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1 **Regulating bacterial dynamics by lime addition to enhance kitchen waste**
2 **composting**

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15 **Abstract**

16 This study examined bacterial dynamics in response to lime addition to enhance
17 kitchen waste composting using modular network analysis. Bacterial communities
18 could be separated into three meta-modules corresponding to the mesophilic,
19 thermophilic, and mature stage of composting. Lime addition at 1% (wet weight)
20 suppressed acidogens and denitrifiers (e.g. *Lactobacillus* and *Acinetobacter*) at the
21 mesophilic stage to reduce greenhouse gas emissions. The matrix pH and temperature
22 were also increased by lime addition via hydrogen reaction to favor bacterial growth
23 and activity. Thus, thermophilic bacteria (e.g. *Thermoactinomycetaceae* and
24 *Planifilum*) were enriched with lime addition to facilitate lignocellulose
25 biodegradation for humus formation at the thermophilic stage. Further lime addition
26 to 1.5% reduced ammonia emission at the thermophilic stage via chemical fixation.
27 Moreover, lime inhibited denitrifiers but proliferated nitrifiers at the mature stage to
28 decrease nitrous oxide emission and enhance nitrate content, respectively. As such,
29 lime addition improved both biotic and abiotic composting performance.

30

31 **Keywords:** Kitchen waste composting, lime, modular network analysis, bacterial
32 community.

33

34 **1. Introduction**

35 Rapid economic development and population growth as well as heightening concern
36 over resource depletion and global warming have placed a spot light on food waste.
37 According to the Food and Agricultural Organization of the United Nations (FAO),
38 globally 1.3 billion tons of food waste is generated each year. The disposal of food
39 waste in landfills entailed many environmental consequences especially greenhouse
40 gas (GHG) emission from the degradation of organic materials. Progressive actions to
41 address the problem of food waste have been taken by authorities around the world.
42 Many European countries have banned the disposal of food waste by landfilling
43 (Nghiem et al., 2017). Australia has set a national target to half the amount of organic
44 waste in landfill by 2030. In April 2021, China has enacted new legislation to prevent
45 food wastage and foster a “resource conserving” society. It is also recognized that a
46 large fraction of food waste is unavoidable. This includes kitchen waste such as
47 banana peel, fruit husks, inedible animal products (e.g. skin, bone, and offal) from
48 food processing and meal preparation. Kitchen waste has a high moisture content
49 and is highly putrescible (Cheng et al., 2020). Thus, the excessive accumulation of
50 kitchen waste or improper disposal can severely deteriorate environmental issues by
51 releasing leachate, generating offensive odors, and proliferating pathogens (Xiong et
52 al., 2019). Kitchen waste, on the other hand, is a valuable resource that can be
53 transformed into biofertilisers and biofuel via several advanced biotechnologies to
54 achieve sustainable development (Yang et al., 2019).

55 Aerobic composting has been verified to effectively convert kitchen waste into high-
56 quality organic fertiliser. In general, kitchen waste composting alone cannot achieve
57 compost maturity, given its unfavorable physiochemical properties, including compact
58 structure, high moisture content, and low matrix pH (Yang et al., 2013). Thus, several
59 pragmatic approaches, such as adding bulking agents and improving operational
60 parameters, have been reported to improve kitchen waste composting from the
61 perspectives of humification and gaseous emissions (Ding et al., 2019; Xu et al.,
62 2021c). Despite their potential for composting performance, these strategies hardly

63 improve acidification characteristic of kitchen waste caused by anaerobic acidogens
64 during storage and transportation. The acidification of kitchen waste could inhibit the
65 metabolisms of aerobic microbes to favor the proliferation of anaerobes, thus,
66 delaying temperature increase and humification as well as exacerbating GHG and
67 malodour emissions during composting (Xu et al., 2021a). Nevertheless, to date, no
68 relevant studies have been dedicated to the development of countermeasures to further
69 ameliorate kitchen waste composting.

70 Lime, which is a calcium containing mineral composed primarily of calcium oxide
71 (CaO), has been widely implemented for acidic soil amelioration (Wang et al., 2021b).
72 Previous studies have evidenced that the addition of lime into acidic soil could
73 significantly increase crop yields and soil organic carbon stocks by enhancing soil pH
74 to optimize microbial diversity and its composition (Li et al., 2019b). In addition,
75 methanotrophic activities and plant nitrogen uptake could also be enhanced with lime
76 addition to control soil GHG emission (Wang et al., 2021b). On the other hand, lime
77 has also been employed in composting of livestock manure or sewage sludge to
78 immobilize toxic heavy metals, disinfect pathogens, and improve humification (Singh
79 & Kalamdhad, 2013; Chen et al., 2021). Singh and Kalamdhad (2013) reported that
80 lime addition decreased water soluble and plant-available heavy metals (including
81 cadmium and lead) to undetectable level during co-composting of cattle manure and
82 water hyacinth. Despite these advantages of lime amelioration, the performance and
83 mechanisms of lime addition to regulate physiochemical characteristics and bacterial
84 dynamics for advanced kitchen waste composting have not been systematically
85 elucidated.

86 High-throughput sequencing technology has been extensively applied to determine the
87 bacterial composition and succession in composting (Zhao et al., 2019). Previous
88 studies have observed remarkable bacterial shifts during composting due to bacterial
89 interactions to alter temperature and substrate components (Wei et al., 2018; Xu et al.,
90 2021c). Nevertheless, recent studies have concentrated mostly on the dominant taxa
91 during composting, which cannot fully reflect the interaction patterns in the bacteria

92 communities and their response to environmental factors (Liu et al., 2018; Wei et al.,
93 2018; Yin et al., 2019). As inspired by previous studies on soil microbial community
94 (Deng et al., 2012; Fernandez-Gonzalez et al., 2020), modular network analysis could
95 be considered as a pragmatic strategy to expand bacterial community analysis. Such
96 network gathers bacteria that have similar ecological functions into identical modules,
97 which then can be used to determine their responses to varying environmental
98 conditions. For instance, Jiang et al. (2015) used modular network analysis to reveal
99 that soil pH and total carbon could significantly enhance the interactions between
100 nematodes and ammonia oxidizers to improve soil nitrogen cycling. However, the
101 application of modular network analysis to decipher microbial dynamics in
102 composting is still limited.

103 This study aims to reveal the performance and mechanisms of lime addition to
104 regulate bacterial dynamics for advanced kitchen waste composting using modular
105 network analysis. Humification and gaseous emissions were determined to evaluate
106 composting performance. Modular network analysis was then conducted to decipher
107 the succession of bacteria communities and identify key functional bacteria for
108 lignocellulose degradation, humification and gaseous emissions. Results reported here
109 will provide valuable understanding on the performance of lime addition to improve
110 kitchen waste composting.

111 **2. Materials and methods**

112 *2.1 Composting materials*

113 Fresh kitchen waste was from a local solid waste collection station (Beijing, China).
114 The kitchen waste contained 40.2% vegetables, 28.7% fruits, 15.2% staple food, 5.1%
115 meat, 5.0% bones, and 5.8% unclassified materials based on their wet weight. The
116 unclassified fraction is likely to be a combination of putrescible materials such as
117 vegetables, fruits, and meats but could not be clearly identified due to rapid
118 degradation during collection, transportation and storage. Garden waste was provided
119 by a landscape company (Jiangsu, China) and used as the bulking agent for kitchen

120 waste composting. The garden waste was air-dried to achieve less than 5% moisture
 121 content and then mechanically cut to the length of 2 – 4 cm. Kitchen and garden
 122 wastes were co-composted at the wet weight ratio of 17:3. This ratio could effectively
 123 facilitate organic biodegradation and reduce gaseous emission to advance kitchen
 124 waste composting (Xu et al., 2021a). Key physiochemical characteristics of these
 125 composting materials were summarized in Table 1. Lime with more than 95% CaO
 126 content was used to amend composting performance.

127 **Table 1:** Key physiochemical characteristics of composting materials (mean value \pm
 128 standard deviation from triplicate measurements). Kitchen and garden wastes were
 129 mixed at the mass ratio of 17:3. # denotes parameters that were determined based on
 130 the dry mass.

Parameters	Kitchen waste	Garden waste	Mixture
pH	4.7 \pm 0.1	7.2 \pm 0.1	5.0 \pm 0.1
Electrical conductivity (mS/cm)	2.1 \pm 0.2	1.2 \pm 0.1	1.5 \pm 0.1
Moisture content (%)	72.8 \pm 3.4	4.9 \pm 1.2	66.8 \pm 1.7
Total carbon (%) #	37.6 \pm 0.8	41.2 \pm 0.3	38.5 \pm 0.2
Total nitrogen (%) #	2.4 \pm 0.1	0.56 \pm 0.1	1.64 \pm 0.1
Total sulphur (%) #	0.57 \pm 0.1	0.14 \pm 0.1	0.41 \pm 0.1
Carbon/nitrogen ratio #	15.6 \pm 0.3	73.5 \pm 0.1	24.0 \pm 0.2
Ammonium nitrogen (g·kg ⁻¹) #	1.6 \pm 0.3	0.33 \pm 0.1	1.39 \pm 0.1
Nitrate nitrogen (g·kg ⁻¹) #	1.3 \pm 0.3	0.12 \pm 0.1	1.12 \pm 0.2

131 2.2 Experimental system and protocols

132 A bench-scale composting system (consisting of multiple 60 L cylindrical composters,
 133 rod-shaped temperature sensors, and an automatic aeration device) was employed in
 134 this study. The details of this system have been described in our previous study (Xu et
 135 al., 2021c). Briefly, each composter with a detachable lid was made of double-layers
 136 stainless steel for heat conversation. Two holes designed in the detachable lid were
 137 used to insert temperature sensor and capture released gases from the composter for
 138 further measurement, respectively. All temperature sensors were wirelessly connected
 139 with a computer for temperature recording. The automatic aeration device was used to

140 provide a continuous aeration of $0.36 \text{ L kg}^{-1} \text{ dry mass min}^{-1}$ from the composter
141 bottom. To ensure homogenous air diffusion to composting piles, a stainless-steel
142 plate with evenly distributed holes was placed at the composter bottom.

143 Four treatments (denoted T1 to T4) were conducted in parallel under identical
144 condition over 5 weeks with the exception of lime addition dosage. “T1 (0%)” is the
145 control treatment without any lime addition. Lime was added to the composting
146 materials on dry mass basis at 0.5%, 1%, and 1.5% corresponding to “T2 (0.5%)”,
147 “T3 (1.0%)”, and “T4 (1.5%)”, respectively. Manual turning and then compost
148 sampling were conducted on day 3 and 7 in the first week and then once per week.
149 Fresh compost samples were preserved at $-80 \text{ }^{\circ}\text{C}$ for bacterial analysis. Moreover,
150 certain fresh samples were air-dried for subsequent analysis of physiochemical
151 properties, lignocellulose degradation, and humification characteristics. Released
152 gases from the composters were captured daily for analysing gaseous emissions.

153 *2.3 Analytic methods*

154 *2.3.1 Gaseous emissions and physiochemical properties*

155 Gaseous emissions were evaluated by monitoring carbon dioxide (CO_2), methane
156 (CH_4), nitrous oxide (N_2O), and ammonia (NH_3) in the off gas. CO_2 was measured by
157 a potable biogas detector (Biogas-5000, Geotech, UK). CH_4 and N_2O were sampled
158 using syringe and then quantified by a gas chromatograph (Beifen, China). NH_3 was
159 examined by 2% boric acid absorption and then titration against 0.1 M sulphuric acid.

160 Water and potassium chloride (KCl) extracts were obtained by completely mixing
161 fresh compost samples with deionized water and 2 M KCl at a mass ratio of 1:10 for
162 evaluating the physiochemical properties of composting materials, respectively. Water
163 extract was used to measure the matrix pH using a pH meter (OHAUS, China). A
164 segmented flow analyser (Technicon Auto Analyser system, Germany) was used to
165 quantify ammonium (NH_4^+), and nitrate (NO_3^-) contents in the composting piles using
166 KCl extract.

167 *2.3.2 Lignocellulose degradation and humification characteristics*

168 The air-dried compost samples were ground to powder to determine lignocellulose
169 composition and humification characteristics following methods previously reported
170 by Vansoest et al. (1991) and Wang et al. (2021a), respectively. For lignocellulose
171 composition analysis, powder samples were treated using neutral-detergent, followed
172 by acid detergent and 72% sulphuric acid to obtain the content of neutral-detergent
173 fibre (*NDF*, %), acid-detergent fibre (*ADF*, %), and acid-detergent lignin (*ADL*, %),
174 respectively. The content of lignocellulose (%) in composting materials was then
175 determined as:

$$176 \text{ Hemicellulose content} = NDF - ADF \quad (1)$$

$$177 \text{ Cellulose content} = ADF - ADL \quad (2)$$

$$178 \text{ Lignin content} = ADL - Ash \quad (3)$$

179 where *Ash* was the ash content of composting materials (%), which was measured at
180 the 550 °C in a muffle furnace for 5 h to remove organic matter as reported previously
181 by Zhang et al. (2020). Lignocellulose degradation rate (*R_d*, %) was defined as
182 follows:

$$183 R_d = \frac{m_0 - m_d}{m_0} \times 100\% \quad (4)$$

184 where *m₀* and *m_x* were the contents of lignocellulose at the beginning and a sampling
185 day (*d*) during composting, respectively.

186 Humic substances (HS) were extracted by blending powder samples with a solution of
187 sodium hydroxide (0.1 M) and sodium pyrophosphate (0.1 M) at a mass ratio of 1:20
188 and then 24 h shaking at 20 °C. The HS extract was adjusted to pH 1 by adding 6 M
189 hydrochloric acid to separate fulvic acid (FA) supernatant from humic acid (HA)
190 precipitate. The HA precipitate was then dissolved in 0.1 M sodium hydroxide to
191 obtain HA extract. HS, FA, and HA were quantified by measuring the total organic
192 carbon (TOC) of their extracts using a TOC analyzer (TOC-V_{CSH}, Shimadzu, Kyoto).
193 Ultraviolet-visible (UV-Vis) spectroscopy was also used to determine the specific
194 ultraviolet absorbance at 280 nm (SUVA₂₈₀) for humification characterization after the

195 HS extract was diluted to the TOC concentration of 10 mg/L. The SUVA₂₈₀ is a
196 representative spectroscopic index to evaluate HS structure in composting (Xu et al.,
197 2021b).

198 2.3.3 *Bacterial community analysis*

199 Genomic DNA materials were extracted from compost samples on day 0, 7, and 35
200 for microbial community analysis using a method previously reported by Li et al.
201 (2019a). Briefly, this method included genomic DNA extraction, polymerase chain
202 reaction amplification of V3 – V4 regions, and high-throughput sequencing on the
203 Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA). Raw sequencing data
204 were processed by Quantitative Insights into Microbial Ecology (QIIME 1.9.1). The
205 optimized sequences were then clustered into operational taxonomy units (OTU)
206 based on the 97% similarity level using the UPARSE pipeline. Ribosomal Database
207 Project Classifier was then employed for taxonomic classification. All raw sequencing
208 data were submitted to the Sequence Read Archive of the National Center for
209 Biotechnology Information (NCBI) with the accession number PRJNA740163.

210 2.3.4 *Statistical and network analysis*

211 The Random Matrix Theory (RMT) based molecular ecological network analysis
212 (MENA) was employed to construct modular network to decipher bacterial dynamics
213 in relation to lignocellulose degradation, humification, and gaseous emissions in
214 composting with and without lime addition. All network construction procedures
215 followed the developer's recommendations on the online pipeline. Only the minimum
216 number of members in each module was changed to 10 for modular network
217 construction to achieve better visualization and discussion under high OTU coverage (>
218 85%). Moreover, the relationships between the modules with physiochemical
219 properties, gaseous emissions, and humification were subsequently determined by the
220 Mantel test to calculate the Euclidean distance matrix and OTU significance matrix.

221 **3. Results and discussion**

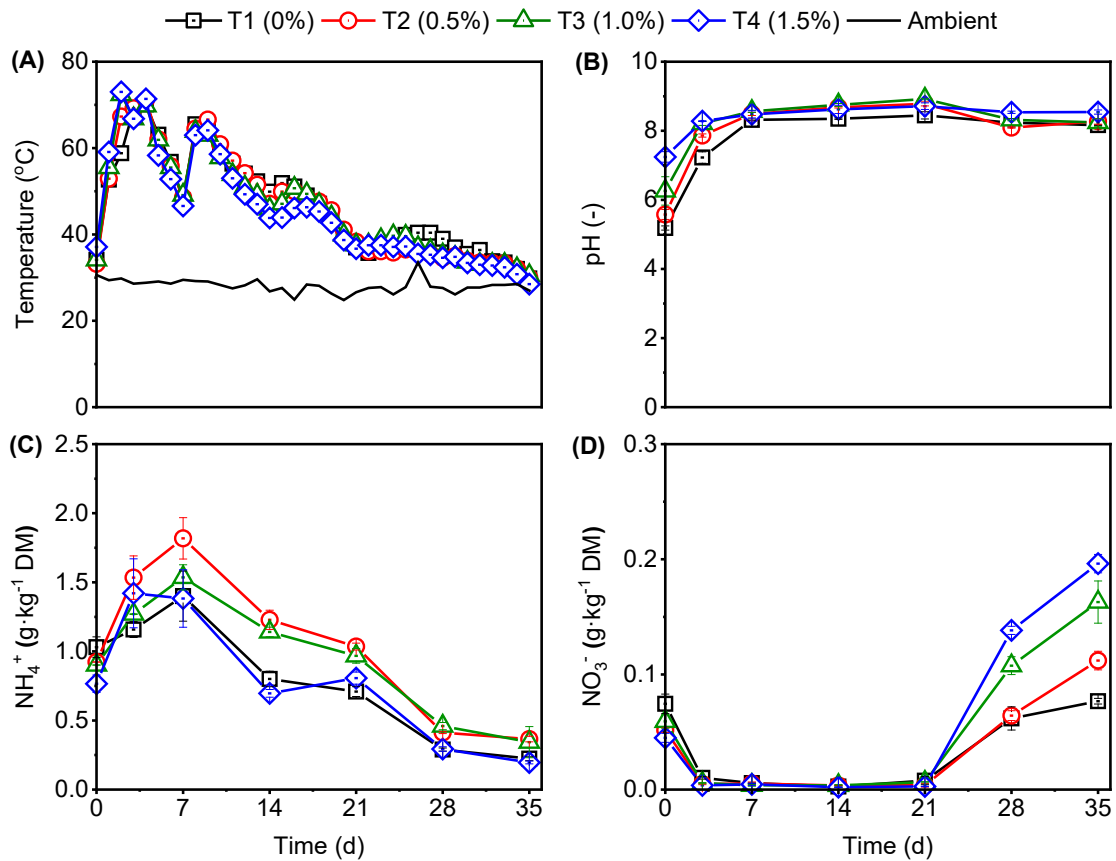
222 3.1 *Effects of lime addition on composting performance*

223 *3.1.1 Physiochemical characteristics*

224 All treatments showed the same temperature profile that was consistent with the
225 sequential mesophilic, thermophilic, and mature stages of composting (Fig. 1A). All
226 treatments achieved their temperature peaks on day 3. The composting temperature
227 then gradually decreased with the depletion of readily biodegradable organic matter.
228 Manual turning of composting piles could reallocate composting substrates and
229 reactivate aerobic microbes (Yang et al., 2019). Thus, the temperature of all
230 treatments increased again immediately after manual turning on day 7. From day 17
231 onward, the impact of manual turning on composting temperature was insignificant.
232 All treatments entered into the mature stage as indicated by the continuous and gentle
233 decrease in temperature to ambient condition.

234 Lime addition resulted in a clearly discernible impact on the temperature profile (Fig.
235 1A). The peak on day 3 was higher and the temperature remained higher with
236 increasing lime dosage in the mesophilic and thermophilic composting stages. Then,
237 treatments with lime addition showed lower temperature in the mature stages. In other
238 words, lime addition accelerated the composting process. The observed acceleration
239 could be attributed to the increased pH to improve the growth and activity of aerobic
240 microbes for organic biodegradation (Onwosi et al., 2017). In addition, CaO in lime
241 can react with water (moisture) to produce heat, thereby further promoting
242 temperature increase.

243 All treatments showed a similar pH profile during composting (Fig. 1B). Kitchen
244 waste is highly putrescible and can be hydrolyzed during transportation and storage to
245 produce organic acids, resulting in the low pH at the beginning of composting (Xu et
246 al., 2021a). During the composting process, organic acids are neutralized by NH₃,
247 which is produced from mesophilic and thermophilic degradation of nitrogen bearing
248 organic matter (Chan et al., 2016). It is noteworthy that the pH increase was more
249 significant with increasing lime dosage as lime is a highly alkaline material.



250

251 **Fig. 1:** (A) Temperature, (B) oxygen content, (C) matrix pH, (D) electrical
 252 conductivity, (E) ammonium nitrogen (NH_4^+), and (F) nitrate nitrogen (NO_3^-) during
 253 kitchen waste composting with different dosages of lime. Values shown in the
 254 parenthesis following each treatment were the addition proportion of lime on dry mass
 255 basis.

256 The NH_4^+ content in all treatments increased in the first week and decreased thereafter
 257 (Fig. 1C). The NH_4^+ increase was possibly due to the mineralization of organic
 258 nitrogen (e.g. protein and amino acids) and the thermophilic inhibition on nitrifiers
 259 (Wang et al., 2017). As the composting process progressed, the NH_4^+ content
 260 continuously declined from day 7 onward, given the continuous NH_3 emission at the
 261 thermophilic stage and the reactivated nitrification at the mature stage. Indeed, an
 262 increase in the NO_3^- content was observed for all treatments after manual turning on
 263 day 21 (Fig. 1D). Moreover, the increase in NO_3^- content was in the order of
 264 increasing lime dosage, possibly due to lime addition to improve the proliferation of
 265 nitrifiers (e.g. the family *Chitinophagaceae*).

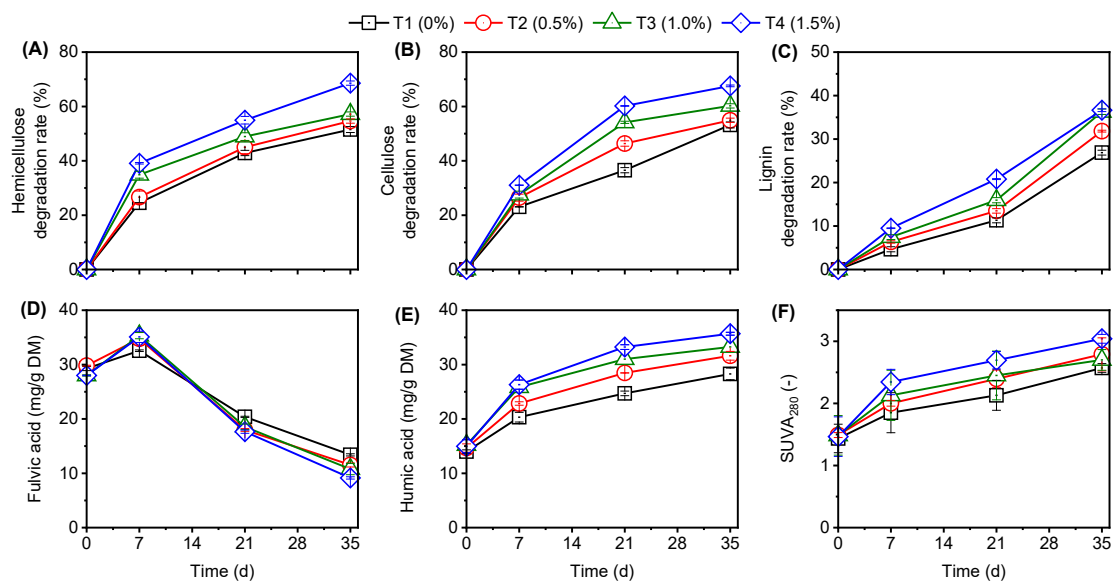
266 It is noteworthy that the increase in NH_4^+ between day 7 and 21 due to lime addition
267 was most significant at the low dosage of 0.5%. As the lime dosage increased, the
268 increase in NH_4^+ production became less significant (Fig. 1C). This result was
269 possibly due to the neutral reaction among $\text{Ca}(\text{OH})_2$ and acid compounds to produce
270 CaCl_2 , which could chemically fix NH_3 as $\text{CaCl}_2 \cdot 8\text{NH}_3$ by complexation
271 (Yamamoto et al., 1990).

272 3.1.2 Lignocellulose degradation and humification characteristics

273 Fig. 2 shows the impact of lime addition on lignocellulose degradation and
274 humification. Hemicellulose and cellulose degradation occurred mostly during the
275 thermophilic stage (Fig. 2A-B), whereas lignin degradation occurred most
276 significantly during the mature stage. Compared to refractory lignin, hemicellulose
277 and cellulose have more simple structure, thus, they can be easily biodegraded by
278 thermophilic microbes in composting (Wei et al., 2019). An improvement in the
279 hemicellulose, cellulose, and lignin degradation was observed for all treatments in the
280 order of increasing lime addition (Fig. A-C). For example, the degradation of
281 lignocellulose (i.e. hemicellulose, cellulose, and lignin) increased from 4.1% to 26.7%
282 as lime content increased from 0.5% to 1.5%. This elevation could be ascribed to the
283 improved matrix pH to facilitate the proliferation of functional microbes (e.g.
284 thermophilic bacteria) for lignocellulose biodegradation (Zhu et al., 2021).

285 All treatments exhibited an increment and then decrease profiles in the FA content
286 during composting (Fig. 2D). The initial increment was possibly due to the
287 degradation of lignocellulose to form humus precursors and thus HS (i.e. HA and FA)
288 (Zhang et al., 2019). However, FA could be employed as carbon resource for
289 microbial metabolisms or polymerized to form stable HA with high aromatic carbon
290 content (Duan et al., 2019). Thus, a continuous increase in HA content and SUVA_{280}
291 value was observed for all treatments during composting (Fig. 2E&F). Compared to
292 the T1 treatment, the other treatments with lime addition notably improved the
293 humification as indicated by their higher HA contents and SUVA_{280} value. It has been
294 reported that the lime addition could accelerate the hydrolysis of C-H bonds in

295 lignocellulose to produce humus precursors during composting (Cai et al., 2019).



296

297 **Fig. 2:** Variations in the degradation rates of (A) hemicellulose, (B) cellulose, and (C)
298 lignin as well as humification indexes, including (D) fulvic acid (FA), (E) humic acid
299 (HA), and (F) SUVA₂₈₀ during kitchen waste composting with different dosages of
300 lime.

301 3.1.3 Gaseous emissions

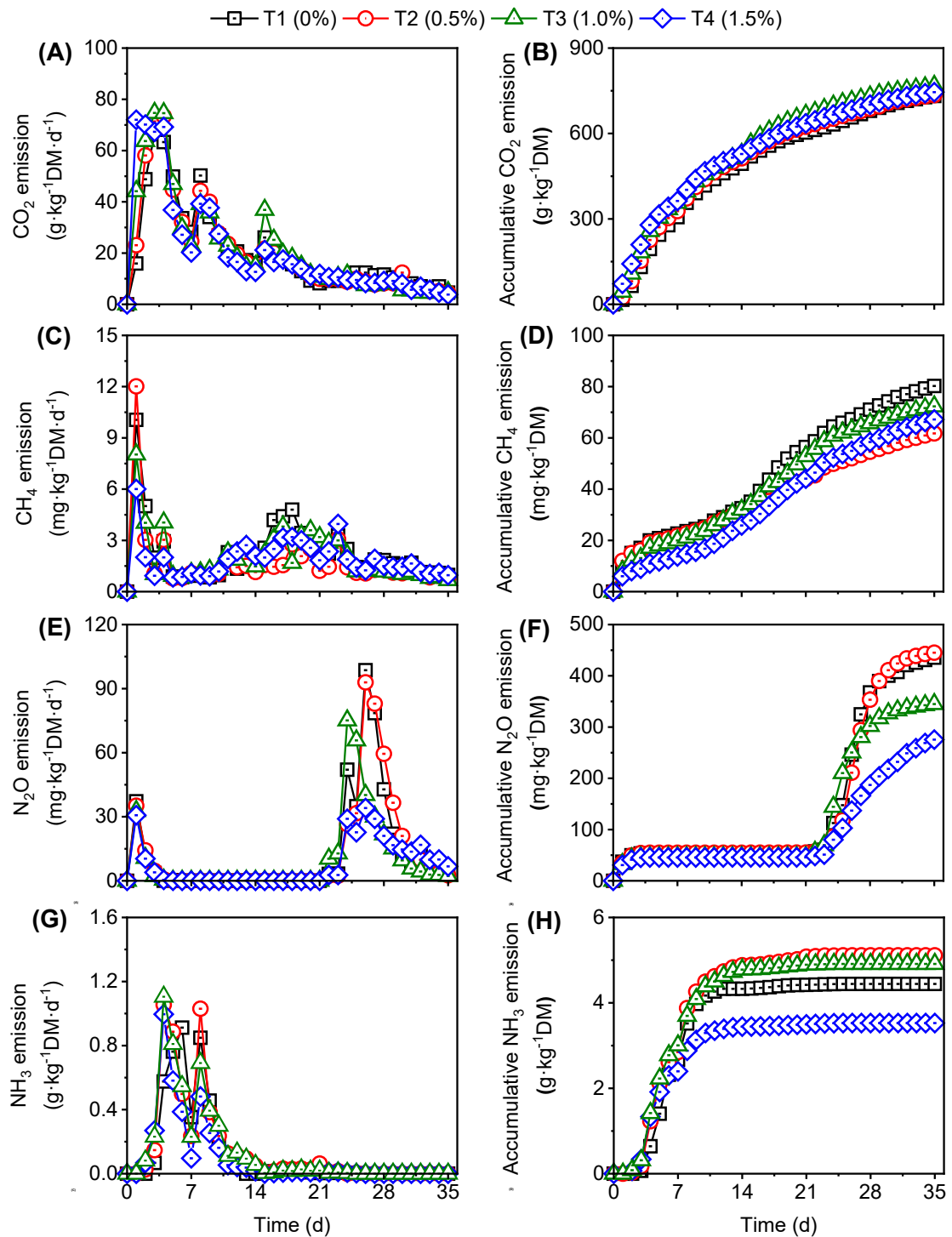
302 All treatments showed a similar CO₂ emission profile (Fig. 3A). CO₂ emission
303 occurred mostly during the thermophilic stage due to organic mineralization. T2 – T4
304 treatments with lime addition had higher CO₂ emission in the initial stage and lower
305 CO₂ emission at later stages. Thus, the accumulative CO₂ emissions of all treatments
306 were similar at the end (Fig. 3B). This result could be attributed to the presence of
307 neutral reaction between Ca(OH)₂ and CO₂ to generate CaCO₃ precipitation to
308 promote carbon fixation in composting substrates.

309 CH₄ emission occurred mostly at the mesophilic and initial mature stages for all
310 treatments (Fig. 3C). It has been reported that acidogenic and methanogenic bacteria
311 could proliferate during the storage and transportation of kitchen waste due to its high
312 moisture and organic contents (Xu et al., 2021a). Thus, all treatments exhibited a
313 dramatic CH₄ emission at the beginning of composting. Given the inhibition of high
314 temperature on methanogens and continuous aeration (Wen et al., 2021), the CH₄

315 emission of all treatments rapidly decreased to a negligible level from day 2 onward.
316 With temperature reduction to mature stage after manual turning on day 14, the CH₄
317 emission emerged again for all treatments. Over the whole composting, the addition
318 of lime slightly alleviated the accumulative CH₄ emission (Fig. 3D), possibly due to
319 the increase matrix pH to facilitate the activities and growth of methanotrophs (Wang
320 et al., 2021b).

321 N₂O emission occurred significantly during the mesophilic and mature stages for all
322 treatments (Fig. 3E). The initial N₂O emission could be ascribed to the occurrence of
323 anaerobic denitrifiers, such as the genus *Acinetobacter* and *Pseudomonas* that could
324 transformed NO₃⁻ to nitrite (NO₂⁻) and then N₂O (Song et al., 2020; Guo et al., 2021).
325 With temperature increase to the thermophilic stage, most denitrifiers could be
326 constricted to remarkably reduce N₂O emission (Yang et al., 2020). Given the
327 temperature decrease to recover the activity of denitrifiers (Xu et al., 2021b), a more
328 dramatic N₂O emission re-occurred for all treatments from day 21 onward. It is noted
329 that both T3 and T4 treatments with lime addition higher than 1% could effectively
330 reduce the N₂O accumulative emission by 26.3% (Fig. 3F). Such reduction was
331 probably related to lime addition to inhibit the activities of denitrifiers and thus reduce
332 NO₃⁻ availability (McMillan et al., 2016), as indicated by the increased NO₃⁻ content
333 with an enhancement in lime dosage by the conclusion of composting (Fig. 1D).

334 All treatments reached to their peaks of NH₃ emission at the thermophilic stage (Fig.
335 3G). As reported previously, organic nitrogen could be mineralized to NH₄⁺ and then
336 NH₃ under high temperature and pH (Pagans et al., 2006). Given its improved organic
337 mineralization and high temperature, the T2 and T3 treatments had a higher NH₃
338 accumulative emission in comparison with the T1 treatment (Fig. 3H). However,
339 compared to the T1 treatment without lime addition, the T4 treatment with lime
340 dosage at 1.5% reduced NH₃ emission by 25.9%, possibly owing to lime regulation of
341 composting materials to drive ammonia fixation as CaCl₂·8NH₃ as discussed above.



342

343 **Fig. 3:** (A) Daily CO₂ emission, (B) accumulative CO₂ emission, (C) daily CH₄
 344 emission, (D) accumulative CH₄ emission, (E) daily N₂O emission, (F) accumulative
 345 N₂O emission, (G) daily NH₃ emission, and (H) accumulative NH₃ emission during
 346 kitchen waste composting with different dosages of lime.

347 *3.2 Bacterial co-occurrence network analysis*

348 3.2.1 Modular co-occurrence network analysis

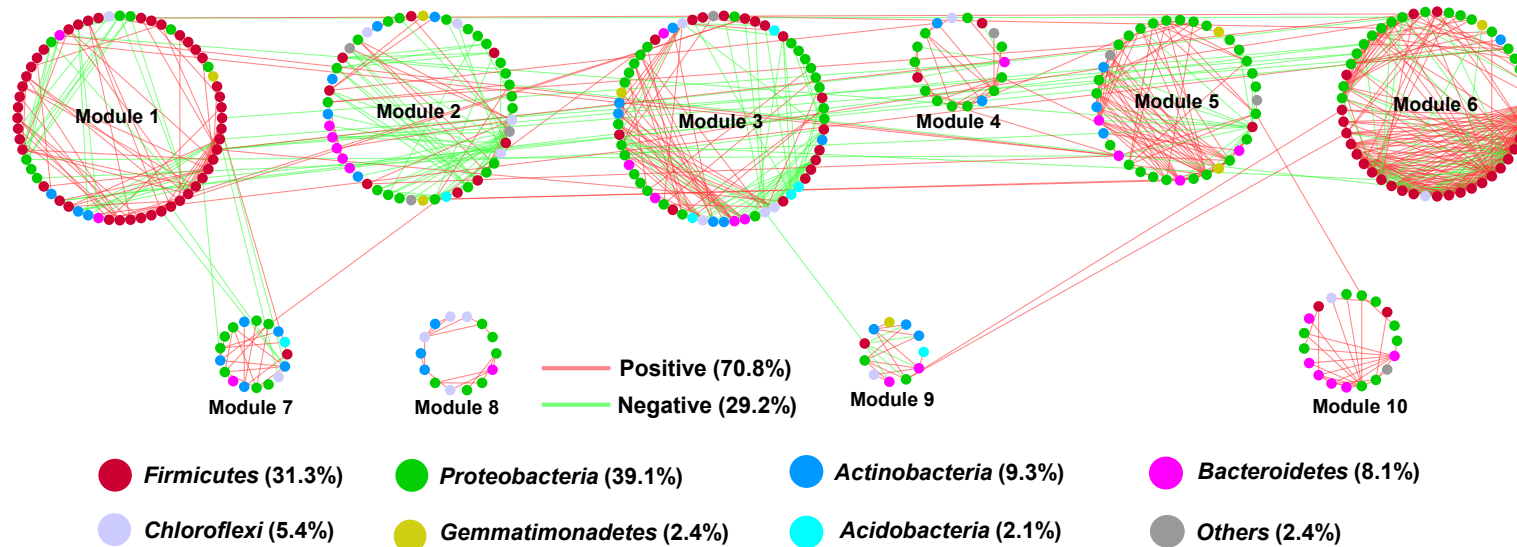
349 The random matrix theory (RMT)-based co-occurrence network analysis was
350 performed to modularize bacterial community for deciphering the underlying effects
351 of lime addition on bacteria dynamics during composting (Fig.4 & Table 2). The
352 constructed network has all fundamental topological characteristics, including scale-
353 free, small-world, modularity. Specifically, the connectivity degree of all nodes in the
354 network had an excellent fitting effect with the power-law model ($R^2 = 0.92$) to
355 exhibit scale-free properties. Moreover, average clustering coefficient, path distance,
356 and modularity of the empirical network were significantly higher than that of its
357 corresponding random network under identical nodes and links, indicating its small-
358 world behavior and modularity structure. As such, further analysis could be conducted
359 given these qualified topological properties of the constructed network.

360 The OTU nodes in the network mainly affiliated to the phylum *Proteobacteria*,
361 *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, and *Chloroflexi* (Fig. 4), which have been
362 identified as dominant contributors to drive composting process (Wei et al., 2018; Yin
363 et al., 2019). Furthermore, the number of positive links was much more than that of
364 negative links, suggesting the predominance of syntrophic and mutual relationships in
365 the bacterial community during composting (Bello et al., 2020). On the other hand,
366 the OTUs detected more than half of all samples were modularized using the greedy
367 modularity optimization method. OTUs in each module were densely connected
368 among one another and commonly possessed similar ecological functions or
369 characteristics (Kong et al., 2019). Thus, each constituted module in the network can
370 be regarded as a functional ecological niche to perform relevant activities at the
371 different stage of composting.

372

373 **Table 2:** Major topological properties of empirical and random molecular ecological networks (MENs) of bacterial community in composting.

R ² of power law	Empirical network					Random network			
	Total nodes	Total links	Average degree	Average clustering coefficient	Average path distance	Modularity	Average clustering coefficient	Average path distance	Modularity
0.92	407	922	4.53	0.23	6.54	0.78	0.026 ± 0.005	3.82 ± 0.04	0.45 ± 0.01



378 3.2.2 Relationships between key modules and their major members

379 Eigengene network analysis was conducted to determine the relationships between
380 key modules and thus unveiled higher-order organization in the constructed network
381 (Fig. 5). All module eigengenes could explain 56% – 76% of total variation in the
382 relative abundance of OTUs during composting. Such results indicate that these
383 eigengenes could effectively represent the module profiles for eigengene network
384 analysis. As shown in Fig. 5A, all modules were clustered into three meta-modules to
385 display a higher-order organization based on their eigengene correlations. The three
386 meta-modules mainly contained the representative OTUs at the mesophilic,
387 thermophilic, and mature stages during composting, respectively (Fig. 5B – D).

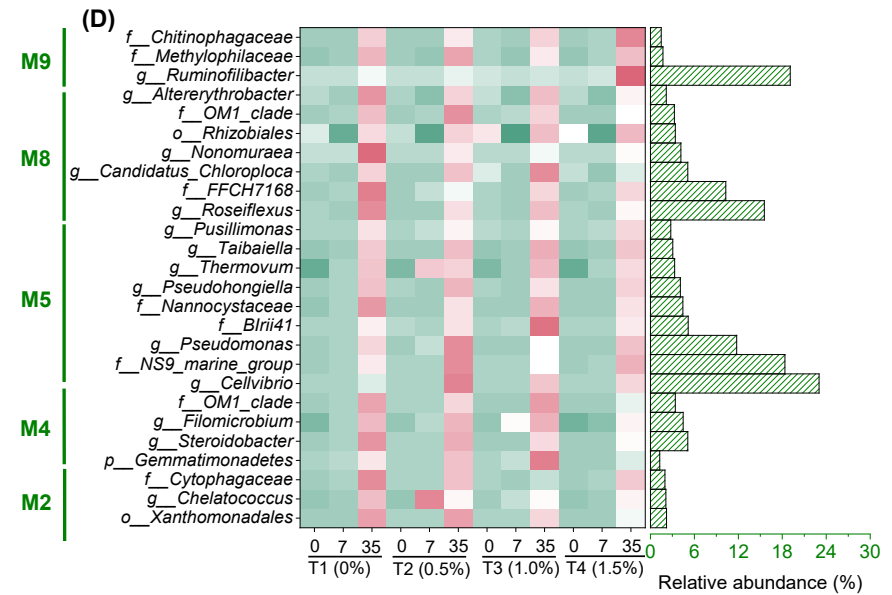
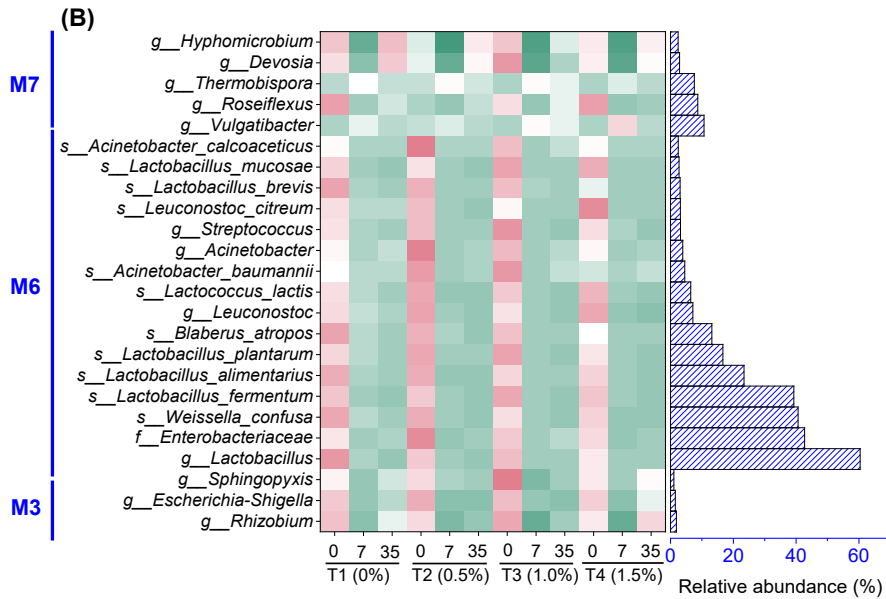
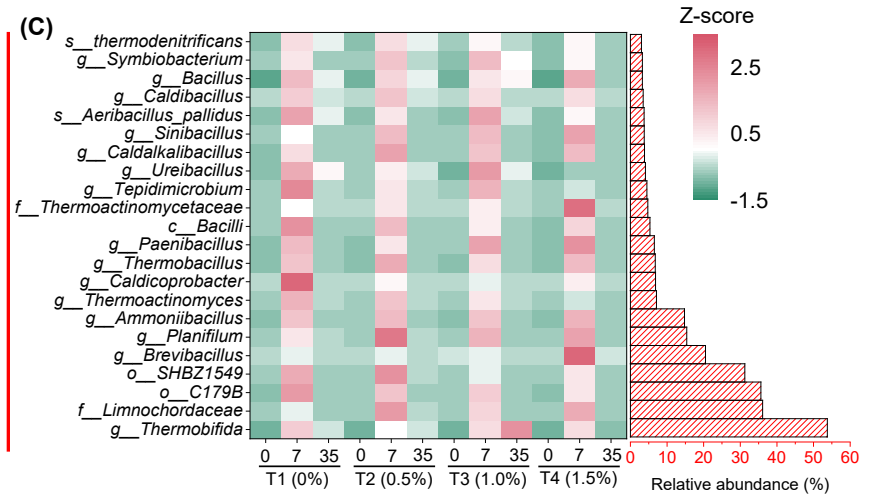
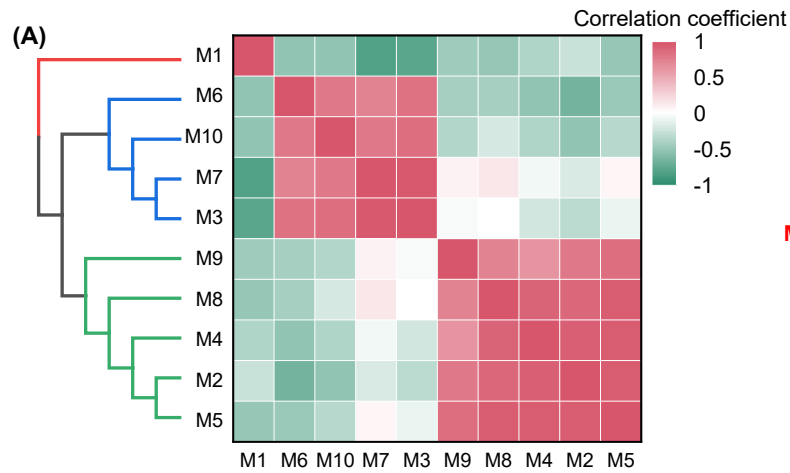
388 Module 3, 6, 7, and 10 constructed the first meta-module, whose members mainly
389 occurred at the mesophilic stage (Fig. 5B). Given their extremely low relative
390 abundance ($< 0.2\%$), all members of module 10 were not visualized for further
391 analysis. The first meta-module was dominated by the anaerobic acidogens, mainly
392 including members belonged to the genus *Lactobacillus* (e.g. the species
393 *Lactobacillus fermentum*, *Lactobacillus alimentarius*, and *Lactobacillus plantarum*),
394 and the species *Lactococcus lactis*, possibly due to their proliferation during the
395 storage and transportation of kitchen waste. These acidogens could produce massive
396 organic acids to contribute to the low matrix pH and thus CH_4 production (Xu et al.,
397 2021c). Moreover, several denitrifiers, such as the genus *Hyphomicrobium*, and
398 *Acinetobacter* as well as its affiliated species, also considerably occurred to use nitrite
399 and nitrate as electron acceptor to trigger N_2O emission at the mesophilic stage (Song
400 et al., 2020). On the other hand, kitchen waste storage promoted the breeding of
401 pathogenic bacteria, including the family *Enterobacteriaceae*, the genus *Escherichia*-
402 *Shigella*, and the species *Weissella confuse* (Aberkane et al., 2017; Colavecchio et al.,
403 2017). It is noteworthy that the relative abundance of most bacteria in this meta-
404 module obviously decreased when lime dosage increased to 1.0%. Indeed, it has been
405 reported that lime addition could effectively inactivate microbes through damaging

406 their outer membrane and nucleic acid before composting (Hijikata et al., 2016). Thus,
407 such inactivation mechanism could be another contributor for the relatively lower
408 emission of CH₄ and N₂O at the mesophilic stage of the T3 and T4 treatments with
409 higher lime addition.

410 As composting temperature increased to the thermophilic stage, most thermophilic
411 bacteria became predominant to enrich in module 1 as the second meta-module (Fig.
412 5C). Almost all of these thermophilic bacteria subordinated to the phylum *Firmicutes*
413 that were thermotolerant and could be responsible for the biodegradation of organic
414 substances, such as lignocellulose and protein (Liu et al., 2018). Compared to the T1
415 treatment, the other treatments with lime addition remarkably increased the relative
416 abundance of the family *Limnochordaceae* and *Thermoactinomycetaceae*, as well as
417 the genus *Brevibacillus*, *Paenibacillus*, *Planifilum*, and *Sinibacillus*. It has been
418 reported that the family *Thermoactinomycetaceae* could secrete dehydrogenase and
419 polyphenol oxidase to decompose cellulose and soluble lignin at the thermophilic
420 stage of composting (Ke et al., 2010; Arab et al., 2017). Furthermore, the genus
421 *Planifilum*, *Sinibacillus*, and *Paenibacillus* could biodegrade macromolecular organic
422 substances (e.g. lignocellulose) into micromolecular humus precursors for further
423 humus polymerization (Zhu et al., 2021). As such, lime addition improved the
424 lignocellulose degradation efficiencies and humification at the thermophilic stage.
425 Despite its higher relative abundance of thermophilic bacteria for organic
426 biodegradation, the T4 treatment exhibited a lower NH₃ emission throughout
427 composting in comparison with other treatments. This result further verified that
428 abiotic process (i.e. chemical reactors between CaCl₂ and NH₃) could be a dominant
429 pathway to reduce NH₃ emission.

430 The main bacteria at the mature stage were distributed into module 2, module 4,
431 module 5, module 8, and module 9 (Fig. 5D), all of which formed the third meta-
432 module and involved in the biodegradation of refractory compounds and
433 denitrification (Maeda et al., 2018; Xu et al., 2021b). The relative abundance of the

434 order *Xanthomonadales*, the family *Cytophagaceae*, the genus *Steroidobacter*,
435 *Roseiflexus*, and *Nonomuraea* in this meta-module gradually declined in response to
436 an increment in the lime contents. Of these declined bacteria, the order
437 *Xanthomonadales* and the genus *Steroidobacter* have been identified as denitrifiers
438 (Maeda et al., 2018), which were probably inhibited under high lime content
439 circumstance (> 6 tonne lime ha^{-1}) (Suzuki et al., 2021; Wang et al., 2021b). Thus,
440 relatively low N_2O emission was observed for the T3 and T4 treatments with lime
441 addition after the thermophilic stage. Moreover, lime addition also facilitated the
442 proliferation of the family *Chitinophagaceae*, the genus *Cellvibrio*, *Pseudomonas*, and
443 *Ruminofilibacter*. These bacteria genera could transform persistent lignocellulose into
444 humus precursors (Yin et al., 2019). Moreover, the family *Chitinophagaceae* as
445 heterotrophic ammonia oxidizing bacteria could promote the NO_3^- formation (Wang et
446 al., 2020). These results demonstrate that lime amendment could effectively enrich
447 functional bacteria to enhance humification and NO_3^- content and meanwhile restrict
448 acidogens and denitrifiers to reduce GHG emissions, thereby improving compost
449 quality.



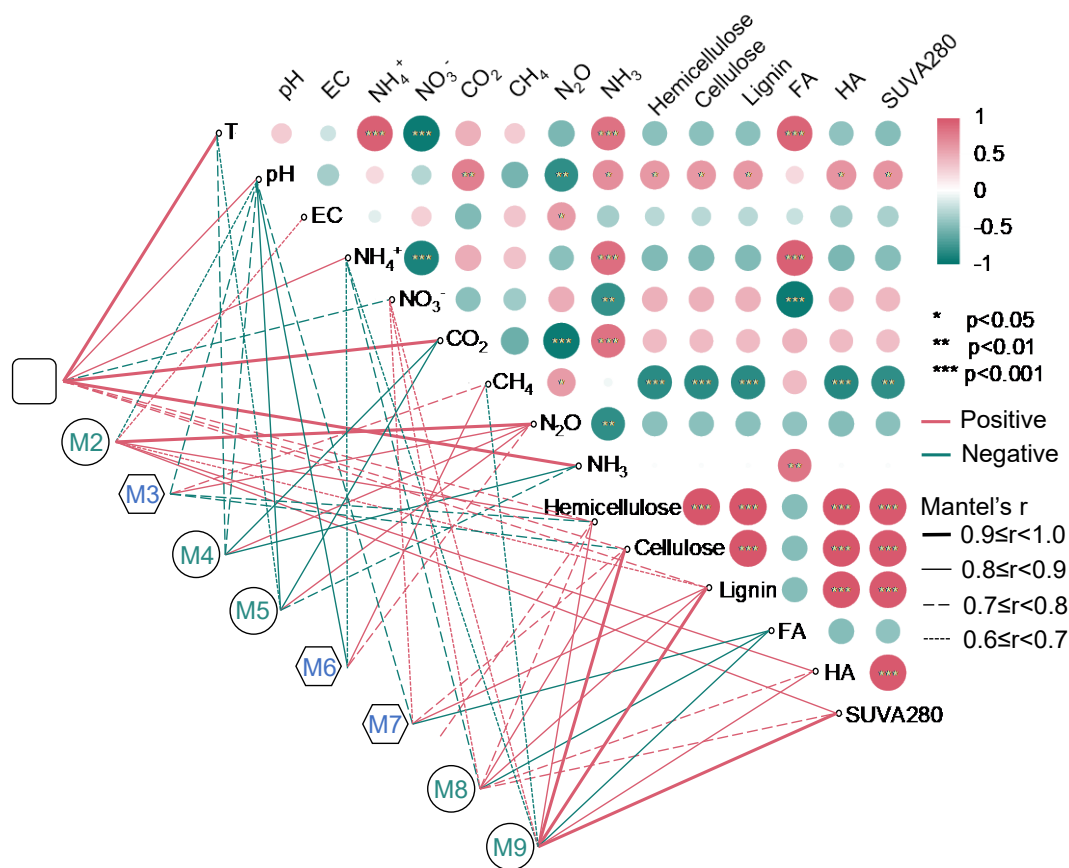
451 **Fig. 5:** Relationships between key modules (A) and their major members (relative abundance > 1%) at the OTU level (B – D) in the co-
452 occurrence network. In the Fig. 5A, the left part shows the hierarchical clustering based on the Pearson correlations among module eigengenes
453 and the right heatmap is their coefficient values. In Fig. 5B – D, the color key represents OTU as standard score (z-score).

454 3.2.3 *Correlations between main modules with physiochemical properties, gaseous*
455 *emissions and humification*

456 The relationships between main modules with physiochemical properties, gaseous
457 emissions, and humification were established by the Mantel test to further reveal the
458 improved composting performance in response to lime addition (Fig. 6). In the first
459 meta-module, module 3 and 6 were significantly positive with CH₄ and N₂O
460 emissions, but negative with matrix pH. As discussed above, most members in both
461 modules were acidogens, methanogens, and denitrifiers, which readily resulted in
462 matrix acidification to reduce matrix pH and produced massive GHGs under oxygen
463 deficiency condition. Similarly, module 7 also exhibited an adverse relationship with
464 the matrix pH in composting. These results suggest that adding lime could increase
465 the matrix pH to inhibit the activities and growth of anaerobic bacteria at the
466 mesophilic stage, thus reducing GHG emission at the beginning of composting.
467 Indeed, a negative association was observed between the matrix pH with the CH₄ and
468 N₂O emissions.

469 Module 1 had a significantly positive correlation with temperature, pH, NH₄⁺, CO₂,
470 NH₃, and lignocellulose degradation (Fig. 6), possibly due to its enrichment of
471 thermophilic bacteria for rapid organic biodegradation and mineralization at the
472 thermophilic stage (Ke et al., 2010; Arab et al., 2017; Liu et al., 2018). It is noted that
473 the correlation coefficients of lignocellulose degradation ($R^2 < 0.80$) were relatively
474 lower than that of other parameters ($R^2 > 0.80$). This result could be ascribed to the
475 fact that organic mineralization for the production of NH₄⁺, CO₂, and NH₃ mainly
476 appeared at the thermophilic stage while the lignocellulose biodegradation was a
477 continuous process over composting. The significantly positive correlations were also
478 observed for matrix pH and CO₂, NH₃, lignocellulose degradation, and humification.
479 Moreover, adding lime could enhance the relative abundance of thermophilic bacteria,
480 such as the family *Thermoactinomycetaceae* and the genus *Brevibacillus*, as discussed
481 above. Thus, the lime amelioration was probably caused by increased pH to

482 strengthen the proliferation of thermophilic bacteria for lignocellulose decomposition
 483 and humification at the thermophilic stage.



484

485 **Fig. 6:** Correlation between network modules with physiochemical properties,
 486 gaseous emissions and humification. Pairwise comparisons of physiochemical
 487 properties, gaseous emissions and humification are shown in the heatmap with a color
 488 gradient to represent Spearman's correlation coefficients. Statistically significant
 489 correlations between network modules with physiochemical properties, gaseous
 490 emissions and humification determined by the Mantel test are shown in the lower-left.
 491 Edge width corresponds to the Mantel's coefficients. Red and green lines represent
 492 positive and negative interactions, respectively.

493 0

494 4. Conclusion

495 Results from this study demonstrate that lime could regulate both biotic and abiotic

496 processes to improve kitchen waste composting. Modular network analysis
497 deciphered that acidogens and denitrifiers as dominant bacteria in the mesophilic
498 stage were suppressed by lime to reduce GHG emission. Lime addition also enhanced
499 matrix pH and temperature via hydrogen reaction to proliferate thermophilic bacteria
500 (e.g. *Thermoactinomyces* and *Planifilum*) for organic biodegradation and then
501 humification. In the mature stage, lime inhibited denitrifiers but enriched nitrifiers to
502 reduce N₂O emission and facilitate NO₃⁻ formation, respectively. Moreover, lime
503 addition at 1.5% exhibited chemical fixation to reduce NH₃ emission.

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