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Managed pasture combines net methane uptake with the lowest soil greenhouse gas balance in a tropical savanna comparison

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Article

Keywords: soil greenhouse gases, methane uptake, nitrous oxide, water-filled pore space, nitrogen-cycle functional genes, tropical pasture

Posted Date: August 11th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10563383/v1

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Additional Declarations: No competing interests reported.

Abstract

Tropical crop-livestock integration is promoted as a route to sustainable intensification, but its direct soil greenhouse gas performance depends on the soil conditions created through management. We compared N_2O and CH_4 fluxes, water-filled pore space (WFPS), mineral N, and N-cycle functional genes across integrated crop-livestock (ICLs), long-established managed pasture (MP), and native vegetation (NV) sites in the Brazilian Cerrado. Annual N_2O emissions were 1.41, 0.77, and 1.68 kg N_2O ha^{-1} in ICLs, MP, and NV, respectively. MP was also a net CH_4 sink (-1.40 kg CH_4 ha^{-1}), and this uptake offset 18.65% of its soil N_2O burden in CO_2-equivalent terms. ICLs and NV were net CH_4 sources. The contrast did not follow a simple gradient of land-use intensity. Higher WFPS at NV coincided with greater N_2O and CH_4 emissions, whereas MP combined lower WFPS, greater *nifH* abundance, and the lowest annual CO_2-equivalent balance. ICLs shifted toward CH_4 uptake after maize harvest, when grass again occupied the soil. These site-level observations point to continuous grass cover, soil aeration, and the timing of N inputs as practical mitigation targets. They do not establish a whole-system greenhouse gas balance because enteric CH_4, productivity, and soil carbon change were outside the study boundary.

1. Introduction

Brazil is globally recognized for its diverse biomes and its capacity to produce food at scale, particularly grains and beef from pasture-based systems. However, an estimated 61.8% of Brazilian pasturelands are currently in some stage of degradation [1]. Pasture degradation reduces plant vigor, compromises animal performance, and undermines the long-term sustainability of production systems. Within this context, the Cerrado biome plays a critical role.

The Cerrado, Brazil's second-largest biome, covers approximately 203.9 million hectares [2]. It is a tropical savanna with edaphoclimatic features comparable to other savannas worldwide [3, 4]. Currently, 47.2% of the Brazilian tropical savanna is occupied by agricultural and livestock activities, with an emphasis on the succession of tropical annual crops (e.g., maize, soybean, cotton, common bean, and sorghum) and extensive livestock production. The latter is characterized by vast areas established with tropical grasses (*Urochloa* spp. and *Megathyrsus* spp.), although with low technology adoption and an average stocking rate of approximately 1.0 AU ha^{-1} [5]. Native forests account for about 44.8% of the Brazilian tropical savanna, while native herbaceous and shrub vegetation comprise 6.4% [5].

The region is characterized by marked seasonality, with distinct rainy and dry periods [2, 6], as well as by deep, acidic, highly weathered, and nutrient-poor soils. Despite these limitations, the tropical savanna has become a hotspot for both crop and livestock production and, consequently, a key contributor to global greenhouse gas (GHG) emissions, especially nitrous oxide (N_2O) and methane (CH_4) [7].

Among agricultural GHGs, N_2O is of particular concern due to its global warming potential (GWP), which is 273 times greater than that of CO_2. Its emissions primarily arise from nitrogen fertilization, animal

excreta, and the decomposition of organic matter [8, 9]. CH_4, with a GWP 28 times higher than CO_2, is mainly produced under anaerobic conditions through the decomposition of plant residues and manure [10]. Although management practices influence GHG fluxes, the ultimate production and release of these gases are largely controlled by soil microbial processes.

Microorganisms mediate the major pathways of N_2O and CH_4 cycling. Nitrifying and denitrifying bacteria, such as those from the genera *Nitrosomonas* and *Nitrobacter*, regulate N_2O production through the expression of genes including *amoA* (the gene encoding the Ammonia Monooxygenase enzyme) in Ammonia-oxidizing Archaea (AOA) and Bacteria (AOB); *nirS* (the gene encoding the Cytochrome cd_1 Nitrite Reductase enzyme); *nirK* (the gene encoding the Copper Nitrite Reductase enzyme); and *nosZ* (the gene encoding the Nitrous Oxide Reductase enzyme) [11, 12]. Biological nitrogen fixation, another critical process influencing N dynamics, is commonly assessed via the *nif*H gene, which encodes the Nitrogenase Reductase enzyme [13]. CH_4 production and oxidation are driven by methanogenic and methanotrophic microbial communities, respectively [10]. The abundance and diversity of these microbial groups are highly sensitive to land use change, agricultural management, and soil degradation [14, 15], making them useful bioindicators of soil quality and ecosystem health [16].

Integrated crop-livestock systems can raise productivity per hectare and improve soil condition relative to degraded or specialized systems [17–19]. Those benefits do not guarantee lower direct soil greenhouse gas fluxes. Rotations alter residue supply, fertilizer N, soil cover, moisture, and aeration throughout the year, so an integrated field can alternate between emission and uptake states. A useful assessment must therefore resolve when fluxes occur and which measurable soil conditions accompany them.

Evidence linking seasonal soil gas fluxes to both soil conditions and microbial functional potential remain limited in tropical savannas [20][21]. We expected the ICL site to have lower annual soil N_2O and CH_4 emissions and a distinct profile of N-cycle functional genes than managed pasture, with native vegetation as an ecological reference. We therefore quantified annual and phase-specific N_2O and CH_4 emissions; related fluxes to soil temperature, WFPS, and mineral N; and compared N-cycle functional genes among the sites. The practical aim was to determine not only which site had the lowest measured balance, but which measurable conditions distinguished it.

2. Results

2.1. Management and weather events punctuated background N_2O and CH_4 fluxes

Across the sites, most N_2O and CH_4 observations remained within the background range defined by Amadori *et al*. [18], but discrete peaks followed fertilization, crop establishment, grazing, or rewetting.

This event-based pattern located the phases in which management or weather coincided with short-lived fluxes that annual means alone would conceal.

In the N_2O fluxes (Fig. 1), we identified periods, hereafter referred to as stages, representing moments of the agricultural cycle and their respective greenhouse gas emission dynamics. Stage 1 corresponds to the period from soybean sowing to the sowing of maize intercropped with grass. During this stage, N_2O fluxes remained predominantly within the background range, with values varying between -6.9 and 32.4 μg N_2O m^2 h^{-1}. The highest mean flux was observed in the NV, followed by MP and, lastly, the ICLs. Stage 2 covers the period between sowing maize intercropped with grass and the maize harvest. During this time, N_2O fluxes mostly remained within the background range, although a peak emission was recorded in the ICLs shortly after maize and grass sowing. Additionally, during this same period, negative N_2O fluxes were observed in the MP system. Stage 3 began after maize harvest and was characterized by an alternation between background fluxes and N_2O emission peaks across all evaluated land uses.

For CH_4, background fluxes predominated throughout the experimental period, particularly in NV (Fig. 1). In the MP system, CH_4 uptake was observed at multiple sampling events, and a similar pattern was observed in ICLs after maize harvest, when only grass remained in the area. A peak emission was recorded exclusively in the ICLs, following nitrogen fertilization in stage 2.

2.2. Managed pasture had the lowest annual greenhouse gas balance

Site contrasts in cumulative N_2O were strongest during the cropland phase (Table 1): NV emitted 0.77 kg N_2O ha^{-1}, ICLs 0.58, and MP 0.11. Pasture-phase totals did not differ ($p \geq 0.05$). Across the year, emissions ranged from 0.77 kg N_2O ha^{-1} at MP to 1.68 at NV; ICLs emitted 1.41. MP was lower than ICLs and NV, which did not differ statistically.

Table 1. Cumulative seasonal emissions of nitrous oxide (N_2O), methane (CH_4), and carbon dioxide equivalent (CO_2eq) in integrated crop-livestock system (ICLs), monoculture pasture (MP), and native vegetation (NV) in the Tropical Savanna Biome.

GHG	Unit	Phase	Land Use			*p*-value
			ICLs	MP	NV	
N_2O	kg ha^{-1}	Cropland	0.58 ± 0.18ab	0.11 ± 0.1b	0.77 ± 0.06a	0.005
		Pasture	0.77 ± 0.15	0.63 ± 0.15	0.85 ± 0.12	0.561
		Annual	1.41 ± 0.19a	0.77 ± 0.07b	1.68 ± 0.10a	< 0.001
CH_4	kg ha^{-1}	Cropland	1.32 ± 0.27ab	-1.33 ± 0.31b	1.09 ± 0.39a	< 0.001
		Pasture	0.19 ± 0.86b	- 0.21 ± 0.76b	1.84 ± 0.95a	0.018
		Annual	1.75 ± 0.25a	-1.40 ± 0.50b	3.07 ± 0.76a	< 0.001
CO_2eq	t ha^{-1}	Cropland	0.19 ± 0.05a	< 0.01 ± 0.03b	0.24 ± 0.02a	< 0.001
		Pasture	0.21 ± 0.05	0.16 ± 0.05	0.28 ± 0.04	0.249
		Annual	0.43 ± 0.05a	0.17 ± 0.03b	0.54 ± 0.04a	<0.001

Values represent means ± standard error. Different letters within the same row indicate significant differences between land uses (Tukey's test, *p*-value < 0.05).

CH_4 separated the sites more clearly. During the cropland phase, ICLs and NV were sources (1.32 and 1.09 kg CH_4 ha^{-1}), whereas MP was a sink (-1.33). During the pasture phase, NV remained a source (1.84), while ICLs was near neutral (0.19) and MP remained slightly negative (-0.21). Annual totals were 1.75, -1.40, and 3.07 kg CH_4 ha^{-1} for ICLs, MP, and NV, respectively; ICLs and NV did not differ, while MP was lower than both.

Expressed as CO_2 equivalents using global warming potentials of 273 for N_2O and 28 for CH_4, MP had the lowest annual balance (0.17 t CO_2eq ha^{-1}), compared with ICLs (0.43) and NV (0.54) (Table 1). MP's CH_4 uptake reduced its N_2O-derived balance by 18.65%; positive CH_4 fluxes increased the balances of ICLs and NV. The measured contrast was therefore driven primarily by N_2O, with CH_4 uptake providing partial additional mitigation at MP.

2.3. Land uses differed in soil moisture, temperature, and mineral nitrogen

For environmental variables, land use significantly influenced key factors, including NH_4^+, NO_3^-, WFPS, and soil temperature (Table 3). The ICLs exhibited the highest levels of mineral nitrogen (NH_4^+ and NO_3^-), particularly during the cropland phase. Regarding soil moisture, NV had the highest WFPS values, followed by MP and ICLs, across all cultivation periods. Finally, soil temperature varied significantly

among the systems, with the highest temperatures observed in ICLs, particularly in the pasture phase (Table 2).

Table 2. Weighted averages of NH_4^+ and NO_3^- concentrations, water-filled pore space (WFPS), and soil temperature (Soil T.) in integrated crop-livestock system (ICLs), monoculture pasture (MP), and native vegetation (NV) in the Tropical Savanna Biome.

Variables	Unit	Phase	Land Use			*p*-value
			ICLs	MP	NV	
NH_4^+	mg kg^{-1}	Cropland	7.96 ± 2.06a	5.32 ± 0.88ab	0.85 ± 0.17b	0.002
		Pasture	1.15 ± 0.32a	0.74 ± 0.50b	0.31 ± 0.15c	< 0.001
NO_3^-	mg kg^{-1}	Cropland	1.39 ± 0.38a	0.05 ± 0.03b	0.45 ± 0.11b	< 0.001
		Pasture	0.94 ± 0.12a	0.25 ± 0.05b	0.39 ± 0.13b	0.003
WFPS	%	Cropland	30.91 ± 1.18b	33.28 ± 0.39b	46.36 ± 0.52a	< 0.001
		Pasture	14.31 ± 0.53b	16.69 ± 0.61b	23.34 ± 0.98a	0.04
Soil T.	°C	Cropland	25.01 ± 0.14	24.99 ± 0.19	24.99 ± 0.12	0.98
		Pasture	27.67 ± 0.2 a	21.10 ± 0.3 c	21.51 ± 0.4 b	< 0.001

Values represent means ± standard error. Different letters within the same row indicate significant differences between land uses (Tukey's test, *p*-value < 0.05)

2.4. Functional-gene contrasts were gene- and phase-specific

Functional-gene responses were selective under ICLs (Table 3). *amoA*-AOA, *nirK*, and *nirS* did not differ among sites in either phase. *amoA*-AOB differed only during the cropland phase, with the highest abundance at MP, followed by ICLs and NV. *nifH* was highest at MP during the cropland phase and remained higher at MP and ICLs than at NV during the pasture phase. *nosZ* differed only during the pasture phase, when abundance was highest at NV and lowest at MP. Thus, each site was distinguished by N-cycle functions; the data does not support a general increase in functional-gene abundance under ICLs.

Table 3. Abundance of functional genes related to nitrogen cycling in integrated crop-livestock system (ICLs), monoculture pasture (MP), and native vegetation in the Tropical Savanna Biome.

Genes	Unit	Phase	Land Use			*p*-value
			ICLs	MP	NV	
AOA	10^7 copies g^{-1} soil	Cropland	10.00 ± 3.37	9.70 ± 3.80	13.00 ± 1.67	0.615
		Pasture	7.42 ± 2.13	4.64 ± 0.60	7.51 ± 1.39	0.193
AOB	10^5 copies g^{-1} soil	Cropland	1.65 ± 0.61ab	3.50 ± 0.96a	0.16 ± 0.08b	0.024
		Pasture	8.34 ± 5.55	2.37 ± 0.87	0.21 ± 0.08	0.090
nifH	10^7 copies g^{-1} soil	Cropland	6.50 ± 0.55b	8.30 ± 0.50a	2.00 ± 0.21c	<0.001
		Pasture	5.99 ± 0.23a	7.58 ± 0.76a	2.83 ± 0.15b	<0.001
nirK	10^4 copies g^{-1} soil	Cropland	2.10 ± 0.85	2.10 ± 0.28	0.77 ± 0.17	0.166
		Pasture	1.94 ± 0.99	0.76 ± 0.47	0.62 ± 0.31	0.515
nirS	10^4 copies g^{-1} soil	Cropland	2.80 ± 0.10	12.00 ± 0.38	2.30 ± 0.11	0.148
		Pasture	8.72 ± 3.07	4.67 ± 2.58	2.49 ± 0.24	0.210
nosZ	10^7 copies g^{-1} soil	Cropland	1.90 ± 0.50	2.90 ± 0.36	2.00 ± 0.46	0.288
		Pasture	1.75 ± 0.29ab	1.01 ± 0.17b	2.71 ± 0.22a	0.002

Values represent means ± standard error. Different letters within the same row indicate significant differences between land uses (Tukey's test, *p*-value < 0.05).

2.5. WFPS and nifH abundance covaried with greenhouse gas emissions

Pearson correlations identified significant associations (Fig. 2). CH_4 correlated positively with WFPS ($r = 0.54$, $p < 0.05$) and *nosZ* abundance ($r = 0.53$, $p < 0.05$), and negatively with *nifH* abundance ($r = -0.75$, $p < 0.01$). N_2O correlated positively with CH_4 ($r = 0.79$, $p < 0.001$) and negatively with *nifH* abundance ($r = -0.54$, $p < 0.05$).

Principal component analysis summarized covariance among gas emissions, soil conditions, and functional-gene abundances (Fig. 3; Table S1). PC_1, PC_2, and PC_3 explained 39.23%, 26.02%, and 19.02% of the total multivariate variance, respectively, or 84.2% collectively.

PC_1 contrasted N_2O, CH_4, WFPS, and *nosZ* with soil temperature, NH_4^+, and *nifH*. NV contributed 62.70% to this axis, followed by ICLs (28.85%) and MP (10.45%); WFPS had the strongest loading. PC_2 aligned N_2O and CH_4 with soil temperature and NO_3^-. MP and ICLs contributed 56.60% and 39.51%, respectively,

compared with 3.89% for NV; NO_3^- had the strongest loading. PC_3 contrasted *nirS*, *nirK*, and *nosZ* with *amoA*-AOA and *amoA*-AOB. ICLs contributed 91.04% to this axis, and *nirK* had the strongest loading.

The ordination placed NV with WFPS, N_2O, CH_4, *nosZ*, and *amoA*-AOA; MP with *nifH*; and ICLs with mineral N, soil temperature, *nirS*, *nirK*, and *amoA*-AOB. These loadings describe covariation among site characteristics. They do not establish that any gene or soil variable caused the observed fluxes.

3. Discussion

3.1. Soil conditions and management events differentiated the site-level gas balances

More integrated system therefore did not emit least. ICLs and NV had similar annual soil greenhouse gas balances, whereas MP emitted 54% less N_2O than NV and was a net CH_4 sink. These contrasts aligned with management-linked pulses and differences in WFPS, soil temperature, and mineral N. Within this comparison, the soil state associated with each management phase was more informative than a simple ranking of land-use intensity.

N_2O fluxes coincided with precipitation, WFPS, and management events. At ICLs, fluxes remained within the background range during soybean growth despite biological N fixation. This pattern is consistent with efficient crop N uptake, but crop uptake and denitrification rates were not measured directly.

In contrast, management practices within the agricultural systems induced emission peaks. In the ICLs, we observed an increase in N_2O emissions following maize planting, which, in turn, can be associated with nitrogen input (via planting fertilization) and the decomposition of soybean crop residues (OM with a low C/N ratio) (Fig. 1). In the MP system, nitrogen fertilization also increased emissions relative to the average period. However, this effect in the MP system was transient, possibly mitigated by split N application, a practice that reduces the immediate substrate availability for denitrifying microorganisms [23]. In the NV area, fluxes were more closely associated with the rainy season, where sustained high soil moisture (WFPS) fostered anaerobic environments conducive to denitrification [24]. Additionally, soil rewetting after dry spells may have triggered the rapid mineralization of organic residues, releasing nitrogenous substrates and causing emission peaks, as was occasionally observed before the onset of the rainy season (Fig. 1) [25].

Annual N_2O emissions were highest at NV, intermediate at ICLs, and lowest at MP. At NV, greater WFPS coincided with greater emissions; litter cover and shading maintained wetter, cooler soil. ICLs reached a similar annual total despite lower WFPS, while mineral N was substantially higher, consistent with greater substrate availability following fertilization [26]. Similar annual totals at NV and ICLs therefore arose alongside distinct soil conditions.

The pasture site combined continuous, well-established forage cover with lower WFPS, conditions consistent with greater soil aeration and plant N capture [27, 28]. Macroporosity and plant N uptake were not measured directly, so the practical inference remains a testable one; maintaining vigorous grass cover and avoiding periods of high mineral N under wet soil may reduce N_2O but replicated management experiments are needed.

Methane flux behavior was dynamic, exhibiting both emission peaks (positive fluxes) and periods of soil uptake (negative fluxes). During the cropping phase, the ICLs recorded the most prominent CH_4 emission peak immediately following maize top-dressing fertilization, representing a substantial increase relative to the previous average (Fig. 3). This effect is attributed to the high availability of nitrogenous compounds, which can inhibit methanotroph activity (CH_4-consuming microorganisms) while favoring methanogens [29]. In NV, the largest CH_4 emission peak occurred at the onset of the rainy season, associated with a weekly precipitation of 68 mm (Figure S2) in supplementary material). This rainfall, combined with high organic matter availability, created an ideal anaerobic environment for methanogenic archaea [30].

Remarkably, the pasture-based system was a CH_4 sink. In the MP system, even nitrogen fertilization led to a reduction in CH_4 emissions, possibly due to the toxicity of excess ammonium to certain Archaea [31]. Following the maize harvest, when the ICL was also under pasture, both systems (ICLs and MP) exhibited periods of CH_4 uptake, thereby reinforcing their capacity as sinks for this gas. A well-established pasture enhances soil structure, improving aeration and drainage. This creates a more aerobic environment that favors methanotrophs over methanogens [32]. According to Bratti et al. [26] pasture areas under intensive grazing, regardless of the N dose (0, 75, 150), act as a methane sink (mean of -1.15 kg CH_4-C ha^{-1} y^{-1} reported by the authors), which is similar to the results found in our study (Table 2).

Across the year, MP remained a net CH_4 sink, whereas NV and ICLs were net sources. The similar cumulative source strengths at NV and ICLs coincided with higher pasture-phase soil temperature at ICLs and higher WFPS at NV (Table 2; Fig. S3). These patterns are consistent with distinct controls on methanogenesis, but the underlying process rates were not measured directly.

3.2. Soil methane uptake partly offset the N_2O burden

At the MP site, soil CH_4 uptake reduced the CO_2-equivalent portion of soil N_2O. This mitigation is meaningful within the measured soil-gas balance, but it should not be interpreted as an offset for enteric CH_4 or as a whole-farm carbon claim, neither of which was measured.

The direction of the CH_4 contribution differed among sites; however, N_2O therefore remained the dominant component in all the balances.

Mitigation priority should therefore not shift from N_2O to CH_4. N_2O accounted for most of the measured CO_2-equivalent balance. Practical trials should test whether matching N supply to crop demand, avoiding fertilizer application before high-WFPS periods, and retaining well-aerated, grass-covered soil can lower N_2O while preserving CH_4 oxidation.

3.3. Functional genes provide relational context

The ordination separated an NV-associated WFPS and gas-emission profile from agricultural-site profiles characterized by mineral N and distinct functional-gene abundances. This pattern indicates covariation among site, soil conditions, and microbial functional potential. It does not identify a land-use mechanism because PCA is descriptive and functional-gene copy represents genetic potential, not process rate.

Nitrogen fertilization and the resulting increase in soil nitrogen compounds promote the growth of ammonia-oxidizing bacteria (AOB), which convert ammonium to hydroxylamine (NH_2OH) as the first step in nitrification [33]. Concurrently, this process leads to an increased abundance of denitrifying microorganisms carrying the *nirS* and *nirK* genes. Although both genes encode enzymes with the same function (the reduction of nitrite to nitric oxide), they are adapted to distinct soil oxygen conditions; *nirS* is associated with low-oxygen environments, while *nirK* is favored in more aerobic soils. Given the dynamic nature of soil profiles, the co-occurrence of both genes is common [34], a finding consistent with our study (Fig. 3).

The MP site was distinguished by *nifH* abundance, which was negatively correlated with both gases. This pattern is consistent with a grass-associated diazotroph community [35], but qPCR did not measure N fixation or demonstrate that *nifH* reduced nitrate availability. Because only a narrow set of functional-gene targets were quantified, this ordination axis represents functional-gene composition.

Long-established tropical grasses can associate with diazotrophs even under N fertilization, and well-developed root systems can support stable carbon inputs [36],[37] Greater *nifH* abundance at MP is therefore biologically plausible. It cannot, however, be interpreted as broader microbial diversity or as evidence of higher N fixation without community-level and process measurements.

The co-occurrence of pasture, greater *nifH* abundance, and lower N_2O identifies a mechanistic hypothesis; grass-associated diazotroph communities may contribute to N retention under these site conditions (Fig. 4). Direct measurements of biological N fixation and transformation rates, together with replicated manipulation, are needed to test this pathway.

3.4. Functional-gene responses were specific to gene and phase

Functional-gene abundance differed by target and phase. The phase-specific contrasts are therefore interpreted separately. The *nifH* showed the clearest and most consistent site contrast. During the cropland phase, abundance was highest at MP, followed by ICLs and NV. During the pasture phase, MP

and ICLs remained higher than NV. This pattern is compatible with grass-associated diazotroph communities [38, 39]., but gene copies do not measure N fixation rate.

The *amoA*-AOA abundance did not differ among sites in either phase. By contrast, *amoA*-AOB differed during the cropland phase, when abundance was highest at MP, intermediate at ICLs, and lowest at NV; it did not differ during the pasture phase. This phase-specific AOB response is consistent with the greater mineral N availability at the agricultural sites and the higher affinity of AOA for low-ammonium environments [40].

The *nirK* and *nirS* abundances did not differ among sites in either phase. *nosZ*, which encodes the final reduction of N_2O to N_2, was more abundant at NV than MP during the pasture phase. This difference indicates greater genetic potential for N_2O reduction at NV, not a measured reduction rate.

Two boundaries are essential. Each land use was represented by one site, so site history and land use are confounded. The research also covers direct soil N_2O and CH_4 only; it excludes enteric CH_4, fertilizer manufacture, energy use, productivity, and soil carbon change. The results identify management hypotheses for replicated trials, not a complete ranking of whole-system sustainability.

Together, the evidence supports a conditional interpretation of land use. The lowest measured soil greenhouse gas balance occurred where lower WFPS, continuous grass cover, CH_4 uptake, and greater *nifH* abundance coincided. ICLs alternated between source and uptake states as crop and pasture phases changed. The practical target is therefore the soil state created through management, especially N supply under wet conditions.

4. Conclusions

At the sampled sites, ICLs did not lower annual soil N_2O or CH_4 emissions relative to NV. MP emitted 54% less N_2O than NV and was the only annual CH_4 sink, producing the lowest measured CO_2-equivalent balance. The evidence therefore does not support inferring mitigation from crop-livestock integration alone.

For practice, the MP site and the grass phase of ICLs point to continuous grass cover and soil aeration as conditions favorable to CH_4 uptake, while the N_2O peaks identify the timing of N inputs relative to WFPS as the principal intervention point. CH_4 uptake partly reduced, but did not neutralize, the soil N_2O burden.

Functional-gene responses were specific; *nifH* was more abundant under pasture, *amoA*-AOB differed during the cropland phase, and *nosZ* was more abundant at NV during the pasture phase. These abundances provide mechanistic context but not direct evidence of microbial activity. Replicated multi-site studies that include enteric CH_4, productivity, and soil carbon accounting are required before whole-system sustainability claims can be made.

5. Methods

5.1. Experimental site

The experiment was conducted from October 2021 to October 2022 at the Forage and Pasture Sector at the Federal University of Goiás, located in Goiás, Brazil. The site is positioned at 16º35’8” south latitude and 49º16’6” west longitude, at an altitude of 727 m. According to the USDA (Agriculture Department, 2014), the soil in the experimental area is classified as Clayey Oxisol.

The prevailing climate at the experimental site, according to Alvares et al. [41] classification, is Aw, described as tropical with a well-defined dry winter season from April to September, and a concentration of rainfall during the summer months (October to March). Total precipitation during the experimental period was 1913 mm, and the mean temperature ranged from 23.43°C to 28.20°C.

5.2. Treatments and experimental design

The treatments consisted of monoculture pasture (MP), integrated crop-livestock systems (ICLs), and native vegetation (NV) (Fig. S4). The MP and ICL sites were each subdivided into five fenced 20 m² paddocks. Five sampling points were demarcated within the NV site while maintaining a buffer distance. These paddocks or points were used as within-site sampling units. Because each land use was represented by one site, the comparison estimates contrasts among the sampled sites and cannot separate land use from site history. The soil chemical properties are presented in Table 4.

Table 4
Soil chemical analysis at 0–20 cm depth in integrated crop-livestock system (ICLs), monoculture pasture (MP), and native vegetation (NV) in the Tropical Savanna Biome.

Variable	Unit	Land Use		
		ICLs	MP	NV
pH ($CaCl_2$)		5.2	5.1	4.1
O.M	g dm^{-3}	36	35	29
P	mg dm^{-3}	2	1.6	0.9
S	mg dm^{-3}	-	-	-
Ca	$mmol_c$ dm^{-3}	35	32	7
Mg	mmolc dm^{-3}	13	12	2
Na	mmolc dm^{-3}	0	0	0
K	mmolc dm^{-3}	3.8	3.03	0.92
Al	mmolc dm^{-3}	0	0	0.05
H + Al	mmolc dm^{-3}	24	26	39
SB	mmolc dm^{-3}	51	47.8	8.9
CEC	mmolc dm^{-3}	75	74	49
BS	V%	67.4	65.3	20.3
Al S	m%	0	0	0.36

pH: potential of hydrogen; O.M: organic matter; P: phosphorus; S: sulfur; Ca: calcium; Mg: magnesium; Na: sodium; K: potassium; Al: aluminum; H + Al: hydrogen + aluminum; SB: sum of bases; CEC: cation exchange capacity; BS: base saturation; Al S: aluminum saturation.

The experimental period was split into two phases, so we used the ICLs as a reference to name the phases across all land uses. The period spanning from soybean sowing to maize harvest was designated as phase 1, while the period following maize harvest was referred to as phase 2. Even for land uses that did not include a cropland phase, such as MP, or that lacked both cropland and pasture phases, such as NV, this nomenclature was maintained for consistency in comparisons. Additionally, we report the total cumulative or annual results, integrating both stratified phases.

The ICLs treatment followed a soybean-maize-grass rotation, involving pasture desiccation, soybean cultivation, and intercropping of second-crop maize with *Urochloa brizantha* 'Marandu'; after maize harvest, the pasture was managed under intensive rotational grazing (4.1 AU ha^{-1}) with 30/15 cm pre/post-grazing heights [42]. All experimental protocols involving animals were conducted under a protocol approved by the Ethics Committee on Animal Use (CEUA No. 2452/21) of the School of Agricultural and Veterinarian Sciences, São Paulo State University (FCAV/UNESP, Jaboticabal Campus). All methods were carried out in accordance with relevant national guidelines and regulations, including Brazilian Federal Law No. 11,794 and CONCEA standards, and the study is reported in accordance with ARRIVE guidelines. The MP treatment consisted of a 23-year established Marandu pasture fertilized with 150 kg N ha^{-1} (urea) split into doses (Nov/21, Jan/22, and Mar/22), sharing the same grazing management as ICLs. As a reference, NV comprised an undisturbed Seasonal Semideciduous Forest [43] with a 15–25 m closed canopy and 20–50% leaf shedding during the dry season. Management practices, yields, nitrogen inputs, and emission factors are detailed in Tables S1, S2, S3, S4, S5 and Table S6.

5.3. N_2O, CH_4 and CO_2eq emissions

Greenhouse gas emissions were quantified using static chambers (0.6 × 0.4 m metal base and 30 cm plastic top), externally coated with 8 mm aluminized thermal insulation [44]. Air samples were collected at 0, 15, and 30 minutes after closure between 09:00 and 10:00 [45]. Sampling was weekly during the wet season (Nov/21–May/22) and fortnightly during the dry season (Jun/22–Sep/22), targeting emission peaks and stable fluxes, respectively [45, 46]. Gas concentrations were determined by gas chromatography (Shimadzu GC-2014). For nitrous oxide, an electron capture detector was used (injector 250°C, column 80°C, detector 325°C, N_2 carrier). For carbon dioxide and methane, a flame ionization detector was used (280°C, H_2 carrier). Flux rates were calculated based on the linear concentration change over time (Alves et al., [45]).

Gas emissions (μg N-N_2O h^{-1} m^{-2} and μg C-CH_4 h^{-1} m^{-2}) were calculated based on the change in gas concentration within the chamber during the incubation period, according to the following equation:

(1) $$F_{GHG} = \frac{\delta C}{\delta t} \times \frac{V}{A} \times \frac{M}{V_m}$$

Where δC/δt represents the change in gas concentration over 30 minutes (μL L^{-1}); V is the chamber volume; A is the soil area covered by the chamber; M is the molecular weight of the gas; and V_m is the molecular volume of the gas, corrected to standard temperature and pressure.

The CO_2-equivalent emissions were calculated over a 100-year horizon using global warming potentials of 28 for CH_4 and 273 for N_2O (IPCC, 2023), consistent with the values used in Table 1.

5.4. Mineral nitrogen, temperature of soil, and water-filled pore space

Mineral nitrogen (NH_4^+ and NO_3^-), air and soil temperature, and the water-filled pore space (%WFPS) were evaluated. To assess these variables, the soil samples were collected from the 0–10 cm layer using an auger, immediately after GHG sampling. Subsamples were taken for the determination of %WFPS and N-mineral concentration.

To calculate WFPS, it was necessary to determine the soil volumetric water content (θv), which represents the amount of water present in each soil volume obtained using the equation:

(2) $\theta_{v=\frac{Water\ mass\ (g)}{Soil\ volume\ \left(cm^3\right)}}$

The water mass was determined by the difference between the wet and dry soil sample weights after drying at 105°C for 24 to 48 hours. Next, the total soil porosity (∅) was calculated, which represents the fraction of the total soil volume occupied by pore spaces (air and water), using the equation:

(3) $\varnothing = 1 - \frac{Soil\ density\ \left(g\ cm^{-3}\right)}{Particle\ density\ (g\ cm^{-3})}$

Where soil bulk density is the mass of soil per unit volume (1.2 ± 0.14 g cm^{-3}), the particle density was assumed to be 2.65 g/cm^3. With the volumetric water content and total porosity determined, %WFPS were calculated using the equation:

(4) $\%WFPS = \frac{\theta_v}{\varnothing}\ x\ 100$

For mineral nitrogen determination, approximately 10 g of fresh soil was mixed with 50 mL of 2 M KCl solution, shaken for 1 hour at 240 RPM, and then filtered [47]. The filtered solution was stored frozen for later quantification of nitrate (NO_3^-+ NO_2^-) and NH_4^+ using spectrophotometry. NH_4^+ was determined using the Berthelot reaction [47], while NO_3^- concentration was quantified via reduction with vanadium (III) chloride, as described by Doane and Horwáth [48]. Additionally, air and soil temperatures were monitored with sensors installed in the experimental area, and daily precipitation was recorded by a weather station located near the study site.

5.5. Soil microbiology

Soil microbiology was assessed after the maize harvest (May 2022) and before soybean planting (October 2022). Nitrogen cycle genes (*amoA*-AOA, *amoA*-AOB, *nirS*, *nirK*, *nifH*, and *nosZ*) were quantified from soil collected at 0.10 m depth and stored at -20°C. Genomic DNA was extracted using the PowerSoil® PowerLyzer Kit, with quality and concentration verified via NanoDrop® ND-2000 and Qubit® 2.0. Gene quantification was performed via qPCR (StepOne Plus) in 10 µL reactions using SYBR Green Master Mix and 10–20 ng of DNA. Specificity was confirmed by melting curves and 1% agarose gel. Standard curves (10^2 to 10^7 copies μL^{-1}) were generated from purified environmental amplicons, with *Paracoccus denitrificans*, *Pseudomonas fluorescens*, and *Bradyrhizobium japonicum* used to ensure R ≥

0.99 and no inhibition. Results were expressed as gene copies per gram of dry soil. Detailed primers, qPCR conditions, and efficiencies are provided in Tables S7 and S8.

5.6. Statistical Analyses

Analyses were conducted in R (R Core Team, 2023). Residual normality and variance homogeneity were evaluated using Shapiro-Wilk and Levene tests. One-way ANOVA with Tukey comparisons ($p < 0.05$) described differences among within-site sampling units for annual and phase-specific greenhouse gas totals and weighted means of soil variables. Pearson correlations quantified pairwise associations among soil variables, gene abundances, and cumulative N_2O and CH_4 emissions. Principal component analysis summarized multivariate covariation after Bartlett's sphericity, KMO, and multicollinearity checks; components with eigenvalues ≥ 1 were retained [49]. Because land use was not replicated across sites, *p*-values describe the sampled-site contrast and should not be interpreted as a general causal effect of land use.

Declarations

Declaration of competing interest

The authors declare that they have no known competing financial or personal interests that could be construed as influencing the results reported in this paper.

Funding

The study received financial support from the São Paulo Research Foundation (FAPESP) along with the Goiás Research Foundation (FAPEG), with approval under grant call No. 2019/24123. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

Author Contribution

VBSJ was responsible for Data curation, Formal analysis, Investigation, Methodology, Resources, Visualization and Writing – original draft. FPAJ contributed to Data curation, Formal analysis, Methodology, Visualization and Writing – review & editing. CESS contributed to Visualization, Writing – review & editing, DSN participated in Data curation, Resources and Visualization. BRAM contributed to Visualization, Writing – review & editing. WML participated in Formal analysis and Methodology. NCS was responsible for Data curation, Investigation, Methodology, Visualization, Writing – review & editing. ASC contributed to Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Validation, Visualization, Writing – review & editing. ACR participated in Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – review &

editing. Finally, WLS was responsible for Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project Administration, Writing – review & editing.All authors reviewed the manuscript.

Acknowledgement

We thank the São Paulo State Research Foundation (FAPESP), the Goiás State Research Foundation (FAPEG), and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for their financial support. We are very grateful to the Federal University of Goiás (UFG) and São Paulo State University (UNESP) for providing the experimental area, laboratory facilities, and equipment for the development of this study. We extend a special thank you to Prof. Dr. Wilton Ladeira da Silva, Prof. Dr. Naiara Caixeta da Silva, Prof. Dr. Ana Claudia Ruggieri, and Dr. Abmael da Silva Cardoso for elaborating and obtaining approval for the proposal that generated the funding for this study.

Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request and subject to approval by the authors.

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Figures

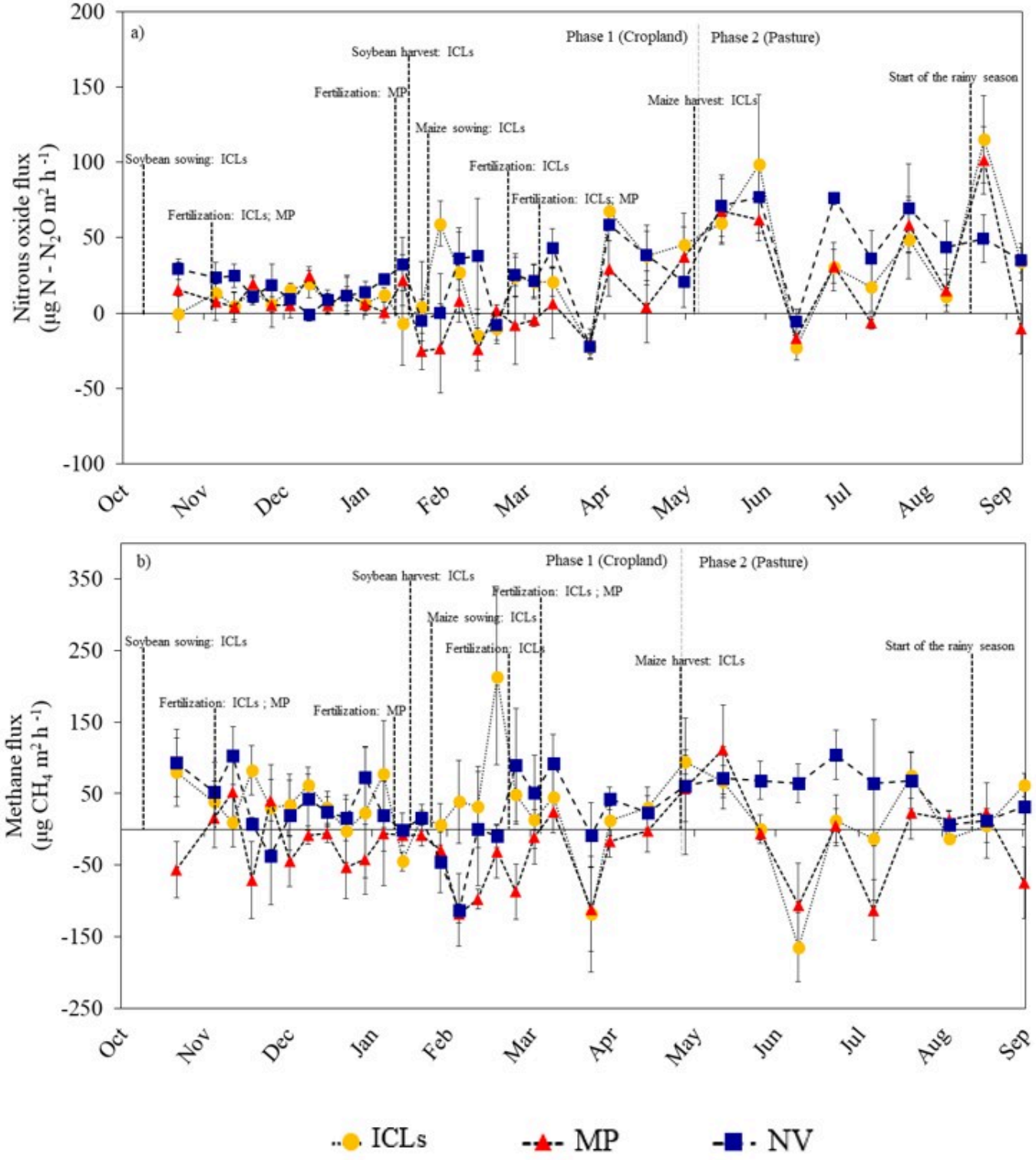


Figure 1

a) Nitrous oxide flux and b) Methane flux in the integration crop-livestock system (ICLs), monoculture pasture (MP) and native vegetation (NV) in the in the Tropical Savanna Biome.

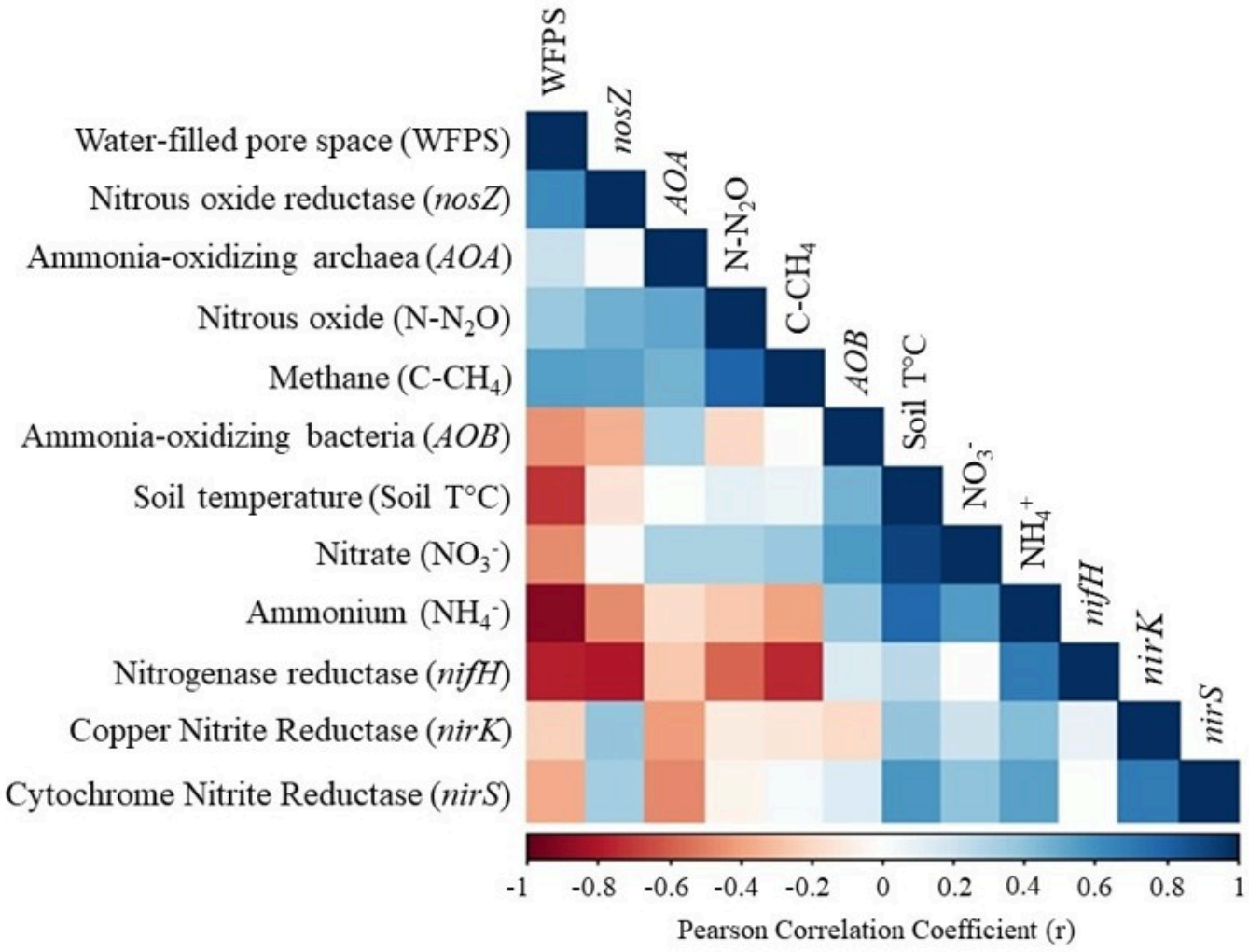


Figure 2

Conditional relationships between the environmental and microbiological variables on N_2O and CH_4 emissions from soil in different land use for in the Tropical Savanna Biome.

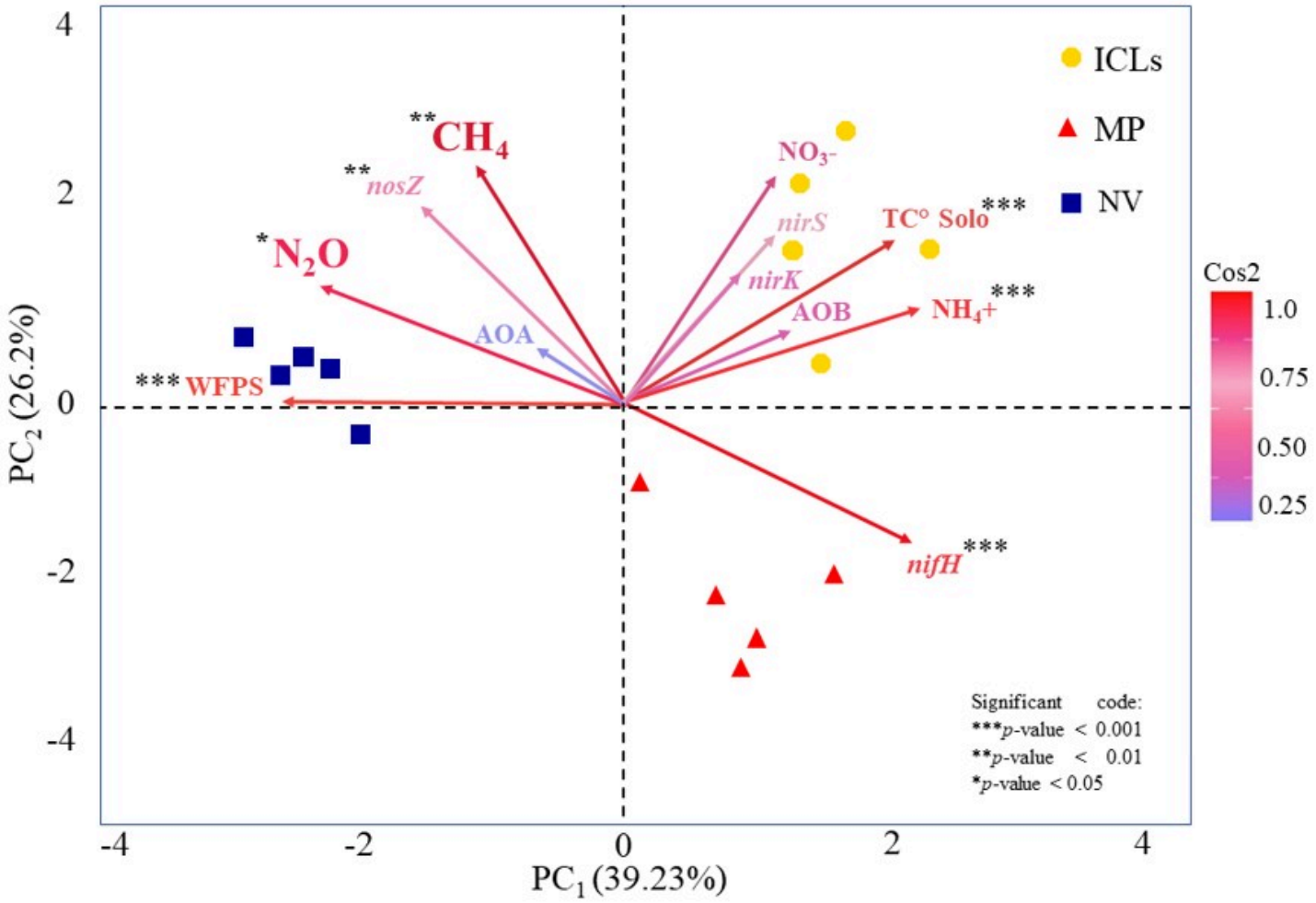


Figure 3

Relationships between environmental, microbiological and land use components. This bi-plot presents a color gradient from blue (indicating less commonality) to red (indicating greater commonality). This visual scale shows how much variance in each variable is captured by common factors. Abbreviations: CH_4 (methane), N_2O (nitrous oxide), WFPS (water-filled pore space), TC° soil (soil temperature), NH_4^+ (ammonium), NO_3^- (nitrate), *nosZ* (nitrous oxide reductase), *nirS* (cytochrome nitrite reductase), *nirK* (copper nitrite reductase), *nifH* (nitrogenase reductase), *AOA* (ammonia-oxidizing archaea) *AOB* (ammonia-oxidizing bacteria). Significant code: ***p-value < 0.001, **p-value < 0.01, *p-value < 0.05. Tropical Savanna Biome.

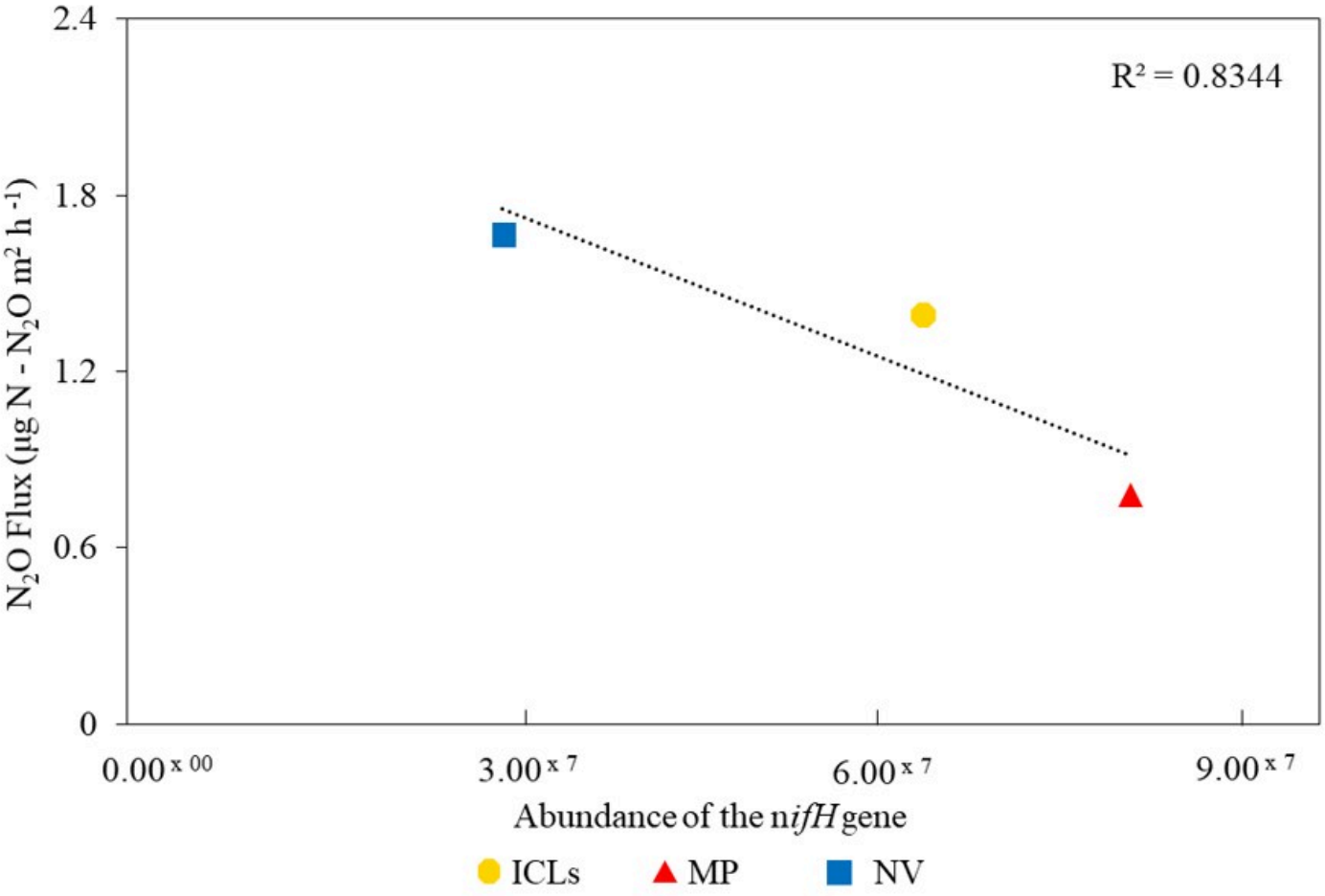


Figure 4

Nitrous oxide (N_2O) emissions as a function of *nifH* gene abundance in the integrated crop-livestock system (ICLs), monoculture pasture (MP), and native vegetation (NV) in the Tropical Savanna Biome.

Supplementary Files

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