

1 **Wild pigs mediate far-reaching agricultural impacts** 2 **on tropical forest soil microbial communities**

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43 **Wild pigs mediate far-reaching agricultural impacts** 44 **on tropical forest soil microbial communities**

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46 **ABSTRACT**

47 Edge effects, the altered abiotic and biotic conditions on the borders of natural areas, rarely extend more
48 than a few hundred meters. Edge effects have rarely been linked to altered soil biota, which shape
49 ecosystem processes including carbon storage, biogeochemical cycling, and plant performance. Here, we
50 investigated if agriculturally-mediated increased wildlife populations affect soil biotic communities at a
51 distance well over that of estimated edge effects when they move between agriculture and natural habitats
52 using a 22-year fenced exclusion experiment in a primary rainforest in Peninsular Malaysia. We found that
53 the presence of wildlife (mainly native pigs (*Sus scrofa*) that crop-raid in nearby oil palm plantations) was
54 associated with higher bacterial diversity, and an altered community composition (mediated by changes in
55 soil pH), and reduced abundances of symbiotic ectomycorrhizal fungi compared to soil in exclosures. There
56 were only minor effects of pigs on soil chemistry or microclimate, so we suggest that changes in soil
57 communities are driven by pigs' leaf litter removal and alterations to plant composition. Our study
58 highlights that indirect effects from agriculture can be transferred by wildlife >1 km into protected areas
59 and this could have important repercussions for ecosystem processes and plant-soil feedbacks.

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61 **INTRODUCTION**

62 Agricultural encroachment into forested areas is a pervasive global phenomenon that has a
63 clear and direct impact on above-(1,2) and below-ground (3) biota and ecosystem processes,
64 especially in tropical forests. It is more challenging to assess how agricultural expansion
65 indirectly affects natural areas over larger spatial scales, such as the cryptic degradation from
66 edge effects. One example is cross-boundary ecological cascades, wherein adjacent ecosystems
67 – first appearing to be distinct – are actually linked through the transport of nutrients (e.g. via
68 floods or mobile animals) or interactions with wildlife that moves across ecotones (4). With over
69 70 % of remaining forests now lying within 1 km of an edge (5), there is an urgent need to
70 understand how edge effects reshape the linkages between above- and below-ground biota and
71 the scale at which they operate.

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73 Wildlife responses to edges are related to their unique habitat preferences, the local hunting
74 intensity and preferences, and some crop-raiding wildlife can even benefit from supplemental
75 foraging in nearby farmlands (4,6-9). Edge effects also produce a range of impacts on soils that
76 are often mediated by microclimate and light (10), as well as plant species composition (e.g.
77 11-13). However, these edge effects have rarely been documented beyond a few hundred
78 meters (10,14,15). Wildlife plays an important role moderating nutrients like nitrogen and
79 phosphorus, and soil biota through deposition of excrement and carcasses (16-19). Wildlife also

80 affects soil physical environments through biopedoturbation (20-22) and plant-soil interactions
81 via herbivory or nest building (23-26).

82

83 Here, we investigate the potential for wide-ranging wildlife to transfer far-reaching edge effects
84 on soil microbial communities in distant 'interior' primary forests at distances >1 km from the
85 nearest edges. We conducted our study in a primary Malaysian rainforest where native forest-
86 dwelling wild boars (*Sus scrofa*) that forage in nearby oil palm plantations (*Elaeis guineensis*)
87 have elevated densities and are known to disturb nearby forest soils and plant communities
88 (4,27,28). Wild boars (hereafter 'pigs') are a key example of a broadly distributed generalist
89 vertebrate that is adaptable to human environments (e.g. forest edges) and strongly affected
90 by humans via hunting (negative) or crop-raiding (positive). Pigs are considered an 'ecosystem
91 engineer' due to the major physical soil disturbances via rooting, grubbing (predating larger soil
92 invertebrates), wallowing, trampling and soil compaction (29-30).

93

94 We focus on pigs' influence on microbial community composition and functioning, which remains
95 largely unknown and has myriad links to biogeochemical processes that in turn shape
96 ecosystem properties including carbon dynamics (31). Using a long-term exclosure experiment,
97 we examined three hypotheses regarding the indirect impacts from oil palm-fed pigs on the
98 forest soil microbial communities based on the ecology of pigs and known relationships between
99 soil disturbances and microbial communities:

- 100 1) First, we predicted that pig-exposed soils would have greater nutrient concentrations
101 due the deposition of urine and faeces and that, together with disturbances caused
102 by removal of understorey plants and leaf litter by pigs, these would be key drivers
103 of microbial community structure in pig-exposed soils.
- 104 2) Second, we predicted that soil bacteria would be more impacted than fungi due to
105 changes in nitrogen deposition from excrement influencing bacteria involved in the
106 nitrogen cycle, and removal of understorey plants and leaf litter, which increases
107 light penetration and likely has a drying effect on soils to which bacteria are more
108 sensitive than fungi (32).
- 109 3) Third, we predicted that whilst fungi would be less influences by the presence of pigs
110 than bacteria, symbiotic ectomycorrhizal (EcM) fungi would be reduced in pig-
111 exposed soils because pigs preferentially remove dipterocarp seedlings (23) that are
112 associated with these fungi.

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114 **RESULTS**

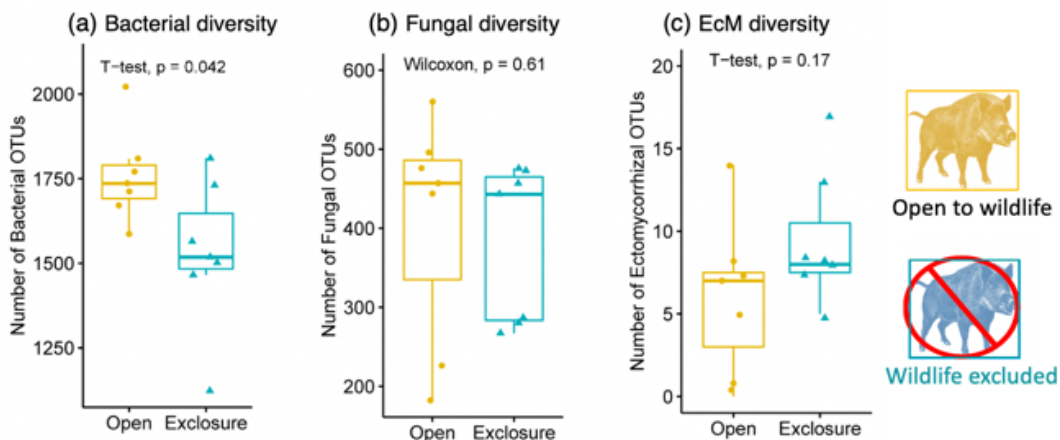
115 There were low concentrations of all soil nutrients measured, which is typical for Southeast
116 Asian rain forests on similar substrates (Table S1). There were no significant differences

117 between the exclosures and the open-control areas with the exception of soil pH that was 0.15
118 pH units more acidic within the exclosures.

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120 Bacterial richness was 13 % lower in exclosure soils (t-test, $P = 0.042$; Fig 1a). The relative
121 abundance of Acidobacteria increased (t test, $P = 0.013$; Fig 2a) but there was lower relative
122 abundance of Proteobacteria, Actinobacteria, Planctomycetes and Gemmatimonadetes (all $P <$
123 0.05 ; Fig. 2). At the subphylum level, we also observed significant differences in the relative
124 abundance of dominant bacterial taxa, for example, the relative abundance of bacterial families
125 Solibacteraceae and Rhabdochlamydiaceae increased, whilst abundance of other families such
126 as Bradyrhizobiaceae and Xanthomonadaceae, including members with biological N_2 -fixation
127 capacities, declined in exclosure soils (Table S2). The bacterial community composition differed
128 between the exclosures and the open-controls (ANOSIM $R = 0.216$, $p = 0.009$; Figs 2 & 3a) and
129 was influenced by soil pH (Fig. 3a). Soil carbon and potassium also influenced the bacterial
130 community composition, but these did not differ between the exclosure and open-control soils
131 (Table S1). There was no clear influence of the exclosures on bacterial community functioning
132 as measured by predicted gene abundance (ANOSIM $R = 0.081$, $p = 0.23$; Fig 3c).

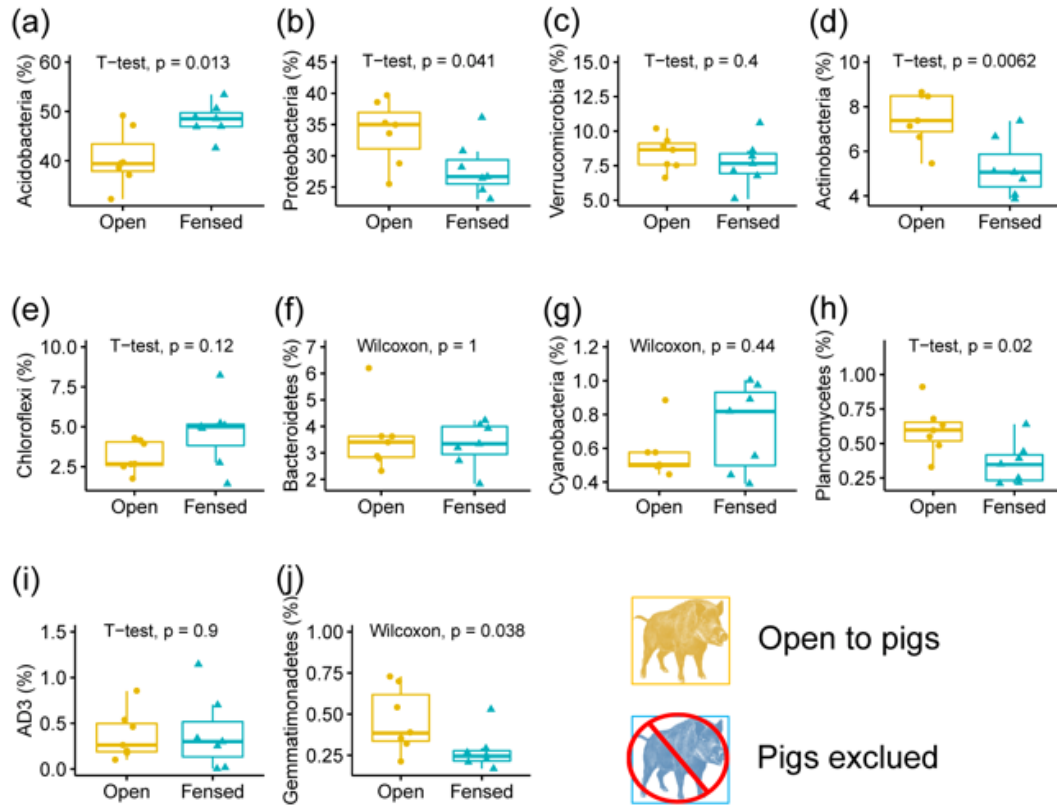
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135 **Figure 1:** The long-term effects of wildlife (primarily pigs) on soil microbial
136 diversity at Pasoh Forest Reserve in Peninsular Malaysia. Samples are separated
137 by whether they were taken from open-control plots where there were many pigs
138 (yellow dots) versus within fenced exclosures without pigs (blue triangles). EcM =
139 Ectomycorrhizal fungi.

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Figure 2: Influence of wildlife (primarily pigs) on the relative abundance of soil bacterial phyla at Pasoh Forest Reserve in Peninsular Malaysia (interpretation is the same as Fig. 1).

The fungal community did not differ significantly between the exclosure and open-control soils in terms of diversity (Wilcoxon test, $P = 0.61$; Fig 1b), phylum abundance (Wilcoxon test, all $P > 0.05$; Fig S1), community composition (ANOSIM: $R = -0.043$, $p = 0.70$; Fig 3b) or guild composition (Fig. S2). However, the ectomycorrhizal (EcM) fungal community composition differed between wildlife treatments (ANOSIM $p = 0.03$; Fig 3d), becoming 1.3 times more diverse (t-test, $P = 0.17$; Fig. 1c) and 2.9 times more abundant in exclosure soils (Wilcoxon test, $P = 0.16$; Fig. S2b). In particular, although the abundance of the most common EcM family Russulaceae was variable it was, overall, 2.5 times more abundant in the exclosure soils (Wilcoxon test, $P = 0.38$). The fungal community composition (including all species) was influenced by soil carbon (Fig. 3b) but none of the soil chemical variables significantly influenced the EcM community structure.

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158 DISCUSSION

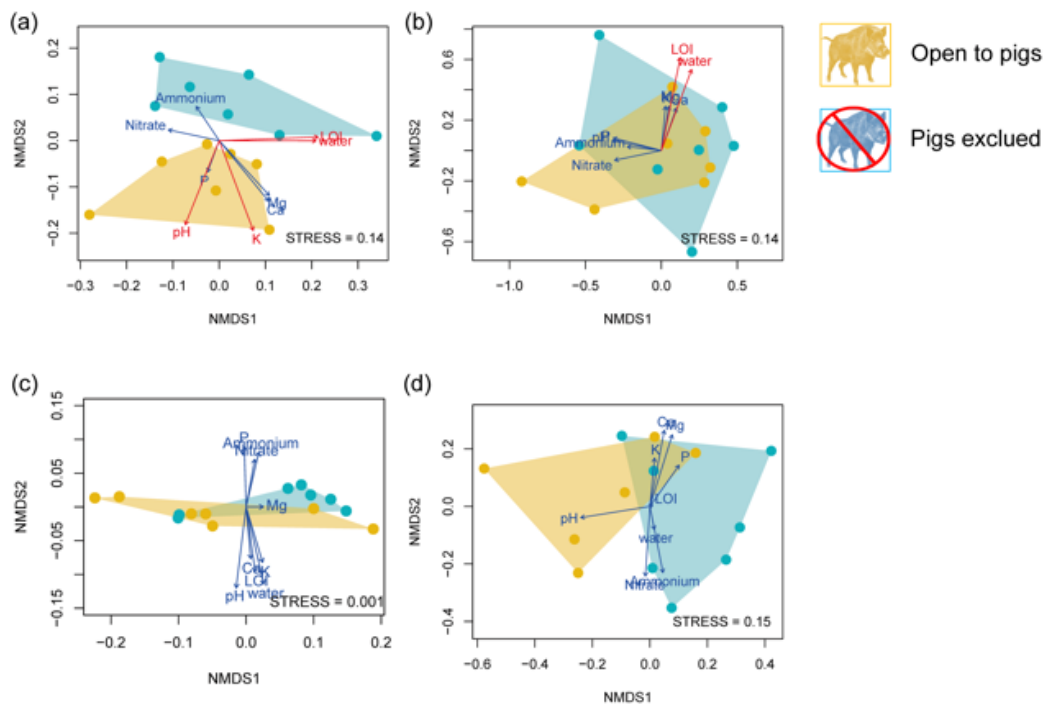
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Our study is the first to link agricultural incursions to altered soil microbial community composition in adjacent habitats at distances >1 km from edges, one of the furthest soil-related edge effects yet recorded. These far-reaching edge effects were mediated by crop-raiding native

162 pigs that fed on oil palm fruits in adjacent plantations and then return to forests. The biotically-
163 driven impacts by pigs that we report are distinct from more well-documented edge effects
164 related to habitat and abiotic conditions, since all our sampling locations were equidistant from
165 edges and microclimatic differences were not detected greater than 100 m from the edge at our
166 site (33). Instead, we posit that pigs impact soil microbial communities by disturbing soils, leaf
167 litter, and understory vegetation and altering the plant community composition (4,23,34). For
168 example, previous work at our site has shown pigs reduce trees with symbiotic root-associated
169 ectomycorrhizal (EcM) fungi (e.g. Dipterocarpaceae) and facilitate lianas that are rarely
170 reported to have EcM associations (35). As predicted, we found wildlife exclusion was
171 associated with altered EcM fungi communities and a greater relative abundance of EcM fungi.
172 Wildlife exclusion was also associated with reduced soil bacterial diversity which led to a greater
173 community change than for the fungal community. Prior work has found invasive pigs reduced
174 soil bacterial diversity in Hawaii (36) – the opposite trend we observed from native pigs – and,
175 in New Zealand, invasive pig grubbing may increase the relative abundance of fungi over
176 bacteria (37) which our results supported.
177



178
179 **Figure 3:** NMDS ordinations showing the influence of wildlife (primarily pigs) on
180 the soil microbial community composition and function at Pasoh Forest Reserve in
181 Peninsular Malaysia. **(a)** Bacterial taxa **(b)** Fungal taxa **(c)** Bacterial gene
182 abundance **(d)** Ectomycorrhizal fungal taxa. Samples are separated by whether
183 they were taken from open-control plots where there were many pigs (yellow dots)
184 versus within fenced exclosures without pigs (blue triangles). Environmental
185 factors that have a significant influence are marked in red. LOI = loss-on-ignition
186 (%).
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188 We did not detect higher soil nutrient concentrations due to pig excrement (urine and faeces)
189 deposition in open-control plots as we had predicted. Such equivocal results align with work
190 finding a wide variety of impacts from wildlife on soil nutrients (17,38,39). It is also possible
191 that additional nutrients from wildlife excrement could have been balanced with loss from leaf
192 litter removal. Soil pH was slightly (0.15 pH units) more acidic in exclosure soils but - as has
193 previously been noted by others (40,41) - this was associated with altered bacterial community
194 composition. As predicted, we found varying trends in relative abundance of bacterial taxa, for
195 example, the family Solibacteraceae (phylum Acidobacteria) dominated in exclosure soils, while
196 the family Vicinamibacteraceae (phylum Acidobacteria) was abundant in open-control soils. The
197 relative abundance of Solibacteraceae is reported to decline with increasing soil pH (42) and
198 organic fertilization (e.g. manure) (43), while that of Vicinamibacteraceae is positively
199 correlated with pH (42) and had a higher prevalence in nutrient-amended soils (44). Contrary to
200 our prediction, the relative abundance of bacterial families such as Bradyrhizobiaceae and
201 Xanthomonadaceae, which include members with biological N₂-fixation capacities, increased in
202 open-control soils. However, N₂-fixing members of these families are also known for their
203 denitrifying abilities (45), which suggest that nitrogen deposition from urine and faeces from
204 the pigs could have increased their abundance together with other denitrifying bacterial taxa
205 such as in Rhodospirillaceae (46). Furthermore, in accordance with our prediction, we found a
206 dominance of drought-tolerant Actinobacterial taxa (47,48) in open-control soils.

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208 We had predicted soil nutrients, water, and soil carbon, which are associated with the energy
209 available for micro-organisms, would drive differences in soil microbial communities. However,
210 while we found that bacterial community composition was associated with soil carbon and
211 potassium, these attributes did not differ between exclosures and open-control soils suggesting
212 these factors did not explain differences in bacterial communities between exclosure treatments.
213 Instead, bacterial community change was more likely associated with abiotic conditions,
214 including leaf litter removal and increased light penetration from a browsed and trampled
215 understory vegetation that may cause increased light penetration and soil drying.

216

217 In support of our third hypothesis, the EcM fungal community differed significantly between
218 treatments, and open-control soils were associated with lower relative abundance and diversity
219 of EcM fungi, although there was borderline statistical significance due to only having seven
220 replicates and the potential to improve fungal guild assignment via FUNGuild (49). Pigs may
221 directly consume some fungi or, more likely, the disproportionate removal of trees with
222 symbiotic relationships with EcM fungi (23) is key here. The decline in EcM fungi may cause
223 plant-soil feedbacks that reduce regeneration of EcM-dependent plant species such as
224 dipterocarps (50) and therefore influence future patterns of forest composition. Future work
225 testing if EcM fungi and their plant symbionts differ in un hunted forests with abundant pigs (e.g.

226 Pasoh) compared to hunted forests where pigs are rare, such as in Lambir Hills in Malaysian
227 Borneo (51) would be of great interest.

228

229 In summary, our study is notable for documenting that cryptic biotically-driven edge effects are
230 mediated by wide-ranging wildlife and affect soil microbial community composition. The
231 magnitude of impacts we observed at our site is linked to elevated pig populations associated
232 with oil palm plantations and low hunting pressure(4), thereby creating a cross-boundary
233 ecological cascade from agriculture to pig to soils that extends > 1 km. Pigs are common
234 throughout Asia and invasive globally (29,30,52,53), so our findings may be generalizable
235 beyond Malaysia, and to other mobile crop-raiding wildlife species besides pigs (7,54). The
236 onslaught of African Swine Fever in Asia, which has spread to wild pigs, may reduce pigs'
237 abundance, and provide opportunities for natural experiments on the ecological impacts of
238 losing pigs (55). Other future research may examine associations between soils and volant
239 animals that are often wider ranging, and examine feedback loops between altered soils and
240 plant composition. We conclude with a warning that far-reaching edge effects may produce
241 consequential changes to ecosystem properties and processes performed by soil microbes, as
242 well as alter plant performance and community composition in the future.

243

244 **METHODS**

245 ***Study site***

246 The study was conducted at the Pasoh Forest Reserve, Negeri Sembilan, Peninsular Malaysia
247 (2°59' N, 102°18' E) where the mean annual precipitation is approximately 1800 mm (56). In
248 the study area, the soils are developed over shale, granite and alluvial parent materials with a
249 generally gentle topography and a fairly homogenous vegetation composition (56). The lowland
250 evergreen rain forest core of the reserve is a 600 ha tract dominated by Dipterocarpaceae and
251 typical of much of the broader region (56). Oil palm plantations surround the reserve on three
252 sides (extending for 2 to 10 km away from the reserve) with the northern side abutting a
253 contiguous area of selectively logged lowland and hill forest. Pasoh supports a diverse wildlife
254 community (4) but pigs (*Sus scrofa*) are by far the most common mammal and present at very
255 high densities of 27-47 per km² (4, 52). Pigs are known to cause soil disturbances in the forest
256 that is tied to their nightly traveling to oil palm plantations to crop-raid (27).

257 ***Wildlife exclosure experiment***

258 Eight open-topped exclosures were constructed in 1996 along the southern edge of the 50-ha
259 permanent Forest Dynamics Plot, and 1.3 km from the nearest forest edge (57). The exclosures
260 were 7 m x 7 m, with 1.5 m tall fences made from 4-cm² chain-link metal and surrounded by
261 barbed wire. Each fenced area was paired with two adjacent open-control areas located at least
262 1 m outside the fences. At the time of this study, seven remained effective and one was

263 damaged by falling trees and was not surveyed. Exclosures are described in more detail by
264 Ickes et al. (34) and Luskin et al. (57).

265 ***Soil sampling and DNA extraction***

266 Surface soil (0-5 cm depth) samples were collected in July 2018 from the seven exclosures and
267 seven of their paired controls. We took soils from four points at the corners of a 1 x 1 m² grid
268 and composited them for further analysis. We avoided sampling areas in the controls that had
269 been grubbed by pigs, as this would have exposed sub-surface soil that is known to have a
270 different microbial community to the upper horizons. Soils were kept chilled for c. 48 hours
271 before DNA was extracted from 0.25 g of each soil sample using a MoBio PowerSoil kit following
272 the manufacturer's instructions.

273 ***DNA sequencing***

274 Extracted soil DNA was PCR-amplified in duplicate using the high-fidelity Phusion polymerase. A
275 single round of PCR was done using "fusion primers" (Illumina adaptors + indices + specific
276 regions) targeting the V6-V8 region of 16S rRNA gene of bacteria and the internal transcribed
277 spacer (ITS) 2 region of fungi using the B969F & BA1406R primers of Comeau et al. (58) and
278 ITS86(F) & ITS4(R) primers of Op De Beeck (59) respectively. The PCR products were cleaned
279 and normalized using the high-throughput Charm Biotech Just-a-Plate 96-well Normalization Kit
280 and pooled to make one library that was quantified fluorometrically before sequencing.
281 Sequencing library construction and Illumina MiSeq sequencing (2 x 300 bp) were performed at
282 the Integrated Microbiome Resource, Dalhousie University, Canada (<https://imr.bio/index.html>).

283 ***Bioinformatics***

284 Forward and reverse sequences were assembled using PANDAseq v.2.8 and further sequence
285 processing was performed following the MiSeq SOP in Mothur v.1.32.1 with chimeric sequences
286 removed using chimera.uchime (60-62). Operational taxonomic units (OTUs) of bacterial 16S
287 rRNA gene sequences were assigned based on the OptiClust algorithm using Mothur v.1.40.5
288 with a 97 % similarity threshold and OTUs of fungal ITS sequences were assigned based on the
289 UCLUST algorithm using QIIME v.1.9.1 with a 97 % similarity threshold (63,64). Singleton
290 sequences were removed. Bacterial sequences were then classified based on EzBioCloud
291 database v.2018.05 for bacteria (65) and the UNITE database v.7.2 for fungi (66). To infer the
292 bacterial functions from 16S rRNA gene sequences, we used Phylogenetic Investigation of
293 Communities by Reconstruction of Unobserved States (PICRUST v. 1.1.2 (67). PICRUST uses
294 extended ancestral-state reconstruction algorithm to generate the composition of gene families
295 for the subset of OTUs present in Greengenes database v. 13.5 (68). The predicted gene
296 families were then classified into Kyoto Encyclopedia of Genes and Genomes (KEGG)
297 orthologues (69). We used FUNGuild v.1.0 for functional guild classification of fungi (49).

298 ***Soil analyses***

299 In the field, c. 2.5 g fresh soil was added to 20 ml of 1 M KCl, shaken and returned to the field
300 laboratory where it was filtered through a 0.2 µm filter after c. 6 hours. It was then diluted 1:4

301 and analysed on a Dionex ICS 6000 ion chromatograph for available ammonium and nitrate.
302 The moisture content of fresh soil was determined by heating subsamples to 105 °C for 24 h
303 and the remainder was air-dried and ground to pass a 1-mm sieve. Soil pH was measured by
304 adding 2.5 g of soil to 6.25 ml of deionised water; the mixture was then shaken and left to
305 equilibrate for 24 h before measurement with a Sartorius PB-11 pH meter. Total carbon and
306 nitrogen were determined on a Vario EL Cube elemental analyser. Cations (P, K, Ca and Mg)
307 were extracted from 2.5 g sub-samples that were shaken with 25 ml of Mehlich 3 solution for
308 ten minutes before being filtered and analysed on a Thermo iCAP 6300 Duo inductively coupled
309 plasma optical emission spectrometer with correction by determining moisture content of the
310 air-dried soil by heating subsamples as above.

311 **Statistical analyses**

312 For diversity analysis, bacterial sequences were subsampled into 23,601 reads and fungal
313 sequences were subsampled into 8,994 reads. To compare relative abundance of phyla and
314 diversity between treatments we used t-tests when data were normally distributed or Wilcoxon
315 rank sum tests when data were not normally distributed. We used Bray-Curtis dissimilarity
316 (based on square-root transformed abundances) to visualize differences in the bacterial and
317 fungal communities between treatments and the KEGG Level 3 gene assignments (69). We
318 drew NMDS (non-metric multidimensional scaling) plots using the 'metaMDS' function in R
319 package 'vegan' (70). Statistical significance between treatments were tested by Analysis of
320 Similarities test (ANOSIM). We assessed if microbial composition was influenced by abiotic
321 conditions by including environmental vectors (covariates) in the NMDS ordinations using the
322 vegan 'envfit' function`.

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325 **LITERATURE CITED**

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478 **Supplementary Materials**

479 **Table S1:** Soil chemical analysis (mean \pm standard error) within and outside exclosures to
480 prevent the influence of wildlife (primarily pigs) on ecological processes at Pasoh Forest Reserve
481 in Peninsular Malaysia.

Soil Property	Exclosure	Open-Control	t-test
pH	4.03 \pm 0.02	4.18 \pm 0.06	t=2.42, p=0.032
Loss-on-ignition (%)	5.71 \pm 0.75	5.29 \pm 0.69	t=0.42, p=0.69
C (%)	2.18 \pm 0.87	2.15 \pm 0.73	t=0.05, p=0.96
N (%)	0.15 \pm 0.02	0.14 \pm 0.02	t=0.30, p=0.77
Ammonium ($\mu\text{g g}^{-1}$)	6.08 \pm 10.90	1.54 \pm 1.13	t=1.09, p=0.30
Nitrate ($\mu\text{g g}^{-1}$)	2.43 \pm 4.70	2.63 \pm 4.80	t=0.08, p=0.94
Available P ($\mu\text{g g}^{-1}$)	9.19 \pm 3.90	9.58 \pm 3.92	t=0.18, p=0.86
Available K ($\mu\text{g g}^{-1}$)	56.8 \pm 19.9	79.7 \pm 43.6	t=1.27, p=0.23
Available Ca ($\mu\text{g g}^{-1}$)	18.0 \pm 14.8	25.9 \pm 16.3	t=0.94, p=0.36
Available Mg ($\mu\text{g g}^{-1}$)	22.1 \pm 10.3	26.0 \pm 9.9	t=0.74, p=0.48

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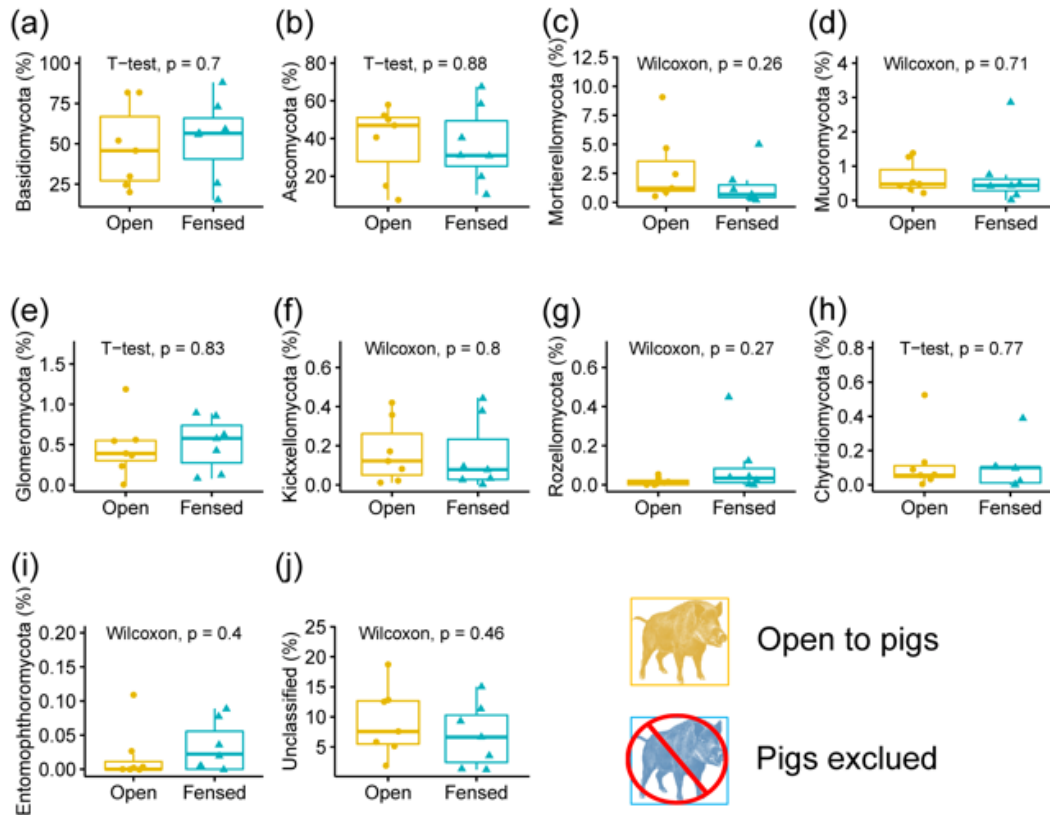
484 **Table S2.** Influence of wildlife (primarily pigs) on the relative abundance of the dominant
 485 bacterial families within the phyla Acidobacteria, Actinobacteria, Chlamydiae and Proteobacteria
 486 at Pasoh Forest Reserve in Peninsular Malaysia. Samples are separated by whether they were
 487 taken from open-control plots where there were many pigs versus within fenced exclosures
 488 without pigs. Only families for which significant differences were observed are shown.

Taxa	Exclosure	Open-Control
Acidobacteria		
Solibacteraceae	8.69 ± 1.16	7.11 ± 1.23
Viciniabacteraceae	1.12 ± 0.25	1.89 ± 0.35
Actinobacteria		
Acidimicrobiaceae	0.36 ± 0.16	0.52 ± 0.08
Conexibacteraceae	0.37 ± 0.14	0.58 ± 0.18
Gaiellaceae	0.11 ± 0.08	0.31 ± 0.11
Propionibacteriaceae	0.04 ± 0.04	0.15 ± 0.12
Sphingobacteriaceae	0.35 ± 0.09	0.58 ± 0.15
Streptomycetaceae	0.42 ± 0.25	1.07 ± 0.28
Chlamydiae		
Rhabdochlamydiaceae	0.17 ± 0.07	0.07 ± 0.06
Alphaproteobacteria		
Bradyrhizobiaceae	3.21 ± 0.63	4.74 ± 0.98
Caulobacteraceae	2.21 ± 0.86	4.04 ± 1.97
Micropepsaceae	1.20 ± 0.32	1.75 ± 0.22
Rhodospirillaceae	3.94 ± 0.90	5.08 ± 0.76
Deltaproteobacteria		
Polyangiaceae	0.93 ± 0.28	1.73 ± 0.72
Gammaaproteobacteria		
Steroidobacteraceae	2.43 ± 0.66	3.34 ± 0.55
Xanthomonadaceae	0.93 ± 0.33	1.33 ± 0.93

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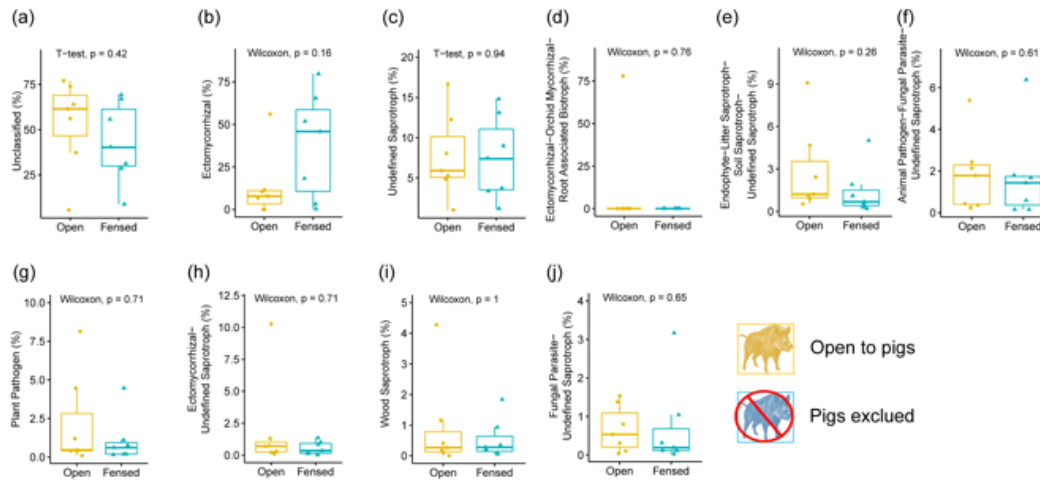
491 **Figure S1:** Influence of wildlife (primarily pigs) on the relative abundance of soil fungal phyla at
492 Pasoh Forest Reserve in Peninsular Malaysia. Samples are separated by whether they were
493 taken from open-control plots where there were many pigs (yellow dots) versus within fenced
494 enclosures without pigs (blue triangles).



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497 **Figure S2:** Influence of wildlife (primarily pigs) on the relative abundance of soil fungal guilds t
498 Pasoh Forest Reserve in Peninsular Malaysia. (determined using FUNGuild) (interpretation same
499 as Fig S1).



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