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Applied Soil Ecology

DOI:

[10.1016/j.apsoil.2020.103637](https://doi.org/10.1016/j.apsoil.2020.103637)

Published: 01/09/2020

Peer reviewed version

[Cyswllt i'r cyhoeddiad / Link to publication](#)

Dyfyniad o'r fersiwn a gyhoeddwyd / Citation for published version (APA):

Qin, L., Tian, W., Freeman, C., & Jiang, M. (2020). Nitrogen availability influences microbial reduction of ferrihydrite-organic carbon with substantial implications for exports of iron and carbon from peatlands. *Applied Soil Ecology*, 153, Article 103637. <https://doi.org/10.1016/j.apsoil.2020.103637>

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1 **Nitrogen availability influences microbial reduction of ferrihydrite- organic carbon with**
2 **substantial implications for exports of iron and carbon from peatlands**

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29 **Abstract:**

30 While iron (Fe) has been proposed to constrain dissolved organic carbon (DOC) export
31 by forming precipitation (Fe-OC) in peatlands, uncertainties remain about the potential
32 interactions between Fe and nitrogen. Such interactions are important for Fe and carbon
33 exports as they can dissolve the Fe-OC through Fe reduction. Here we studied the reduction
34 of ferrihydrite-OC (Fh-OC) following incubation with microbes from agricultural and natural
35 peatlands under differing nitrogen availability, with high-throughput sequencing to identify
36 microbial mechanisms. Our results showed that in agricultural peatlands, high nitrogen levels
37 ($>100\text{mg kg}^{-1}$) modified the composition and abundance of iron-reducing bacteria, increasing
38 reduction rates of Fh-OC ($0.09\text{-}0.20\text{ kg day}^{-1}\text{ ha}^{-1}$ of Fh-OC) above the low N treatments
39 ($<100\text{ mg kg}^{-1}$) ($p<0.05$). However, reduction rates of Fh-OC and iron-reducing community in
40 natural peatlands were far less affected. Our findings suggest that N-induced microbial
41 reduction of Fh-OC may create the potential for greater iron and carbon exports from
42 agricultural peatlands to aquatic systems.

43 **Key words:** Iron-reducing bacteria; ferrihydrite-organic carbon; nitrogen; peatlands;
44 agriculture.

45 **1. Introduction**

46 Peatlands represent a global major carbon (C) store, and a dominant source of dissolved
47 organic carbon (DOC) to aquatic systems (Fenner et al., 2007). Climate change and human
48 activity have been found to increase DOC export to freshwater from peatlands (Freeman et al.,
49 2001; Evans et al., 2005; Fenner et al., 2007; Bjorneras et al., 2017). Iron (Fe) has been noted
50 to constrain DOC export through coprecipitation or adsorption, forming immobilized
51 complexes (Fe-OC) in peatland (Riedel et al., 2013), with studies showing that Fe stabilizes
52 8-17 % of organic carbon (Wen et al., 2019; Zhao et al., 2019). However, recent studies also
53 report that increased DOC concentrations are synchronous with dissolved Fe concentrations
54 in peat catchment (Ekstrom, et al., 2016; Bjorneras et al., 2017).

55 Under global warming, rising temperatures could stimulate Fe reduction (Knorr, 2013),
56 leading to DOC and Fe release from Fe-OC (Pan et al., 2016). Peatlands contain an
57 abundance of phenolics, which contribute to forming stable dissolved complexes with Fe(III)
58 or Fe(II) (Wan et al., 2018). These complexes could offer protection against forming Fe-
59 oxyhydroxide precipitation, further supporting the unimpeded transport of Fe and carbon
60 from peatlands to aquatic ecosystems (Krachler et al., 2015; Wan et al., 2018). There is
61 further possible influence from anaerobic ammonium oxidation coupled to iron reduction
62 (Feammox), which is known to be widespread in natural anaerobic conditions (Yang et al.,
63 2012; Ding et al., 2017). Moreover, long-term N fertilization in soil has also been found to
64 enhance Fe reduction rate (Ding et al., 2015). Peatlands belong to nutrient-poor ecosystems
65 (Bragazza et al., 2006), which are threatened by increased atmospheric nitrogen deposition
66 (Bragazza et al., 2006; Li et al., 2019). Furthermore, about 14-20 % of peatlands have been
67 drained for agriculture worldwide (IPS, 2008), in which fertilization significantly increases N
68 content (Ruckauf et al., 2004). It is clearly important to determine the extent to which
69 increased N availability will affect reduction of Fe-OC, due to the consequent implications for
70 iron and DOC export from peatlands to aquatic system.

71 The incubation experiment with microbes from environmental soil and synthesized
72 ferrihydrite or ferrihydrite-OC (Fh-OC), has been widely applied in studies of iron
73 biogeochemistry as a way of minimising wider environmental influences on the targeted iron
74 transformation processes (Zhuang et al., 2015; Cooper et al., 2017). Here, we observed the
75 Fh-OC reduction with microbes from agricultural and natural peatlands under different N
76 availability and used high-throughput sequencing to reveal microbial mechanisms. According
77 to N availability in agricultural and natural peatlands, we set up two high N (>100mg N kg⁻¹)
78 and four low N (<100mg N kg⁻¹) treatments. We hypothesized that (1) high N contents would
79 increase microbial reduction of Fh-OC in agricultural peatlands only as long-term N
80 application may increase microbial N demand. (2) Increased N availability would increase
81 microbial reduction of Fh-OC in natural peatlands.

82 2. Materials and method

83 2.1. Experiment design

84 Soil samples were collected from Jinchan Peatland, a temperate fen in Northeast China
85 (42°21'-42°22' N, 126°21'-126°22'E), where areas of peatland have been converted to paddy
86 fields since the 1960s. In general, N fertilizer (urea) is applied mid-May, and throughout June
87 for rice growth, totalling 260 kg N ha⁻¹ year⁻¹. In contrast, P fertilizer is applied in mid-May
88 only, amounting to 70 kg P ha⁻¹ year⁻¹ (Shi., 2019). Soil properties in agricultural and natural
89 peatlands are shown in table 1. Atmospheric N deposition in northeast China is about 1.4 g N
90 m⁻² yr⁻¹, which is significantly higher than in other global areas at a similar latitude (< 0.6 g
91 N m⁻² yr⁻¹) (Li et al., 2019).

92 According to Zhang et al. (2016)'s field investigation data, in August 2018, we selected
93 eight random sample sites in flooded agricultural and natural peatlands. After removing litter
94 and vegetation, we collected three random soil cores using a soil core sampler at 0-15 cm
95 depth each site, then mixed and transported to a laboratory on ice under anoxic conditions
96 (N₂). We extracted microbes from each site within 72 h using the standard protocol of Cooper
97 et al. (2017). Fresh soil (equal to dry mass 2.0 g) was added to sterile, anoxic 100 ml of 0.85
98 % NaCl solution and 8 g sterile glass beads in serum bottles (eight replicates), and the slurry
99 was shaken at 4 °C overnight. After centrifugation at 900 g for 5 min, the supernatant was
100 transferred to fresh sterile tubes and centrifuged again at 1200 g for 10min. Finally, the solid
101 phase was resuspended in 10 ml anoxic 0.85 % NaCl solution and we used microscopy (XDS-
102 2BI, China) to confirm the presence of microorganisms in the suspension.

103 Fh-OC was synthesized as described by Pan et al. (2016) with minor modifications. A
104 water extract of freeze-dried soil litter, from natural peatlands with 30:1 ratio, filtered with
105 0.22 μm and diluted in 200 mg L⁻¹ DOC, and as a molar ratio 1:1.04 of C with Fe (FeCl₃)
106 observed in the field (4 g kg⁻¹ DOC and 18 g kg⁻¹ SRO). While constantly stirring, 0.1 M
107 NaOH was added to pH6 then centrifuged 5000 rpm and the supernatant decanted (repeated
108 three times). Solid Fh-OC with final C/Fe 0.35 was formed after freeze-drying. Based on N

109 availabilities in agricultural and natural peatlands, we set up high N (as 100, 400 mg N kg⁻¹)
110 and low N content (as 0, 1, 10, 50 mg N kg⁻¹) treatments. The 40 ml medium consisted of 30
111 ml L⁻¹ of sterile 1 M NaHCO₃ (autoclaved, CO₂), 10 ml L⁻¹ Wolfe's vitamin solution (ATCC,
112 1957) and 10 ml L⁻¹ modified Wolfe's minerals (ATCC, 1957), a combination of filter-
113 sterilized electron donors (sodium acetate, 2 mM; sodium lactate, 2 mM; and glucose, 2 mM),
114 1 mM KH₂PO₄, 80 mg Fh-OC, 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic
115 (Hepes, pH=6), 2.5 ml bacterial suspensions (equal to 0.5 g dry soil) and NH₄Cl as nitrogen
116 treatment in 50 ml vials (Cooper et al., 2017). The vials were sealed with a rubber septa and
117 aluminium crimp caps after being purged with ultrapure helium for 20 min at a rate of 10 ml
118 min⁻¹. All treatments were performed in triplicate, at a constant temperature of 25 °C, under
119 dark conditions. Reduction of Fh-OC was monitored as Fe (II) every 24 h using the
120 phenanthroline assay (Cooper et al., 2017). At the end of incubation (240 h), we sampled the
121 microbial community.

122 **2.2. DNA Extraction and 16S rRNA Gene Sequencing**

123 DNA from the enrichment culture was collected by centrifugation (8000 rpm and 4 °C
124 for 10 min) and then extracted using a Power Soil™ DNA isolation kit (MO BIO
125 Laboratories, USA), according to the manufacturer's instructions. Bacterial 16S rRNA gene
126 fragments were amplified from DNA samples using the primers F341 and R806 (Mori et al.,
127 2014). Then the samples were sent to Novogene (Beijing, China) for amplicon sequencing
128 using an Ion S5 XL platform. The bioinformatics analysis was performed following
129 previously described methods (Yang et al., 2019). All raw sequences have been deposited into
130 a NCBI Sequence Read Archive with the accession number PRJNA576710.

131 **2.3. Statistical analysis**

132 Linear regression was performed to identify reduction rate of Fh-OC over 240h, and we
133 estimated microbial reduction rate of Fh-OC at field scale (kg day⁻¹ ha⁻¹) based on soil bulk
134 density and reduction rate of Fh-OC in cultures. Differences between treatments were
135 evaluated by one-way analysis of variance. Significant differences between means were

136 established by Duncan test at $p < 0.05$. These were performed by the statistical package
137 SPSS23.0. Furthermore, we used the Functional Annotation of Prokaryotic Taxa
138 (FAPROTAX) database to annotate microbial functional groups based on 16S rRNA
139 sequences (Louca et al., 2016). Nonmetric multidimensional scaling (NMDS) based on Bray-
140 Curtis distance was performed to predict iron-reducing bacterial composition by Canoco 5.0.

141 **3. Results**

142 With microbes from agricultural peatlands, low N treatments (0, 1, 10, 50 mg kg⁻¹) did
143 not affect the reduction rates of Fh-OC during the 240h incubations, and these were
144 significantly lower than those of the high N treatments (100, 400 mg kg⁻¹) ($p < 0.05$), with the
145 higher rate in 400 mg kg⁻¹ treatment than in 100 mg kg⁻¹ treatment ($p < 0.05$). Interestingly, the
146 100 mg N kg⁻¹ treatment triggered a more rapid rise of Fe (II) from 120 h to 168 h, and then
147 the rate subsided (Figure 1a). However, with microbes from natural peatlands, we found little
148 variation in the reduction rates of Fh-OC across the any of the N treatments (Figure 1b).

149 We also estimated the microbial reduction rates of Fh-OC at a field scale across the N
150 treatments. Results showed that high N treatments significantly increased rates by 0.09-0.20
151 kg day⁻¹ ha⁻¹ compared with low N contents treatments in agricultural peatlands ($p < 0.05$)
152 (Figure 2), which were all significantly higher than all the N treatments in natural peatlands.
153 Overall, average microbial reduction rate of Fh-OC (1.21 kg day⁻¹ ha⁻¹) in agricultural
154 peatlands was about 13 times more than that in natural peatlands (Figure 2).

155 By using the FAPROTAX database, the effects of N on iron-reducing bacteria
156 composition were revealed by NMDS (Figure 3). Results showed that the 400 and 100 mg N
157 kg⁻¹ treatments induced a similar iron-reducing bacteria composition in cultures with
158 microbes from agricultural peatlands. However, the composition in corresponding low N
159 treatments were similar to those in all the N treatments in the natural peatlands. Furthermore,
160 a heat map showed a shift in relative abundance of the key microbial functional groups
161 (Figure 4). There was a noticeably high relative abundance of iron-reducing bacteria under
162 100 and 400 mg N kg⁻¹ treatment in agricultural peatlands, and groups associated with the

163 degradation of aromatic-hydrocarbon, hydrocarbon, plastic, aromatic compounds, and
164 fumarate reduction under 400 mg N kg⁻¹ treatment in natural peatlands.

165 **4. Discussion**

166 Our findings support the hypothesis that high N can increase reduction rates, but only in
167 agricultural peatlands. Iron-reducing bacteria composition from cultures under low N contents
168 were similar to those in all the N treatments in natural peatlands. However, in agricultural
169 peatlands, high N content resulted in a modified iron-reducing bacteria composition and
170 increased relative abundance (Figure 3&4). These findings support previous observations that
171 long-term N fertilization can change iron-reducing bacteria community in mineral soils and
172 likewise increase iron reduction rates in paddy soils (Ding et al., 2015). Furthermore,
173 reduction rate in 100 mg N kg⁻¹ treatment was significantly lower than 400 mg N kg⁻¹ ($p < 0.05$),
174 and reduction rate of Fh-OC tended to decrease from 168 to 240h (Figure 1a). This also
175 implies that a shift of iron-reducing bacteria has a high demand in nitrogen, which then
176 mediates reduction of Fh-OC. However, results disagree with hypothesis in natural peatlands,
177 as iron-reducing composition and relative abundance were far less affected by N treatments in
178 natural peatlands (Figure 3&4). Moreover, N-replete conditions (400 mg kg⁻¹ N) could
179 promote microbial groups associated with the degradation of aromatic-hydrocarbon,
180 hydrocarbon, plastic and aromatic compound (Figure 4). These findings support previous
181 studies that high N availability could increase phenol oxidase activity, a specialized enzyme
182 for degrading recalcitrant materials in peatlands (Bragazza et al., 2006; Song et al., 2019).
183 These also suggest that N is not the determining nutrient for iron-reducing bacteria during
184 short-term incubations.

185 Although laboratory control experiments revealed the effects of single N on microbial
186 reduction of Fh-OC, field soil conditions also influence the microbial composition and
187 function (DeAngelis et al., 2010), which could determine the microbial reduction of Fh-OC.
188 In our study, compared with natural peatlands, soil short-ranged iron, soil total phosphorus
189 and soil bulk density increased, but soil organic carbon decreased in agricultural peatlands

190 (Table1). First of all, iron-reducing bacteria mainly use short-ranged Fe oxides (Lovley, 1987),
191 which could influence iron-reducing bacteria composition and increase their abundance (Liu
192 et al., 2019). This implies that a shift of iron-reducing bacteria composition is also related to
193 field short-ranged iron contents. Secondly, P limitation is common in fens (Hill et al., 2014),
194 while Li et al. (2019) also reported that P plays a key role in soil biochemical cycling in fens
195 located in Northeast China. Therefore, sufficient P in incubated cultures might cause
196 overestimation of microbial reduction rate of Fh-OC in natural peatlands as reduction of P
197 limitation (Bongoua-Devisme et al., 2013). Thirdly, agricultural practice could increase peat
198 bulk density and decrease soil organic carbon in drained peatlands (Kasimir-Klemedtsson et
199 al., 1997). The reduction rates of Fh-OC appeared no different between cultures with
200 microbes from agricultural and natural peatlands (Figure 1a&b), but average microbial
201 reduction rate of Fh-OC ($1.21 \text{ kg day}^{-1} \text{ ha}^{-1}$) in agricultural peatlands was about 13 times more
202 than that in natural peatlands at field scale (Figure 2). These also emphasize that the increased
203 soil bulk density in agricultural peatlands can enhance total production of Fh-OC reduction
204 per area. Although carbon contents and different organic carbon as electron donor could also
205 influence microbial Fe reduction (Yang and Liptzen, 2015; Su et al., 2020), soil organic
206 carbon (27.2%) is still at high levels in agricultural peatlands. Therefore, further work is
207 needed on organic carbon composition and its effect on reduction of Fh-OC in agricultural
208 peatlands.

209 Globally, 14-20% of peatlands have been drained for agriculture (IPS, 2008). Mineral
210 soil addition is widespread during peatlands reclamation for trafficability and crop yields
211 (Saurich et al., 2019), which also creates abundant iron resource (Banik et al., 2016). A recent
212 study also reported that high percentages of agricultural land correlated with high
213 concentrations of Fe in river water (Palviainen et al., 2015). According to our study, the N-
214 induced microbial reduction of Fh-OC would increase dissolved iron and DOC release from
215 precipitation of iron and organic carbon in long-term fertilized agricultural peatlands.
216 Increased dissolved Fe could form dissolved complexes with phenolic (Wan et al., 2018),

217 hence creating the potential for greater exports of DOC and Fe from agricultural peatlands to
218 aquatic systems.

219

220 **Acknowledgments**

221 This work was supported by the National Natural Science Foundation of China
222 (41771120), the CAS President's International Fellowship Initiative (2020DC0012), and UK-
223 China Joint Research and Innovation Partnership Fund PhD Placement Programme
224 (201802527016).

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379 **Table**

380 Table1 Soil properties of agricultural and natural peatlands.

	TN (g kg ⁻¹)	SOC (%)	TP (g kg ⁻¹)	SRO (g kg ⁻¹)	BD (g cm ⁻³)	NH ₄ ⁺ -N (mg kg ⁻¹) [†]	pH [‡]
Agricultural Peatlands	14.5±1.53	27.22±0.36	1.19±0.13	18.03±1.20	1.60±0.12	115-225	6.0
Natural Peatlands	18.4±0.61	40.41±0.15	0.80±0.03	7.20±1.65	0.12±0.03	25-60	5.4

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382 TN: soil total nitrogen, SOC: Soil organic carbon, TP: Soil total phosphorus, SRO: soil short ranged iron, BD: soil
 383 bulk density. These were measured according to method of Carter (1993). Values are means ± deviations (n=3).

384 [†] Average soil ammonium nitrogen contents (NH₄⁺-N) from May to September, which are from Shi (2019).

385 [‡] Average soil pore water pH, which are from Shi et al. (2017).

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401 **Figure Captions**

402 **Figure 1** Characteristics of microbial reduction of Fh-OC in cultures under different N
403 availability. (a) With microbes from agricultural peatlands. (b) With microbes from natural
404 peatlands. k represents the reduction rate of Fh-OC, evaluated as changes of Fe(II) ($\mu\text{mol h}^{-1}$)
405 over 240 h using linear fit. N0, 1, 10, 50, 100, 400 represent N concentration treatments (mg
406 kg^{-1}), respectively. Value and error bars represent mean \pm standard deviations (n=3) Different
407 letters represent significant differences among N treatments ($p < 0.05$).

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409 **Figure 2** Comparison of microbial Fe reduction rates in Fh-OC at field scale under different
410 N availability in agricultural and natural peatlands. The error bars represent mean \pm standard
411 deviations (n=3). N0, 1, 10, 50, 100, 400 represent N concentration treatments (mg kg^{-1}),
412 respectively. Different letters represent significant differences among N treatments ($p < 0.05$).

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414 **Figure 3** Nonmetric multidimensional scaling (NMDS) predicts iron-reducing bacteria
415 composition in cultures from agricultural and natural peatlands with different N availability.
416 Red and blue represent agricultural and natural peatlands, respectively. N0, 1, 10, 50, 100,
417 400 represent N concentration treatments (mg kg^{-1}), respectively. Each contains three
418 replicates.

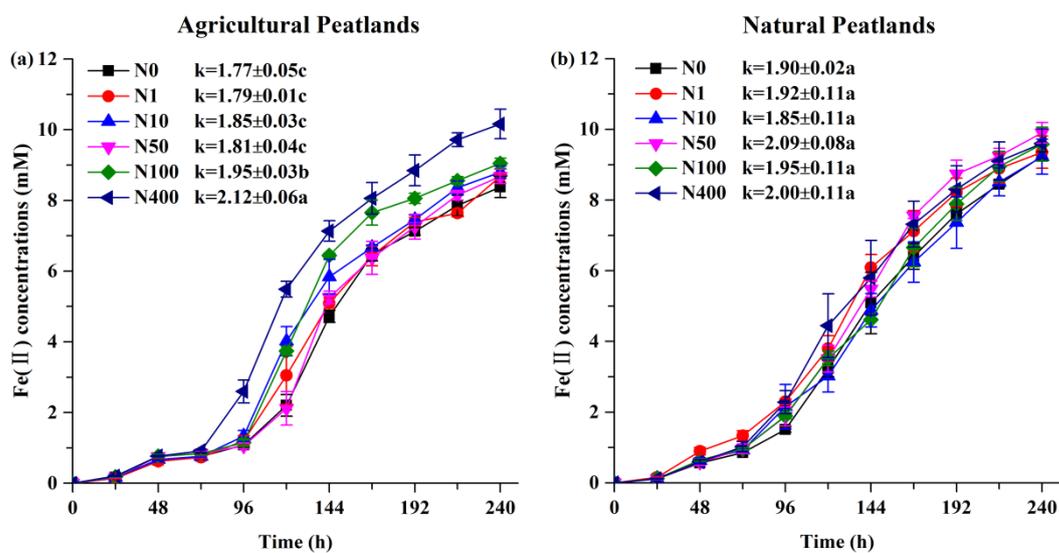
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420 **Figure 4** Heat map analysis of the highly represented bacterial functional groups in
421 incubation cultures from natural and agricultural peatlands. The blue denotes low relative
422 abundance and the red denotes high relative abundance. The colour key for the Z score
423 indicates correspondence between blue-red colouring and standard deviations from the mean
424 abundance of each functional group. A: agricultural peatlands; P: Natural peatlands; N0, 1, 10,
425 50, 100, 400 represent nitrogen concentrations (mg kg^{-1}), each contains three replicates.

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428 **Figure1**



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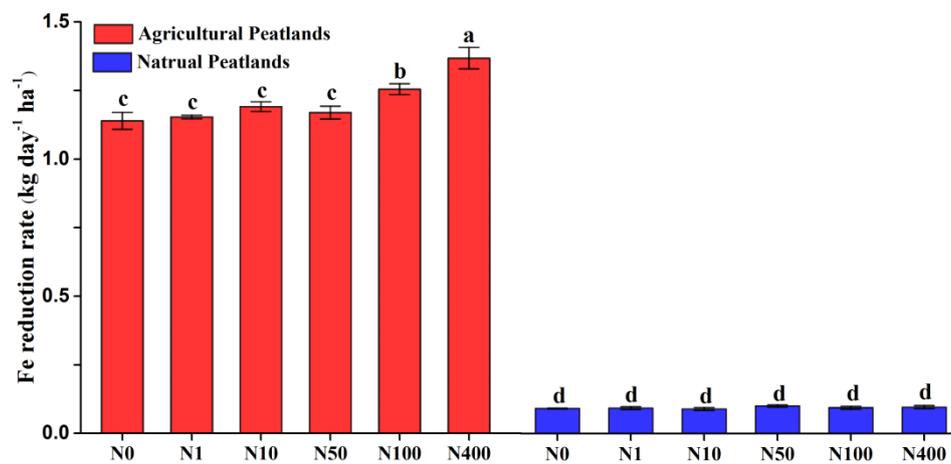
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449 **Figure 2**



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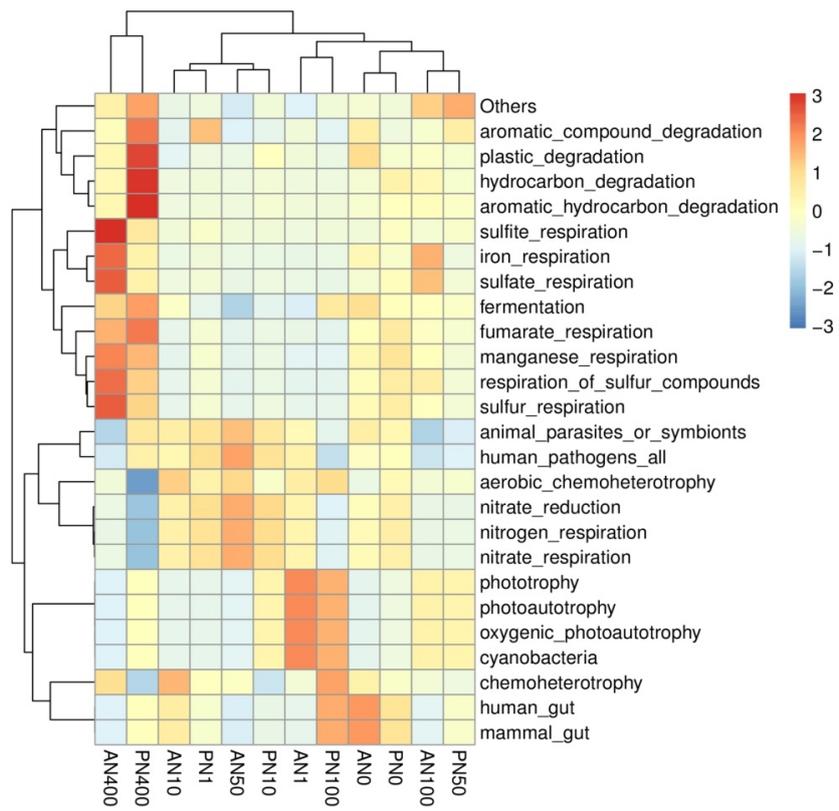
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494 **Figure 4**



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