites, wastewater treatment plants, and other sources have become a significant societal issue due to their negative impact on human well-being (Daniel 2020). These odors are shown to annoy nearby residents (Aatamila et al. 2011; Cavalini et al. 1991) and cause physical irritation to the respiratory system (Schiffman and Williams 2005). Long-term exposure to malodors can even lead to serious health issues, including respiratory problems and stress-related diseases such as cardiovascular disease and depression (Cohen et al. 2007; Horton et al. 2009; Kivimäki and Steptoe 2018). The perception of odor is complex and influenced by, among other factors, the concentration of an odorous compound and its odor detection threshold, i.e., the concentration at which the compound can be detected by a human. To quantify the possible perception of an odor, the Odor Activity Value (OAV) that accounts for both these factors was developed (Feilberg et al. 2010; Wu et al. 2017). For odor mixtures, the sum of OAVs (SOAV) is used. As they aim to reflect the effect of an odorant on human perception, the OAV and SOAV are widely used (Wu et al. 2017). However, the use of SOAV also has limitations as it does not fully account for the complexity of human odor perception, including individual differences in detection thresholds and interactions between different odorants (Wu et al. 2016). For instance, olfactory sensitivity significantly deteriorates with increasing age, and women possess lower thresholds and perform slightly better than men in odor discrimination and identification (Bliss et al. 1996; Sorokowski et al. 2019). Therefore, when studying the impact of odors, it is crucial to consider not only chemical methods of odor detection, as this greatly underestimates the complexity of odor perception in humans.

In recent decades, wastewater treatment plants (WWTPs) have faced increasing pressure to reduce odor emissions (Daniel 2020; Lebrero et al. 2011). Anaerobic microbial activity in primary settling tanks and sludge dewatering units in WWTPs produces odorous compounds such as H_2S and volatile organic compounds (VOCs) such as mercaptans, amines, and fatty acids (Lebrero et al. 2011). The final stages of sludge processing and sludge composting contribute in large part to odor emissions (Gebicki et al. 2016; Naddeo et al. 2012), since the elevated concentrations of organic matter in dewatered sewage sludge lead to higher microbial activity (Lebrero et al. 2011).

The presence of rotting organic matter in WWTPs provides a favourable environment for a multitude of invertebrate species, including moth flies (Learner and Chawner 1998). *Clogmia* albipunctata (Williston 1893) (Diptera, Psychodidae, Psychodinae, Paramormiini, syn. *Psychoda* albipunctata, *TelmatoScopus* albipunctatus), commonly known as the drain fly, is one of the most common species present in WWTPs (Boumans et al. 2009; Hribar et al. 2004). This cosmopolitan species originally occurred worldwide between 40° southern and 42° northern latitude, but in recent decades it has spread throughout Europe (Boumans et al. 2009; Global Biodiversity Information Facility (GBIF) 2024). The larvae thrive in shallow, turbid, semi-aquatic environments, feeding on biofilm, including bacteria, in WWTPs, drains, toilets, and rubbish bins (Boumans et al. 2009; Oosterbroek 2006; Zhang et al. 2020). In natural habitats, they are found in ponds, bogs, and rot holes in trees (Boumans et al. 2009; Englund et al. 2007; Harlan and Paradise 2007; Oosterbroek 2006). In Europe, this species can also thrive outside of synanthropic environments during summer. It is not clear if this is also the case year-round (Boumans et al. 2009). The larvae of this holometabolous insect are cylindrical with chewing mouthparts and respiratory siphons (Farrag et al. 2019). Adults are nocturnal, lack functional mouthparts, and stay near larval habitats (Oosterbroek 2006). They have long, water-repellent hairs on their wings, legs, and body, which protect them from drowning (Akhoundi et al. 2022; Boumans et al. 2009). Adults are about 2.2 mm long and hold their wings horizontally at rest (Boumans et al. 2009; Williston 1893). The life cycle includes four stages: eggs hatch in three days, followed by larval development in four stages, with a total duration of 16–18 days. Then there is a five- to six-day pupal stage, and adults are ready to lay eggs within three days after emergence (Jiménez-Guri et al. 2014; Sehgal et al. 1977). In laboratory conditions, the complete cycle spans 27 ± 5 days, with the total lifespan being approximately 35 days (Figure 1; Jiménez-Guri et al. 2014). Due to their short development time, *C. albipunctata* is used in evolutionary development research (e.g., Farrag et al. 2019; García-Solache et al. 2010).

Clogmia albipunctata larvae are known to unclog biofilters used to alleviate odor emissions from WWTPs, which could help in the maintenance of these filters (Won et al. 2004; Zhang

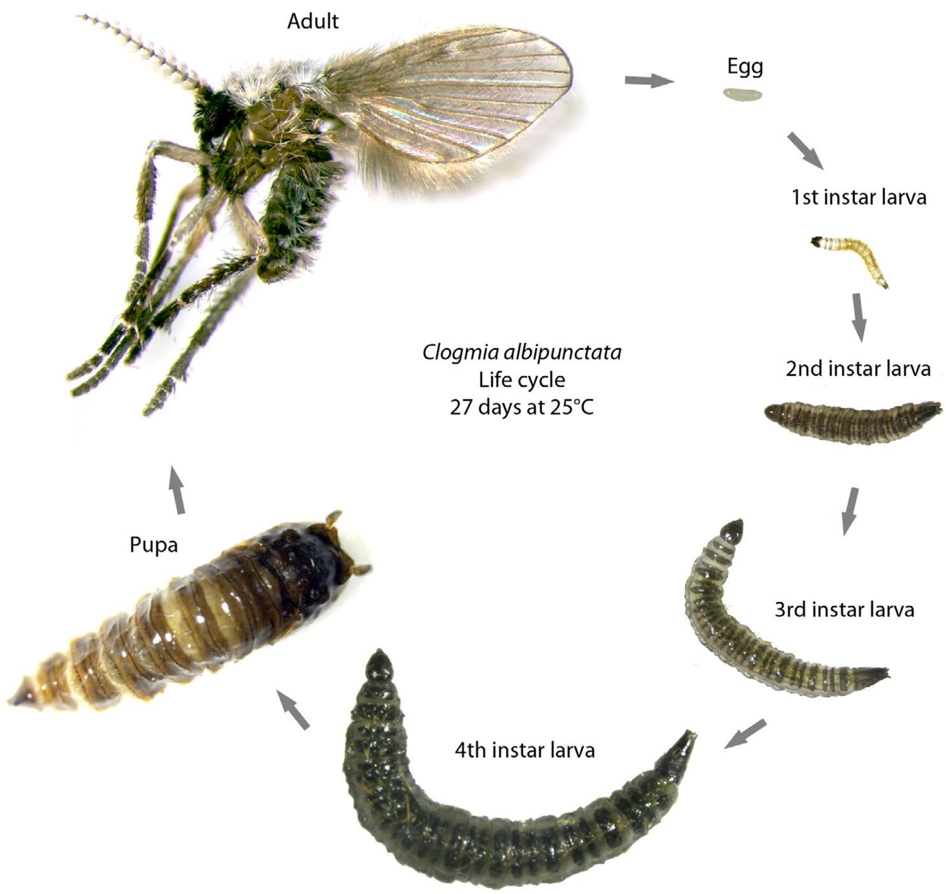

FIGURE 1 | The life cycle of *Clogmia albipunctata*. The duration of the life cycle is 27 ± 5 days at 25°C. Figure adopted with permission from Jiménez-Guri et al. (2014).

et al. 2023). It may also be possible for larvae to reduce malodor directly by consuming bacteria and biofilm, with the potential of lowering malodor-producing bacteria populations. Additionally, odors might be alleviated due to larvae increasing the concentration of oxygen into the system, which could reduce the anaerobic bacterial community (Cruz-García et al. 2019; Won et al. 2004). Recent studies demonstrated that inoculating rotting food waste and cattle manure with larvae of another dipteran, the black soldier fly, *Hermetia illucens* (L. 1758) (Diptera, Stratiomyidae), significantly reduced the production of volatile organic sulphur compounds (Beskin et al. 2018; Michishita et al. 2023). On the other hand, the detachment of the biofilm and the physical agitation of the medium by larvae could increase the release of VOCs, thereby increasing the malodor.

Moth flies, particularly *C. albipunctata*, are common in wastewater treatment plants and homes, living in rotting media. The impact of moth flies on malodors from these environments is not well studied. Understanding their impact on malodor is crucial for addressing the growing problem of environmental odor nuisance. This study, therefore, estimated the effect of *C. albipunctata* on malodor in humid rotting organic matter. We used two different media, sludge made from blended nettles (*Urtica dioica*) and dewatered sewage sludge collected from a WWTP. Ten replicates of treatment (with larvae) and control (without larvae) were prepared for each type of medium. These replicates were all assessed for odor by using non-professional human participants to rate the hedonic tone of the smell and by using PTR–ToF-MS analysis to assess the difference in concentration of odorous compounds between the treatment groups. We hypothesised that *C. albipunctata* larvae would have an effect on malodor, but we were not certain in which direction.

2 | Materials and Methods

2.1 | Experiment Preparation

2.1.1 | Drain Fly Cultivation

Nettles were blended with water to prepare a breeding medium, which was stored in a plastic container at room temperature for a week. Nettle sludge was used because it was easy to gather, and it is known that it causes malodor when rotting. 52 adult *C. albipunctata* flies were collected at a wastewater treatment plant and transferred to a mesh breeding cage with a container of nettle sludge. This cage was kept in a climate room (25°C, 65% RH, 12 h day/night cycle). The breeding setup was managed by spraying water to maintain high humidity levels. Larvae were observed after 11 days, and the cultivation was expanded by transferring larvae to larger containers with additional nettle sludge. Later, rotting sewage sludge was added to the containers and mixed with the nettle sludge, establishing a stable population over six to seven generations.

2.1.2 | Setting Up Media for Egg-Laying

In the experiments two medium types have been used (Figure S1). Nettle sludge was prepared by blending nettles with water (1.5:1 water: nettle w/w), and dewatered sewage sludge was collected from the wastewater treatment plant Leiden Zuidwest. Both media were incubated in separate 1-l containers in a climate room for seven days to let the media rot. The nettle sludge was later rehydrated with additional water to counter dryness, so that the initial water: nettle w/w ratio would be 2:1. In the first step of the experiment, each medium type was incubated with adult drain flies, enabling egg-laying in the medium (Figure 2). After incubation, two 1-l containers per medium were set up, one sealed with fine mesh (control) and one left open (treatment). One control and one treatment container per medium type were placed inside two breeding cages. Adult drain flies were introduced to the breeding cages, and the cages were managed in a climate room with periodic spraying for humidity.

2.1.3 | Setting Up Test Containers

New nettle sludge (prepared in a 2:1 water-to-nettle ratio w/w) and sewage sludge were prepared and then stored in the climate room to promote decomposition. Six days later, 20 1L containers for each medium were filled with 150 g medium. Fifty larvae were added to each of 10 containers of each medium (treatment), while the remaining 10 containers of each medium were not inoculated and served as a control group. This resulted in two treatment and two control groups, all with 10 replicates. To the sewage sludge treatment containers, 15 g of treatment egg-laying medium was added. To the sewage control containers, 15 g of control medium was added. The egg-laying medium was not added to the nettle treatment containers, since the medium was too dry and had no visible movement of larvae. The next day, additional larvae were added to all treatment containers, rendering a total of 130 larvae per treatment container (Figure 2). After this, a toothpick was used to transfer a small amount of the general breeding container sludge into all of the control containers to spread the bacterial community. Containers were incubated in the climate room for another week before the start of the measurements.

2.2 | Chemical Analysis

Twelve days after the inoculation with larvae, a chemical analysis was performed using a Proton-transfer-reaction mass spectrometry instrument (model PTR–ToF 8000, Ionicon Analytik, Innsbruck, Austria) at Radboud University. Air holes in the test containers were sealed with painter's tape 3 h before the measurements. Before measuring, the system was calibrated using a gas mixture consisting of 1 ppm methanol, acetaldehyde, ethanol, acetone, isoprene, benzene, toluene, xylene, and α-pinene. Each container was sampled separately, with 25 mL of air drawn per minute from a closed container through a needle and tube connected to the PTR–ToF-MS instrument. Each measurement lasted 2 min and started after compound intensity levels had reached a stable equilibrium. The instrument was flushed with ambient air for 30 s between samples. The drift tube pressure was 2.3 mbar, the temperature was 80°C, the voltage was 600 V, and the E/N ratio was ~140 Td. The scan range of the mass spectrometer was m/z 0–234, and resolution was ~8000 m/Δm. Concentrations were determined using reaction constants, which were assumed to be 2×10^{-9} cm^3/s for each component.

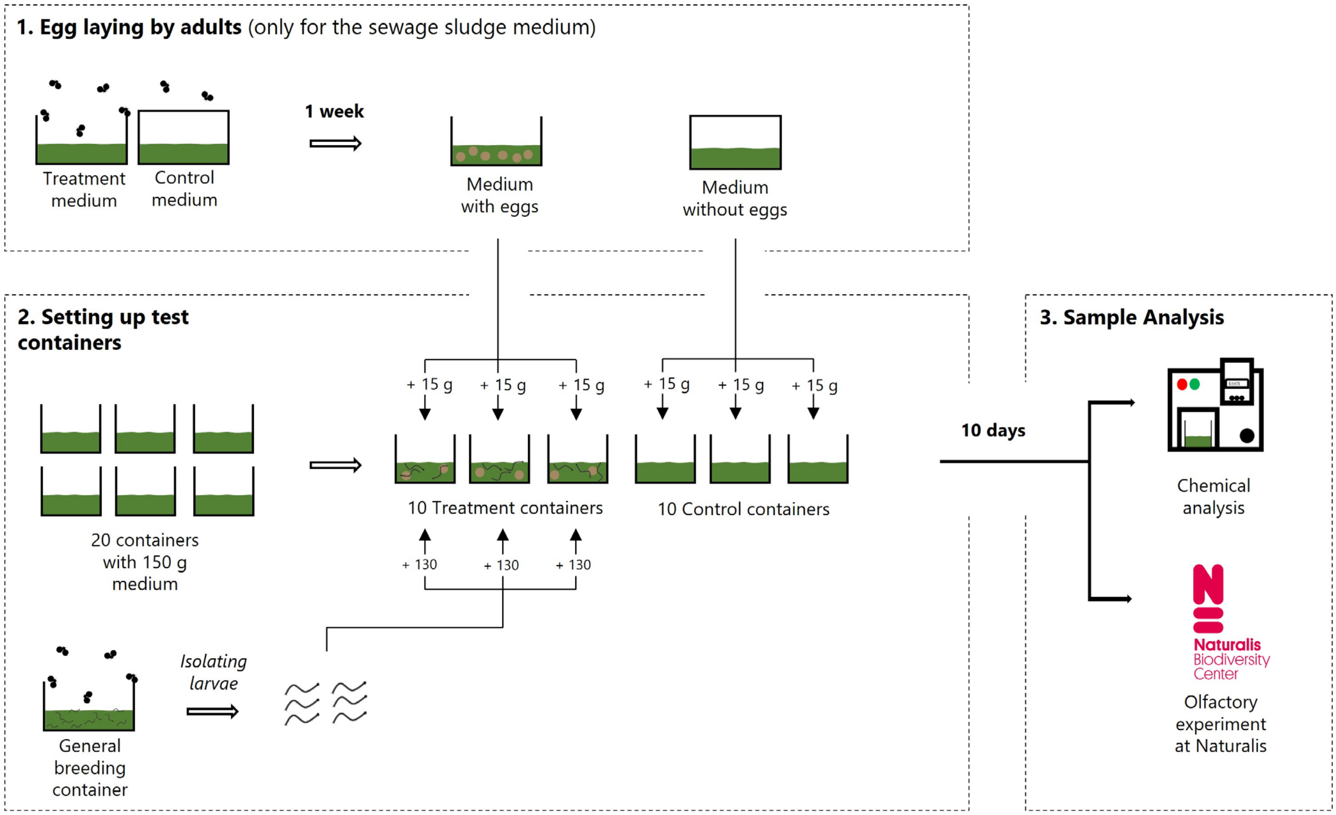

FIGURE 2 | Simplified flowchart of the experiment preparation. Except for step 1, every step was performed for each medium type. Step 1 was only performed for the sewage sludge medium. The olfactory measurements were done over nine days, and the chemical analysis was performed a day after the first four days of the olfactory measurements.

These calculations were performed in PTR–MS Viewer (Ionicon Analytik, Austria).

Mass-to-charge ratio (m/z) values from the chemical analysis were identified within a 0.01 error margin using a supplementary spreadsheet from Pagonis et al. (2019). Odor threshold concentrations and descriptions were sourced from Murnane et al. (2013); Nagata and Takeuchi (2003), PubChem, and Haz-Map. The lowest value was used if discrepancies existed. Anomalies, such as an exceptionally low methanethiol value, were handled with caution, using the second-lowest reported threshold instead. Compounds that were unidentifiable, non-odorous, or had incomplete information were excluded from analysis.

2.3 | Hedonic Scaling Experiment

2.3.1 | Experimental Setup

The hedonic scaling experiment started on day 8 after the incubation period and was performed at Naturalis Biodiversity Center over nine days, including an interval of three days. Test containers were numbered randomly to ensure a double-blind setup. Before the experiment, the physical characteristics of the media in the containers were recorded. Each day, containers were opened and blown on in order to release emerged adults. A selected container was placed in a larger box with a sniffing hole for half an hour, during which it was presented to participating museum visitors. Visitors rated the hedonic tone of the odor on a scale from −5 (very unpleasant) to 5 (very pleasant), with 0 being neutral. In addition to the hedonic tone, participants were asked to write down their age and sex. To avoid influencing others, participants were asked not to react visibly. The sniffing hole was closed between participants, and the box was aerated every 30 min before a new container was randomly selected. Each container was tested twice, and some three times. At the end of the measurements, all containers were opened for visual inspection before disposal.

Data from the hedonic scaling experiment forms was digitised, excluding entries from children under the age of 10 due to potential bias from parents influencing their children's choices.

2.3.2 | Data Analyses

Statistical analyses and graph creation were performed using R (v4.5.1). An α level of 0.05 was used for determining statistical significance.

On the data of the hedonic scaling experiment, a cumulative link mixed model was applied using the ordinal package (Christensen 2023), This was used to assess the effect of treatment, participant sex, age, and experiment day on hedonic tone ratings, with container number as a random effect. Non-significant interactions were excluded, and the model with the lowest Akaike Information Criterion (AIC) was chosen. Model assumptions were checked with the brant (Schlegel and Steenbergen 2020) and MASS (Ripley 2024) packages. Two separate models were created for each media type, and both were structured as follows:

$$\text{Rating} \sim \text{Treatment} + \text{Sex} + \text{Age} + (1 \mid \text{Nr})$$

For the analysis of the chemical data, the odor activity value (OAV) for each identified compound was calculated as follows:

$$\text{OAV} = \text{concentration} / (\text{odor threshold concentration})$$

Mean OAVs for each compound were calculated per treatment. The contribution to overall odor nuisance was approximated by dividing each compound's mean OAV by the total mean OAV sum in each treatment. Considering a deviation from the assumption of normality, the differences in compound concentrations and SOAVs between treatment groups were analysed using Wilcoxon Signed-Rank tests with Benjamin–Hochberg correction.

Lastly, the effect of treatment on the mean concentration of all compounds detected above the odor concentration threshold was analysed using a two-way ANOVA (Analysis of Variance). A log-transformation was applied to the data to meet the assumptions of normality and homoscedasticity. Following the ANOVA, a post hoc analysis was conducted using Estimated Marginal Means from the *emmeans* package (Lenth 2025) to identify significant differences for individual compounds. The Benjamini–Hochberg procedure was used for multiple testing correction of the *p*-values. A separate ANOVA was performed for each medium type.

3 | Results

3.1 | Drain Fly Larvae Increased the Total Odorant Concentration of Sewage Sludge

To assess the effect of drain fly larvae on malodor emission, the difference in total odorant concentrations between treatment and control containers was determined. In Figure 3A, the distribution of log-transformed total compound concentrations in containers across treatment and medium groups is depicted. The sum of container compound concentrations was 4.36 times higher in sewage sludge treatment containers (M = 1036 ppb, SD = 782 ppb) than in the control containers (M = 243 ppb, SD = 151 ppb) (Wilcoxon signed-rank test: W = 18, $p = 0.03$). There was no significant difference between nettle sludge treatment (M = 488 ppb, SD = 259 ppb) and control containers (M = 827 ppb, SD = 802 ppb) (Wilcoxon signed-rank test: W = 58, $p = 0.58$).

A total of 32 compounds were detected and identified across all containers. Of these, 20 are considered malodorous in isolation, while the other compounds are either pleasant or have a variable or objectionable odor. All compounds were VOCs, except for nitrogen dioxide Figure 4 illustrates the concentrations of compounds detected above the odor threshold for each treatment and medium combination. The two-way ANOVA revealed a significant main effect of treatment on the mean concentration (Sewage sludge: $F(1, 180) = 9.24$, $p = 0.003$; Nettle sludge: $F(1, 144) = 10.66$, $p = 0.001$), and this effect was dependent on the identity of the compound (Sewage sludge: $F(9, 180) = 2.56$, $p = 0.009$; Nettle sludge: $F(7, 144) = 5.94$, $p < 0.001$). In sewage sludge containers, the mean concentration of nitrogen dioxide ($p = 0.056$), methanethiol ($p = 0.056$), dimethylsulphide ($p = 0.056$), dimethylamine ($p = 0.056$), and 2-methylpyridine ($p = 0.056$) was higher in treatment compared to control containers, while dimethyl disulphide concentrations ($p = 0.056$) were lower, but these effects were marginally non-significant after Benjamin–Hochberg correction (Table S1A). In contrast, in nettle sludge containers, the concentrations of trimethylamine ($p = 0.03$), pentanal ($p < 0.001$), and dimethylamine ($p < 0.001$) were lower relative to control (Table S1B).

3.2 | Drain Fly Larvae Increased the SOAV of Sewage Sludge Containers

The difference in the calculated odor nuisance, represented by the SOAV, between treatment and control containers was

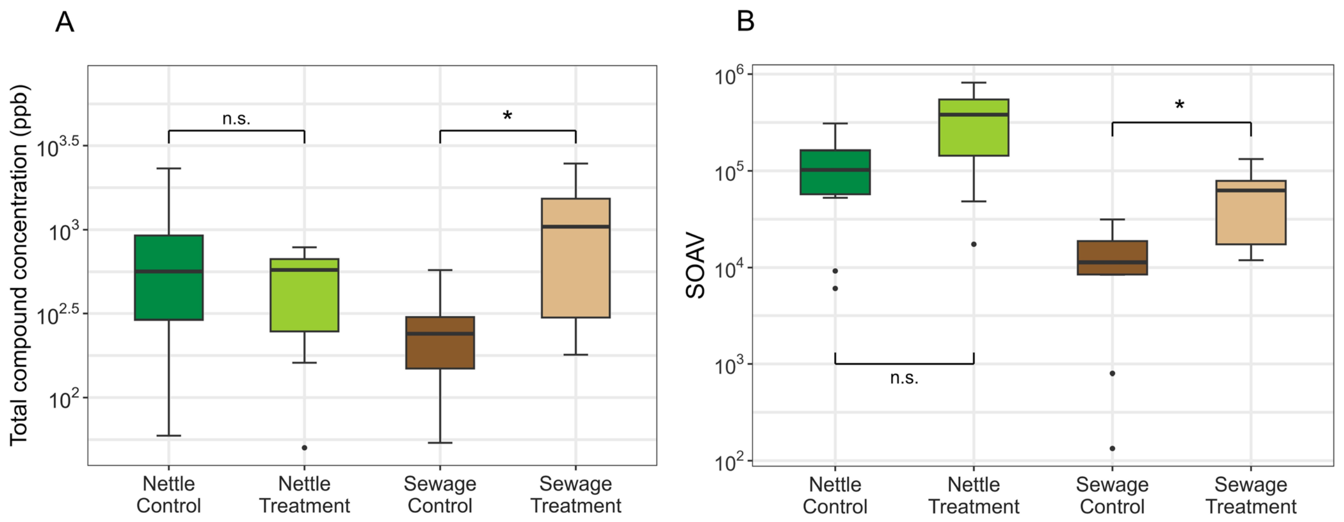

FIGURE 3 | Boxplots illustrating the difference in distribution of the log-transformed sum of malodorous compound concentrations (ppb) (A) and the log-transformed sum of OAVs (SOAVs) (B) between containers of different treatments for nettle sludge and sewage sludge media. The middle line shows the median, the box represents the interquartile range, and the whiskers indicate the minimum and maximum values with points marking outliers. A non-significant difference revealed by Wilcoxon Signed-Rank tests is noted with "n.s." and a significant difference ($0.01 < p < 0.05$) with "*".

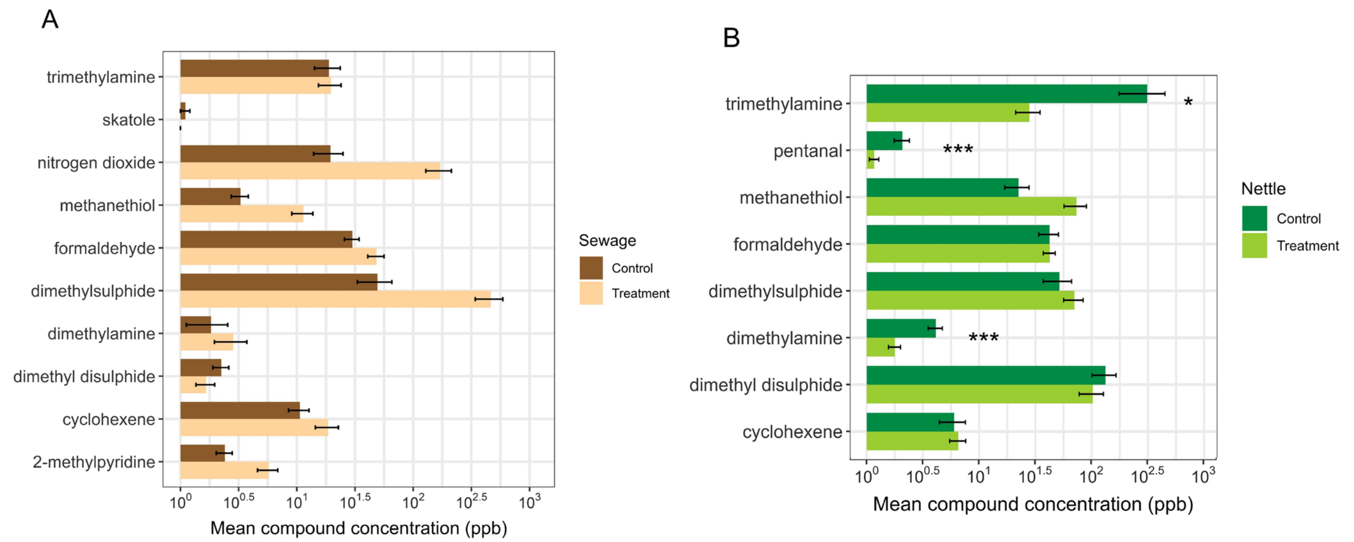

FIGURE 4 | Bar plots depicting the mean compound concentrations (ppb) on a log scale of all detected compounds above the odor threshold concentration found in containers with sewage sludge (A) and nettle sludge (B). Each bar represents the mean concentration of a compound within a control or a treatment group, and the error bars represent the Standard Error. Asterisks show significant differences, as revealed by a two-way ANOVA, between the treatment and control bar of a compound, with $*p<0.05$, $**p<0.01$ and $***p \leq 0.001$. For visualisation purposes, a value of 1 was added to the mean concentration of all compounds. Summary statistics can be found in Table S1.

determined to provide insight into the effect of drain flies on odor nuisance. In Figure 3B, the distribution of log-transformed container SOAVs across treatment and medium groups is depicted. The container SOAVs significantly increased with a factor of 4.45 in sewage sludge treatment containers (M = 56 930, SD = 40 688) compared to control containers (M = 12 798 ppb, SD = 9582) (Wilcoxon signed-rank test: W = 14, $p = 0.02$). The SOAV of nettle sludge treatment containers (M = 364 047, SD = 262 395) was elevated, but not significantly different compared to the control containers (M = 123 125, SD = 100 397) (Wilcoxon signed-rank test: W = 23, $p = 0.06$). Methanethiol contributed the most to the total OAV, with proportions ranging from 87% to 99%. The dominant odorants after methanethiol were trimethylamine for both media and dimethyl sulphide for sewage sludge.

3.3 | Drain Fly Larvae Changed the Medium Appearance and Inhibited Fungal Growth

To provide insight into the mechanisms by which drain flies could affect malodor, the physical appearance of the media was recorded. After being incubated for 10 full days, the medium in the containers with and without larvae differed in their appearance. A notable difference between sewage sludge containers with and without larvae that stood out during the visual observation was that sewage sludge containers with larvae were more watery. This difference was not observed in nettle sludge containers. We also noticed fewer larvae in nettle sludge treatment containers than in sewage sludge treatment containers, possibly due to the fact that eggs were only added to the sewage sludge treatment. During visual observation before the experiments, we noticed that mold covered all the sludge in the nettle control containers, and no mold grew on the nettle treatment containers. The mold quickly disappeared from all the nettle sludge during the experiments, possibly because the containers were moved and shaken during the experiments. Two of the sewage sludge control containers also contained visible fungal growth, which did not disappear during the experiments and was not present in treatment containers.

3.4 | Control and Treatment Containers Showed Similar Hedonic Tone Ratings

The hedonic scaling experiment was performed to assess whether drain fly larvae influence the hedonics of the odors in the media as experienced by humans. The ratings of 1244 individuals were used in the analysis. The average age of the contributing participants was 35.9 and ranged from 10 to 82. At least 75% of the odor ratings, regardless of medium or treatment type, indicated some degree of unpleasantness (Figure 5). The cumulative link mixed model estimated that the chance of a nettle treatment container receiving a higher hedonic tone rating (higher pleasantness) of at least one category than a nettle control container was 22% higher, but this estimate was not significant (Table S2). There was also no significant difference between treatment and control container ratings of the sewage sludge medium. Males gave significantly higher ratings than females, and younger participants gave significantly higher ratings than older ones. The difference in ratings of males and females was not significantly influenced by the treatment type. Additionally, there were no significant interactions between treatment type and sex and age, so these variables were not included in the model.

4 | Discussion

This study aimed to examine the effect of *C. albipunctata* on malodor from rotting organic matter. It was hypothesised that there would be an effect, though the direction was unknown. Containers of nettle sludge and sewage sludge were prepared with and without drain fly larvae. The difference in odor between

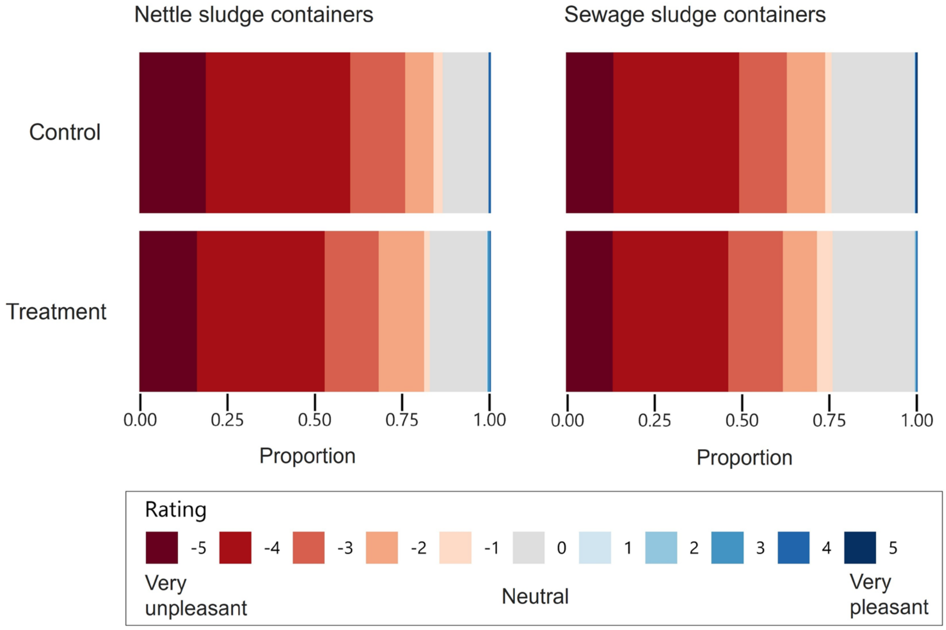

FIGURE 5 | Bar plots representing the proportion of each rating category out of the total ratings per medium and treatment type combination. In total, 1244 ratings of human volunteers are incorporated. A cumulative link mixed model showed no significant difference between ratings of treatment and control containers for both nettle sludge and sewage sludge.

the two treatments per medium type was assessed by using both human participants and mass spectrometry techniques. Some results supported the hypothesis, while others were unclear. Neither medium yielded a significant difference in hedonic tone ratings by humans. However, drain fly larvae did have a visual effect on both media, by inhibiting fungal growth, and for the sewage sludge, rendering the medium more fluid. In sewage sludge, the larvae increased the concentration of odorants by a factor of 4.26 and the mean SOAV by 4.45. Organic sulphur compounds, including dimethyl sulphide and methanethiol, were the most critical compounds. These compounds are known to be important odorants that arise from sewage treatment processes, partly due to their low odor concentration thresholds (Devai and DeLaune 1999; He et al. 2018; Lebrero et al. 2011). The observed effect of the drain fly larvae on the chemical composition of the sewage sludge odor supports the hypothesis.

In this study, the concentration of odorous compounds and the resulting mean SOAV were significantly higher in sewage sludge containers with larvae than in those without. This deviates from earlier research on black soldier fly larvae, where VOC concentrations were lower in containers with larvae (Beskin et al. 2018; Michishita et al. 2023). In contrast to black soldier fly larvae, drain fly larvae feed on microbes, which could change the microbial community structure differently when compared to black soldier fly larvae. This could explain the difference in microbial odor emissions between media with black soldier flies and drain flies. We hypothesised that the larvae's movement influenced odor emissions by enhancing oxygen distribution, potentially inhibiting odor-producing anaerobic bacteria. Given the increased odor in the presence of larvae, this seems unlikely. Another possibility is that the larvae's movement caused already-formed odorants to evaporate more readily, explaining the higher odor levels observed. However, we observed that the sewage sludge medium with larvae appeared more watery than the sewage sludge without larvae. If an increased evaporation was the cause of higher odor emission, the medium would appear drier rather than more liquid, since evaporation of water would also have been increased. Interestingly, carbon dioxide, resynthesised organic compounds (such as VOCs), and water are released during the decomposition of organic matter (Yadav et al. 2021). A plausible explanation for both the increase in VOC concentrations and the possible increase in water content in sewage sludge containers with larvae could therefore be that the larvae have enhanced the decomposition of the medium. Research has shown that earthworms can effectively aid in the composting of dewatered sludge from wastewater treatment plants, as shown in numerous examples from New Zealand (Quintern and Morley 2017). It is reasonable to suggest that drain fly larvae may possess similar decomposing abilities, considering their comparable diets.

Unlike sewage sludge, nettle sludge's total odor concentration and resulting SOAV were not significantly affected by drain fly larvae, although the larvae prevented fungal growth on the medium's surface. This difference may be due to varying medium characteristics such as nutrient concentration and composition. Schreven et al. (2022) noted that black soldier fly larvae's impact on microbial communities depended on the substrate type, suggesting differences in moisture, nutrients, microorganisms, and plant metabolites could explain this. The inhibitory effect of larvae on fungal growth in nettle sludge also suggests medium-dependent effects. Preventing fungal growth in some media might increase odor emissions by removing the fungal barrier, while in others, it might reduce odorants produced by fungi. Therefore, the relationship between larvae, fungal growth, and odor emissions needs more study. Additionally, larval density may explain the differences. Nettle sludge had fewer larvae per volume compared to sewage sludge. Schreven et al. (2022) found that higher larval densities affected bacterial group abundance, suggesting that varying larval densities between the media influenced microbial composition and odor production. Possibly, these medium-dependent variations also caused the observed decrease in the concentrations of trimethylamine, pentanal, and dimethylamine in response to the larval activity in nettle sludge.

In the hedonic ratings, no difference was detected between control and treatment containers, despite the chemical analysis showing significantly higher odorant concentrations in sewage sludge with larvae. This discrepancy could have arisen because the chemical differences, although measurable, were too subtle for human detection. Odorous compound concentrations were more than four times higher in sewage treatment containers than in sewage control containers, but still they were only 1036 ppb on average. The SOAV, which accounts for human odor thresholds, was significantly higher in sewage containers with larvae, but this simplified measure may not fully capture the complexity of odor perception, since this is influenced by complex interactions between different odorants and differences in sensitivities between people, factors the SOAV does not take into account (Wu et al. 2017). Possibly treatment containers also produced more good-smelling compounds, which could impact the bad odors in the hedonic ratings, while they were not accounted for in the SOAV. Additionally, high variability between both containers and participants' cultural, social, and biological backgrounds likely contributed to the experiment's insensitivity (Bliss et al. 1996; Griep et al. 1996; Murnane et al. 2013; Sorokowska et al. 2013; Sorokowski et al. 2019). These factors, along with methodological limitations such as the small sample size and high variation in container conditions, suggest that the chemical differences observed were not sufficient to produce a detectable difference in the hedonic ratings.

The societal relevance of our findings extends to both public health and urban living standards. The discovery that drain flies may increase sewage sludge odor emissions has implications for sewage sludge treatment methods, marking the first indication that these insects could play a significant role in sludge odor dynamics. However, due to the many environmental factors involved, further research is necessary to better assess their influence on the treatment process. Despite their potential to worsen odors, the larvae also offer benefits. They can effectively unclog biofilters and drains, thus helping reduce odors by improving airflow and drainage (Won et al. 2004; Zhang et al. 2023). Additionally, by potentially accelerating the decomposition of sewage sludge, they may mitigate long-term odor production during sewage sludge composting, though this could lead to a temporary increase in odor. Moreover, this potential might also yield economic benefits for WWTPs. Further research is needed to fully understand the balance between these benefits and drawbacks, especially in static sludge environments where odor issues could worsen.

To further understand the effects of *C. albipunctata* on malodor, we suggest that future researchers use this study as a starting point, but improve the research design by increasing the sample size and ensuring that the treatment of all media types is the same. To investigate the mechanisms behind changes in malodor production, a metagenomic analysis of the microbial community present in the samples is recommended as well. Finally, we also recommend the use of an olfactometry panel, specifically trained to characterise and rate odors, to more accurately assess the effect of the larvae on odors as experienced by humans.

5 | Conclusion

This study examined the impact of the drain fly *C. albipunctata* on odor production in rotting organic matter. The activity of larvae resulted in alterations of the physical structure of the sewage sludge medium, leading to increased total odor emissions. This contrasts with studies on black soldier flies, which observed decreased emissions. The ability of drain fly larvae to graze on bacteria and thereby potentially influence the microbial community structure may be a key factor in explaining this disparity. However, the exact cause of increased odor emissions remains unclear, since larvae could both induce greater odorant production by bacteria and accelerate decomposition, leading to more concentrated emissions over a shorter period. While the hedonic scaling experiment with human participants and chemical analysis on rotting nettle sludge were inconclusive, the results suggest that drain flies can alter the odor dynamics of rotting organic matter. The results also indicate that this effect is dependent on many environmental factors, which requires further research. Although no firm conclusions can be drawn, these findings are of considerable importance for further research into the effect of drain flies on odor emissions from sewage sludge treatment and other environments containing decaying organic matter. Future research with a larger sample size, metagenomic analysis, and olfactometry is necessary to confirm these findings and clarify the biological mechanisms involved.

Author Contributions

Niels J. E. van Hof: conceptualization, methodology, data curation, visualization, investigation, formal analysis, writing – original draft, writing – review and editing, project administration. **Luuk Torn:** conceptualization, methodology, data curation, visualization, investigation, formal analysis, writing – original draft, writing – review and editing, project administration. **Jan J. Wieringa:** conceptualization, methodology, investigation, supervision, writing – review and editing, resources, validation. **Aglaia M. Bouma:** conceptualization, methodology, funding acquisition, investigation, supervision, writing – review and editing, resources, validation.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The raw data for this paper has been made available at https://doi.org/10.5281/zenodo.16634413.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Supplementary Table 1** Summary statistics of the two-way ANOVA post hoc using *emmeans* package on the compound concentrations data. Only compounds above the odor threshold concentration are analysed. The mean concentration per treatment group, lower and upper 95% confidence limit (CL), and the *p*-value is shown. *p*-values at or below the chosen α of 0.05 are in bzold. Table A shows the statistics for the sewage sludge medium, and B for nettle sludge. Supplementary Table 2. Ordinal mixed model odds ratio's (OR), 95% confidence intervals (95% CI) and *p*-values of different comparison variables from the hedonic scaling experiment data. The OR represents the relative likelihood of receiving a rating at least one category higher (higher pleasantness) for treatment containers compared to control containers, males compared to females, or younger people compared to older people. An odds ratio of 1 indicates no difference between treatment and control ratings. An odds ratio greater than 1 indicates a higher likelihood, while an odds ratio less than 1 indicates a lower likelihood. *p*-values at or below the chosen α of 0.05 are in bold. Supplementary Figure 1: Pictures of the nettle sludge (A) and sewage sludge (B) media that were used in the experiments. The picture of the nettle sludge was taken before the rotting and inoculation with drain fly, while the picture of the sewage sludge was taken after drain fly inoculation and just before the hedonic scaling experiment.