

Microbiome Evolution of Brewer's Spent Grain and Spent Coffee Ground Solid Sidestreams Under Industrial Storage Conditions

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S1. Experimental setup and physical measurements of simulated storage trials

Photographs of the experimental setup of simulated storage of BSG is shown in Figure S1. The physical measurements of temperature and humidity by dataloggers for each tray and drum containing BSG are shown in Figures S2 to S5. The starting temperature for BSG is above the ambient 30 °C, ranging from about 35 °C to 45 °C. This represents residual heat from the mashing process, typically in the temperature range of 65 °C to 76 °C [17]. The temperature in drums decline to and maintain at the ambient level within 2-3 days, while for trays it has mostly dissipated already by the start of datalogging, presumably due to the smaller amount, larger surface area and direct air contact. However, the temperature of BSG in trays does not maintain at the ambient level, but fluctuate above 32.5 °C between day 1 to day 10, reaching temperatures near or above 40 °C, before declining to ambient by day 14. The elevated temperature is linked with microbial metabolism, and visual inspection confirmed extensive mould growth occurring from day 3 onwards in the trays. The relative humidity measured by dataloggers is generally above 90%, as is expected from a sensor inserted into a wet material. Several of the humidity probes malfunctioned during the simulated storage, especially in the drums, likely due to exposure to free water.

The physical measurements for SCG are shown in Figures S6 to S9. In contrast to BSG, the temperature of SCG starts near the ambient, but then rapidly climbs during day 1 as high as 45 °C for trays, and 38.5 °C for drums, confined to the top and middle sections. Temperatures in trays then drop to ambient by day 2, before again starting to increase from day 4 to day 10, peaking at around 37°C, but more typically around 35 °C. Day 4 also corresponds to the first visual observation of a white colour mould in the trays. The top of the drums generally maintained an elevated temperature around 35 °C from day 1 to around day 10, likely similarly caused by surface growth of fungi as in the trays. The bottom of the drums closely followed ambient temperature conditions, with the middle somewhat in between. The humidity of SCG was generally high and above 90% RH similar to BSG.

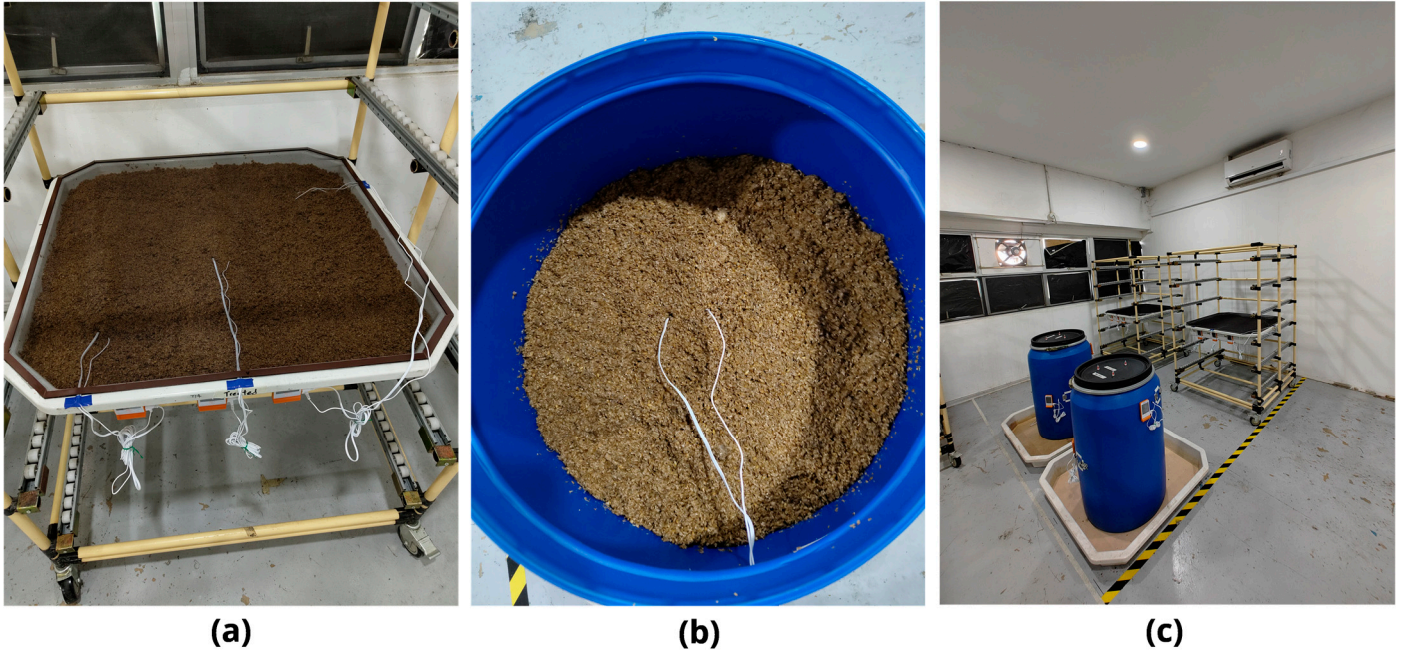


Figure S1. Experimental setup used for simulated storage trials: (a) aerobic trays with 40 kg BSG, (b) anaerobic drums with 100 kg BSG, and (c) trial area at Tiong Lam Supplies with finalised experimental setup.

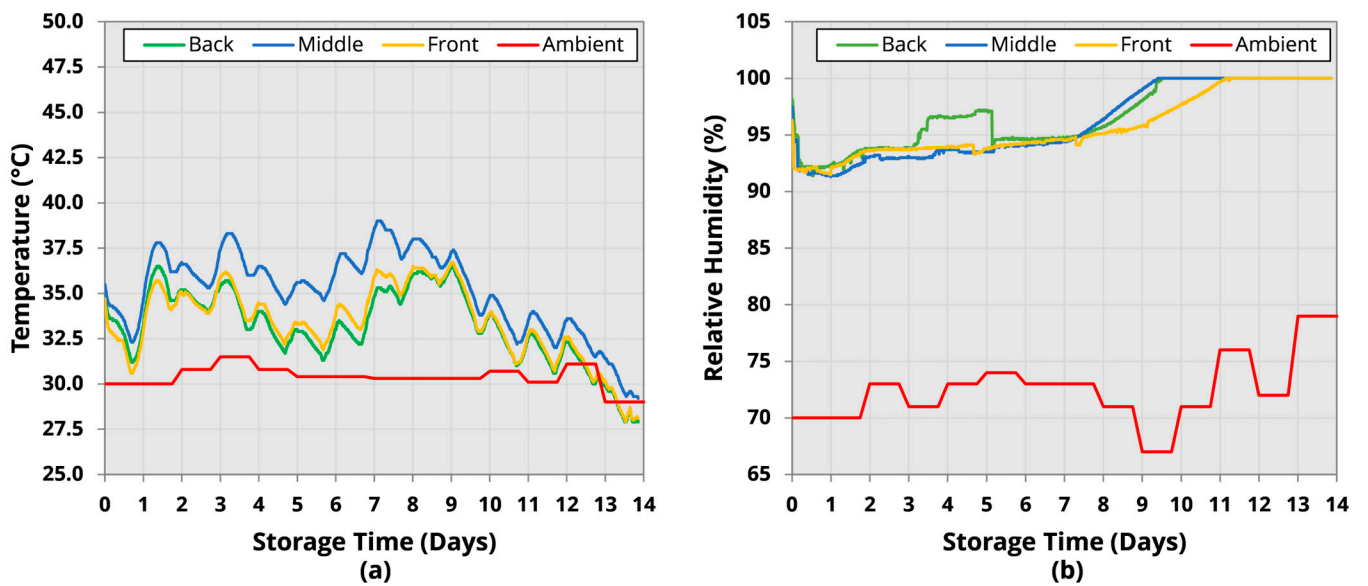


Figure S2. Physical measurements of (a) temperature and (b) relative humidity of BSG in Tray 1 and ambient conditions during simulated storage.

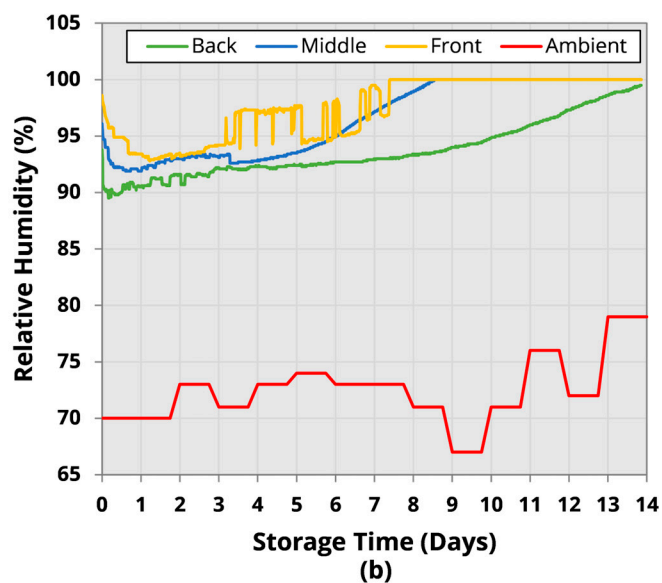
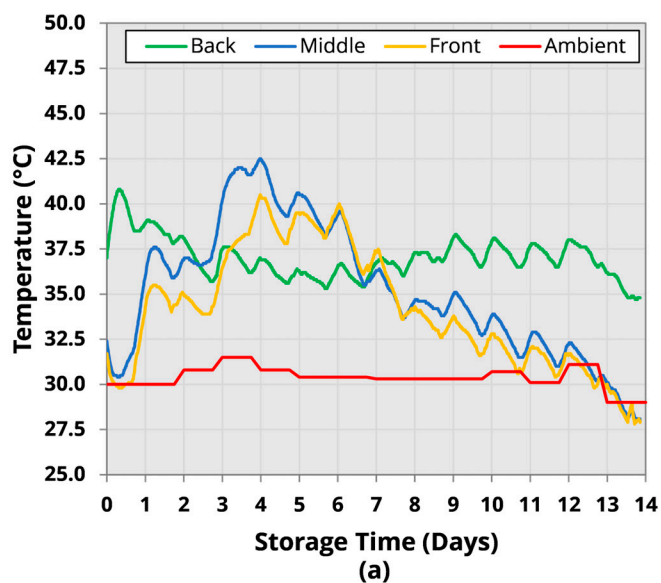


Figure S3. Physical measurements of (a) temperature and (b) relative humidity of BSG in Tray 2 and ambient conditions during simulated storage.

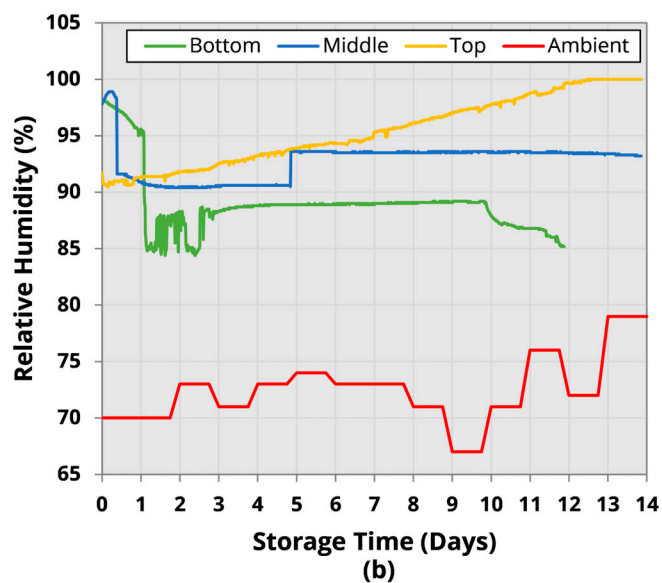
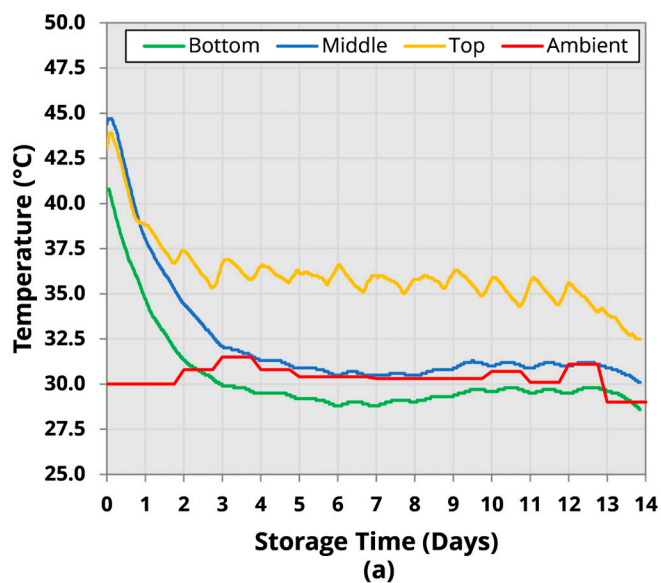


Figure S4. Physical measurements of (a) temperature and (b) relative humidity of BSG in Drum 1 and ambient conditions during simulated storage. Discontinuous lines are caused by malfunction of the respective datalogger sensor.

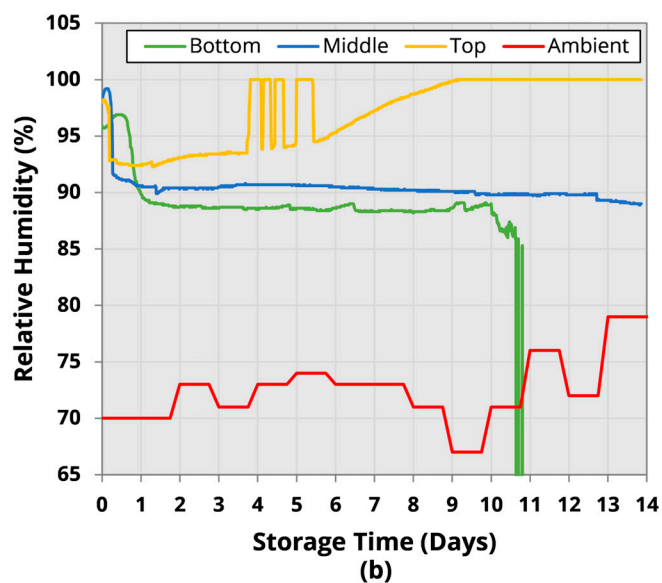
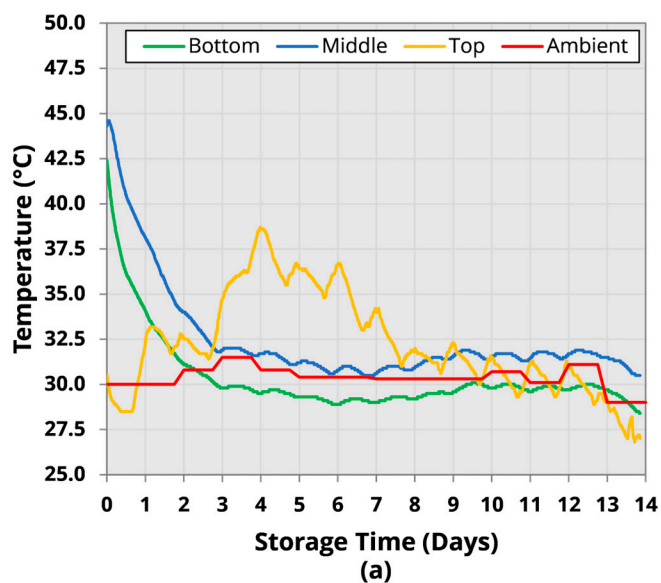


Figure S5. Physical measurements of (a) temperature and (b) relative humidity of BSG in Drum 2 and ambient conditions during simulated storage. Discontinuous lines are caused by malfunction of the respective datalogger sensor.

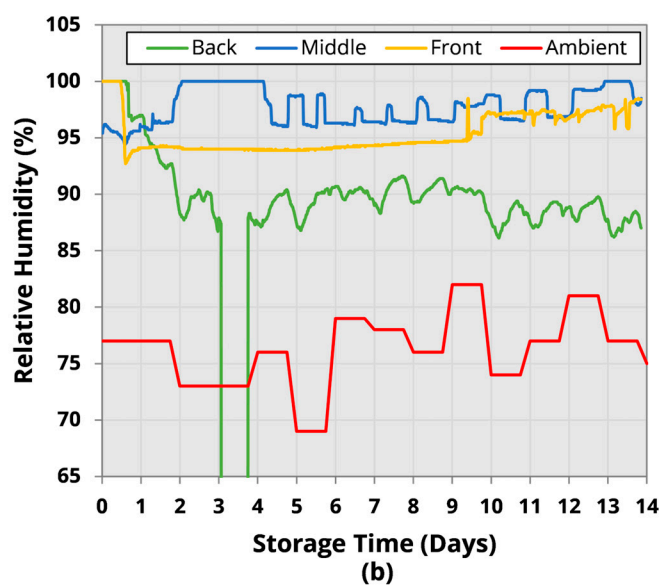
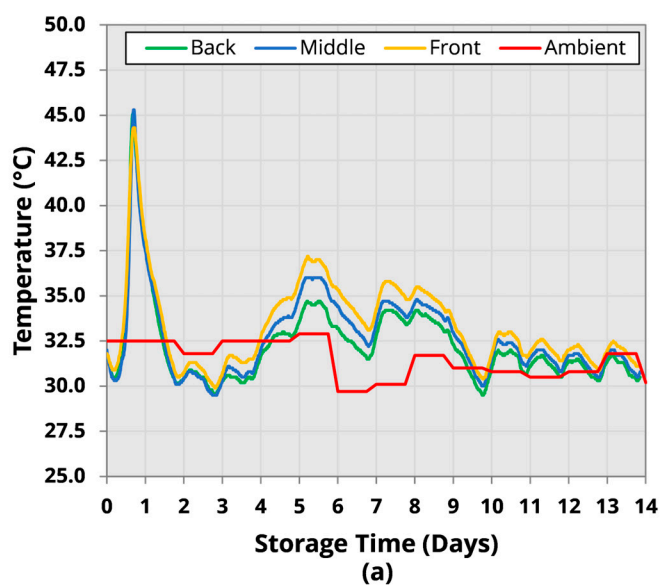


Figure S6. Physical measurements of (a) temperature and (b) relative humidity of SCG in Tray 1 and ambient conditions during simulated storage. Discontinuous lines are caused by malfunction of the respective datalogger sensor.

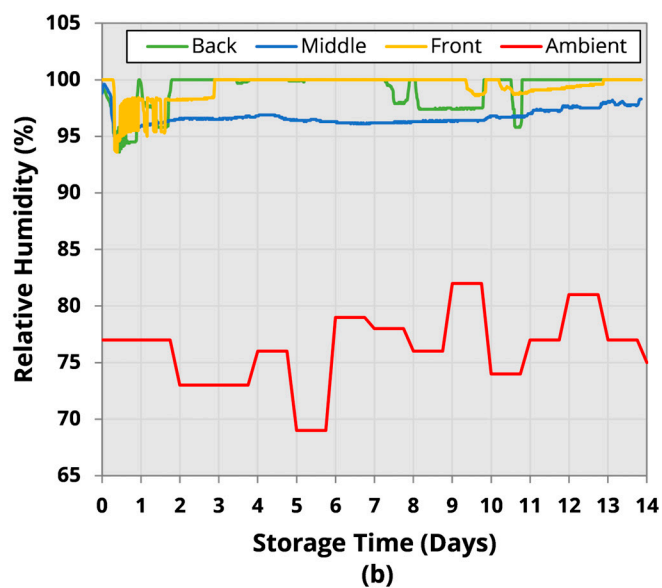
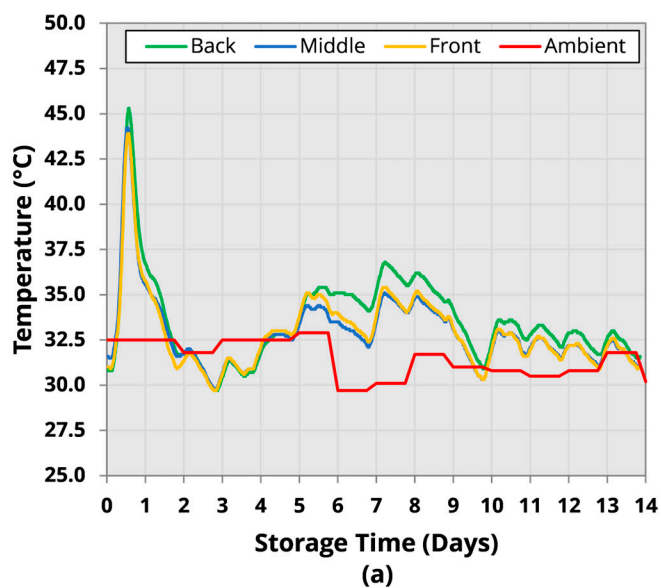


Figure S7. Physical measurements of (a) temperature and (b) relative humidity of SCG in Tray 2 and ambient conditions during simulated storage. Discontinuous lines are caused by malfunction of the respective datalogger sensor.

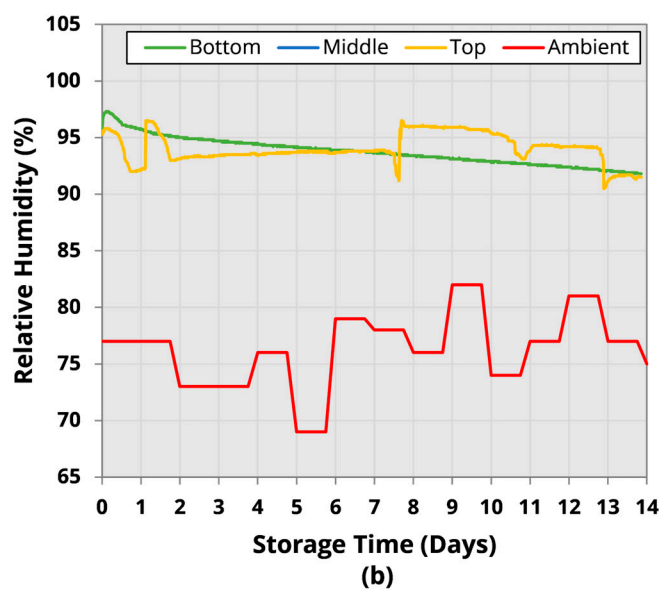
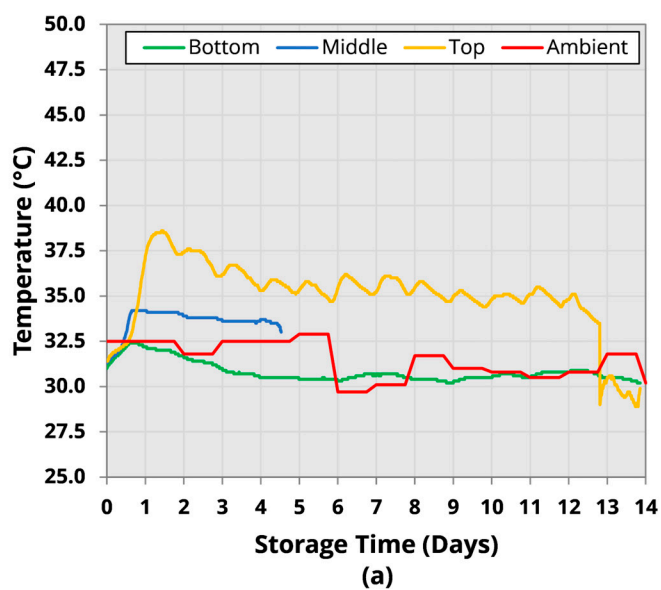


Figure S8. Physical measurements of (a) temperature and (b) relative humidity of SCG in Drum 1 and ambient conditions during simulated storage. Discontinuous lines are caused by malfunction of the respective datalogger sensor.

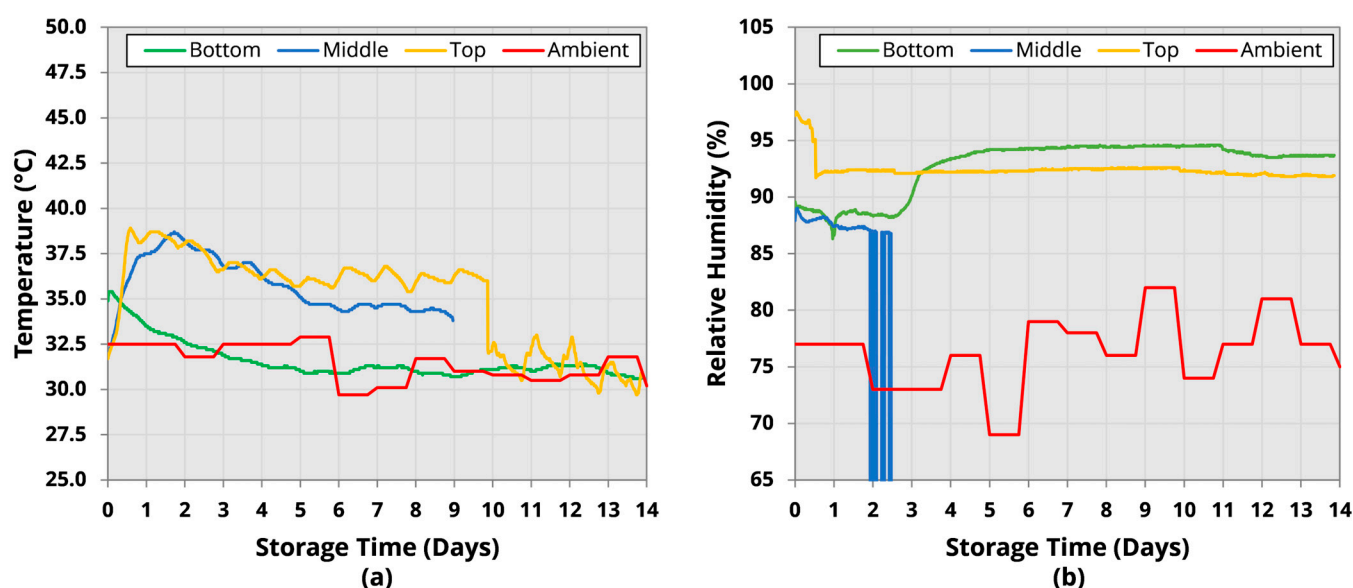


Figure S9. Physical measurements of (a) temperature and (b) relative humidity of SCG in Drum 2 and ambient conditions during simulated storage. Discontinuous lines are caused by malfunction of the respective datalogger sensor.

S2 Quantitative Polymerase Chain Reaction (qPCR) during simulated storage

The microbial dynamics during simulated storage were investigated using qPCR at day 0, 3, 7 and 14. The measured inverse cycle threshold was normalised to that of the day 0 sample for BSG and SCG under each storage condition, indicating a fold-increase in measured DNA for bacterial and fungal primer pairs. It was hypothesised that microaerophilic LAB would play a prominent role on simulated storage in drums, and conversely fungi in trays, and the primers selected accordingly as in Table S1.

DNA extraction was performed as described in for Amplicon metagenomic sequencing in section 2.4 for BSG, and SCG was treated with DNeasy PowerSoil Pro Kit (Qiagen), and the samples subjected to DNA extraction according to the manufacturer's instruction. DNA concentrations were then quantified in duplicate via qPCR on Applied Biosystems QuantStudio 3 Real- Time PCR System using SYBR FAST qPCR master mix (KAPA) in a 96-well qPCR reaction plate prepared as per the manufacturer's instructions. The respective primers and qPCR conditions are summarized in Table S1.

Table S1. PCR primers and run conditions for qPCR of BSG and SCG samples.

Primer	5'-3' Sequence	Target Region	Run conditions
15f	GCTCAG-GAYGAAC-GCYGG	Bacterial 16S rRNA gene with high specificity for lactic acid bacteria [65]	95 °C for 3 min, 40 cycles at 95 °C for 10 s, 57 °C for 15 s, 72 °C for 30 s
687r	CACCGC-TACACAT-GRADTTC		
ITS1	TCCG-TAGGTGAAC-CTGCGG	Yeast 18S gene, the internal transcribed spacer (ITS) segment, and most of the 5.8S gene in ribosomal (r)DNA	95 °C for 3 min, 40 cycles at 95 °C for 10 s, 57 °C for 15 s, 72 °C for 20 s
ITS2	GCTGCGTTCTT-CATCGATGC		

The qPCR results are illustrated in Figure S10. A rapid increase in LAB is observed for BSG stored in trays already by day 3, not significantly different from that of day 14 of 1.11 ± 0.03 fold increase, with an apparent drop in between at day 7. Drum storage does not exhibit a significant change in LAB from day 0 to day 14, which may be linked larger

differences between the drum sampling positions, as also evidenced by the large standard error for drum storage. BSG stored in trays also show growth of fungi, but with a more gradual increase, culminating in a 1.23 ± 0.10 to 1.27 ± 0.12 fold increase by day 14. This is in contrast to the decreasing trend for fungi under drum storage, with 0.84 ± 0.15 fold decrease at day 14.

SCG stored in trays exhibit a slower increase in LAB as compared to BSG, ending at 1.10 ± 0.03 fold increase at day 14, while drum storage has more pronounced growth with 1.28 ± 0.19 fold increase by day 14. qPCR for fungi shows larger increments, with trays ending at 1.98 ± 0.03 fold increase and drums 1.98 ± 0.36 fold increase compared to day 0.

The qPCR data show little variance between the duplicate simulated storage conditions, and the trend of the agrees well with the TPC data obtained at day 14, yes does not capture the 2-3 orders of magnitude difference typically found for bacteria, and over 6 for fungi in tray storage of BSG. The only major deviation from the TPC trend is the high qPCR value for fungi drums, even when compared to the qPCR data for trays.

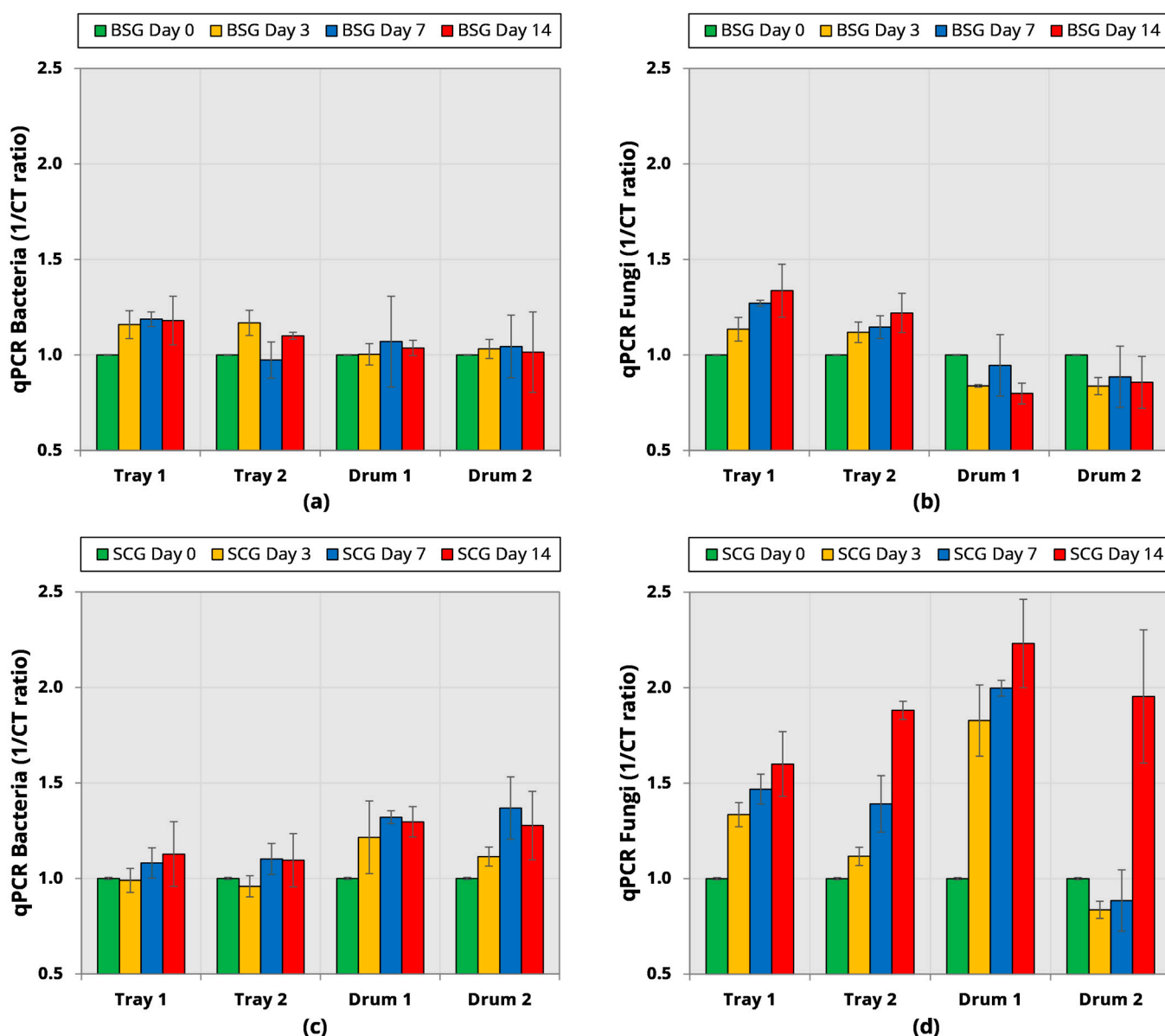


Figure S10. Quantitative polymerase chain reaction (qPCR) of BSG and SCG during 14 days of simulated storage in trays (BSG-2 & SCG-2) and drums (BSG-2 & SCG-3): (a) Bacteria primer pair inverse cycle threshold normalised to day 0 for BSG; (b) Fungi primer pair inverse cycle threshold

normalised to day 0 for BSG. (c) Bacteria primer pair inverse cycle threshold normalised to day 0 for SCG; (d) Fungi primer pair inverse cycle threshold normalised to day 0 for SCG.

S3 Taxonomic abundance cluster heatmaps of BSG and SCG bacterial microbiome

Taxonomic abundance cluster heatmap hierarchical clustering of samples for the top 35 bacterial species are provided for BSG in Figure S11 and SCG in Figure S12. These taxonomic heatmaps indicate the relative prevalence of bacterial species across samples, and therefore storage conditions. The absolute value of 'Z' represents the distance between the raw score and the mean of the standard deviation. 'Z' is negative when the raw score is below the mean, and vice versa. Red indicates a species with skewed distribution, with the presence of a species concentrated in few samples; white indicates an even distribution among samples; and blue represents low relative abundance or absence. Moreover, the colours on the cluster dendrogram correspond to the species phylum as shown.

The taxonomic heatmaps highlight that the variable microbiome of fresh BSG and SCG depending on the source, but also that the initial microbiome does not persist on simulated storage. The most similar microbiomes are found in the tray samples at different locations, but even between the two tray replicates there are significant variations in the microbiome at the species level. Drum microbiomes at the middle and bottom sampling positions show large differences, as also found by comparison at the genus-level. Drum microbiomes show a higher degree of similarity at the same sampling positions in the two replicates, consistent with the results, and hypothesis that the physical environment, (moisture content, pH, oxygen availability) are significantly different.

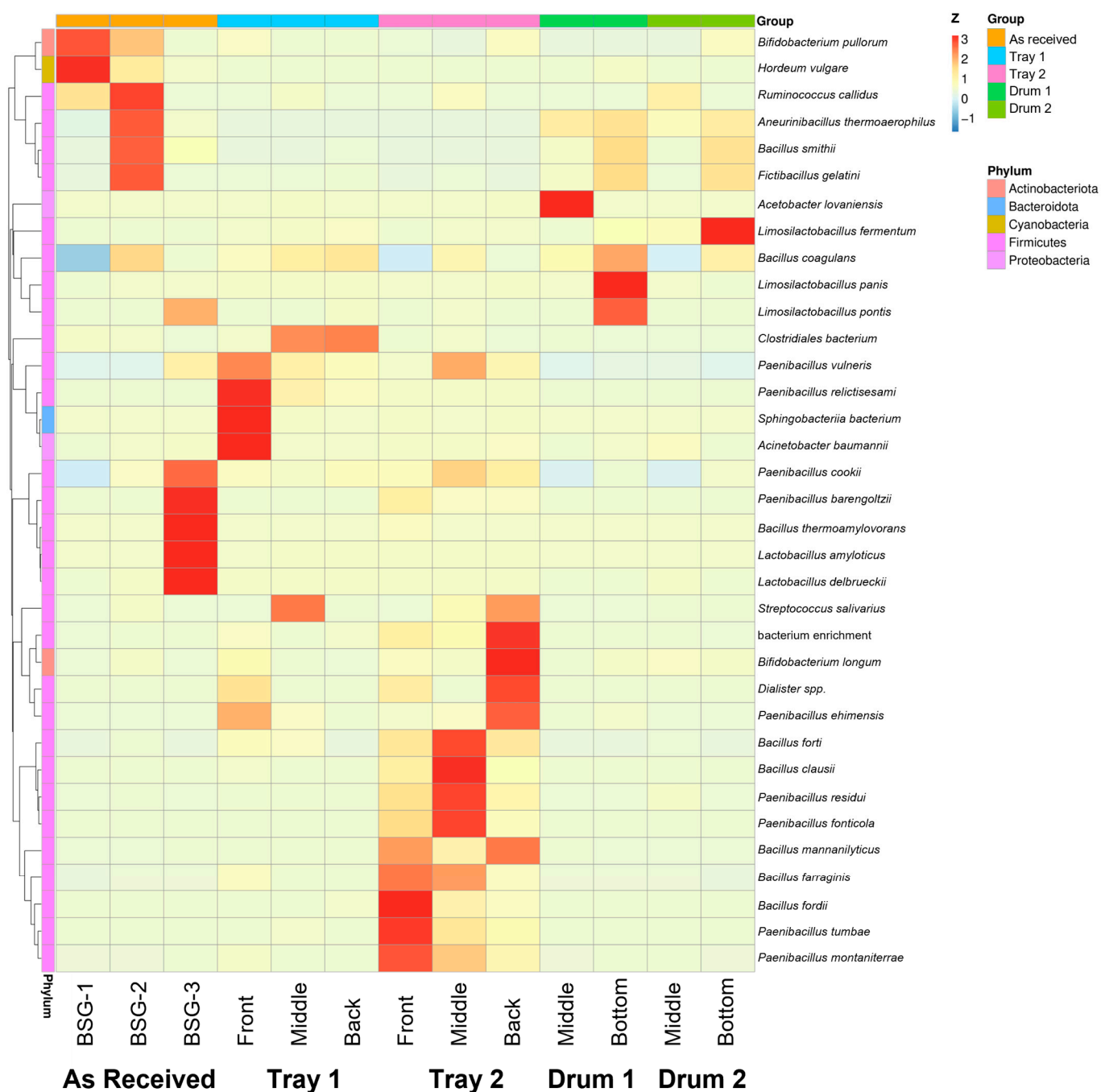


Figure S11. Taxonomic abundance cluster heatmap hierarchical clustering of samples for the top 35 bacterial species of BSG-1, BSG-2 and BSG-3 as received and after 14 days of simulated storage in trays (BSG-2) and drums (BSG-2).



Figure S12. Taxonomic abundance cluster heatmap hierarchical clustering of samples for the top 35 bacterial species of SCG-2 and SCG-3 as received and after 14 days of simulated storage in trays (SCG-2) and drums (SCG-3).