

## Reviewer 2

Thank you for the opportunity to review the paper by Adame et al., titled 'Soil disturbance in wetlands by feral pigs increases greenhouse gas emissions', submitted to European Geosciences Union - biogeosciences.

*This study investigates whether soil disturbance caused by invasive hoofed mammals in tropical wetlands of northern Australia enhances greenhouse gas emissions, specifically carbon dioxide, methane, and nitrous oxide. The authors combined field measurements of greenhouse gas fluxes with some laboratory incubations of soil and root respiration, alongside measurements of various environmental parameters, to determine the drivers of changes in fluxes. The study found that the highest emissions occurred near disturbed sites, suggesting that feral pigs have a measurable impact on wetland greenhouse gas cycling.*

*Overall, this is an interesting and timely study addressing an important and underexplored topic. The manuscript is generally well written and presents novel findings with potential implications for wetland management and greenhouse gas accounting. However, several methodological details require clarification, and some interpretations should be presented more cautiously.*

### **Specific comments to address:**

**Line 69** – “Old disturbed” reads awkwardly and should be rephrased.

We have changed the term “old disturbed” for “past disturbance” throughout the manuscript

**Figure 1** – Please indicate what the grey shaded areas represent.

We have clarified as follows:

Fig 1. “Grey areas represent the extent of the floodplain (modified from Ward et al. 2014 )”

Ward et al. 2014. Floodplain inundation and vegetation dynamics in the Alligator Rivers region (Kakadu) of northern Australia assessed using optical and radar remote sensing. Remote Sensing of Environment. 147, 43-55.

**Line 120** – *It is not clear at what depth soil physicochemical characteristics were measured. Please provide more details on how these measurements were conducted. For example, were soil cores collected and redox measured along a depth profile? Soil redox potential changes substantially with depth, particularly in wetlands where soils transition from aerobic surface layers to increasingly anaerobic subsurface conditions. This redox gradient strongly influences the production and distribution of microbial greenhouse gases, meaning hotspots for CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O may differ with depth. Please clarify whether soil redox potential values were adjusted relative to the Standard Hydrogen Electrode (SHE) and specify the reference electrode used. This information is important for interpreting the redox conditions associated with greenhouse gas production. Understanding where and how soil redox was measured is important for interpreting these results.*

**Figure 6** – *As mentioned above, were soil redox potential values adjusted to a standard hydrogen electrode/reference electrode? Please also specify the depth at which redox was measured and indicate what the lightly shaded area represents.*

We measured surface soil redox, which was calibrated according to the instrument instructions with a ZoBell’s solution. We have clarified as follows:

L120: "At each plot, surface reduction-oxidation potential (redox; 3-5 measurements) and soil temperature were determined with a redox meter (HQ 11d ORP- meter, Hach, calibrated against ZoBell's Solution)."

**Line 134** – *More methodological detail regarding greenhouse gas sampling is required eg: How much gas was removed from the chamber headspace at each sampling interval? Were vials over-pressurised? Please clarify whether ambient/background atmospheric samples were collected alongside chamber measurements as including atmospheric reference samples is useful for quality assurance of GC measurements, identifying potential contamination or instrument drift, and contextualising chamber concentrations relative to ambient greenhouse gas concentrations. This is particularly important for low-flux measurements and GHG 'uptake' measurements to see if your starting concentrations are near atmospheric (start measurement) and final concentrations (end measurement) ~below atmospheric levels. What were the precision and detection limits of the GC system used? Were any samples outside the calibration range? What is meant by "linearity of fluxes within each chamber was checked"? eg was an  $r^2$  threshold used to reject poorly linear fluxes? If so, what threshold was applied? How are airtight seals with soil confirmed? When were chambers/collars installed? Eg were they pre-installed and revisited, or inserted immediately before measurements? Both approaches have important caveats that should be acknowledged. Long-term collar installation can alter soil biogeochemistry, whereas installing chambers immediately prior to measurements may disturb soils and release naturally accumulated gases, potentially altering measured fluxes. How was temperature measured for use in Flux Equation 1?*

We have clarified the requested information as follows:

L132: "Instantaneous gas fluxes ( $n = 5$  per plot) were measured in dark, closed chambers (Hutchinson & Mosier, 1981) made of polyvinyl chloride (4L, 15 cm diameter and 30 cm in height; Fig. 2). The chambers were installed right before the measurements, hammered into the soil at least 3 cm deep, acknowledging some disturbance to the soil. After 15 minutes of deployment, gas samples were collected from the headspace of the chambers using a plastic 30 mL syringe and a 26G needle and transferred to 12mL vacuumed glass containers (flat bottom, 103 mm, diameter 15.5 mm, Labco Exetainer). The air samples were collected at 0 and 60 min at all chambers and at 0, 20, 40, and 60 min in one chamber per plot. During each sampling, the headspace was mixed with the syringe three times before the air sample was retrieved.

Back in the laboratory, the  $\text{CO}_2$ ,  $\text{CH}_4$ , and  $\text{N}_2\text{O}$  concentrations within each sample were measured in a gas chromatograph (Shimadzu GC-2010 Plus, DESITI, Queensland Government). The analyses had accuracies of 0.2%, 0.5%, and 1.5% for  $\text{CO}_2$ ,  $\text{CH}_4$ , and  $\text{N}_2\text{O}$ , respectively. Air temperature next to the chambers was monitored throughout the experiment. The chamber with continuous measurements was checked for linearity using a regression of time for each gas within each plot. Values for the linear regression and the  $R^2$  values are shown in Table S1; chambers with low linearity ( $R^2 < 50$ ) were identified in sites with very low  $\text{N}_2\text{O}$  fluxes or very high  $\text{CH}_4$  emissions. Because  $\text{CH}_4$  is characterized by nonlinearity and spikes due to ebullition, outliers were retained in the calculations, although median values along with interquartile ranges were used to provide conservative estimates. Only one emission value of  $\text{CH}_4$  in a disturbed site by pigs, which was three orders of magnitude higher than the rest of the chambers within the same plots, was removed from calculations (Coolaboo, chamber D, during Kunumeleng season). Fluxes ( $F$ ) were calculated per hour from the net changes in gas concentrations ( $N$ , mol) during the incubation time ( $t$ , hours), per volume of the chamber ( $V$ ,  $\text{m}^3$ ), at a given air temperature ( $T$ , °K), assuming a pressure of one atmosphere"

Additionally, a new supplementary Table (S1) has been included with all the parameters of the regression equations for each plot. Finally, all data will be available to the public through the Griffith Research Repository Dataset to support accessibility and transparency.

**Line 157** – *The manuscript extrapolates emissions to fluxes per hectare, but it may also be useful to cautiously estimate the impact per feral animal, or cautiously use reference satellite data, or published estimates to try upscale to the NT region and see what effect this disturbance could be having regionally or nationally. This could help contextualise the broader regional impact of feral pig disturbance.*

*It may also be worthwhile to provide a preliminary estimate of the broader magnitude of these processes. As noted above, this could potentially be achieved by combining measured disturbance impacts with satellite imagery, aerial mapping, or existing estimates of feral pig disturbance extent across the region.*

Thank you for the idea. We have included an estimate (acknowledging the limitations) of the potential of greenhouse gas reductions at the regional scale:

L366: “We cautiously estimate annual emissions from pig disturbance using our data, supplemented by published estimates of emissions during flooding (Bass et al. 2014). We consider emissions from flooded Melaleuca swamps to be representative of reference sites and open-water areas without vegetation to represent sites damaged by pigs. Seasonal differences were included in our estimations, considering that the wet seasons Kudjewe and Bangkerrent seasons span from 20 Dec-30 April, the cool seasons, Yekke and Wurrkeng, from 1st May- 15th August (data from this study), and the hot dry seasons, Kurrung and Kunumeleng seasons, span from 16th Aug to 19th December (data from this study). We estimate that annual emission reductions by comparison (reference vs disturbed) are 13.2 Mg CO<sub>2</sub> ha<sup>-1</sup> yr<sup>-1</sup>, 1.1 Mg CH<sub>4</sub> ha<sup>-1</sup> yr<sup>-1</sup>, and 0.004 Mg N<sub>2</sub>O ha<sup>-1</sup> yr<sup>-1</sup>, equivalent to reductions of 43.4 Mg CO<sub>2-eq</sub> ha<sup>-1</sup> yr<sup>-1</sup>. We can then extrapolated these emissions to the Kakadu and adjacent river floodplains (See Fig 1; 356,800 ha, Ward et al. 2014) considering that about 10% of the area is disturbed by pigs (unpublished data). We estimate emissions of 0.47, 1.04, and 0.04 Mt CO<sub>2-eq</sub> yr<sup>-1</sup> for CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O, respectively, or a total of 1.5 Mt CO<sub>2-eq</sub> yr<sup>-1</sup>. These levels of emissions are equivalent to 5.6% of the Northern Territory's emissions in Australia, or 57.4% of the Territory's agricultural emissions. Although we have acknowledged the limitations of our calculations and the challenge of removing the thousands of pigs inhabiting these wetlands, it is clear that there is significant potential to reduce greenhouse gas emissions through feral pig control.”

**Table 1** – *There is a substantial soil temperature difference between reference and disturbed sites. I would have expected the opposite trend, where disturbed sites lacking vegetation cover would exhibit higher soil temperatures due to increased solar exposure and radiation heating bare/darker soil surfaces. Could this reflect a time-of-day sampling artefact? For example, were disturbed sites sampled in the morning and reference sites later in the day? Please consider and discuss.*

**Line 291** – *Disturbed plots are colder? Are these soil temperatures or air temperatures? This is currently unclear. Was this time-of-day artefact? Seems counterintuitive.*

The plots affected by pigs were consistently colder as they tend to dig into the soil until they reach the groundwater level. They need to do this to stay cooler during the hottest parts of the day. The plots (disturbed and reference) were measured simultaneously. See Discussion:

L316: “The disturbed plots were also colder and filled with fresh groundwater, as pigs used some of these areas to wallow or coat themselves with mud to avoid overheating (Bracke, 2011).”

**Figures 3 and 4** – Nitrous oxide fluxes in Figure 3 range from approximately 200–800  $\mu\text{g}/\text{m}^2/\text{h}$  in disturbed sites, whereas Figure 4 appears mostly within  $\pm 5 \mu\text{g}/\text{m}^2/\text{h}$ . This represents a difference of roughly two orders of magnitude, while methane and carbon dioxide fluxes appear comparable between figures. Please check whether this discrepancy is correct or due to an error.

Figure 3 shows data from the Yekke season, while Figure 4 shows data from Kunumleng, where  $\text{N}_2\text{O}$  emissions were much lower. During Kunumeleng, pigs move closer to the waterholes, leaving behind highly anoxic soils where methanogenesis dominates over denitrification (see Fig. 7).

**Line 306** – “ $^{13}\text{C}$ ” is incorrectly formatted and should be written as  $\delta^{13}\text{C}$  throughout. I also do not think the statement that “disturbed soils showed slightly reduced  $\delta^{13}\text{C}$  values” is fully supported by the data. Four out of six sites showed essentially the same/ non-significantly different  $\delta^{13}\text{C}$  values between treatments, while only two disturbed sites were statistically more negative, and only by a small fractionation ( $\sim 2\%$ ). Therefore, the broader claim appears overstated. Furthermore, bulk soil  $\delta^{13}\text{C}$  is only an indirect indicator of methanogenic processing and integrates multiple carbon pools and decomposition pathways. Small differences in  $\delta^{13}\text{C}$  could simply reflect pig disturbance-driven mixing of soil organic matter, altered litter inputs, or differences in carbon turnover rather than enhanced methanogenesis directly

Good point, we have removed this statement and also corrected the formatting of  $\delta^{13}\text{C}$

**Line 303** – The discussion regarding oxygen limitation in soils is also somewhat speculative because oxygen concentrations were not directly measured; only soil redox was assessed, and respiration rates and possibly only at the surface. This does not necessarily indicate that subsurface soils were aerobic too. Wetlands occupy low landscape positions and commonly feature shallow water tables, so deeper anaerobic conditions may still occur irrespective of surface measurements.

Agree. We have clarified that our measurements are redox, not directly oxygen concentrations. We have also specified that we are referring to surface processes.

e.g.

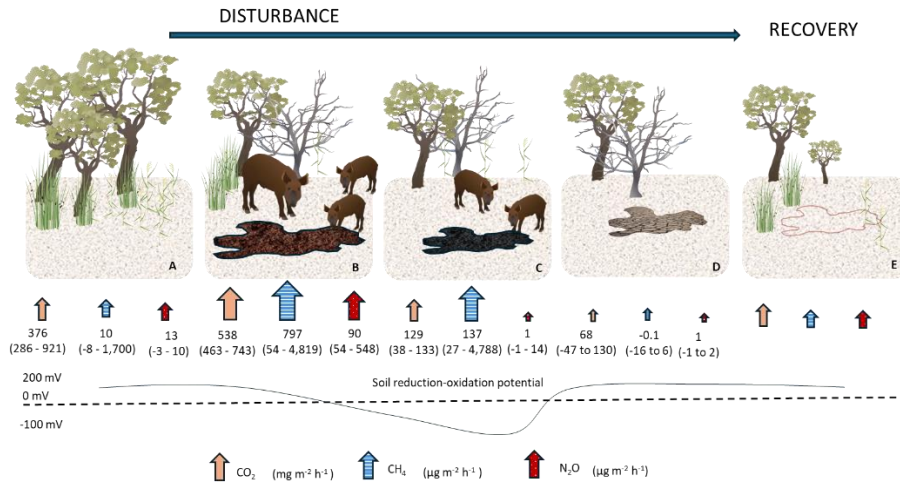
L22: “Soil surface redox values were correlated with emissions in plots disturbed by pigs, with negative values associated with high  $\text{CH}_4$  emissions.”

L317: “The soil redox potential at all disturbed plots was significantly reduced, suggesting low oxygen conditions. Low redox soils favour microbial respiration through nitrification-denitrification when nitrate ( $\text{NO}_3^-$ ) is available.”

**Figure 7** – Consider adding an arrow indicating 'time' progression from left to right along the x-axis. Also consider using different colours or line styles for  $\text{CO}_2$  and  $\text{N}_2\text{O}$ , as they are difficult to distinguish in black-and-white (and in colour) printing. It may also be worth discussing the concept of ecosystem recovery/re-establishment after disturbance as inset 'e'. While this was not directly investigated here,

it could represent an interesting direction for future studies examining post-disturbance dynamics once feral pigs move on.

Thanks for the idea. We have changed the format for each arrow to have a different pattern. We have also included a potential recovery panel and added an arrow to show progression with time.



**Line 331** – I recognise the body of Australian literature may be limited but are there additional recent studies in tropical wetlands beyond Iram et al. (2022) that support this claim that seasonal differences in GHG fluxes are low which could be discussed here? A couple of other relevant papers that could be considered on seasonal Australian wetland GHGs are:

<https://agupubs.onlinelibrary.wiley.com/doi/full/10.1029/2023JG007556>

<https://aslopubs.onlinelibrary.wiley.com/doi/abs/10.1002/lno.11158>

<https://link.springer.com/article/10.1007/s13157-014-0522-5>

and this undulate research on Australian alpine wetland GHGs:

<https://www.sciencedirect.com/science/article/pii/S0301479723018224>

Thanks for sharing. These are very helpful. We have incorporated them in our Introduction and Discussion. The data from Bass et al. (2014) was especially helpful to conduct the landscape extrapolation.

L34: “Emissions from wetlands increase when soils are disturbed, for example, by the trampling, grubbing, and digging of invasive ungulates or hoofed mammals (O’Bryan et al., 2021; Traby and Grover 2023).”

L331: “These results complement previous studies showing the effect of hoofed animals in increasing CH<sub>4</sub> emissions in wetlands (Treby and Grover, 2023).”

L347: “Emissions of *Melaleuca* wetlands during flooding periods in the region are higher for CH<sub>4</sub> but lower for CO<sub>2</sub> (Bass et al. 2014), and year-to-year variations in emissions are likely under different pre-flooding conditions, with lower emissions in floods preceded by dry periods (Jeffrey et al. 2019).”

L366: “We cautiously estimate annual emissions from pig disturbance using our data, supplemented by published estimates of emissions during flooding (Bass et al. 2014).”

**Conclusion:** *The introduction discusses national greenhouse gas accounting schemes or conservation programs to support feral pig exclusion as a mechanism to reduce greenhouse gas emissions. I suggest reconnecting this concept in the conclusion, albeit cautiously given this is the first study of its kind, nevertheless, it represents an important potential policy implication of this work.*

We have added a conclusion statement with the potential applications of this study; however, we have purposefully limited our discussion in management issues, as the Traditional Owners, who are co-authors of this manuscript, have requested to leave that topic aside for their own decision-making.

L381: “In conclusion, we demonstrate that feral pigs pose a significant threat to soil carbon integrity and significantly modify soil biogeochemical processes, leading to increased greenhouse gas emissions. These effects, measured only in this study in tropical floodplains in Australia, are likely to occur in wetlands worldwide. Physical activity in the soil and the increase in nitrogen from pig excreta are significantly increasing emissions, especially of CH<sub>4</sub> and N<sub>2</sub>O. Tackling these two potent greenhouse gases exacerbated by feral pigs has the potential to significantly contribute to climate change mitigation (Ocko et al., 2021), while also improving biodiversity and culture.”