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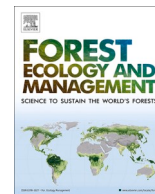
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## Wild pigs influence tropical forest soil microbial communities in a forest-agriculture mosaic landscape

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### ABSTRACT

Edge effects, the altered abiotic and biotic conditions on the borders of natural areas, have rarely been linked to altered soil biota, which shape ecosystem processes including carbon storage, biogeochemical cycling, and plant performance. Here, we investigated if increased wildlife populations (their increase mediated by foraging in nearby oil palm plantations) affect soil biota when they move between plantations and natural habitats. We used a 22-year fenced exclusion experiment in a primary rain forest in Peninsular Malaysia. We found that the presence of wildlife (mainly native pigs; *Sus scrofa*) was associated with greater bacterial diversity, an altered bacterial community composition, and indications of a reduced abundance of symbiotic ectomycorrhizal fungi. There were only minor effects of pigs on soil chemistry or microclimate, so we suggest that changes in soil communities are driven by pigs' leaf litter removal and alterations to plant composition. Our study highlights that indirect effects from agriculture can be induced by wildlife more than 1 km into protected areas and this could have important repercussions for ecosystem processes and plant-soil feedbacks.

### 1. Introduction

Encroachment of industrial agriculture into forested areas is a pervasive global phenomenon that has a clear and direct impact on above- (Gibbs et al., 2010; Gibson et al., 2011) and below-ground (Brearley and Thomas, 2015) biota and ecosystem processes, especially in the tropics. It is challenging to assess how agricultural

expansion indirectly affects natural areas over larger spatial scales, such as the degradation through cryptic edge effects. One example is cross-boundary ecological influences, wherein adjacent ecosystems – first appearing to be distinct – are actually linked through the transport of nutrients, or interactions with wildlife that moves across ecotones (Luskin et al., 2017). With over 70 % of remaining forests now lying within 1 km of an edge (Haddad et al., 2015), there is an urgent need to

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understand how edge effects reshape the linkages between above- and below-ground biota and the scale at which they operate.

Wildlife responses to edges are related to their habitat preferences, and local hunting activities. Some crop-raiding wildlife can actually benefit from supplemental foraging in nearby farmlands (Yahner, 1988; Rand et al., 2006; Prugh et al., 2008; Luskin et al., 2017). In the context of this study, Luskin et al. (2017) showed that wild boar (hereafter 'pigs') found in primary forest in Malaysia increased in abundance when fruit production in surrounding oil palm plantations was high, with cascading impacts on ecological processes in their primary forest habitat; we consider this an agriculturally-mediated edge effect. Edge effects also produce a range of impacts on soils that are often mediated by microclimate and light (Murcia, 1995), as well as plant species composition (e.g. Flores-Rentería et al., 2015; Forbes et al., 2019). However, these edge effects have rarely been documented beyond a few hundred meters (Murcia, 1995; Laurance, 2000; Ewers and Banks-Leite, 2013). Wildlife plays an important role moderating soil nutrients like nitrogen and phosphorus, and soil biota through deposition of excrement and carcasses (Bueno et al., 2013; Brodie and McIntyre, 2019; Villar et al., 2021). Wildlife also affects soil physical environments through biopedoturbation (Rosin et al., 2017; Lamperty et al., 2020; Tuomi et al., 2021), and plant-soil interactions via herbivory or nest building (Ickes et al., 2005; Wirth et al., 2008; Porensky, 2011; Andriuzzi and Wall, 2017).

Soil microbes have myriad links to biogeochemical processes that, in turn, shape ecosystem properties including carbon dynamics (Cavicchioli et al., 2019). Soil microbes are strongly affected by the edaphic environment (Lladó et al. 2017). However, given that pigs impact upon soil properties and processes (Bueno et al., 2013; Eldridge and Soliveres, 2023), their influence on soil microbial communities is little studied. Parkes et al. (2015) found indications that fungal communities were proportionally more abundant in pig-disturbed soil, although the opposite pattern was seen by Lundgren et al. (2023). Wehr et al. (2019) showed that an increase in pig numbers reduced bacterial diversity but they did not examine the bacterial community composition in further detail.

Here, we investigate the potential for wide-ranging wildlife (primarily pigs) to cause far-reaching edge effects on soil microbial communities in the distant 'interior' of primary forests more than 1 km from the nearest edge. We conducted our study in a primary Malaysian rain forest dominated by the *Dipterocarpaceae* (Brearley et al., 2016) that are associated with symbiotic ectomycorrhizal fungi (Brearley, 2012). In this forest, native forest-dwelling wild pigs (*Sus scrofa*) that forage in adjacent oil palm (*Elaeis guineensis*) plantations have increased population densities and are known to disturb nearby forest soils and plant communities (Luskin et al., 2017, 2019, 2021; Fujinuma and Harrison, 2012). They also have a preference for using dipterocarp saplings for their nests (Ickes et al., 2005). Wild pigs are a key example of a broadly distributed generalist vertebrate that is adaptable to human-disturbed environments and are considered 'ecosystem engineers' due to the major physical soil disturbances via rooting, grubbing (predating larger soil invertebrates), wallowing, trampling and soil compaction (Barrios-García and Ballari, 2012; Bevins et al., 2014).

In this paper, we focus on pigs' influence on soil microbial community composition and functioning, which remains largely unknown. Using a long-term enclosure experiment, we investigated how edge effects develop deep within primary forest as a result of the influence of oil-palm fed pigs on microbial communities. We examined three hypotheses based on the ecology of pigs and known relationships between soil disturbances and microbial communities:

1) First, we predicted that pig-exposed soils would have greater nutrient concentrations due to the deposition of urine and faeces (Wirthner et al., 2012; Bueno et al., 2013) and that, together with disturbances caused by removal of understorey plants and leaf litter

by pigs (Singer et al., 1984), would be key drivers of microbial community structure in pig-exposed soils.

- 2) Second, we predicted that soil bacteria would be more impacted than fungi due to changes in nitrogen deposition from excrement influencing bacteria involved in the nitrogen cycle, and removal of understorey plants and leaf litter, which increases light penetration and likely has a drying effect on soils to which bacteria are more sensitive than fungi (de Vries et al., 2018).
- 3) Third, we predicted that whilst fungi would be less influenced by the presence of pigs than bacteria, symbiotic ectomycorrhizal (EcM) fungi would be reduced in pig-exposed soils because pigs preferentially remove dipterocarp saplings (Ickes et al., 2005) that are associated with these fungi.

## 2. Methods

### 2.1. Study site

The study was conducted at the Pasoh Forest Reserve, Negeri Sembilan, Peninsular Malaysia (2°59' N, 102°18' E) where the mean annual precipitation is approximately 1800 mm (Davies et al., 2003). In the study area, the soils have developed over shale, granite and alluvial parent materials with a generally gentle topography and a fairly homogeneous vegetation composition (Davies et al., 2003). The lowland evergreen rain forest core of the reserve is a 600 ha tract dominated by *Dipterocarpaceae* and typical of much of the broader region (Davies et al., 2003). Oil palm plantations surround the reserve on three sides (extending for 2–10 km away from the reserve) with the northern side abutting a contiguous area of selectively logged lowland and hill forest. Pasoh supports a diverse wildlife community (Luskin et al., 2017) but pigs (*Sus scrofa*) were, prior to the recent outbreak of African swine fever (Luskin et al., 2023), by far the most common mammal and present at very high densities of 27–47 per km<sup>2</sup> (Ickes, 2001; Luskin et al., 2017).

### 2.2. Wildlife enclosure experiment

Eight open-top enclosures were constructed in 1996 along the southern edge of the 50-ha permanent Forest Dynamics Plot, and 1.3 km north from the nearest forest edge where the plantations were located (Ickes et al., 2001). The enclosure replicates were spaced at 50 m intervals along a 400 m east-west transect and were 7 m × 7 m, with 1.5 m tall fences made from 4-cm<sup>2</sup> chain-link metal and surrounded by barbed wire. Each fenced area was paired with two adjacent and equal-sized open-control areas located at least 1 m outside the fences on the sides that most closely resembled the vegetation structure within the enclosure at the time of construction (Ickes et al., 2001). At the time of this study, seven remained effective as one had been damaged by falling trees and was not surveyed. Enclosures are described in more detail by Ickes et al. (2001) and a map is provided in Luskin et al. (2019).

### 2.3. Soil sampling and DNA extraction

Surface soil (0–5 cm depth) samples were collected in July 2018 from the seven enclosures and seven of their paired open-controls. We took soils from four points at the corners of a 1 m<sup>2</sup> grid and composited them for further analysis. We avoided sampling areas in the open-controls that had been grubbed by pigs, as this would have exposed sub-surface soil that is known to have a different microbial community to the upper horizons (e.g. López-Mondéjar et al., 2015), thus confounding our results. Soils were kept chilled for c. 48 h before DNA was extracted from 0.25 g of each soil sample using a MoBio PowerSoil kit following the manufacturer's instructions.

### 2.4. DNA sequencing

Extracted soil DNA was PCR-amplified in duplicate using the high-

fidelity Phusion polymerase. A single round of PCR was done using "fusion primers" (Illumina adaptors + indices + specific regions) targeting the V6-V8 region of 16S rRNA gene of bacteria and the internal transcribed spacer (ITS) 2 region of fungi using the B969F + BA1406R primers of Comeau et al. (2011) and ITS86(F) + ITS4(R) primers of Op De Beeck (2014) respectively. The PCR products were cleaned and normalized using the high-throughput Charm Biotech Just-a-Plate 96-well Normalization Kit and pooled to make one library that was quantified fluorometrically before sequencing. Sequencing library construction and Illumina MiSeq sequencing (2 × 300 bp) were performed at the Integrated Microbiome Resource, Dalhousie University, Canada (<https://imr.bio/index.html>).

## 2.5. Bioinformatics

Forward and reverse sequences were assembled using PANDAseq v.2.8 (Masella et al., 2012) and further sequence processing was performed following the MiSeq SOP in Mothur v.1.32.1 (Schloss et al., 2009) with chimeric sequences removed using chimera.uchime (Edgar et al., 2011). Operational taxonomic units (OTUs) of bacterial 16S rRNA gene sequences were assigned based on the OptiClust algorithm using Mothur v.1.40.5 with a 97 % similarity threshold and OTUs of fungal ITS sequences were assigned based on the UCLUST algorithm using QIIME v.1.9.1 with a 97 % similarity threshold (Edgar, 2010; Westcott and Schloss, 2017). Singleton sequences were removed. Bacterial sequences were then classified based on EzBioCloud database v.2018.05 for bacteria (Yoon et al., 2017) and the UNITE database v.7.2 for fungi (Abarenkov et al., 2010). To infer the bacterial functions from 16S rRNA gene sequences, we used Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt v. 1.1.2 (Langille et al., 2013). PICRUSt uses extended ancestral-state reconstruction algorithm to generate the composition of gene families for the subset of OTUs present in Greengenes database v. 13.5 (De Santis et al., 2006). The predicted gene families were then classified into Kyoto Encyclopedia of Genes and Genomes (KEGG) orthologues (Kanehisa et al., 2012). We used FUNGuild v.1.0 for functional guild classification of fungi (Nguyen et al., 2016).

## 2.6. Soil analyses

In the field, c. 2.5 g fresh soil was added to 20 ml of 1 M KCl, shaken and returned to the field laboratory where it was filtered through a 0.2 µm filter after c. 6 hours. It was then diluted 1:4 and analysed on a Dionex ICS 6000 ion chromatograph for available ammonium and nitrate. The moisture content of fresh soil was determined by heating subsamples to 105 °C for 24 h and the remainder was air-dried and ground to pass a 1-mm sieve. Soil pH was measured by adding 2.5 g of soil to 6.25 ml of deionised water; the mixture was then shaken and left to equilibrate for 24 h before measurement with a Sartorius PB-11 pH meter. Total carbon and nitrogen were determined on a Vario EL Cube elemental analyser. Cations (P, K, Ca and Mg) were extracted from 2.5 g sub-samples that were shaken with 25 ml of Mehlich 3 solution for ten minutes before being filtered and analysed on a Thermo iCAP 6300 Duo inductively coupled plasma optical emission spectrometer with correction by determining moisture content of the air-dried soil by heating subsamples as above.

## 2.7. Statistical analyses

For diversity analysis, bacterial sequences were rarefied to 23,601 reads and fungal sequences were rarefied to 8994 reads. To test the effects of exclosures on the relative abundance of bacterial and fungal taxa, fungal functional guilds and soil chemical properties, we performed mixed-effects models in the 'nlme' package (Pinheiro et al., 2018), with each exclosure/paired open-control included as a random factor. We used Bray-Curtis dissimilarity (based on square-root

transformed abundances) to visualize differences in the bacterial and fungal community composition between treatments and the KEGG Level 3 predicted gene assignments (Kanehisa et al., 2012). We drew NMDS (non-metric multidimensional scaling) plots using the 'metaMDS' function in the R package 'vegan' (Oksanen et al., 2013). Statistical significance between treatments were tested by Analysis of Similarities tests (ANOSIM). To determine relationships between edaphic factors and microbial community composition/function, we fitted environmental variables onto the NMDS plot using the 'envfit' function in the R package 'vegan'.

## 3. Results

There were low concentrations of all soil nutrients measured, which is typical for Southeast Asian rain forests on similar substrates (Table 1). There were no significant differences between the exclosures and the open-control areas, with the exception of soil pH that was 0.15 pH units more acidic within the exclosures (Table 1).

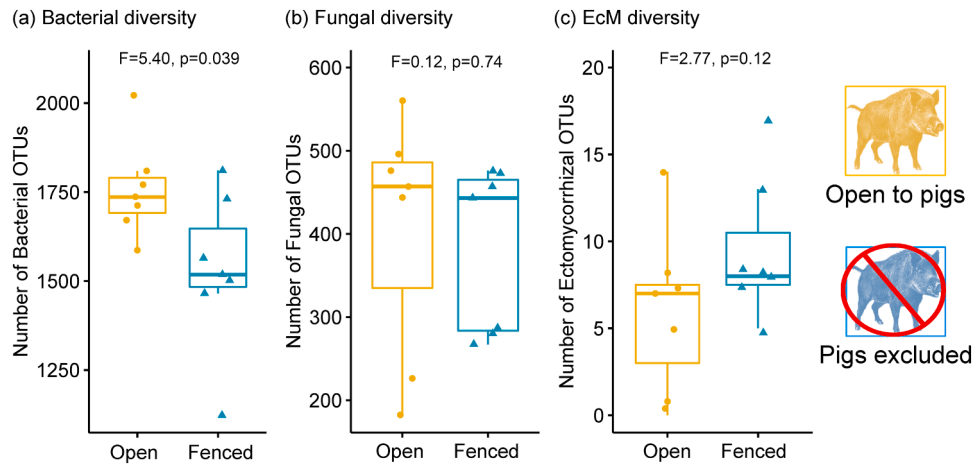
Bacterial richness was 13 % lower in exclosure soils ( $p = 0.039$ ; Fig. 1a). In the exclosures, the relative abundance of Acidobacteria increased ( $p = 0.010$ ; Fig. 2a) but there was lower relative abundance of Proteobacteria, Actinobacteria, Planctomycetes and Gemmatimonadetes (all  $p < 0.05$ ; Fig. 2). At the subphylum level, we also observed significant differences in the relative abundance of dominant bacterial taxa, for example, the relative abundance of bacterial families *Solibacteraceae* and *Rhizobiales* increased, whilst abundance of other families such as *Bradyrhizobiaceae* and *Xanthomonadaceae*, including members with biological N<sub>2</sub>-fixation capacities, declined in exclosure soils (all  $p < 0.05$ ; Table S1). The bacterial community composition differed between the exclosures and the open-controls (ANOSIM  $R = 0.216$ ,  $p = 0.009$ ; Figs. 2 & 3a) and was correlated with soil pH (Fig. 3a). Soil carbon and potassium were also correlated with the bacterial community composition, but these did not differ between the exclosure and open-control soils (Table 1). There was no clear association between the exclosures and bacterial community functioning as measured by predicted gene abundance (ANOSIM  $R = 0.081$ ,  $p = 0.23$ ; Fig. 3c).

The fungal community did not differ significantly between the exclosure and open-control soils in terms of diversity ( $p = 0.74$ ; Fig. 1b), phylum abundance (all  $p > 0.20$ ; 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 exclosure treatments (ANOSIM:  $R = 0.21$ ,  $p = 0.03$ ; Fig. 3d), and there were indications of increases in diversity ( $p = 0.12$ ; Fig. 1c) and abundance in exclosure soils ( $p = 0.10$ ; Fig. S2b) (note both these p-values are greater than 0.05). The fungal community composition (including all species) was correlated with soil carbon (Fig. 3b) but none of the soil chemical variables were significantly correlated with the EcM community structure (Fig. 3d).

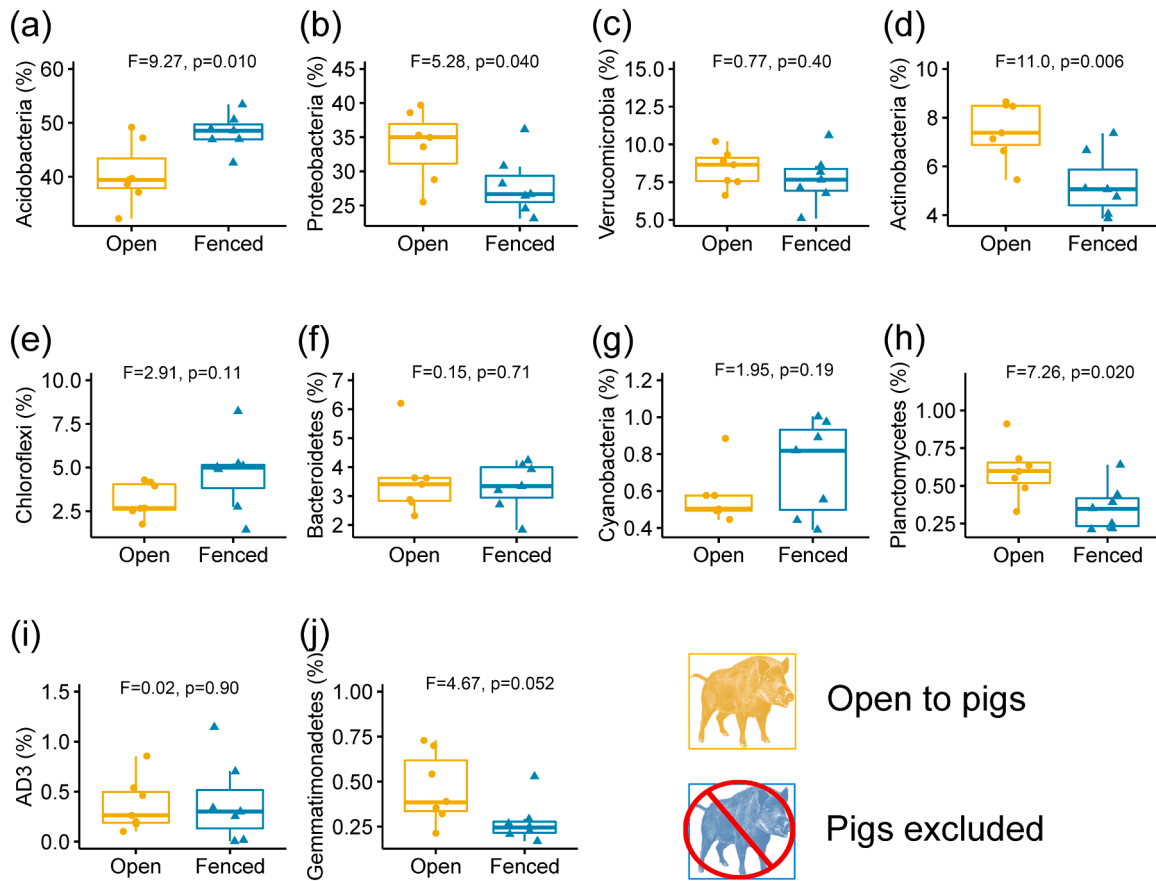
**Table 1**

Soil chemical properties (mean ± standard error) within and outside exclosures to prevent the influence of wildlife (primarily pigs) on ecological processes at Pasoh Forest Reserve in Peninsular Malaysia.

Soil Property	Exclosure	Open-Control	
pH	4.03 ± 0.02	4.18 ± 0.06	F = 9.58, p = 0.009
Loss-on-ignition (%)	5.71 ± 0.75	5.29 ± 0.69	F = 0.17, p = 0.69
C (%)	2.18 ± 0.87	2.15 ± 0.73	F = 0.03, p = 0.87
N (%)	0.15 ± 0.02	0.14 ± 0.02	F = 0.02, p = 0.89
Ammonium (µg g <sup>-1</sup> )	6.08 ± 10.90	1.54 ± 1.13	F = 1.20, p = 0.30
Nitrate (µg g <sup>-1</sup> )	2.43 ± 4.70	2.63 ± 4.80	F = 0.01, p = 0.94
Available P (µg g <sup>-1</sup> )	9.19 ± 3.90	9.58 ± 3.92	F = 0.03, p = 0.86
Available K (µg g <sup>-1</sup> )	56.8 ± 19.9	79.7 ± 43.6	F = 1.60, p = 0.23
Available Ca (µg g <sup>-1</sup> )	18.0 ± 14.8	25.9 ± 16.3	F = 0.89, p = 0.36
Available Mg (µg g <sup>-1</sup> )	22.1 ± 10.3	26.0 ± 9.9	P = 0.54, p = 0.48



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

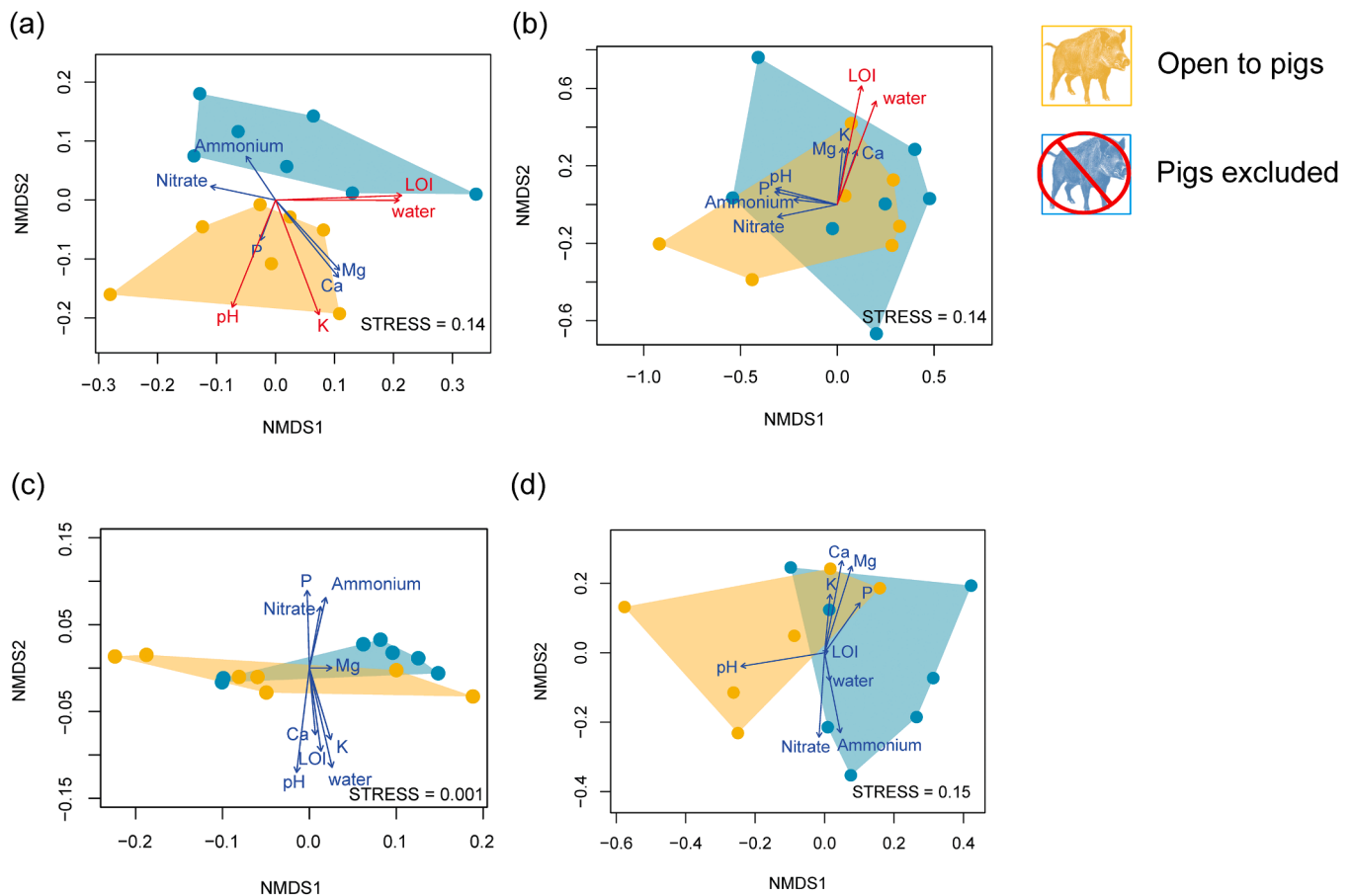


**Fig. 2.** Influence of wildlife (primarily pigs) on the relative abundance of soil bacterial phyla at Pasoh Forest Reserve in Peninsular Malaysia (interpretation is the same as Fig. 1).

**4. Discussion**

Our study is the first to link agricultural impacts on altered soil microbial community composition in adjacent habitats via the impacts of wildlife that travels distances more than 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 pigs that fed on oil palm fruits in adjacent plantations and then returned to the forest. The

biotically-driven impacts by pigs that we report are distinct from more well-documented edge effects related to habitat and abiotic conditions (Haddad et al., 2015), since all our sampling locations were equidistant from edges and microclimatic differences were not detected greater than 100 m from the edge at our site (Luskin and Potts, 2011). Instead, we posit that pigs impact soil microbial communities by disturbing soils, leaf litter, and understory vegetation and altering the plant community composition (Ickes et al., 2001, 2005; Luskin et al., 2017,2019). For



**Fig. 3.** NMDS ordinations showing the influence of wildlife (primarily pigs) on the soil microbial community composition and function at Pasoh Forest Reserve in Peninsular Malaysia. (a) Bacterial taxa (b) Fungal taxa (c) Bacterial gene abundance (d) Ectomycorrhizal fungal taxa. Samples are separated by whether they were taken from open-control plots where there were many pigs (yellow dots) versus within fenced enclosures without pigs (blue triangles). Environmental factors that have a significant influence are marked in red. LOI = loss-on-ignition (%).

example, previous work at our site has shown pigs reduce the abundance of saplings with symbiotic root-associated ectomycorrhizal (EcM) fungi (i.e. *Dipterocarpaceae*) (Ickes et al., 2005) and facilitate lianas (Luskin et al., 2019) that are rarely reported to have EcM associations. As predicted, we found wildlife exclusion was associated with altered EcM fungi communities and indications of an increased relative abundance of EcM fungi. Additionally, wildlife exclusion led to a greater community change in the bacterial community than the fungal community. Prior work has found invasive pigs reduced soil bacterial diversity in Hawai'i (Wehr et al., 2019) and, in New Zealand, invasive pig grubbing increased the relative abundance of fungi over bacteria (Parkes et al., 2015) which our results supported. However, our sampling design could have been finessed by establishing enclosures with increasing distance from the forest edge to assess how changing densities of pigs might influence the patterns shown here – this would have also allowed us to determine the impact of increasing pig densities over and above the ‘background’ impact of wildlife on soil microbial communities.

We did not detect greater soil nutrient concentrations due to deposition of pig excrement (urine and faeces) in open-control plots as we had predicted. Such equivocal results align with work finding a wide variety of impacts from wildlife on soil nutrients (Singer et al., 1984; Bruinderink and Hazebroek, 1996; Wirthner et al., 2012; Bueno et al., 2013; Lundgren et al., 2023). Soil pH was slightly more acidic (0.15 pH units) in enclosure soils but this was associated with altered bacterial community composition as has previously been noted by others (Fierer and Jackson, 2006; Tripathi et al., 2012). As predicted, we found varying trends in relative abundance of bacterial taxa, for example, the family *Solibacteraceae* (phylum Acidobacteria) dominated in enclosure

soils, while the family *Vicinamibacteraceae* (phylum Acidobacteria) was abundant in open-control soils. The relative abundance of *Solibacteraceae* is reported to decline with increasing soil pH (Jones et al., 2009) and organic fertilization (e.g. manure) (Soman et al., 2017), while that of *Vicinamibacteraceae* is positively correlated with pH (Jones et al., 2009) and had a greater prevalence in nutrient-amended soils (van der Bom et al., 2018). Contrary to our prediction, the relative abundance of bacterial families such as *Bradyrhizobiaceae* and *Xanthomonadaceae*, which include members with biological N<sub>2</sub>-fixation capacities, increased in open-control soils. However, N<sub>2</sub>-fixing members of these families are also known for their denitrifying abilities (Shapleigh, 2013), which suggest that nitrogen deposition from urine and faeces from the pigs could have increased their abundance together with other denitrifying bacterial taxa such as in *Rhodospirillaceae*. Furthermore, in accordance with our prediction, we found a dominance of drought-tolerant Actinobacterial taxa in open-control soils (Barnard et al., 2013; Bouskill et al., 2013).

We had predicted soil nutrients, water, and soil carbon, which are associated with the energy available for micro-organisms, would drive differences in soil microbial communities. However, while we found that bacterial community composition was associated with soil carbon and potassium (as determined by ‘envfit’ on the NMDS), these attributes did not differ between enclosures and open-control soils suggesting these factors did not explain differences in bacterial communities between enclosure treatments. Instead, bacterial community change was more likely associated with abiotic conditions, including leaf litter removal and increased light penetration from a browsed and trampled understorey vegetation that may cause increased irradiance and soil drying.

In support of our third hypothesis, the EcM fungal community differed significantly between treatments, and open-control soils appeared to have a lower relative abundance and diversity of EcM fungi, although this did not reach statistical significance due to only having seven replicates and the potential to improve fungal guild assignment via FUNGuild (Nguyen et al., 2016). Pigs may directly consume some fungi or, more likely, the disproportionate removal of trees with symbiotic relationships with EcM fungi (Ickes et al., 2005) is key here. A decline in EcM fungi may cause plant-soil feedbacks that reduce regeneration of EcM-dependent plant species such as dipterocarps (Brearley, 2012) and therefore influence future patterns of forest composition. Future work testing if EcM fungi and their plant symbionts differ in un hunted forests with abundant pigs (e.g. Pasoh) compared to hunted forests where pigs are rare, such as in Lambir Hills in Malaysian Borneo (Harrison et al., 2013) would be of great interest. Indeed, since we conducted this study, the onslaught of African Swine Fever in Asia, which has spread to wild pigs, has markedly reduced pig abundance at Pasoh (Luskin et al., 2023), providing clear opportunities for natural experiments on the ecological impacts of losing pigs; although populations are already on the rebound (Vanar, 2023; Newey, 2024).

In summary, our study is notable for documenting that cryptic biotically-driven edge effects are mediated by wide-ranging wildlife and affect the soil microbial community composition. The magnitude of impacts we observed at our study site is linked to elevated pig populations associated with oil palm plantations and low hunting pressure, thereby creating a cross-boundary ecological cascade from agriculture to pigs to soils that extends more than 1 km. Pigs are common throughout Asia and invasive globally, so our findings may be generalizable beyond Malaysia, and to other mobile crop-raiding wildlife species besides pigs. The recent impact of African swine fever on reducing pig populations may reverse some of the results found here and are therefore worth follow-on studies. Other future research may examine associations between soils and volant animals that are often wider ranging, and examine feedback loops between altered soils and plant composition. We conclude with a warning that far-reaching edge effects may produce consequential changes to ecosystem properties and processes performed by soil microbes, as well as alter plant performance and community composition in the future.

#### Author declaration

FQB, JMA & MSL conceived the ideas, FQB, MSL, KI & JMA designed the methodology, FQB & MSL conducted fieldwork, FQB conducted soil analyses, ARAR & KD conducted molecular laboratory analyses, HS & BMT conducted the bioinformatics, KI & NMZ provided experimental and molecular resources, respectively, NMZ, MSL, KI & FQB obtained funding, HS, BMT & FQB analysed the data, HS prepared the graphs, FQB & MSL wrote the manuscript with editorial input from all authors except KI.

#### CRedit authorship contribution statement

**Francis Brearley:** Writing – review & editing, Writing – original draft, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Hokyung Song:** Writing – review & editing, Visualization, Investigation, Formal analysis. **Binu Tripathi:** Writing – review & editing, Validation, Software, Investigation, Formal analysis, Data curation. **Ke Dong:** Writing – review & editing, Resources, Investigation, Formal analysis, Data curation. **Noraziah Zin:** Writing – review & editing, Resources, Project administration, Funding acquisition. **Abdul Rahim:** Writing – review & editing, Resources, Investigation, Data curation. **Kalan Ickes:** Resources, Methodology, Investigation, Conceptualization. **Jonathan Adams:** Writing – review & editing, Project administration, Methodology, Conceptualization. **Matthew Luskin:** Writing – review & editing, Writing – original draft, Resources, Project administration,

Methodology, Investigation, Funding acquisition, Conceptualization.

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#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data availability

Data is available via GenBank Bioproject ID PRJNA748839.

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#### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.foreco.2024.122320](https://doi.org/10.1016/j.foreco.2024.122320).

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