COVID-19 face masks attracted \textit{Cellulomonas} and \textit{Acinetobacter} bacteria and provided breeding haven for red cotton bug (\textit{Dysdercus suturellus}) and house cricket (\textit{Acheta domesticus})

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Abstract
This study investigated the possibility of COVID-19 medical face masks to affect bacterial and macrofaunal communities in open soil environment. An estimated 1.24 trillion of face masks have been used and discarded as a result of the COVID-19 pandemic, with a significant part of this ending up in the soil environment, where they degrade gradually over time. Because bacteria and macrofauna are sensitive indicators of changes in soil ecosystem, we investigated possible impacts of face masks on population, distribution, and diversity of these soil species. Effect on soil bacterial community was studied by both culture-based and advanced molecular (metagenomics) approach, while impact on macrofauna was investigated by examining monoliths around heap of masks for soil insects. In both cases, control soil experiments without face masks were also set up and monitored over a period of 48 weeks. The study found that the presence of face masks led to a more diverse bacterial community, although no influence on overall bacterial population was evidenced. More importantly, bacteria belonging to the genera \textit{Cellulomonas} and \textit{Acinetobacter} were found prominently around face masks and are believed to be involved in biodegradation of the masks. The bacterial community around the masks was dominated by Proteobacteria (29.7–38.7%), but the diversity of species increased gradually with time. Tiny black ants (\textit{Monomorium invidium}) were attracted to the face masks to take advantage of water retained by the masks during the period of little rainfall. The heaps of face masks also provided shelter and breeding “haven” for soil insects, notably the red cotton bug (\textit{Dysdercus suturellus}) and house cricket (\textit{Acheta domesticus}), thereby impacting positively on the population of insect species in the environment. This study provides insights into the actual impacts of face masks on soil organisms under normal outdoor environmental conditions.

Keywords COVID-19 · Medical face masks · Soil environment · Impacts · Microorganisms · Macrofauna

Introduction
Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), belonging to the family \textit{Coronaviridae}. Members of this family are known to cause common colds in humans. The World Health Organization (WHO) detected COVID-19 in China in December 2019 and declared it a global pandemic and public health emergency of international concern (Murray et al. 2020). A meta-analysis study by Coccia (2022) showed that COVID-19 virus most probably originated from laboratory-developed hazardous viral agents and consequent laboratory accidents involving such viruses. The SARS-CoV-2 spread rapidly through person-to-person transmission, and via other means such as international trade and travels, and presence in excreta releases,
which then reach watercourses and other environmental compartments, including soil, air, surfaces, and groundwater (Nunez-Delgado et al. 2021; Bontempi and Coccia 2021). Data from WHO COVID-19 dashboard revealed that the number of infected persons globally stands at 544 million, with over 6 million deaths as of June 30, 2022.

Since the detection of COVID-19, various measures have been used in different countries across the world to prevent the transmission of the virus. These include lockdown, social and physical distancing, travel restrictions, isolation, good hand hygiene (handwashing, use of hand sanitizers), avoiding public or crowded spaces, and the use of personal protective equipment (PPE), e.g., disposable medical face masks, face shield, and hand gloves (Wilder-Smith and Freedman 2020; Lin et al. 2020). Medical face masks were primarily made for the protection of healthcare workers (HCWs), who are well trained on the usage and disposal, to prevent occupational hazards. However, COVID-19 pandemic necessitated many authorities to recommend the use of face masks to stem the transmission of the virus, especially from person-to-person. This is because the masks are capable of reducing the number of times a person touches the face or mouth or nose with unwashed hands, thereby reducing the chances of infection (Xu and Li 2020). However, the wearing of medical face masks by the general public, who are not well trained on the usage and disposal, has created a challenge and added to the burden of solid waste management in the environment (Elachola et al. 2020).

The mass usage of the face masks brought about a phenomenal rise in the volume of face masks manufactured worldwide (Aragaw 2020). WHO had estimated that approximately 89 million of medical face masks were needed to respond to COVID-19 each month (WHO 2020). The high demand resulted in an unprecedented rise in the global production of face masks, with the major mask producers scaling up their outputs. For example, China produced 200 million face masks per day in June 2020, while orders of face masks in Japan rose sharply to over 600 million per month in April 2020 (METI 2020). Many countries have now removed lockdown measures, due to the adverse effects on the economy and mental health. Other measures such as social and physical distancing, travel restrictions, isolation, and avoiding public or crowded spaces have also been relaxed in most countries. The only measures still in active use especially in highly affected countries are the use of face masks and maintenance of good hand hygiene. Worldwide, approximately 52 billion of face masks were reportedly used and discarded in 2020 alone (Turkmen 2022). A later study by Benson et al. (2021) indicates that the total face masks used during the COVID-19 pandemic could be put at an estimated 1.24 trillion. Without doubt, the terrestrial (soil) environment has been the first receptacle for the indiscriminately disposed medical face masks. Furthermore, many face masks have been permanently abandoned on soil areas designated as dumpsites or landfills.

Soil is the foundation of all terrestrial ecosystems, containing a vast diversity of bacteria which influence most ecosystem services (Dominati et al. 2010; Aislabie and Deslisle 2013). For instance, phosphorus solubilizing functions are performed by Proteobacteria (especially Pseudomonas), Actinobacteria, Firmicutes, and Bacteroidetes (Mander et al. 2012). Bacteroidetes also help to degrade recalcitrant carbon compounds and complex organic molecules such as starch and proteins, while nitrogen fixation is carried out by Azotobacter, Burkholderia, Clostridium, and some methanogens (Aislabie and Deslisle 2013). In addition to providing homes for the various bacterial species, soil also supports the existence of a large number of macrofauna (invertebrate and insect) species, which perform functions ranging from aeration and burrowing for plant growth, to pollination of flowering plants and control of fungi abundance in the soil environment (Behiep et al. 2012).

Given the enormous amounts of face masks that have ended up in the environment due to the COVID-19 pandemic, there is the likelihood that the presence of such unprecedented levels of polymer material upsets the soil ecosystem, especially in developing nations where hospital and public wastes are poorly managed, and are merely dumped in soil environments. Previous studies on the possible environmental effects of the face masks did not address these issues. A study by Turkmen (2022) examined the potential of the masks to impact the terrestrial environment and estimated the amount of dichlorobenzene and heavy metals (chromium, mercury, and vanadium) that could potentially be released into the soil. Other studies by Ma et al. (2021), Chen et al. (2021), and Meier et al. (2022) demonstrated the release of nanoplastics and microplastics from face masks, while Ma et al. (2021) further reported that the plastic particles adsorbed on diatom surfaces and were ingested by marine organisms. The current study investigated how presence of face masks may affect soil bacteria and macrofauna species in terms of diversity and population, which may consequently affect soil ecosystem functioning and services. The study was also aimed at understanding how soil bacterial and macrofaunal species may respond and interact with face masks dumped in the soil environment.

Materials and methods

Materials

Disposable medical face masks were obtained from a public pharmaceutical store (Matador) in Akure city, Nigeria. The 3-ply face masks (blue and white in color) consist of three layers. The masks were the commonly used type, having
samples were put into sterile transparent cellophane bags (F), with the aid of a soil auger at depths 0–10 cm. The soil cores from radius (20, 50, and 100 cm) from the face masks (approximately 100 g) were obtained by mixing four of 4 weeks over a period of 48 weeks, composite soil samples were taken around the center of plots (n = 3) which lacked face masks and were treated exactly as for the samples from plots containing masks.

**Determination of culturable bacteria population and characterization**

Approximately 10 g of each soil sample was weighed into a sterile 250 ml Schott bottle containing 90 ml phosphate buffer saline (PBS) solution and mixed thoroughly at 260 r/min for 15 min. Thereafter, tenfold serial dilutions were carried out and aliquots from $10^{-5}$, $10^{-6}$, and $10^{-7}$ were plated on sterile tryptic soy agar (TSA), prepared according to manufacturer’s specifications in triplicate. The plates were incubated at 25 ± 2 °C for 24 to 72 h and colonies were counted, calculated, and recorded as colony-forming units per gram of soil (CFU/g). Isolates were sub-cultured repeatedly to obtain pure isolates and then stored in agar slants at 4 °C. The bacterial species that were isolated from the soil samples were characterized further using morphological, physiological, and biochemical properties that included Gram reaction, catalase test, motility test, oxygen relation, sulfide test, indole production test, and carbohydrates (glucose, lactose, fructose, dextrose, maltose, tryptose, and sucrose) utilization test. Thereafter, the isolates were identified using Bergey’s Manual of Determinative Bacteriology (Holt et al. 1994).

**Bacterial genomic DNA extraction from soil samples**

For metagenomic analysis, soil samples were collected quarterly (i.e., January, April, July, and October, 2021) from the closest radius (20 cm) to the face masks or marked centers in control experiments, all of which were set up in triplicate. Genomic bacterial DNA was extracted from the soil sample using cetyl trimethyl ammonium bromide (CTAB) and sodium dodecyl sulfate (SDS) buffers. Briefly, 5 g of the soil sample was transferred into a well labeled 50 mL sterile falcon tubes. Thereafter, 30 mL of CTAB extraction buffer consisting of 200 mM Tris, pH 7.5; 50 mM EDTA, pH 8.0; 2 M NaCl; 2% CTAB; 1% beta-mercaptoethanol was added to each tube. SDS (20%, 3 mL) was also added and vortex thoroughly. The mixture was incubated in the water bath at 65 °C for 30 min. During the incubation, the sample tubes were inverted at intervals to ensure homogenization. After incubation, 20 mL chloroform-isooamyl alcohol (24:1) was added, mixed thoroughly, and centrifuged at 1000 rpm for 10 min using Allegra 25R centrifuge. The supernatant was transferred into sterile empty labeled falcon tubes and 30 mL ice-cold (4 °C) isopropanol was added, and the mixture was incubated at –80 °C for 15 min. Again, the sample tube was centrifuged at 10,000 rpm for 10 min. The supernatant was

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nose strips consisting of a thin metal piece, coated on the outside with a non-woven fabric material. This medical face mask type was used in all the experiments.

Chemical reagents including isopropanol, ethanol, betamercaptoethanol, sodium hexametaphosphate (Calgon), disodium ethylenediamine tetra-acetic acid (EDTA), cetyl trimethyl ammonium bromide (CTAB), sodium dodecyl sulphate (SDS), and phosphate buffer saline (PBS) ingredients were of analytical grade and obtained from Sigma-Aldrich (UK). Tryptic soy agar (TSA) and carbohydrate reagents (glucose, lactose, fructose, dextrose, maltose, tryptose, and sucrose) were obtained from Loba Chemie, India. Chloroform-isooamyl alcohol (24:1) for DNA extraction was obtained from Sigma-Aldrich (UK).

**Description of the study area**

The research was conducted on an allocated garden within the Research Farm at the Federal University of Technology Akure (FUTA), Nigeria (approximately 28–32 m above sea level, and between longitude 5–20° E and latitude 7–9° N). The area is characterized by the tropical monsoon climate, with high temperature and high humidity for the most part of the year. The annual average temperature is about 32 °C during the day and approximately 23 °C at night. In addition, the annual rainfall ranges from 1300 to 1650 mm, with two distinct rainfall peaks within a year. The rainy season normally begins in March and lasts till the end of July with a peak in June. This first rainy period is followed by a short dry break in August. The rain then returns early September and lasts till mid-October with a peak period at the end of September. This second rainy period is followed by a long dry season from late October until early March the following year. Throughout the period of this study (February–December, 2021), hourly data on temperature, rainfall, relative humidity, wind speed, solar power (irradiance), and atmospheric pressure were recorded at a meteorological station located on the research farm.

**Experimental design and collection of soil samples**

A portion of the experimental garden was divided into several small plots of 5 m by 5 m size. To investigate the impacts of the face masks on soil bacteria, 30 face masks were stacked on one another directly on the ground to form a small heap at the center of a plot. This was set up in triplicate. The heaps were exposed to the ambient environmental factors, causing the masks to degrade gradually. At intervals of 4 weeks over a period of 48 weeks, composite soil samples (approximately 100 g) were obtained by mixing four cores from radius (20, 50, and 100 cm) from the face masks (F), with the aid of a soil auger at depths 0–10 cm. The soil samples were put into sterile transparent cellophane bags

and labelled appropriately. The samples were transported to the laboratory for analysis within 1 h. Control soil samples (C) were also taken around the center of plots (n = 3) which lacked face masks and were treated exactly as for the samples from plots containing masks.
discarded and the pellet was washed in 30 mL 70% ethanol, centrifuged at 10,000 rpm for 10 min, and the supernatant was discarded. The pellet was air dried at ambient temperature and resuspended using sterile distilled water. The quality of the DNA was examined using Nanodrop (ND-1000 spectrometer) and gel electrophoresis method. In order to prevent DNA extraction bias, three replicate DNA isolation of each soil sample were pooled to provide the template for amplification and sequencing.

16S rRNA gene amplification and sequencing

The 16S rRNA genes were amplified with primers targeting the V4 region at fragment length approximately 390 bp with sequence (5′–3′) 515F (Parada) forward (GCMGCGCCGCTTAA) and 806R (Apprill) reverse (GGA CTACNVGGGTWTCTAA) (Apprill et al. 2015; Parada et al. 2016). The PCR reaction mixture contained a final volume of 25 µL: 13 µL PCR-grade water (Sigma, cat. no. W3500), 10 µL platinum hot start PCR master mix (2x) (ThermoFisher, cat. no. 13000014), 0.5 µL forward primer (10 µM), 0.5 µL reverse primer (10 µM), and 1 µL template DNA. A negative control (i.e., PCR mixture without template DNA) was included in all PCR amplification. The thermocycling conditions included: one cycle of initial denaturation (enzyme activation step) at 94 °C for 3 min, then 35 cycles run at 94 °C for 45 s, 50 °C for 60 s and 72 °C for 90 s, followed by a final extension step at 72 °C for 10 min. The PCR mixture was then held at 4 °C. The 16S V4 bacterial community metagenomic sequencing was carried out on the Illumina platform, following the Earth Microbiome protocol. The Illumina Basespace 16S metagenomics software was then used to generate taxa abundance tables.

Monitoring of soil macrofauna species

To investigate the impact of the face masks on soil macrofauna species, heap of masks was placed at the centre of 5 m × 5 m plots as described under experimental design. Three transects, each passing through the center, were marked on the plot. The transects (T1, T2, and T3) drawn from different sides of the plot were used to collect macrofauna after 3, 6, and 9 months of placing the masks on the plot, respectively. The macrofauna were collected from monoliths dug along the transects at 20, 50, and 100 cm distances from the masks, and on both sides of the masks. Each monolith was divided into two layers (surface—10 cm depth and 10–20 cm depth) for the collection of the macrofauna species. Soil was excavated from each layer of the monolith and examined in situ on a white board (1.5 m × 1.5 m size). The macrofauna found were counted, preserved in 70% ethanol and transported to the biology laboratory for taxonomic identification. The experiment was set up in triplicate. Macrofauna were also collected from control experiments (without face masks in the centre of plots) and were treated as for the plots that had face masks.

Shannon indices and statistical analysis

Shannon diversity index (H), a measure of the diversity of species in a community, was calculated for the macrofauna species, using the relationship \( H = -\sum p_i \ln(p_i) \), where \( \Sigma \) is the Greek symbol for summation; \( \ln \) is natural logarithm; \( p_i \) is the proportion of an entire community made up of species \( i \). Subsequently, the Shannon equitability index, \( E_H = \frac{H}{\ln(S)} \) was determined, with \( S \) being the total number of unique species observed.

Non-parametric Mann–Whitney U test was performed to compare the population of ants in plots with face masks to that of the plots without face masks. Statistical Package for Social Sciences (SPSS, version 24) was used for the analyses; \( P \) values \( \leq 0.05 \) was considered as statistically significant.

Determination of soil physicochemical parameters

Soil samples were taken at the beginning of the study (prior to setting up of masks on the sites) and at 3-month interval (in April, July, and October). Composite samples (\( n = 5 \)) were obtained at depths up to 20 cm from both garden sections used for face masks experiments and the adjacent one used for the controls. Each sample was air-dried in the laboratory for 5 days and a subsample (180–200 g) was obtained by quartering technique. The subsampled amount was used for the physicochemical analyses. The soil pH was determined after homogenization with distilled-deionized water as described previously (Idowu et al. 2020). Total organic matter content was determined by loss on ignition method described by Mudroch et al. (1997). Particle size distribution (i.e., % sand, % silt, and % clay) was determined by dispersion and sedimentation method developed by Kettler et al. (2001). Total nitrogen was determined by measurement of near infrared (NIR) spectra according to the method of Sun et al. (2006).

Results and discussion

Effects of medical face masks on soil bacteria population

Effects of face masks on the soil bacterial community was investigated by both conventional microbiological techniques, involving culturing of the soil bacteria on agar media, and by metagenomics approach, whereby the entire culturable and non-culturable bacteria were identified.
through the sequencing of genetic (DNA) materials recovered directly from the soil samples. As a measure of the bacterial population in the soil around the medical face masks and around the marked centers in control plots (which lacked face masks), the number of colony forming units per gram of soil (CFU/g) was determined. Figure 1 shows the results (in logarithmic form) for soil samples taken at 20, 50, and 100 cm radii from the masks/center, at 4-week interval over a period of 48 weeks. The figure includes result for the period designated as “0,” corresponding to the initial samples taken prior to placing face masks at the center of the plots (for the experimental plots that required masks). Despite absence of face masks in all plots at this initial stage and despite the identical nature of the soils in both experiments with and without face mask, as revealed by their physicochemical properties—pH, total nitrogen, organic matter content, and soil particle size distribution (Supplementary Information, Table S1), the mean number of CFU at the initial stage differs in the two experiment types, at all distances from the centre. It is noteworthy also that the CFU values for this initial stage were not consistently higher in either of the experiments—at 50 cm radius from the center, mean CFU value was higher in plots reserved for face mask experiments ($5.1 \times 10^6$ CFU/g) than in plots that were to receive no face masks ($3.2 \times 10^6$ CFU/g), whereas the reverse was the case for CFU values at 20 cm and 100 cm radii.

Following the introduction of face masks to the plots that were to receive them, the trend of CFU values from week 4 to week 48 was revealed for the soil samples from both experiment types, and for the various distances from the face masks or centre. At 20 cm from the face masks/center, a continuous exchange of “high” and “low” was seen between mean CFU values in plots with face masks (F20) and the control plots which lacked face masks at the center (C20) (Fig. 1a). This was observed between weeks 4 and 32. It was followed by higher mean CFU values in plots that lacked face masks, from weeks 36 to 44, which then went lower than the mean CFU value of plots with face masks at the terminal week 48. Overall, the mean CFU values ranged from $1.2 \times 10^5$ to $2.9 \times 10^7$ CFU/g at F20, while it ranged from $1.3 \times 10^6$ to $6.9 \times 10^7$ CFU/g at C20 over the entire monitoring period. For soil samples taken at 50 cm radius, mean CFU values for plots with face masks (F50) were initially higher than those without masks (C50), over the first 12 weeks, and followed by exchanges between “high” and “low” for the rest of the study period (Fig. 1b). It is very unlikely that the higher CFU values observed in F50 at the initial weeks were due to the presence of the face masks in the plots. This is because similar consistent higher CFU values were not seen in soil samples obtained from 20 cm radius (F20), which were much closer to the face masks than F50. Much larger differences would have been expected between CFU values of F20 and C20, with F20 being higher, assuming the presence of the masks was responsible for the higher mean CFU values observed early in F50 samples. For the same reason, the higher mean CFU values observed later for F50 (between weeks 24 and 32) may not be attributed
to the presence of face masks. As observed for the 20 cm radius, a consistent exchange of “high” and “low” was seen between the mean CFU values of soil samples F100 and C100, obtained at 100 cm radius of plots with face masks and those without masks, respectively (Fig. 1c). Overall, the results indicate that the presence of face masks had no effect on culturable soil bacteria population. Rather, the highly dynamic nature of culturable bacteria, plus the tendency to be easily influenced by many environmental factors (including wind and water that carry materials), may be responsible for the various trends in CFU values observed in soils near face masks and those without face masks, over the entire period of study.

Bioinformatics analysis performed on the 16S metagenomics data of the soil sample obtained prior to placing face masks on the garden plots and those obtained around the face masks at quarterly intervals gave insights into the number of bacterial genomes associated with the different experimental setups. Kingdom level classification resulted in 94.48–97.87% of reads in the metagenome data-sets being identified as bacteria, 0.57–2.59% identified as archaea, while 1.56–2.93% was unclassified. In terms of the number of sequenced bacterial genomes that passed 100% quality filtering, a total of 17,968 genomes was obtained from initial soil sample taken prior to setting up of the face masks. Total number of genomes obtained from soil samples taken around the face masks was 61,348 after 3 months, 80,932 after 6 months, and 188,227 after 9 months. For the counterpart soil samples, taken from marked centers in the control experiments (lacking face masks), total number of genomes obtained was 67,854 after 3 months, 84,105.43 after 6 months, and 152,883 after 9 months. While it appears that the soil from plots that lacked the face masks had higher number of sequenced bacterial genomes after the period of 3 months and 6 months, this trend was overwhelmingly contradicted by the 9-month results, in which the number of genomes obtained for soil samples around face masks was far higher than that obtained from the control plots. Thus, it may not be concluded on the basis of the number of sequenceable bacterial genomes that the presence of face masks caused an increase or a decrease in the soil bacterial population. Nonetheless, the period of 9 months, at which much higher number of genomes (188,227) were found around face masks, correspond to a time when the face masks had undergone significant degradation to release their component materials into the soil. The face masks materials (especially polypropylene and cellulose) are carbon-containing, and may have therefore raised the carbon content of the soil surrounding the face masks at this period. Availability of this carbon/food source may be responsible for the increase in bacterial activity (indicated by increased bacterial genomic DNA) around the face masks after 9 months. Indeed, it is well established in nutrient cycling that bacteria are responsible for utilizing soil carbon, which they consequently convert to the atmospheric carbon (IV) oxide (de Vries and Shade 2013; Gougoulias et al. 2014).

Effects of medical face masks on soil bacterial diversity

Morphological, biochemical, and carbohydrate utilization tests were employed to characterize and identify bacterial isolates from soil samples around face masks and around centers of plots (controls) which lacked face masks. Tables 1 and 2 show the bacterial species isolated from soil with face masks and the controls, respectively, at intervals of 4 weeks. The “0” week isolates are the species present in the soil prior the introduction of face masks to the experimental plots that required them. Despite the absence of face masks at this initial stage, only Clostridium butyricum and species from the Gram positive and catalase positive Micrococcus genus were common to the plots designated for the two experiment types. M. luteus and M. nishinomiyaiensis were isolated from the plots reserved for masked experiments, whereas M. halobius and M. varians were isolated from the control plots. Other bacterial species found in the plots reserved for face masks at this initial stage were not present in the control plots, and vice-versa; again demonstrating the dynamic nature of soil bacterial community, even within the same garden area. This notwithstanding, the initial results provide basis to monitor changes in isolable bacterial species around the face masks and around designated center of plots lacking the face masks.

From week 4 (after placement of the masks) and following, various bacterial types and species were isolated from the two experiment types (Tables 1 and 2). With the exception of only four species, all the species isolated from soil around the face masks were also isolated from the control plots, either at the same or different periods during the 48-week monitoring exercise. The four species found only around the face masks were Actinobacter johnsonii (first isolated at week 4), Cellulomonas flavigena (first isolated at week 12), as well as Cellulomonas fimii and Desulfotomaculum antarcticum (both first isolated at week 16). These bacterial species are indicated with superscript numerals in Table 1 and were absent completely in the control plots (Table 2). Furthermore, the species were seen very frequently in subsequent isolation experiments following their first appearance. For instance, following the first isolation of C. fimii at week 16, the species was found consistently at the 4-week interval up to week 44. Similar results were obtained for the other three species, with the maximum non-occurrence time for any of the species being 2, after the time of its first isolation.

Literature search into the nature of these species provide insight into their possible functional roles around the face.
Bacterial species isolated from soil samples around medical face masks at intervals of 4 weeks

| Time (weeks) | Arthrobacter globiformis | Clostridium butyricum | Bacillus subtilis | Micrococcus luteus | Micrococcus nishinomiyaensis | Terrabacter tamescens | Rhodococcus rhodnii | Pimelobacter simplex | Paracoccus halodenitrificans |
|--------------|--------------------------|-----------------------|------------------|-------------------|-----------------------------|-----------------------|-------------------|---------------------|--------------------------|
| 0            |                          |                       |                  |                   |                             |                       |                   |                     |                          |
| 4            | Pimelobacter                | Acinetobacter 1 johnsonii | Terrabacter tamescens | Micrococcus halobius | Micrococcus luteus | Rhodococcus rhodnii | Pimelobacter simplex |                   |                          |
| 8            | Micrococcus halobius        | Acinetobacter calcoaceticus | Acinetobacter 1 johnsonii | Marinococcus halophilus | Cellulomonas flavigena | Cellulomonas butyricus |                   | Cellulomonas flavigena |                          |
| 12           | Arthrobacter globiformis    | Acinetobacter 1 johnsonii | Phenylaceticobacter immobile | Aeromicrobium erythreum | Cellulomonas 2 flavigena | Aeromicrobium erythreum | Acinetobacter 1 johnsonii | Cellulomonas flavigena |                          |
| 16           | Cellulomonas 2 flavigena    | Acinetobacter calcoaceticus | Cellulomonas 2 flavigena | Desulfotomaculum 4 antarcticum | Cellulomonas 2 flavigena | Aeromicrobium erythreum | Acinetobacter 1 johnsonii | Cellulomonas flavigena |                          |
| 20           | Thermoan aerobium brockii  | Desulfotomaculum 4 antarcticum | Cellulomonas 3 flimi | Cellulomonas 2 flavigena | Cellulomonas 2 flavigena | Deinococcus radiodurans | Acinetobacter 1 johnsonii | Cellulomonas flavigena |                          |
| 24           | Vagococcus flavialis       | Cellulomonas 3 flimi | Aeromicrobium erythreum | Terrabacter tamescens | Cellulomonas 2 flavigena | Cellulomonas 3 flimi | Acinetobacter 1 johnsonii | Cellulomonas flavigena |                          |
| 28           | Aeromicrobium erythreum    | Cellulomonas 2 flavigena | Desulfotomaculum 4 antarcticum | Acinetobacter 1 johnsonii | Cellulomonas 2 flavigena | Clostridium butyricum | Acinetobacter 1 johnsonii | Cellulomonas flavigena |                          |
| 32           | Acinetobacter calcoaceticus | Desulfotomaculum 4 antarcticum | Cellulomonas 3 flimi | Clostridium butyricum | Cellulomonas 2 flavigena | Acinetobacter 1 johnsonii | Cellulomonas flavigena | Terrabacter tamescens | Derxia gummosa |
| 36           | Trichococcus flocculiformis | Desulfotomaculum 4 antarcticum | Cellulomonas 2 flavigena | Cellulomonas 3 flimi | Pimelobacter simplex | Acinetobacter 1 johnsonii | Cellulomonas flavigena | Terrabacter tamescens |                          |
| 40           | Acinetobacter 1 johnsonii | Desulfotomaculum 4 antarcticum | Arthrobacter globiformis | Cellulomonas 2 flavigena | Cellulomonas 3 flimi | Bacillus subtilis | Acinetobacter 1 johnsonii | Cellulomonas flavigena |                          |
| 44           | Thermoan aerobium brockii  | Cellulomonas 3 flimi | Micrococcus halobius | Cellulomonas 2 flavigena | Acinetobacter calcoaceticus | Clostridium butyricum | Acinetobacter 1 johnsonii | Aeromicrobium erythreum |                          |
| 48           | Thermoan aerobium brockii  | Clostridium butyricum | Pediococcus damnosus | Desulfotomaculum 4 antarcticum | Cellulomonas 2 flavigena | Clostridium butyricum | Acinetobacter 1 johnsonii |                          |                          |

Superscripts 1, 2, 3, 4 indicate species found in soil samples around face masks, but not in the control soil.

A. johnsonii, like other species in the genus Acinetobacter, is innately saprophytic, and normally lives on dead or decaying food and organic matters (Kämpfer 2014; Montaña et al. 2016). It is therefore likely that this species has taken the advantage of the face masks decomposing in the environment to derive food and support, while at the same time accelerating the decay of the face mask’s materials. More importantly, the Cellulomonas species (C. flavigena and C. fimi), also isolated from soil around the face masks, belong to a genus of Gram-positive rod-shaped bacteria, known particularly for their ability to degrade cellulose, through the help of a series of endoglucanase and exoglucanase enzymes (Chaudhary et al. 1997; Pourcher et al. 2001; Lakhundi et al. 2015). The presence of these Cellulomonas species near the face masks is highly consistent with the nature and composition of the medical face masks, which are made from cellulose, joined with polypropylene and polyester fibers (Leonas and Jones 2003; Aragaw 2020). It is therefore apparent that the Cellulomonas bacteria have appeared around the masks to degrade the cellulose component of the face mask’s material. The fourth bacteria found consistently in soil around the face masks are of the genus Desulfotomaculum, which are...
Gram-positive obligately anaerobic sulfate-reducing bacteria (Vandieken et al. 2006; Aüllo et al. 2013). While it is very unlikely that this species is directly involved in degrading medical face masks (since face masks do not contain sulphate or any sulfur compound, upon which the bacteria may act), the stacking of masks on top of one another to form heaps may have created an anaerobic condition under the masks, by limiting oxygen supply, thereby providing suitable atmosphere for this obligate anaerobic organism to thrive. The Desulfotomaculum bacteria may then act on sulphate that is normally released into the soil from oxidation of soil organic matter.

In agreement with the result from culturable bacteria, comparative examination of taxonomic data from metagenomics results of the soils around face masks and that of the control, led to the identification of species belonging to the Cellulomonas, Acinetobacter, and Desulfotomaculum genera, present only in the metagenomics data of soil samples from around the face masks (Table 3). As expected, due to the versatility and sensitivity of the metagenomics method, the number of bacterial species detected were six, nine, and eight, respectively for Cellulomonas, Acinetobacter, and Desulfotomaculum genera, as against the maximum of two species found for any of the genera in the cultivable bacteria data. Of the three genera, the Acinetobacter species were most prominent, being found at all the quarterly samples, with an indication of increasing abundance around the face masks. Cumulative percentage abundance of the Acinetobacter rose from 0.026% at 3 months to 1.14% at 9 months. The presence of the Acinetobacter species around the face masks may have implications for the management of face masks dumped on soil environment over a long period, with respect to the health and safety of humans. In particular, A. baumannii (found in the soil samples) is an etiological agent of diseases ranging from diarrhea and wound infections, to more severe illnesses such as pneumonia, meningitis, bacteremia, and urinary tract infections (Fournier et al. 2006; Eliopoulos et al. 2008; Antunes et al. 2014). Many strains of the bacteria are also multi-drug resistant. Indeed, infections caused by carbapenem-resistant A. baumannii was considered by the WHO as the number one critical priority pathogen for which new drugs were urgently needed (Shales and Bradford, 2018; Morris et al. 2019). Evidence is also emerging that some Acinetobacter species, previously susceptible to many antibiotics, such as the A. Johnsonii (also found around the masks), are now acquiring drug-resistance genes (Feng et al. 2016; Montañà et al. 2017).

### Table 2 Bacterial species isolated from control soil samples (without face masks) at intervals of 4 weeks

| Time (weeks) | Bacterial species |
|--------------|------------------|
| 0            | Clostridium butyricum | Desulfotomaculum nigrificans | Micrococcus halobius | Micrococcus varians | Acinetobacter calcoaceticus |
| 4            | Bacillus subtilis | Derxia gummosa | Micrococcus halobius | Rhodococcus rhodnii | Pimelobacter simplex |
| 8            | Marinococcus halophilus | Bacillus subtilis | Marinococcus halophilus | Alcaligenes xylosoxidans | Terrabacter tamescens |
| 12           | Pimelobacter simplex | Bacillus subtilis | Acinetobacter calcoaceticus | Aeromicrobium erythreum | Clostridium butyricum |
| 16           | Alcaligenes xylosoxidans | Clostridium butyricum | Aerobacterium barkeri | Terrabacter tamescens | Bacillus subtilis |
| 20           | Vagococcus flavidis | Pimelobacter simplex | Deinococcus radiodurans | Aeromicrobium erythreum | Clostridium butyricum |
| 24           | Aeromicrobium erythreum | Pimelobacter simplex | Trichococcus flocculiformis | Vagococcus flavidis | Clostridium butyricum |
| 28           | Arthrobacter globiformis | Bacillus subtilis | Pediococcus damnosus | Clostridium butyricum | Aeromicrobium erythreum |
| 32           | Aeromicrobium erythreum | Clostridium butyricum | Pimelobacter simplex | Alcaligenes xylosoxidans | Terrabacter tamescens |
| 36           | Bacillus subtilis | Pimelobacter simplex | Arthrobacter globiformis | Clostridium butyricum | Clostridium butyricum |
| 40           | Bacillus subtilis | Phenylbacterium immobile | Aerobacterium barkeri | Micrococcus luteus | Terrabacter tamescens |
| 44           | Thermoanaerobium brockii | Clostridium butyricum | Micrococcus luteus | Rhodococcus rhodnii | Clostridium butyricum |
| 48           | Acinetobacter calcoaceticus | Deinococcus radiodurans | Pediococcus damnosus | Thermococcus brockii | Clostridium butyricum |
2016), thereby becoming more potentially dangerous. Increased soil level of these pathogens around the masks may also have implications for other animal species interacting with the soil environment.

In general, the metagenomics and bioinformatics results provide insights into the changes in the diversity of bacterial community in the soil around face masks over the period of study. A total of 35 phyla were found in the soil prior to the introduction of the face masks, while the number was 38, 43, and 44 after placing the masks for 3, 6, and 9 months, respectively (Supplementary Information, Table S2). Figure 2 shows the phyla that constitute the majority of the bacterial community (i.e., those with 1% abundance and above) in each case. Prior the introduction of face masks, *Firmicutes* dominated the bacterial community in the soil, with 44.84% abundance. The *Firmicutes* were predominantly of the class *Clostridia* (40.45%), followed by *Negativicutes* and *Bacilli* which constitute only 0.74% and 0.71% of this phylum in the soil sample. *Clostridia* are obligately anaerobic and saprophytic bacteria which form very resistant endospores, that enable them to survive elevated temperatures and other harsh environmental conditions (Kator and Rhodes 2003). Together with the *Firmicutes*, six other phyla defined the bacterial community in the soil prior the placement of face masks. These are *Proteobacteria* (29.58%) > *Acidobacteria* (7.173%) > *Bacteroidetes* (6.174%) > *Verrucomicrobia* (2.144%) > *Gemmatimonadetes* (2.048%) > *Planctomycetes* (1.137%).

The initial dominance of *Firmicutes* phylum shifted after placement of the masks, as the *Proteobacteria* phylum became the most abundant thereafter, constituting 38.68%, 30.94%, and 29.67% of all the phyla at 3, 6, and 9 months, respectively (Fig. 2). Overall, the diversity of the bacterial community at phylum level, increased gradually over the study period (Table S2), and this increase in diversity compensated for the decrease in percentage abundance of the most dominant phylum at the quarterly observations.

Changes in diversity of the bacterial community around the face masks were more apparent at the genus level. Whereas the *Symbiobacterium* genus was predominant in the soil (27.32%) prior the placement of face masks, the genus did not constitute up to 1% of the community after 3, 6, or 9 months. Not only did the *Symbiobacterium* lose dominance of the bacterial community, no single genus also constituted up to 5% abundance thereafter, implying a greater diversity of genera in the soil around the face masks. The three most abundant genera after 3 months were *Tepidisphaera* (2.596%), and *Cellulomonas*, *Acinetobacter*, and *Desulfotomaculum* genera identified in metagenomics data of soil samples around face masks

| Table 3 Species of the Cellulomonas, Acinetobacter, and Desulfotomaculum genera identified in metagenomics data of soil samples around face masks |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Bacterial species               | Number of genomes (percentage abundance) |
|                                | 3 months | 6 months | 9 months |
| **Cellulomonas sp.**            | -        | 36 (0.043%) | 11 (0.006%) |
| **Cellulomonas pakistanensis**  | -        | 30 (0.036%) | 11 (0.006%) |
| **Cellulomonas massiliensis**   | -        | 6 (0.007%)  | 8 (0.004%)  |
| **Cellulomonas hominis**        | -        | 3 (0.004%)  | 6 (0.003%)  |
| **Cellulomonas persica**        | -        | 1 (0.001%)  | -           |
| **Cellulomonas bogoriensis**    | -        | -         | 1 (0.001%)  |
| **Acinetobacter sp.**           | 6 (0.01%) | 155 (0.184%) | 2005 (1.006%) |
| **Acinetobacter baumannii**     | 9 (0.014%) | 3 (0.004%)  | 14 (0.007%)  |
| **Acinetobacter rudis**         | -        | -         | 182 (0.091%) |
| **Acinetobacter seohaensis**    | -        | -         | 32 (0.016%)  |
| **Acinetobacter schindleri**    | -        | -         | 22 (0.011%)  |
| **Acinetobacter qingfengensis** | -        | -         | 5 (0.003%)   |
| **Acinetobacter variabilis**    | -        | -         | 5 (0.003%)   |
| **Acinetobacter johnsonii**     | -        | -         | 4 (0.002%)   |
| **Acinetobacter albensis**      | 1 (0.002%) | -         | 1 (0.001%)   |
| **Desulfotomaculum sp.**        | 172 (0.274%) | 12 (0.014%) | 31 (0.016%) |
| **Desulfotomaculum thermoacetoxidans** (Y11573) | - | 1 (0.001%) | 4 (0.002%) |
| **Desulfotomaculum alkaliphilium** (AF097024) | - | 1 (0.001%) | - |
| **Desulfotomaculum acetoxidans** (Y11566) | 2 (0.003%) | 1 (0.001%) | - |
| **Desulfotomaculum thermosapovorans** (Z26315) | - | - | 2 (0.001%) |
| **Desulfotomaculum aeronauticum** (X98407) | - | - | 1 (0.001%) |
| **Desulfotomaculum ruminis**    | -        | -         | -           |
| **Desulfotomaculum indicum**    | -        | -         | -           |
Flavisolibacter (1.736%), and Ramlibacter (1.732%), while the most abundant at 6 months were Gemmatimonas (4.704%), Gaiella (1.429%) and Zavarzinella (1.402%). Tepidisphaera is a recently identified genus of facultatively aerobic, moderately thermophilic bacteria (surviving at temperatures up to 56 °C) and utilizing carbohydrates and sugar for growth (Kovaleva et al. 2015). The genus Gemmatimonas that dominated the soil at 6 months composed of only two species—G. auran-tiaca and G. phototrophica. Gemmatimonas are aerobic or semi-aerobic organisms that reproduce by binary fission at temperatures between 20 and 37 °C. G. auran-tiaca is chemoheterotrophic (Zhang et al. 2003), while G. phototrophica is unique for possessing bacteriochlorophyll photosynthetic reaction centers, which enable it to harness light radiation as an additional energy source for metabolism (Koblížek et al. 2020). Gaiella (2.68%), Bacillus (2.67%), and Gemmatimonas (2.53%) were the three most abundant genera in the soil after 9 months. The nature of the dominant bacterial genera truly reflects the tropical conditions of the study area, with high environmental temperatures (mostly between 18 and 39 °C) recorded during the period of study.

Effects of medical face masks on soil macrofauna population and diversity

Fifteen soil macrofauna species were identified within the experimental plots for monitoring the effects of medical face mask on the population and diversity of soil invertebrate species. The collected macrofauna were all classified taxonomically to species level, with the exception of termite larvae (Kalotermitidae), jumping spider (Salticidae) and mil-lipedes (Julidae) which were identified to the family level.

Fig. 2 Dominant bacterial phyla in the soil: (a) initially before placing face masks, (b) 3 months after placing face masks, (c) 6 months after placing face masks, (d) 9 months after placing face masks
The surface of the garden soil was characterized mainly by two macrofauna species—carpenter ant (Camponotus haereticus) and tiny black ants (Monomorium invidium), which were seen prominently without digging of monoliths or disturbing the garden soil. The carpenter ants and the tiny black ants' population were at an average of 83.6 ± 9.21 and 62.9 ± 11.5, respectively, per the 25 m² of the experimental plot. Apart from the fifteen macrofauna types which were used to monitor the effects of the presence of face masks, three other macrofauna species were found on the field and identified, but not seen consistently enough to be included among the species monitored periodically. They are antlion (Distoleon tetragrammicus), stonfly (Eusthenia sp.), and red cotton bug (Dysdercus suturellus).

Table 4 presents data on the population and distribution of the macrofauna species collected from monoliths dug at 20, 50, and 100 cm around a middle reference point, prior to the setting up of the medical face masks on the experimental plots. Red velvet mites (Trombidium sp.) and fire ants (Solenopsis geminata) were not found in all the monoliths at this initial stage, although they were later seen in subsequent quarterly macrofauna collections. Earthworm (Eudrilus eugeniae) was also not found in this initial macrofauna set, reflective of the nature of this species which are usually active during the rainy season (Owa et al. 2003; Okoye et al. 2019), as against the peak dry season (January 2021) when this initial macrofauna observation was conducted. With the exception of red velvet mites, fire ants, and earthworms, all the macrofauna species were present in the top layer (0-10 cm depth) of the monoliths, in at least one of the distances away from the center reference. In contrast to the top layer of the monoliths, the millipedes (Julidae) were the only macrofauna type not seen in the bottom (10-20 cm) layers in the initial data collection. Overall, carpenter ants and tiny black ants were highest in population, with range of mean values as 1.33–11.67 and 1.67–7.5, respectively, across all monoliths. Most of the other macrofauna species were scanty at various depths in the monoliths and were absent in some, giving rise to mean values below unity when averaged over the 6 replicates (Table 4). It should be noted that a concrete statement could not be made regarding the distribution of the species at this initial stage, since the middle of the plots were only used as the reference point, from which the various distances were measured. However, the results provide indication of the natural condition of the garden soil, with respect to the population of the invertebrate species, prior the introduction of medical face masks to the plots.

Results of macrofauna presence and distribution on the experimental plots, after placing the face masks in the center of plots for a period of 3, 6, and 9 months are presented in Table 5 and Tables S6 and S7, respectively. These quarterly macrofauna data correspond to the time of early rainy season (in April), heavy rainy season (in July) and early dry season (in October). A number of redistribution patterns of the macrofauna species became noticeable, following the presence of medical face mask on the plots for 3 months and other subsequent quarterly observations. The tiny black ants (Monomorium invidium) were found around and on top of the medical face masks placed at the center of the experimental plots. Thus, a high concentration of this species, at an average of 47.5 ± 5.76 individuals, was observed in the closest monolith (20 cm) to the face masks in the month of April.

Table 4 Soil macrofauna population and distribution around the center of an experimental plot before introduction of medical face masks (data represent mean ± standard deviation of 6 replicates)

| Scientific name       | Common name           | 20 cm (t)       | 20 cm (b)       | 50 cm (t)       | 50 cm (b)       | 100 cm (t)      | 100 cm (b)      |
|-----------------------|-----------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Camponotus haereticus | Carpenter ant         | 8.33 ± 0.56     | 1.33 ± 0.16     | 11.67 ± 1.03    | 2.50 ± 1.14     | 7.17 ± 1.32     | 2.83 ± 1.63     |
| Paederus sp.          | Rove beetles          | 0.16 ± 0.08     | 0.17 ± 0.08     | -               | 0.17 ± 0.05     | 0.50 ± 0.22     | -               |
| Melanotus communis    | Wire worm             | -               | -               | 0.16 ± 0.02     | -               | 0.16 ± 0.04     | 0.17 ± 0.05     |
| Chelifer cancrivorus  | Pseudoscorpion        | 0.33 ± 0.31     | 0.17 ± 0.41     | 0.50 ± 0.27     | 0.67 ± 0.26     | 0.50 ± 0.27     | 1.33 ± 0.86     |
| Monomorium invidium   | Tiny black ant        | 7.50 ± 3.61     | 2.50 ± 0.35     | 3.33 ± 1.36     | 2.50 ± 1.68     | 5.70 ± 1.21     | 1.67 ± 0.04     |
| Kalotermitidae        | Baby termite          | -               | 2.83 ± 1.94     | 0.67 ± 0.21     | 0.33 ± 0.16     | 2.83 ± 0.57     | -               |
| Eudrilus eugeniae     | Earthworm             | -               | -               | -               | -               | -               | -               |
| Salticidae            | Jumping spider        | 0.33 ± 0.17     | 0.33 ± 0.07     | 0.83 ± 0.32     | 0.35 ± 0.16     | 0.33 ± 0.06     | 0.17 ± 0.04     |
| Gymnus sp.            | Ground beetle         | -               | -               | 1.33 ± 0.75     | 0.67 ± 0.13     | 0.50 ± 0.36     | 0.16 ± 0.01     |
| Solenopsis invicta    | Red and black ant     | 0.33 ± 0.13     | -               | 0.17 ± 0.03     | 0.17 ± 0.08     | 0.83 ± 0.04     | 0.16 ± 0.02     |
| Julidae               | Millipedes            | -               | -               | 0.16 ± 0.02     | -               | -               | -               |
| Trombidium sp.        | Red velvet mite       | -               | -               | -               | -               | -               | -               |
| Hermetia illucens     | Black soldier fly     | -               | 0.67 ± 0.21     | 0.16 ± 0.07     | -               | -               | 1.17 ± 0.42     |
| Solenopsis geminata   | Fire/red ants         | -               | -               | -               | -               | -               | -               |
| Acheta domesticus     | Field cricket         | -               | 0.16 ± 0.08     | 1.50 ± 0.57     | 0.83 ± 0.42     | 0.67 ± 0.09     | -               |

* t top soil layer from surface to 10 cm depth, b bottom soil layer from 10 to 20 cm
Table 5 Soil macrofauna population and distribution around medical face masks after a period of 3 months (data represent mean ± standard deviation of 6 replicates)

| Scientific name            | Common name   | 20 cm (t)       | 20 cm (b)       | 50 cm (t)       | 50 cm (b)       | 100 cm (t)      | 100 cm (b)      |
|----------------------------|---------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| *Camponotus haereticus*    | Carpenter ant | 22.3 ± 3.52     | 5.23 ± 0.51     | 28.6 ± 4.03     | 2.67 ± 1.14     | 17.2 ± 3.33     | 1.83 ± 0.72     |
| *Paederus sp.*             | Rove beetles  | 0.17 ± 0.41     | 0.16 ± 0.08     | -               | 0.16 ± 0.04     | 0.5 ± 1.225     | -               |
| *Melanotus communis*       | Wire worm     | -               | -               | 0.16 ± 0.08     | -               | 0.16 ± 0.05     | 0.17 ± 0.12     |
| *Chelifier cancroideis*    | Pseudoscorpion| 0.33 ± 0.52     | 0.33 ± 0.82     | -               | 0.33 ± 0.21     | -               | 0.50 ± 0.84     |
| *Monomorium invidium*      | Tiny black ant| 47.5 ± 5.76     | 3.67 ± 0.82     | 3.17 ± 1.40     | 2.17 ± 1.68     | 2.67 ± 0.21     | 0.67 ± 0.04     |
| *Kalotermididae*           | Baby termite  | -               | 2.83 ± 1.94     | 0.67 ± 0.22     | 0.33 ± 0.16     | 2.83 ± 1.57     | -               |
| *Eudrilus eugeniae*        | Earthworm     | -               | -               | -               | 0.33 ± 0.11     | 0.17 ± 0.41     | -               |
| *Salticidae*               | Jumping spider| 0.33 ± 0.52     | 0.33 ± 0.21     | 0.83 ± 0.38     | 0.33 ± 0.07     | 0.33 ± 0.16     | 1.50 ± 0.62     |
| *Gyrinus sp.*              | Ground beetle | -               | -               | 1.33 ± 0.75     | 0.67 ± 0.15     | 0.50 ± 0.36     | 0.17 ± 0.09     |
| *Solenopsis invicta*       | Red and black ant | 0.33 ± 0.26 | -               | 0.16 ± 0.41     | 0.17 ± 0.08     | 0.83 ± 0.04     | 0.67 ± 0.12     |
| *Julidae*                  | Millipedes    | -               | -               | 0.17 ± 0.09     | -               | -               | -               |
| *Trombidium sp.*           | Red velvet mite| -               | -               | -               | 0.17 ± 0.11     | 0.17 ± 0.15     | -               |
| *Hemiptera sp.*            | Black soldier fly| -               | 0.67 ± 0.21     | 0.17 ± 0.08     | -               | -               | 0.16 ± 0.08     |
| *Solenopsis geminata*      | Fire/red ants | -               | -               | -               | -               | -               | -               |
| *Acheta domesticus*        | Field cricket | 0.50 ± 0.31     | 0.33 ± 0.17     | 0.17 ± 0.06     | -               | 0.83 ± 0.25     | 0.16 ± 0.41     |

*top soil layer from surface to 10 cm depth, bottom soil layer from 10 to 20 cm

(Table 5). Such concentration around the center was not seen in the control experimental plots which lacked medical face masks at the middle (Supplementary Information, Table S2). It is worth emphasizing that the high number of the ants occurred only at the top layer of the 20 cm monoliths, while further monoliths from the face masks (50 cm and 100 cm) had numbers similar to the background or initial values of the species at all the layers. This result clearly points to the influence of the presence of the medical face masks on the garden soil. The high number of the *Monomorium* species found around the heap of face masks may be attributed to the retention of water by the masks. Rainfall was little and intermittent during the months of March and April, falling several days apart, and causing the ground to return to a dry state after short periods of wetness. While the surrounding soils were dry, the heap of face masks on experimental plots were observed to retain water and remained wet for longer periods. Many species of ants are known to be abundant near a source of water, particularly in water-stressed environments (Tschinkel et al. 2012; Pringle et al. 2013). Thus, in this case, the *Monomorium invidium* species, appear to take the advantage of water retained by the medical face masks during the period of little and irregular precipitation. Retention of water by the face masks may have therefore contributed to the survival of this ant species during the water-scarce part of the year. No clustering of the tiny ants around face masks was observed during the heavy precipitation period, when water was abundant throughout the environment and the ground was wet for the most part.

In an effect similar to that elicited by the *Monomorium* species, the carpenter ants (*Camponotus haereticus*) were found to respond to the presence of medical face masks on the experimental plots. At all quarterly inspection of the monoliths (after 3, 6, and 9 months), the carpenter ants were observed to be more abundant in the plots that had masks compared to those without masks. Average population in top layers of monoliths that had face masks ranged from 17.2–28.6, 15.7–23.7, and 13.7–23.5 after 3, 6, and 9 months, respectively (Table 5 and Tables S6–S7), compared to the range of 2.00–6.35, 1.00–4.5, and 2.25–3.50 recorded for the top layers of plots that had no face masks (Supplementary Information, Tables S3–S5). Indeed, a non-parametric Mann–Whitney *U* test performed to compare the population distribution of the carpenter ants in plots with and without face masks, showed that there was significant difference in the two population sets at 3, 6, and 9 months (*p* ≤ 0.05 in all cases). These results notwithstanding, it should be noted that the *Camponotus* species were not specifically abundant at the closest monoliths to the face mask (unlike the *Monomorium* species in dire need of water), but were generally more visible across each plot that contained masks, compared to non-masked plots. Thus, it is not immediately clear how the presence of the face masks influenced the abundance of the *Camponotus* species.

More importantly, the heaps of medical face masks were found to serve as shelter for some of the soil macrofauna. Two species—the red cotton bug (*Dysdercus suturellus*), and house cricket (*Acheta domesticus*)—were found living and breeding under the face masks in June and July of the experimental year (2021). This period corresponded to 16–24 weeks after the masks were placed on the garden soil and fell within the high temperature–heavy rainfall period.
of the year. The red cotton bugs are known to be attracted particularly to cotton plants, which they destroy by puncturing and feeding on young cotton bolls, preventing them from attaining full maturity. It is noteworthy that the main component of the cotton plant that provides nutrient to the bug is cellulose, also a key component of the medical face masks as noted earlier on. The attraction and presence of the red cotton bugs under the face masks are therefore highly plausible. The timing of the appearance of the bugs, after 4 months of the presence of the masks on the soil, is also consistent with the fact that the biotic components of the environment (sunlight radiation, precipitation, humidity, etc.) have already caused some physical attack and degradation on the outer synthetic polymer materials of the masks, thereby exposing the inner cellulosic layer, to which the cotton bugs are attracted. Figure 3 shows the image of nymph and adult stages of the red cotton bugs (D. suturellus) found under the heap of face masks. Image of two nymphal stages of the house cricket (A. domesticus) captured under the face masks is also shown in the figure (See also supplementary materials (videos 1 and 2). Like the red cotton bugs, the house crickets are hemimetabolous—having only egg, nymphal and adult stages with no larvae or pupae (Oppert et al. 2020; Vaga et al. 2021). The ability of the crickets to feed on fabric materials also explains why they are attracted to the medical face masks. Given that an enormous number of face masks has ended up in soil environments globally, occasioned by the COVID-19 pandemic, the provision of breeding sites by face masks for insect fauna species may have contributed silently to an increase in population of insects. Previously, many studies have demonstrated an accelerating decline in the population of insects, with concerns raised for ecosystems functions and biodiversity, as well as humans’ quality of life and survival (Hallmann et al. 2017; Forister et al. 2019; Seibold et al. 2019; Janzen and Hallwachs, 2019). Thus, the COVID-19 pandemic and the huge amounts of face masks utilized during the period appear to have had a positive environmental and ecological impact in this regard. While results presented herein are from localized field observations, an important point for research would be to determine whether there has been any increase in insect population regionally or globally in the post-COVID-19 pandemic era, and the extent to which face masks dumping in soil environments may have contributed to the increase.

In contrast to attraction and potential beneficial impacts of the face mask on the garden soil, some of the macrofauna species appear to be repelled by the presence of the masks. A pseudoscorpion insect (Chelifer cancroides) was seen more in the control plots (lacking face masks), than in the plots that had masks. The range of means across all layers in the monoliths was 1.50–5.50 and 1.25–5.50 in the control plots, after 3 and 6 months of the presence of the masks, respectively, compared to 0–0.5 and 0–0.67 in the plots that had masks for the same period (Tables 5 and 6; Supplementary Information, Tables S3 and S4). Similarly, the fire ants (Solenopsis geminata) and the red velvet mites (Trombidium sp.) exhibited repulsive effects towards the face masks. The fire ants were found more in the control plots than in the masked plots, after 6 and 9 months of the presence of the masks. Influence on the velvet mites was observed earlier, as they were seen more in the control plots than in the plots with face masks, after 3 months of placement of the masks. It is noteworthy that in instances where the red velvet mites were found in the masked plots after 3 months, they were present only in the farthest (100 cm) monoliths from the center/face masks (Table 5), suggesting that they are sensitive to the presence of the masks. This contrasts with the control plots, where the mites were found at all monoliths across the transects, irrespective of the distance from the center (Supplementary Information, Tables S3 and S4). In general, the red velvet

![Fig. 3](image-url) Macrofauna found breeding under heap of medical face masks on a garden soil: (a) Nymphal and adult stages of the red cotton bug (D. suturellus). (b) Nymphal stages of the house cricket (A. domesticus)

| Table 6 | Shannon diversity index (H) and Shannon equitability index (E_H) of species on experimental plots after 3, 6, and 9 months |
|---------|---------------------------------------------------------------|
| Treatments | 3 months | 6 months | 9 months |
| Plots with face masks | H | 1.282 | 1.503 | 1.249 |
| | E_H | 0.473 | 0.555 | 0.461 |
| Plots without face masks | H | 1.732 | 1.913 | 2.050 |
| | E_H | 0.639 | 0.706 | 0.757 |

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mites were seasonal in appearance—they were absent in the dry periods and only found in the months of April and July during the rainy period. This observation was consistent with the mites being also referred to as rain mites, with the tendency to appear during rainy season when their foods/prey insects are more abundant in the environment (De, 2020).

Species such as the earthworm (Eudrilus eugeniae), rove beetle (Paederus sp.), and wire worm (Melanotus communis) showed no regard for the presence of the face masks, i.e., they neither migrated towards or away from the masks, and there were no clear differences between their numbers found in the main experimental plots and the control plots (lacking face masks) at the same periods. These species appeared to be influenced mainly by seasonal effects. They were found mostly during the wet season, corresponding to the period 3 and 6 months after placement of the face masks on the garden soil. These findings provide an indication of the impact of huge number of medical face masks, particularly those made of chemical polymers, which may disrupt endocrine systems and cause reproductive impairments in the species. A probable limitation of this study is the fact that the effects on soil bacterial and macrofaunal endocrine systems and cause reproductive impairments in the species.

Prior to placing the masks on each 25 m² plot of the garden soil, Shannon diversity index ($H$) and Shannon equitability index ($E_H$) determined for macrofauna species were 1.677 and 0.619, respectively. Table 6 shows that values of $H$ were higher in plots that had no face masks compared to those with face masks at all corresponding quarterly observations, implying that the presence of the medical face masks caused an overall reduction in the diversity of species in the plots. $H$ values in the plots with face masks were also lower than the $H$ value obtained for the plots before the face masks were introduced (1.677). Similarly, values of $E_H$, a measure of evenness/equality of abundance of species, were lower in plots with face masks than in plots without face masks at corresponding quarterly recordings, and also lower than the value determined prior the introduction of the masks. Lower $E_H$ value means lesser evenness of abundance, and this result again suggests that the presence of face masks may have upset the evenness of abundance of macrofauna species on the garden soil. These findings provide an indication of the impact of huge number of medical face masks, particularly those used for COVID-19 management, that may have been placed/abandoned on soil ecosystems in various parts of the world.

Table 6 presents Shannon indices, calculated based on total population of the various macrofauna species observed on the experimental plots after 3, 6, and 9 months of placing medical face masks. Prior to placing the masks on each 25 m² plot of the garden soil, Shannon diversity index ($H$) and Shannon equitability index ($E_H$) determined for macrofauna species were 1.677 and 0.619, respectively. Table 6 shows that values of $H$ were higher in plots that had no face masks compared to those with face masks at all corresponding quarterly observations, implying that the presence of the medical face masks caused an overall reduction in the diversity of species in the plots. $H$ values in the plots with face masks were also lower than the $H$ value obtained for the plots before the face masks were introduced (1.677). Similarly, values of $E_H$, a measure of evenness/equality of abundance of species, were lower in plots with face masks than in plots without face masks at corresponding quarterly recordings, and also lower than the value determined prior the introduction of the masks. Lower $E_H$ value means lesser evenness of abundance, and this result again suggests that the presence of face masks may have upset the evenness of abundance of macrofauna species on the garden soil. These findings provide an indication of the impact of huge number of medical face masks, particularly those used for COVID-19 management, that may have been placed/abandoned on soil ecosystems in various parts of the world.

Conclusions

This study examined the possible impacts of medical face masks on soil environment, particularly the effects on soil microorganisms and macrofauna (insect) species. Face masks influenced the diversity of bacteria in soil, with the Cellulomonas spp. (C. fimi and C. flavigena), Acinetobacter johnsonii, and Desulfotomaculum antarcticum being the culturable species found most frequently around the masks. Due to their saprophytic nature, the Cellulomonas spp. and A. johnsonii are likely involved in the degradation of the masks under the prevailing tropical conditions. This is the first report of bacterial species that may be involved in the decomposition of medical face masks dumped in soil environment during the COVID-19 pandemic. Initial dominance of Symbiobacterium genus in the soil was lost, to give way for Tepidisphaera, Gemmatimonas, and Gaiella after 3, 6, and 9 months of introducing the face masks, respectively. Although the presence of face masks led to a more diverse bacterial community in the soil, the study provided no evidence that the masks influenced bacterial population—the number of colony forming units (CFU) of bacteria species were higher around the masks at some instances, while they were higher in the control soils (which lacked face masks) at other instances, during the 48-week monitoring period.

Presence of face masks had a repelling effect on some soil macrofauna species, especially fire ants (Solenopsis geminata), red velvet mites (Trombidium sp.), and pseudoscorpion (Chelifer cancroides). Presence of face masks also reduced diversity and evenness of abundance of macrofauna species in soil environment around the masks. However, the masks retained water and attracted ant species (Monomorium invidium) during the period of low water availability in the environment. More significantly, the masks provided shelter and breeding haven to red cotton bugs (D. saturellus) and crickets (A. domesticus), which were attracted by the cellulosic component of the masks, that served as food for them while sheltering under the masks. This study suggests that the insect faunas, where available, may have contributed to the degradation of the face masks. Thus, a combined force of biotic factors (microbial and insect actions), in addition to normal abiotic effects (sunlight, precipitation, and humidity), acts to cause the degradation of face masks abandoned in soil environments.

The provision of breeding sites by face masks may contribute to an increase in population of soil insects. This may be regarded as a major positive impact of COVID-19 face masks dumped in soil environments, given that there are concerns about the declining population of many insect species globally. However, eating and munching of face masks by insect species may have deleterious biological effects in the long term, as the masks are made of chemical polymers, which may disrupt endocrine systems and cause reproductive impairments in the species.
communities were monitored for 48 weeks only, whereas monitoring for longer periods could potentially reveal any secondary effects on species and ecosystem services. This notwithstanding, the current study has offered deep insights, as to how COVID-19 face masks may affect and influence organisms, in the open and natural soil environment.

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ing acquisition; project administration; study design; formal analyses; investigation; data curation; methodology; supervision; writing—original draft; writing—review and editing.

Data availability Research data used in this study are provided in Tables 1–5 and in the Supporting Information online. Metagenomic data of the soil bacteria sequenced in this study have been deposited with the National Center for Biotechnology Information (NCBI), accession number PRJNA861236 (available online at [https://www.ncbi.nlm.nih.gov/sra/PRJNA861236](https://www.ncbi.nlm.nih.gov/sra/PRJNA861236) as from 01/01/2023 and on reasonable request to guidown@futa.edu.ng).

Declarations

Ethics approval and consent to participate Not applicable.

Consent to publish Not applicable.

Conflict of interest The authors declare no competing interests.

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