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1 **Assessment of the use of compost stability as an indicator of alkane and**
2 **aromatic hydrocarbon degrader abundance in green waste composting**
3 **materials and finished composts for soil bioremediation application**

4

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ABSTRACT

Green waste composting materials and finished composts were collected from different commercial ex situ composting sites all treating source segregated green waste feedstocks. Stability of each material was determined using the standard ORG0020 dynamic respiration test. To assess whether stability could be used as an indicator for the potential suitability of green waste composting materials and finished composts as amendments for soil bioremediation, comparison was made with alkane and aromatic hydrocarbon degrader abundance determined using a quantitative PCR (qPCR) approach. Specifically, primers targeting *alkB* and, polyaromatic hydrocarbon ring-hydroxylating dioxygenases genes (PAH-RHD) of Gram positive (GP) and Gram negative (GN) populations were used for qPCR analysis. The results showed no direct correlation between compost stability and gene abundance. Further, increase in *alkB* gene abundance was not linked to PAH-RHD gene abundance. The results support the use of qPCR as a tool for screening organic amendments on a site by site basis for soil bioremediation treatment.

KEYWORDS: Compost; stability; bioremediation; alkane; total petroleum hydrocarbons

51 1. INTRODUCTION

52 The active 'composting' of soils contaminated with (poly)aromatic
53 hydrocarbons (PAHs) and alkanes in combination with significant quantities of
54 composting feedstock materials has for a time received attention as an effective
55 bioremediation treatment approach (Antizar-Ladislao et al., 2004; Antizar-Ladislao et
56 al., 2007; Sasek et al., 2003; Semple et al., 2001; Tran et al., 2018). By comparison,
57 the addition of smaller quantities of compost to contaminated soil has been less
58 widely studied (Semple et al., 2001) with reporting of full scale (>1000 m³)
59 application scarcer still and lacking comparative controls due to live project economic
60 and time pressures (Aspray, 2006).

61 Known benefits of compost (or other organic residue) addition to
62 contaminated soils include improved porosity, pH, oxygen diffusion (Semple et al.,
63 2001) and contaminant desorption (Wu et al., 2013). Composts are also generally
64 rich in nutrients such as phosphorus, nitrogen, and carbohydrates which are
65 potentially important for achieving optimal activity of pollutant degraders indigenous
66 to contaminated soils (Komilis & Timotheatou, 2011; Sarkar et al., 2005; Roldán-
67 Martín et al., 2007). A further benefit of composts is their potential in augmenting
68 soils with populations of microorganisms which may degrade organic contaminants
69 present (Scelza et al., 2007).

70 A few examples of the use of 'mature' compost for soil bioremediation
71 application can be found in the literature. For example, Gomez & Sartaj (2013) used
72 'mature' compost for the bioremediation of total petroleum hydrocarbon (TPH)
73 contaminated soil in cold conditions. The authors concluded that the addition of
74 mature compost along with inoculation of a microbial consortium was an effective
75 approach. Similarly, Sayara et al. (2010) used agricultural soil spiked with PAH

76 contamination, testing five different composts with varying stabilities. These authors
77 found the more stable composts, with higher humic acid content, were more effective
78 at PAH removal than less stable composts. Finally, Wallisch et al., (2014) compared
79 two composts of differing maturity. Although compost addition influenced soil
80 microbial community composition, and *alkB* gene abundance and diversity, there
81 was no enhancement in alkane degradation relative to the control (soil without
82 compost amendment) at the end of the experiment. Unfortunately, with the exception
83 of Sayara et al., (2010) the above studies lack important information on the
84 characteristics of the composts used. Further, where composts have been compared
85 these have been largely limited to a maximum of two composts from different
86 composting sites processing very different feedstock types.

87 In many instances, alkane and PAH contaminants are commonly found
88 together in soils with remediation targets for both TPH fractions and total or specific
89 PAH compounds. The objective of this work was to assess whether compost stability
90 could be used as an indicator of alkane and aromatic hydrocarbon degrader
91 abundance in green waste composting materials and finished composts for soil
92 bioremediation application. To avoid potential issues of pathogen microorganisms as
93 previously highlighted (Wallisch et al., 2014), our focus was on samples post-
94 sanitisation (considered here as 'composting material') and finished composts.
95 Compost stability was measured using the ORG0020 dynamic respiration test
96 (Aspray et al., 2015) and hydrocarbon degrader abundance assessed using a
97 quantitative polymerase chain reaction (qPCR) approach and focusing on genes
98 relevant to both alkane and PAH contaminants.

99

100 2. MATERIALS AND METHODS

101 *2.1. Compost samples*

102 Green waste composting materials and finished composts were collected from
103 four Scottish PAS100 certified sites (numbers 1-4) during this work. Green waste
104 composting materials were 3-6 weeks post-sanitisation, whereas, finished composts
105 were taken 10 or more weeks post-sanitisation and post-screening. Samples were
106 collected in February and May reflecting winter and early spring feedstocks, as well
107 as, June and July reflecting late spring feedstocks. All sites were ex-situ windrow
108 processes taking household and commercial green waste (GW) only feedstocks.

109 As green waste composting materials contained oversize material to aid
110 aerobic degradation, these samples were screened to 20 mm prior to
111 characterisation and testing. Finished composts were either already screened onsite
112 to 0-20 mm by the composting site operator as part of the full-scale process or
113 screened to this grade in the laboratory following sample recovery.

114

115 *2.2. Sample physicochemical characterisation*

116 Physicochemical characterisation of compost samples included dry matter
117 (DM) and laboratory compacted bulk density as per BS EN 13040:2007, electrical
118 conductivity (EC) as per BS EN 13038:2000 (with the exception of using unfiltered
119 extracts), pH as per BS EN 13037:2011 and organic matter (OM) as per BS EN
120 13039:2000.

121

122 *2.2. Compost stability determination*

123 Compost stability was determined using the ORG0020 dynamic respiration
124 test and setup as previously described (Guillen Ferrari et al., 2017).

125

126 *2.3. Genomic DNA extraction*

127 Genomic DNA was extracted from green waste composting materials and
128 finished compost samples in duplicate (0.25 g fresh weight of compost) using the
129 PowerSoil DNA Isolation Kit (MoBIO Laboratories, Inc., USA) according to the
130 manufacturer's protocol. Duplicate sample extracts were 'pooled' and DNA quantified
131 spectrophotometrically using a NanoDrop 2000 instrument (Thermo Scientific).

132

133 *2.4 Quantitative PCR*

134 Quantitative PCR (qPCR) was carried out on an Applied Biosystems Step
135 One instrument using the primer pairs listed in Table 1.

136

137 Table 1. qPCR primer pairs used

Primer name	Primer sequence (5'-3')	Reference
alkB F	AAY ACI GCI CAY GAR CTI GGI CAY AA	Kloos et al., 2006
alkB R	GCR TGR TGR TCI GAR TGI CGY TG	
PAH-RHD α GN F	GAG ATG CAT ACC ACG TKG GTT GGA	Cebren et al., 2008
PAH-RHD α GN R	AGC TGT TGT TCG GGA AGA YWG TGC MGT T	
PAH-RHD α GP F	CGG CGC CGA CAA YTT YGT NGG	Cebren et al., 2008
PAH-RHD α GP R	GGG GAA CAC GGT GCC RTG DAT RAA	

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139 The qPCR reactions were performed in duplicate for each sample with the AB
140 software quality control (QC) check used to accept/reject replicate homogeneity.
141 Reactions were performed in 20 μ l volumes containing; 10 μ l PerfeCTa SYBR®
142 green ROC (Quantabio), 2 μ l each of forward and reverse primer (concentrations as
143 previously reported, see references in Table 1), 0.8 μ l of 20 mg ml⁻¹ bovine serum
144 albumin (Roche), 3.2 μ l nuclease-free water (Ambion®) and 2 μ l of extracted sample
145 DNA (section 2.3), standard DNA or nuclease-free water in the case of negative

146 controls. Further quality controls included verification of melt curves and gel
147 electrophoresis to confirm single amplification products with all three primer sets and
148 samples.

149 Standards for qPCR were prepared by extracting DNA from *Pseudomonas*
150 *putida* strain PG (9816, NCIMB Ltd, Aberdeen UK) and *Rhodococcus* sp. strain
151 MJL100 (12038, NCIMB Ltd, Aberdeen, UK) following growth of monocultures under
152 aseptic conditions using QIAamp DNA Mini Kit (Qiagen, Germany) according to the
153 manufacturer's protocol. Amplification of single products was verified by standard
154 endpoint PCR and gel electrophoresis approaches. The products were cleaned
155 using the Wizard SV Gel and PCR Clean-Up System (Promega Corporation, USA)
156 according with manufacturer's protocol.

157

158 *2.5 Culture based microbial population abundance*

159 Replicated sample aliquots (1 g fresh weight) were suspended in 9 ml of
160 0.85% (w/v) NaCl and shaken for one min. Ten-fold dilutions were made of each
161 replicate in 0.85 % NaCl and spread on Actinomycete Isolation Agar (Fluka-Sigma
162 Aldrich) and *Pseudomonas* Isolation agar (Fluka-Sigma Aldrich). Plates were
163 incubated in the dark at 25 °C for 48 h. Dilutions giving 30-300 colonies were
164 counted and results reported as colony forming units (CFU) per gram dry weight
165 using the previously determined sample moisture content.

166

167 *2.6 Statistical analysis*

168 Pearson correlation coefficient analysis was carried out in R version 3.4.0.
169 Non-metric multidimensional scaling (NMDS) was performed with the 'metaMDS'
170 function of the 'vegan' package in R version 3.4.0. using Bray–Curtis similarity

171 indices. Environmental variables were fitted to the NMDS ordinations as vectors with
172 the 'envfit' function of the 'vegan' package (Oksanen *et al.*, 2018). The NMDS plot
173 was generated with the ggplot2 library in R. Significant compost parameters ($p \leq$
174 0.05) were plotted onto the graph as arrows. Image editing was performed in
175 Inkscape (version 0.91).

176

177 3. RESULTS AND DISCUSSION

178 *3.1 Compost stability and sample characterisation*

179 Stability of green waste composting materials and finished composts was
180 determined in triplicate for each sample using the ORG0020 dynamic respiration test
181 (Guillen Ferrari *et al.*, 2017). Composting materials and finished composts, all from
182 green waste only composting sites (numbers 1-4), were found to have varying levels
183 of stability (Table 2); ranging from active composting material (sample K), just below
184 the current BSI PAS100:2018 limit of $16 \text{ mg CO}_2 \text{ g}^{-1} \text{ OM d}^{-1}$, to very stable finished
185 composts (samples B, H and L) with less than $3 \text{ mg CO}_2 \text{ g}^{-1} \text{ OM d}^{-1}$ activity. The six
186 finished composts studied here were all below $6 \text{ mg CO}_2 \text{ g}^{-1} \text{ OM d}^{-1}$. Where green
187 waste composting material and finished compost samples were collected from a site
188 at the same time, the composting material was in all cases less stable (i.e. more
189 active) than its finished compost counterpart. For example, sample D (composting
190 material) was more active than sample E (finished compost).

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195 Table 2. Compost site, sample month and status and physicochemical characterisation

Sample ID	Site number	Sample month	Sample Status	Stability (mg CO ₂ g ⁻¹ OM d ⁻¹) ^b	EC (mS cm ⁻¹)	DM (%) ^a	OM (%) ^a	pH	BD (g ml ⁻¹) ^b
B	1	May	Finished compost	2.70 (0.22)	0.92	43.9	43.6	7.4	0.52 (0.01)
D	2	Feb	Composting material	7.96 (0.04)	0.81	36.2	44.1	8.1	0.56 (<0.01)
E	2	Feb	Finished compost	2.39 (0.31)	1.15	42.9	33.7	8.4	0.68 (0.01)
I	3	June	Composting material	6.50 (0.84)	0.30	48.2	41.3	7.1	0.38 (0.02)
H	3	June	Finished compost	3.25 (0.07)	1.16	49.5	46.2	7.9	0.46 (<0.01)
J	4	June	Finished compost	3.62 (0.10)	1.56	54.1	33.9	8.7	0.59 (<0.01)
K	2	July	Composting material	14.69 (1.77)	1.28	43.9	31.6	8.5	0.57 (0.01)
L	2	July	Finished compost	2.11 (0.48)	1.18	51.0	33.9	7.2	0.61 (0.02)
M	1	July	Finished compost	5.67 (0.40)	0.90	63.5	39.2	7.5	0.49 (0.01)

196 BD – bulk density; DM – dry matter; EC – electrical conductivity; OM – organic matter

197 ^a n=2; ^b n=3; values in parenthesis are standard deviation of triplication values to demonstrate precision for different samples

198 Despite taking samples from different commercial composting sites, key
199 measured parameters support the fact that these sites were processing similar green
200 waste feedstocks (supporting our research objective). Specifically, EC and OM fell
201 within narrow ranges of 0.33 – 1.56 and 31.6 – 46.2 respectively. This contrasts with
202 the previous study by Sayara et al., (2010) where five composts had a much wider
203 EC range (4.91 – 7.13) and slightly wider OM range (44.48 – 61.63). Interestingly, in
204 the work by Sayara et al., (2010) the samples with lower OM content (closer to our
205 samples) were more effective at PAH removal than those with higher OM content.

206 For pH, green waste composting materials and finished composts tested here
207 were all neutral and so unlikely to support neutralisation of acidic contaminated soils
208 as previously suggested (Kashner & Miltner, 2016). In addition, we have previously
209 found that soils contaminated with hydrocarbons from brownfield sites in the UK may
210 in fact be neutral or even slightly alkaline (Aspray et al., 2007; Aspray et al., 2008).
211 Further, as composts should on most commercial projects typically be added in low
212 concentrations, to ensure they are appropriate for end use application, pH
213 adjustment is unlikely to be a significant driver for using green waste composting
214 materials and finished composts in practice.

215

216 *3.2 Relationship between degrader gene abundance and stability*

217 As previously stated, hydrocarbon contaminated soils are often contaminated
218 with both alkane and aromatic hydrocarbons (the latter including but not limited to
219 PAHs). Despite this, studies on compost bioremediation to date have focused on
220 either alkane (Wallish et al., 2014) or PAH (Sayara et al., 2001; Wu et al., 2013)
221 contaminants in isolation. In these previous studies, soils have been amended with
222 composting materials or finished composts and contaminant removal monitored

223 compared to unamended controls. However, limitations of these previous studies are
224 the low number of compost samples tested and lack of compost characterisation.
225 The complexity of these tests typically leads to the use of spiked rather than real
226 contaminated soil which further impacts on their direct relevance. Therefore, here we
227 decided on a novel approach of assessing the green waste composting materials
228 and finished composts themselves individually for this application. The main
229 objective of this research was to assess whether stability would be a good indicator
230 of the suitability of green waste composting materials and finished composts for
231 bioaugmentation of soils contaminated with alkanes and/or PAHs.

232 For alkane degrader abundance, the *alkB* primer set developed by Kloos et al.
233 (2006) and as used in the Wallish et al. (2014) study was chosen. The use of this
234 primer set was further supported by Jurelevicius et al., (2013) who found it had the
235 greatest coverage (48.8%) when tested alongside other *alkB* primer sets on known
236 alkane degrading strains. In all nine of our samples, *alkB* gene abundance was
237 greater than the corresponding PAH gene abundance (Table 3). This supports the
238 fact that the ability to degrade alkanes is widely distributed in bacteria in terrestrial
239 environments.

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253 Table 3. Degradative gene abundance in compost samples with paired samples
 254 indicated

Sample ID	Site number	'Paired' samples	Sample Status	Gene copies (g ⁻¹ compost _{dry weight})		
				<i>alkB</i>	PAH RHD GP	PAH RHD GN
B	1		Finished compost	7.47 x 10 ⁷	2.43 x 10 ⁷	1.79 x 10 ⁶
D	2	Y	Composting material	3.13 x 10 ⁸	3.34 x 10 ⁷	2.34 x 10 ⁶
E	2		Finished compost	8.34 x 10 ⁷	3.39 x 10 ⁷	1.75 x 10 ⁶
I	3	Y	Composting material	1.18 x 10 ⁸	1.14 x 10 ⁷	6.57 x 10 ⁵
H	3		Finished compost	1.35 x 10 ⁸	5.10 x 10 ⁶	3.75 x 10 ⁵
J	4		Composting material	8.52 x 10 ⁷	3.02 x 10 ⁶	6.21 x 10 ⁵
K	2	Y	Composting material	3.97 x 10 ⁶	1.04 x 10 ⁶	4.32 x 10 ⁵
L	2		Finished compost	3.31 x 10 ⁷	4.20 x 10 ⁶	3.57 x 10 ⁵
M	1		Finished compost	7.11 x 10 ⁷	1.87 x 10 ⁶	1.66 x 10 ⁵

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 256 Comparing *alkB* gene abundance and sample stability showed no significant
 257 correlation when considering the nine materials from different commercial
 258 composting sites. As no correlation was found between stability and *alkB* gene
 259 abundance across the nine samples, samples were also considered on an individual
 260 site basis where green waste composting material and finished compost were
 261 collected on the same sampling occasion. Considering samples on this basis
 262 showed differences in *alkB* abundance between green waste composting material
 263 and finished composts samples; however, no trend was observed. For example, not
 264 all green waste composting materials had higher *alkB* gene abundance than finished
 265 samples. The challenge with this approach is likely to be variability in the feedstock
 266 at the batch level. Therefore, as the results show variability in *alkB* abundance in
 267 composting materials and finished composts processing similar feedstocks it

268 suggests the need to evaluate materials on a case by case basis depending on the
269 desired properties. Although *alkB* genes have been shown to be already abundant
270 (2.9×10^8 copies g^{-1} soil) in heavily ($10,000 \text{ mg kg}^{-1}$) hydrocarbon contaminated soils
271 (Heiss-Blanquet et al., 2005); the abundance of *alkB* in the green waste composting
272 materials and finished composts tested here should directly increase *alkB* gene
273 abundance when added to soils with lower ($2,000 \text{ mg kg}^{-1}$) hydrocarbon
274 concentrations (Heiss-Blanquet et al., 2005). Our experimental design of course
275 does not allow for the potential of *alkB* genes increasing in abundance in green
276 waste composting materials or finished composts themselves after addition to
277 contaminated soil and responding to available substrate.

278 For PAH degraders, two primer sets developed by Cebon et al., (2006),
279 widely applied by others, were used to target Gram-negative and Gram-positive
280 degraders respectively. As with *alkB* gene abundance, there was no significant
281 correlation between gene abundance and compost stability.

282

283 *3.3 Relationships between degrader gene abundance other measured parameters*

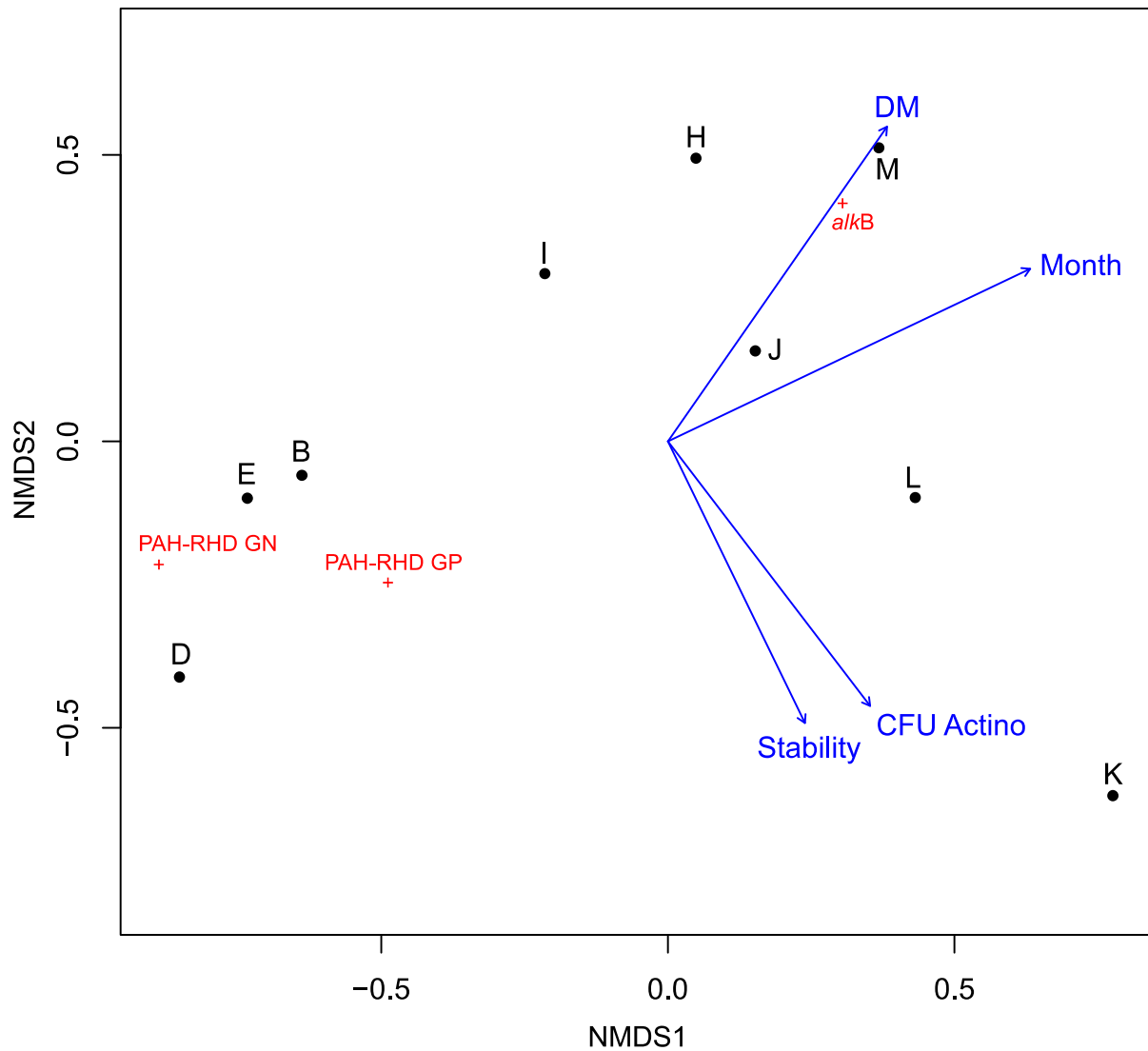
284 To test for any significant covariance between the degrader gene abundances
285 and the other measured compost parameters we log-transformed the data and
286 performed a simple Pearson correlation coefficient analysis (Supplementary table 1).
287 The results showed a significant correlation ($p \leq 0.001$) between the PAH-RHD GN
288 and PAH-RHD GP degrader gene abundances and the sampling month ($r^2 = -0.95$
289 and $p = 0.00001$ for PAH-RHD GN, $r^2 = -0.91$ and $p < 0.0007$ for PAH-RHD GP),
290 supporting the argument that changes in feedstock with season, which is known to
291 be the case for green waste composting processes (Reyes-Torres et al., 2018), is
292 likely to be a stronger factor than stability. A strongly significant positive correlation

293 was also found between PAH-RHD GN and PAH-RHD GP degrader gene
294 abundance ($r^2 = 0.96$ and $p < 0.00002$) suggesting conditions needed by both
295 degrader populations are similar, matching findings of Cebren et al., (2008) for PAH-
296 RHD GN and PAH-RHD GP degrader abundance in soils and sediments.

297 The PAH-RHD GN and PAH-RHD GP degrader gene abundances showed a
298 significant negative correlation with compost dry matter (DM) content ($r^2 = -0.75$ and
299 $p = 0.012$ for PAH-RHD GN, $r^2 = -0.78$ and $p = 0.013$ for PAH-RHD GP). By
300 comparison, no significant correlation was found between the culture-based
301 *Pseudomonas* spp. or Actinomycetes plate counts and DM content (culture-based
302 counts also displayed in Supplementary table 2).

303 A weak positive correlation could also be observed between compost stability
304 and actinomycete plate counts ($r^2 = 0.79$ and $p = 0.006$), and between compost
305 conductivity and pH ($r^2 = 0.67$ and $p = 0.03$). In the case of actinomycete abundance,
306 this supports previous studies showing increased actinomycetes abundance in post
307 peak temperature composting stages (Xiao et al., 2011) and latter stages (Steger et
308 al., 2007).

309 The above observations were further supported by fitting of the compost
310 parameters to the NMDS ordination (Figure 1), which showed a correlation between
311 the observed pattern of degrader gene abundance with sampling month ($r^2 = 0.86$, p
312 $= 0.002$) and dry matter ($r^2 = 0.79$, $p = 0.006$). There was also a weaker correlation
313 with compost stability ($r^2 = 0.53$, $p = 0.045$) and actinomycete plate counts ($r^2 = 0.6$,
314 $p = 0.023$), like the Pearson correlation analysis.



315

316

317 Figure 1. Nonparametric multidimensional scaling (NMDS) plot generated using log-
 318 transformed degrader gene abundance qPCR data for nine compost samples.
 319 Arrows are correlation vectors of gene abundance differences and compost
 320 parameters with significance factors $p < 0.05$. DM – dry matter; CFU Actino -
 321 *Actinomyces* plate counts; PAH-RHD GN - PAH degrader gene abundance Gram-
 322 negative; PAH-RHD GP - PAH degrader gene abundance Gram-positive.

323

324 CONCLUSIONS

325 Although compost stability is routinely used to test composts in industry as
 326 part of compost quality assurance schemes, we conclude that on its own it is not a
 327 good indicator of alkane and PAH degrader abundance in green waste composting

328 materials and finished composts. Comparative analysis of a wider range of
329 physicochemical parameters indicate that month is a more significant factor in
330 predicting degrader abundance reflecting the changing nature of green waste
331 feedstock during winter/early spring and late spring.

332 The benefits of green waste composting material or finished compost
333 amendment to contaminated soil is not limited to bioaugmentation of degraders but
334 potentially includes contaminant absorption to organic matter, improved soil structure
335 and nutrient supply (beyond the scope of our study). The study does suggest that
336 qPCR is a rapid and effective tool to rapidly screen the abundance of degraders in
337 specific materials for alkane and PAH mixed contaminant scenario. Therefore,
338 individual contaminant scenarios will influence the selection of specific organic
339 amendments based on desired benefits (e.g. nutrient addition, improved porosity,
340 contaminant adsorption, microbial augmentation) of the amendment.

341

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Supplementary table 1. Pearson correlations between degrader gene abundances and compost parameters. Significant correlations ($p \leq 0.001$) to weaker correlations ($p \leq 0.05$) are marked in red. A) Correlations between degrader gene abundances and *Pseudomonas* and *Actinomyces* plate counts and compost parameters. B) Correlations between compost parameters.

A

	<i>alkB</i>		PAH RHD GN		PAH RHD GP		CFU Pseudo		CFU Actino	
	P	V	P	V	P	V	P	V	P	V
pH	0.719	-0.13	0.653	0.16	0.649	0.18	0.246	-0.40	0.270	0.39
Site	0.165	0.47	0.704	-0.14	0.509	-0.25	0.777	-0.10	0.894	0.05
Month	0.836	-0.08	0.000	-0.95	< 0.001	-0.91	0.157	0.48	0.266	0.39
EC	0.504	-0.24	0.456	-0.27	0.603	-0.20	0.647	0.17	0.357	0.33
DM	0.697	0.14	0.012	-0.75	0.013	-0.78	0.391	0.30	0.761	-0.11
OM	0.259	0.39	0.618	0.18	0.437	0.30	0.694	0.14	0.443	-0.27
BD	0.415	-0.29	0.249	0.40	0.402	0.32	0.456	-0.27	0.830	-0.08
Stability	0.071	-0.59	0.446	-0.27	0.787	-0.10	0.876	-0.06	0.007	0.79
CFU Pseudo	0.699	-0.14	0.147	-0.49	0.205	-0.47			0.560	0.21
CFU Actino	0.229	-0.42	0.191	-0.45	0.375	-0.34	0.506	0.21		
Sample status	0.373	0.32	0.673	0.15	0.832	-0.08	0.736	0.12	0.253	-0.40

<i>alkB</i>			0.872	0.06	0.826	-0.08	0.699	-0.14	0.229	-0.42
PAH RHD GN					0.000	0.96	0.147	-0.49	0.191	-0.45
PAH RHD GP	0.826	-0.08					0.205	-0.47	0.375	-0.34

BD – bulk density; DM – dry matter; EC – electrical conductivity; OM – organic matter; CFU Pseudo - *Pseudomonas* plate counts; CFU Actino - *Actinomyces* plate counts; PAH-RHD GN - PAH degrader gene abundance Gram-negative; PAH-RHD GP - PAH degrader gene abundance Gram-positive

B

Parameter	pH		Site		Month		EC		DM		OM		BD		Stability		Sample status	
	P	V	P	V	P	V	P	V	P	V	P	V	P	V	P	V	P	V
pH			0.231	0.42	0.291	-0.38	0.034	0.67	0.374	-0.31	0.193	-0.45	0.070	0.59	0.518	0.23	0.494	-0.25
Site					0.938	-0.03	0.489	0.25	0.737	-0.12	0.565	-0.21	0.739	0.12	0.645	-0.17	0.371	-0.32
Month							0.739	0.12	0.018	0.73	0.736	-0.12	0.174	-0.47	0.523	0.23	0.883	-0.05
EC									0.751	0.11	0.117	-0.53	0.060	0.61	0.821	-0.08	0.749	0.12
DM											0.943	-0.03	0.263	-0.39	0.762	-0.11	0.715	0.13
OM													0.035	-0.67	0.711	-0.13	0.895	0.04
BD															0.476	-0.25	0.391	0.30
Stability																	0.030	-0.68
Sample status																		

BD – bulk density; DM – dry matter; EC – electrical conductivity; OM – organic matter

Supplementary table 2. Selective microbial plate counts of the ten compost materials

Sample ID	Log colony forming units (CFU) g ⁻¹ dry weight	
	<i>Pseudomonas</i> spp.	Actinomycetes
B	5.24 (0.13)	7.94 (0.19)
D	5.67 (0.07)	8.09 (0.38)
E	4.01 (0.64)	7.76 (0.18)
I	6.28 (0.04)*	8.29 (0.06)
H	7.14 (0.26)	8.86 (0.14)
J	5.42 (0.08)	8.46 (0.19)
K	6.63 (0.10)	9.31 (0.11)*
L	7.31 (0.04)*	8.22 (0.11)
M	4.56 (0.58)	6.59 (0.04)*

*Averages of duplicate measurements, the rest in triplicate