Original Research Paper

Changes in the Microbial Succession During Sewage Sludge Composting and its Correlation with Physico-Chemical Properties

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ABSTRACT

Sewage sludge composting is a process entailing a continuous succession of microorganisms. To understand the microbial mechanisms involved in sewage sludge composting, we performed an aerobic static composting of sewage sludge and sawdust (ratio = 3:1 m/m) in medium-scale bioreactor systems. The associated changes in physico-chemical parameters (i.e., temperature, organic matter, pH, ammonium nitrogen) were studied parallelly to those in the microbial (i.e., bacteria, fungi, archaea) succession. Additionally, we discussed correlations between these physico-chemical parameters and the microbial communities. The results showed that the pile temperature went through mesophilic phase, thermophilic phase, and cooling phase. The pile temperature reached a maximum of 78.68°C by day 3 and remained above 55°C for more than 6 days, complying with the harmless composting requirements. The organic matter content decreased gradually, the pH increased after a first decrease, and the NH_4^+-N content showed a consistent trend. The dominant bacteria during composting were *Ureibacillus*, *Bacillus*, *Sphaerobacter* and *Thermobifida*, while the dominant fungi were unclassified_f_*Trichocomaceae*, unclassified_d_*Eukaryota*, *Hypocrea* and *Thysanophora*; finally, the dominant archaea were *Methanobrevibacter*, *Methanosaeta*, *Methanobacterium* and unclassified_k_norank. The composting stages were characterized by different microbial compositions. The mesophilic phase presented a relatively uniform proportion of bacterial genera, while the thermophilic and cooling phases were dominated by *Ureibacillus* and *Bacillus*, respectively. The fungus unclassified_f_*Trichocomaceae* played a major role during the mesophilic, thermophilic, and cooling phases, while unclassified_d_*Eukaryota* played a major role during the mesophilic and thermophilic phases. For what concerns the archaea, *Methanobrevibacter* played a major role in the mesophilic, thermophilic, and cooling phases, *Methanosaeta* during the mesophilic and thermophilic phases, and *Methanobacterium* during the thermophilic and cooling phases. Additionally, the bacterium *Ureibacillus* and the archaea *Methanospirillum* were positively correlated with temperature, while the bacteria norank_Pem15, norank_JG30-KF-CM45, and the archaea *Methanosphaera* were negatively correlated with temperature. The fungi *Thysanophora*, unclassified_d_*Eukaryota*, and unclassified_p_*Ascomycota* were negatively correlated with pH. Moreover, the bacterium norank_c_1-20, the fungi *Trichosporon*, norank_o_*Saccharomycetales*, unclassified_o_*Pleosporales,* and the archaea *Methanosaeta*, *Methanomethylovorans* were positively correlated with organic matter. On the other hand, the bacteria *Bacillus*, *Thermobifida*, the fungus unclassified_f_*Trichocomaceae*, and the archaea *Methanobrevibacter* were negatively correlated with organic matter. Finally, the bacteria *Bacillus*, *Thermobifida*, the fungus unclassified_f_*Trichocomaceae*, and the archaea *Methanobrevibac*ter were positively correlated with ammonium nitrogen, while the bacterium norank_c_1-20, the fungi *Trichosporon*, norank_o_*Saccharomycetales*, unclassified_o_*Pleosporales*, and the archaea *Methanosaeta*, *Methanomethylovorans* were negatively correlated with ammonium nitrogen. This paper provides new solid bases to understand changes in microbial composition and their correlation with physico-chemical parameters during sewage sludge composting.

INTRODUCTION

Due to the high moisture content and biodegradability of sewage sludge (SS), its treatment is considered a pressing environmental challenge, especially in developing countries (Onwosi et al. 2017). Aerobic composting is an eco-friendly and inexpensive method to convert SS into a relatively steady humus, which can be used to improve soil structure, fertility, and quality (Han et al. 2019, Yuan et al. 2016). Composting is a biological process, through which aerobic microorganisms consume organic matter (OM) as food and convert it into CO_2 and H_2O, releasing heat (Bernal et al. 2009) and generating stabilized organic compounds (e.g., humic

compounds) (Jalili et al. 2019). Microorganisms (mainly bacteria, fungi, and archaea) play a key role during the SS composting process (Zhao et al. 2019). Bacteria and fungi are both functional communities in this process, although bacteria are more influential, due to their metabolic versatility. Some researchers have tried to evaluate the influence of bacterial, but not of fungal communities, on the composting performance (López-González et al. 2015). Overall, fungi occur in relatively low abundances during composting and their role in the process is considered minor compared to that of bacteria (Bonito et al. 2010); however, fungi tend to dominate during the maturation or mesophilic phase, perhaps due to lignin degradation and to the maturity of sludge composting (Amir et al. 2008). On the other hand, archaea are important participants to the aerobic composting process and play an important role in CH_4 production, although their dynamic change is still not well understood. Hence, the monitoring of bacterial, fungal, and archaea population structure dynamics can provide comprehensive information on the microbial community succession during SS composting.

This paper focuses on several aspects of the SS composting process: 1) dynamic changes in the physico-chemical parameters; 2) dynamic changes in the bacterial, fungal, and archaeal communities; 3) correlations between the physico-chemical parameters and the bacterial, fungal, and archaeal communities. The results of our study contribute to the understanding of microbial mechanisms during SS composting.

MATERIALS AND METHODS

Compost Materials and Environmental Design

Dewatered SS was collected from the Zhengzhou Wulongkou municipal wastewater treatment plant (China). The sawdust used for our experiment consisted of pine wood particles (1-2 mm). The SS and sawdust were used as raw materials and homogenized to obtain a mixture (ratio of 3:1 m/m, fresh weight), for a total weight of 132.5 kg. The initial

Table 1: Initial physico-chemical properties of the raw materials and of the mixture.

Parameters	SS	Sawdust	Mixture
pH	8.12	5.55	8.30
Moisture content	80.52%	8.23%	51.34%
Total carbon	28.11%	52.82%	35.79%
Total nitrogen	2.05%	0.41%	3.23%
C/N ratio	13.32	140.30	35.51

physico-chemical properties of the raw materials and of the mixture are shown in Table 1.

Composting System and Sampling

The SS aerobic composting was performed in a medium-scale (400 L) bioreactor, described in detail in one of our previous studies (Du et al. 2019). This study focused on the first fermentation stage of aerobic composting, which usually takes 10-12 days and indicated the microbial metabolic process. The sampling was done on days 1, 3, 5, 7, and 10 (after aerobic fermentation), so to include the composting mesophilic (sample 1d), thermophilic (samples 3d, 5d, 7d), and cooling (sample 10d) phases. Three samples of composting material were obtained from each pile at a depth of ~ 50 cm. Approximately 500 g of material was collected during each sampling and divided into two parts: one part was used for enzyme activity measurements, while the other part was homogenized and kept in a refrigerator at -80°C for subsequent DNA analysis (Du et al. 2019).

The diversity of bacterial, fungal, and archaeal communities during the composting process were calculated based on the microbial DNA extracted from each pile (on days 1, 3, 5, 7, and 10), using a FastDNA® SPIN Kit for Soil (Mpbio, USA) according to the manufacturer's protocols. The DNA was hence purified and its concentration was determined using a NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA). The V3-V4 hyper variable regions of the bacterial 16S rRNA gene were amplified with primers 338F (ACTCCTACGGGAGGCAGCAG) and 806R (GGACTACHVGGGTWTCTAAT), while the V5-V7 regions of the fungal 18S rRNA gene were amplified with primers SSU0817F (TTAGCATGGAATAATRRAATAGGA) and 1196R (TCTGGACCTGGTGAGTTTCC); finally, the archaeal gene were amplified with primers 524F10extF (5′-TGYCAGCCGCCGCGGTAA-3′) and Arch958RmodR (5′-YCCGGCGTTGAVTCCAATT-3′).

Analysis Methods

The moisture content (MC) of the raw materials and composting mixtures were determined by drying samples at 105°C for 24 h (Tiquia & Tam 1998). The OM content was determined instead by measuring the loss of dry solid mass after ignition at 550°C in a muffle furnace for 24 h (Awasthi et al. 2015). The change in NH_4^+-N content was determined according to the method used by Fang et al. (1999). The pH value was measured at a ratio of 1:5 (wet weight of composting sample/water volume), after shaking equilibration for ~30 min, using a combination pH meter (E-201-C, Lei-ci, Shanghai, China). The total carbon and nitrogen contents were instead detected using a Vario EL elemental analyser (Elemental Vario MICRO, Germany).

RESULTS AND DISCUSSION

Dynamic Changes in Physico-Chemical Parameters During Composting

Temperature: Temperature is one of the most important parameters for the composting process (Zhou 2016), since it reflects the degradation of organic components and is used to evaluate the composting efficiency (Wu et al. 2017). As shown in Fig. 1, the pile experienced three stages: a mesophilic phase, a thermophilic phase, and a cooling phase. Due to the decomposition of degradable components during the mesophilic phase (Guo et al. 2012), the pile temperature rapidly increased to over 50°C by day 2 and reached a maximum of 78.68°C by the day 3 (heating rate = 26.43 °C/day). During the thermophilic phase (i.e., ≥ 50°C), which lasted for 8 days, the temperature remained >55°C for more than 6 days; these high temperatures destroyed the pathogens (Sadef et al. 2014), complying with the harmless composting requirements (United States Environmental Protection Agency, 1994). On day 8 the pile entered the cooling phase, during which temperatures dropped from 65.04°C (day 8) to 41.19°C (day 10), at a rate of 11.9°C/day.

Organic matter (OM): Readily biodegradable OM gets degraded by microorganisms during composting into CO_2 and H_2O, generating heat and promoting the thermophilic phase (Petric et al. 2012), which results in a stable final product. The stability of a compost is determined in relation to the rate of OM decomposition (Xiao et al. 2009). As shown in the Fig. 2a, the OM content decreased continuously during the whole composting process, consistently with the results obtained by Liu et al. (2019). Between days 1-3, due to the

decomposition of easily degradable OM, its content significantly ($p < 0.05$) decreased from 84.42% (day 1) to 81.72% (day 3). Subsequently, the degradation rate of OM began to decrease, due to the almost complete decomposition of easily degradable OM and to the start of refractory OM (Lignin and Cellulose) degradation. Between days 3-10, the degradation rate of the OM decreased gradually, but its content did not decrease significantly (from 81.72% on day 3, to 77.44% on day 10).

NH_4^+-N: The change in NH_4^+-N content during composting reflects the nitrogen transformation process (Fang et al. 1999). As shown in Fig. 2b, the NH_4^+-N content increased during the whole composting process: from a minimum of 0.3 mg/g (day 1) to a maximum of 1.68 mg/g (day 10). The maximum rate of NH_4^+-N increase was between the fifth day to the seventh day of thermophilic phase, reflecting significant increases in the NH_4^+-N content of all samples ($p < 0.05$). NH_4^+-N contents increased due to the degradation of urea, uric acid, and proteins during the composting process. The results of our experiment are similar to those of Ren et al. (2010). In this study, the NH_4^+-N content increased during the composting process, reached its maximum at the end; these results were attributed to the occurrence of ammonification and to the particularly strong inhibition of nitrification at high temperatures (Thompson et al. 2004).

pH: The pH of the compost material tends to be directly related to the amount of NH_3 released as well as to the microbial activity (Spencer et al. 2013). As shown in the Fig. 2c, the pile pH first decreased and then increased during the composting process. The pH of the first day to the third day of compost showed a significant ($p < 0.05$) decreased from
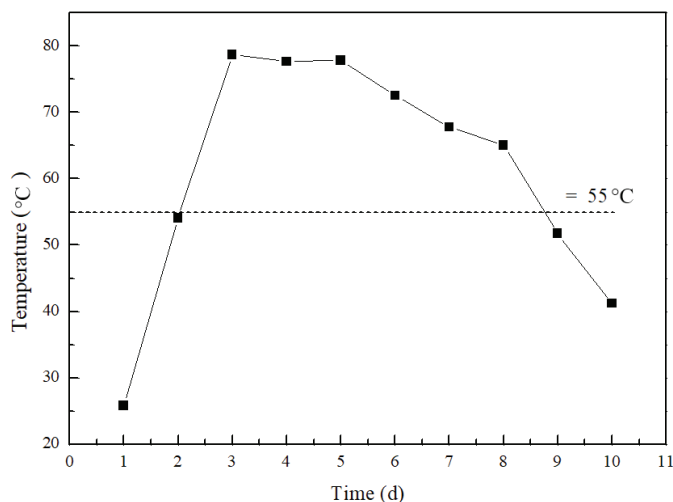

Fig. 1: Evolution of temperature during the composting process.

day 1 (8.3) to day 3 (8.13), and then fell to a minimum of 8.11 on day 5, due to the formation of volatile fatty acids (Gajalakshmi & Abbasi 2008). The release of ammonia during ammonification and the mineralization of organic nitrogen during composting caused an increase of the composting mixture pH (Wang et al. 2013). Between days 5-10 the pile pH increased significantly ($p < 0.05$), from 8.11 to 9, and then remained at alkaline levels (Hosseini et al. 2013).

Microbial Composition

The bacterial community dynamics at the genus level are shown in Fig. 3a. *Ureibacillus*, *Bacillus*, *Sphaerobacter*, and *Thermobifida* were overall the dominant genera within the bacterial community. On the first day of composting, the bacterial community was relatively homogeneous: the bacterial genera showed similar relative abundances. The thermophilic phase was dominated by *Ureibacillus* and *Bacillus*; however, while *Ureibacillus* decreased gradually, *Bacillus* increased gradually with time (Zhang et al. 2018). These two genera accounted for 46.4% and 2.2% of the bacterial community, respectively, on day 3 (early stage of the thermophilic phase). Between days 3-7 (late stage of the thermophilic phase), the proportion of *Ureibacillus* decreased to 8.8%, while that of *Bacillus* increased to 27.7%. The relative abundance of other bacteria did not change significantly during the thermophilic phase. On day 10 (cooling phase), the abundance of *Ureibacillus* and *Bacillus* decreased slightly (to 7.1% and 16.3%, respectively), while that of *Sphaerobacter* and *Thermobifida* increased significantly (from 0% and 0.7%, to 7% and 6.6%, respectively): *Ureibacillus*, *Bacillus* (Vieira et al. 2019), *Sphaerobacter*, and *Thermobifida* were the dominant bacterial general during the cooling phase.

Fungi are considered an important microbial group in composting materials, due to their superior ability in decomposing recalcitrant acid (Langarica-Fuentes et al. 2014a) and low-nitrogen substrates compared to bacteria (Langarica-Fuentes et al. 2014b). Unclassified_f_*Trichocomaceae*, unclassified_d_*Eukaryota*, *Hypocrea*, and *Thysanophora* represented the major fungal genera, whose dynamic changes are shown in Fig. 3b. Unclassified_f_*Trichocomaceae* was overall the most abundant fungus in our samples. Its relative abundance increased during the composting process (from 30.2% on day 1, to 90.8% on day 10). The relative abundances of unclassified_d_*Eukaryota* and *Thysanophora* accounted for a higher proportion during the mesophilic and thermophilic phase of composting. They reached 17.5% and 8.0% on day 5 (during the middle thermophilic phase), respectively; during the cooling phase they corresponded instead to only 0% and 1.4%, respectively. These results
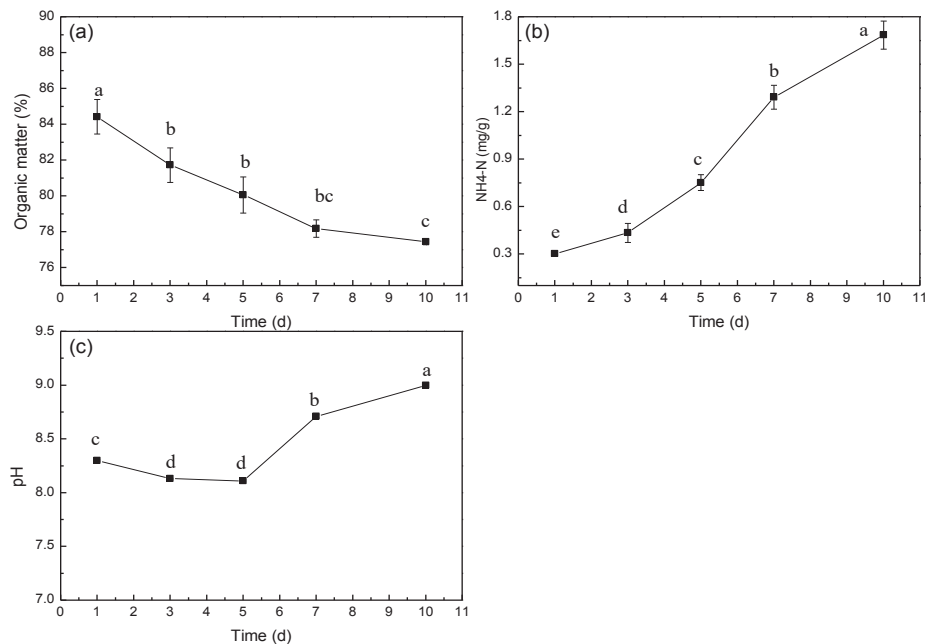

Fig. 2: Evolution of the physico-chemical parameters during the sludge composting process. Note：The letters indicate the occurrence of significant differences ($p < 0.05$) among samples. The same below.

indicate that unclassified_d_*Eukaryota* and *Thysanophora* were among the major fungal genera during the mesophilic and thermophilic phases. *Hypocrea* increased gradually from 5.2% (day 1) to 11.7% (day 7), reaching a minimum of 2.2% during the cooling phase: it also represented a major fungal genus during both the mesophilic and thermophilic phases.

The archaeal community composition is shown in Fig. 4c. Throughout the composting process, the archaeal communities were dominated by (from the most to the less abundant): *Methanobrevibacter*, *Methanosaeta*, *Methanobacterium*, and unclassified_k_norank. During the mesophilic phase, *Methanobrevibacter*, *Methanosaeta*, *Methanobacterium*, and unclassified_k_norank accounted for 30.0%, 44.7%, 7.3%, and 3.2% of the total archaeal community, respectively. Between days 2-10 the relative abundance of *Methanobrevibacter* rose gradually: from 20.3% (during the early period of the thermophilic phase) to 55.8% (during the cooling phase).

The temporal evolution of *Methanosaeta* was completely opposite to that of *Methanobrevibacter*: *Methanosaeta* gradually decreased between days 2-10: from 54.4% (during the early period of the thermophilic phase) to 4.0% (during the cooling phase). *Methanobacterium* gradually increased between days 2-7: from 7.0% (during the thermophilic phase) to 21.9% (during the late thermophilic phase); then, it slightly decreased to 14.6% (during the cooling phase). Unclassified_k_norank showed an increasing and then decreasing trend: increased from 3.2% (on day 1) to 12.7% (on day 7, during the middle of the thermophilic phase); then, it gradually decreased to 8.2% (during the cooling phase). *Methanosaeta* was most active during the mesophilic phase and the early stage of the thermophilic phase, while *Methanobrevibacter*, *Methanobacterium*, and unclassified_k_norank were more active during the middle and late stages of the thermophilic and cooling phases.
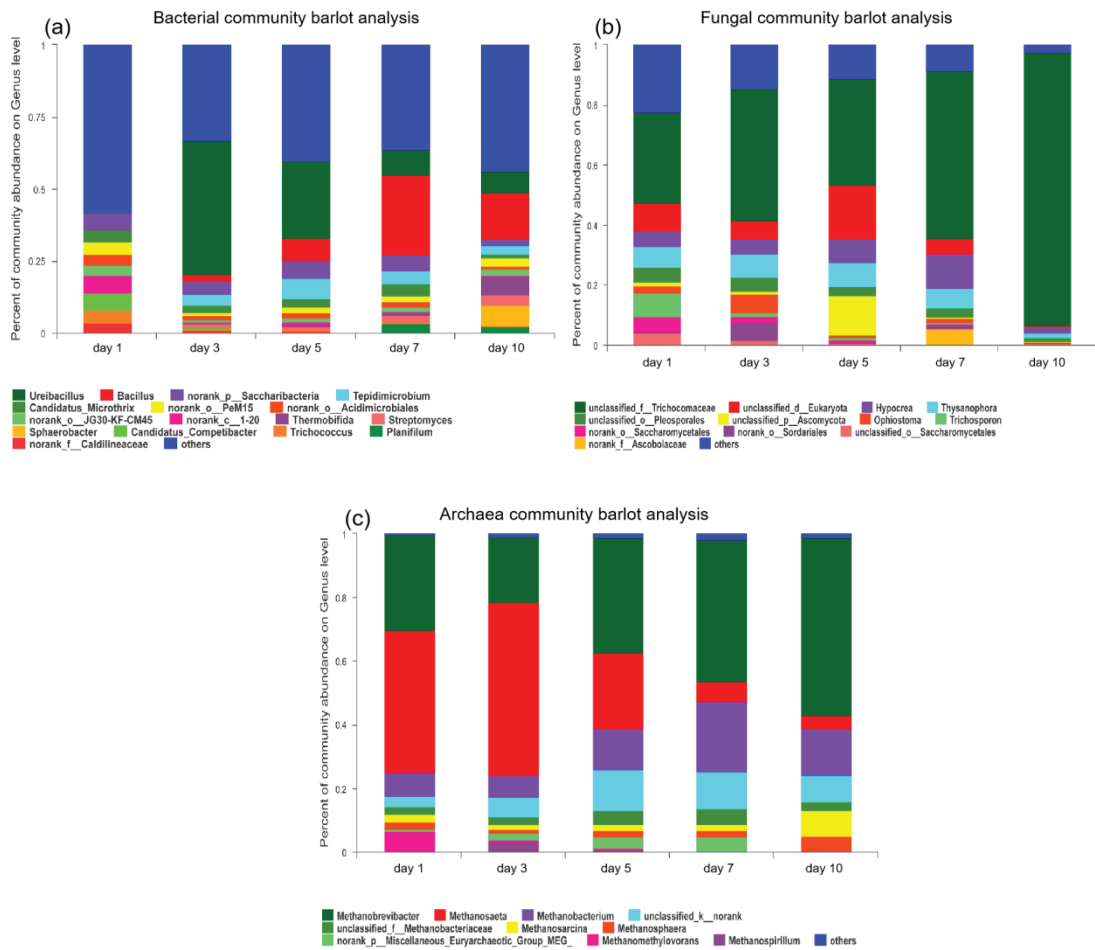

Fig. 3: Evolution of the microbial composition during sludge composting.

Correlation Between Physico-Chemical Parameters and Microorganisms

The diversity of the microbial community was closely correlated with the physico-chemical properties of the compost (Hosseini et al. 2013). A correlation heat map (Fig. 4a) showed that several physico-chemical parameters influenced the functional bacterial community. It is known that microbial activities can be enhanced by increasing temperatures (Yuan et al. 2016). In the studied bacterial communities, *Ureibacillus* (r = 1, *p* = 0) was significantly and positively correlated with temperature, while norank_Pem15 (r = -1, *p* = 0) and norank_JG30-KF-CM45 (r = -1, *p* = 0) were negatively correlated. Additionally, we observed that norank_c_1-20 (r = 0.9, *p* = 0.037) was significantly and positively correlated with OM, while *Bacillus* (r = -0.9, *p* = 0.037) and *Thermobifida* (r = -0.975, *p* = 0.005) were negatively correlated. Moreover, *Bacillus* (r = 0.9, *p* = 0.037) and *Thermobifida* (r = 0.975, *p* = 0.005) were significantly and positively correlated with

NH_4^+-N, while *norank_c_1-20* (r = -0.9, *p* = 0.037) was negatively correlated to NH_4^+-N.

Fungi have the ability to survive in extremely adverse habitats, due to their great physiological adaptability (Sarsaiya et al. 2018). Our correlation heat map (Fig. 4b) shows that several physico-chemical parameters should have influenced the functional fungal community. Firstly, *Thysanophora* (r = -1, *p* = 0), unclassified_d_*Eukaryota* (r = -0.9, *p* = 0.037), and unclassified_p_*Ascomycota* (r = -0.9, *p* = 0.037) significantly and negatively correlated with pH. Secondly, *Trichosporon* (r = -1, *p* = 0), norank_o_*Saccharomycetales* (r = -1, *p* = 0), and unclassified_o_*Pleosporales* (r = -1, *p* = 0) were significantly and negatively correlated with NH_4^+-N, while unclassified_f_*Trichocomaceae* (r = 0.9, *p* = 0.037) was positively correlated with NH_4^+-N. Thirdly, *Trichosporon* (r = 1, *p* = 0), norank_o_*Saccharomycetales* (r = 1, *p* = 0), and unclassified_o_*Pleosporales* (r = 1, *p* = 0) were significantly and positively correlated with OM,
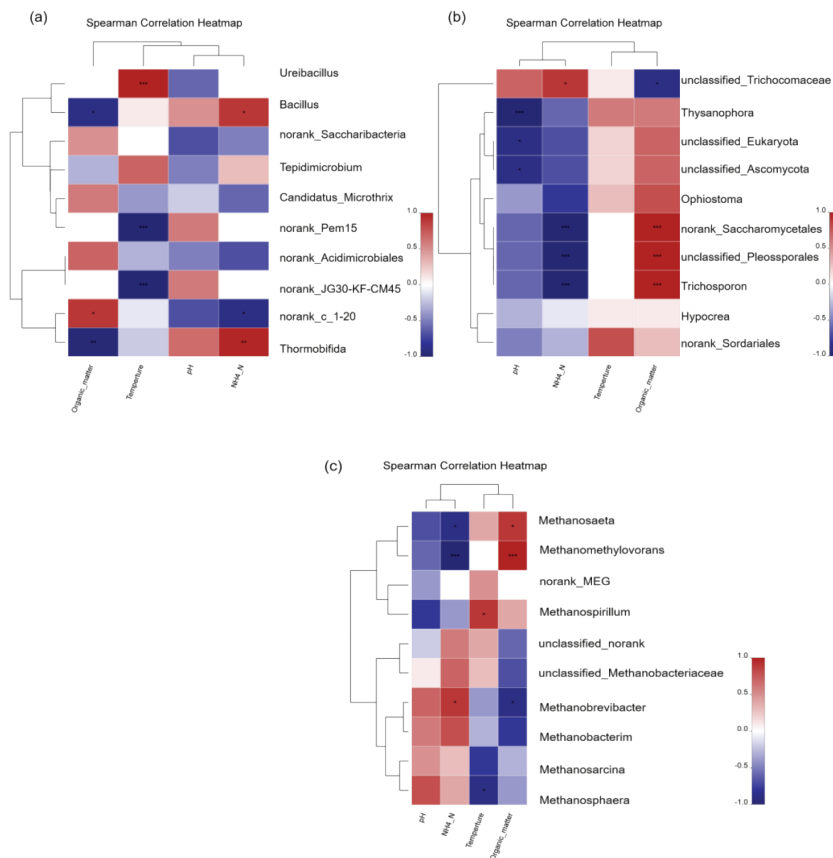

Fig. 4: Spearman correlation maps, showing the correlations between several physico-chemical parameters and the microbial community at the genus level.

while unclassified_f_*Trichocomaceae* (r = -0.9, *p* = 0.037) was negatively correlated with OM.

The archaeal community is known for being strongly influenced by environmental factors (e.g., temperature, pH, and various forms of nitrogen; Sun et al. 2019). Our correlation heat map (Fig. 4c) showed that several physico-chemical parameters should have influenced this functional archaeal community during the composting process. Firstly, *Methanobrevibacter* (r = 0.9, *p* = 0.037) was significantly and positively correlated with NH_4^+-N, while *Methanomethylovorans* (r = -1, *p* = 0) and *Methanosaeta* (r = -0.9, *p* = 0.037) were negatively correlated with NH_4^+-N. Secondly, *Methanosaeta* (r = 0.9, *p* = 0.037) and *Methanomethylovorans* (r = 1, *p* = 0) were significantly and positively correlated with OM, while *Methanobrevibacter* (r = -0.9, *p* = 0.037) was negatively correlated with OM. Thirdly, *Methanospirillum* (r = 0.9, *p* = 0.037) was significantly and positively correlated with temperature, while *Methanosphaera* (r = -0.9, *p* = 0.037) was negatively correlated with temperature.

CONCLUSION

The microbial succession during the SS composting process and its correlation with the analysed physico-chemical parameters are complex. The OM content of the composting material decreased gradually with time, while its pH increased after a first decrease, and the NH_4^+-N gradually increased. The composting stages were characterized by different microbial compositions; nevertheless, bacteria generally dominated the community, followed by fungi and, finally, archaea. The most abundant bacterial genera were *Ureibacillus*, *Bacillus*, *Sphaerobacter*, and *Thermobifida*. They showed different temporal evolutions; in particular, the proportion of *Ureibacillus* gradually decreased, while that of *Bacillus* gradually increased. The most abundant fungal genera were unclassified_f_*Trichocomaceae*, unclassified_d_*Eukaryota*, *Hypocrea*, and *Thysanophora*. The proportion of unclassified_f_*Trichocomaceae* showed a gradual increase with time. Finally, the most abundant archaeal genera were *Methanobrevibacter*, *Methanosaeta*, *Methanobacterium*, and unclassified_k_norank. Within this group, we noticed that the proportion of *Methanobrevibacter* gradually increased with time, while that of *Methanosaeta* gradually decreased. The bacterial communities were strongly correlated with temperature, while fungi were strongly and negatively correlated with pH; additionally, fungi and archaea were strongly correlated with OM and ammonium nitrogen. The results of this study provide new information about not only the structure and diversity of microbial communities during the SS composting process, but also about the interrelationships between microorganisms and various physico-chemical parameters. Overall, this information improves our understanding of microbial mechanisms during SS composting.

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