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Research article

How sustainable agricultural management practices mitigate greenhouse gas emissions in paddy fields

Min Wang [a], Junjiang Chen [a], Matthias C. Rillig [b,c], Josep Peñuelas [d,e], Stavros D. Veresoglou [a,f,*]

[a] State Key Laboratory of Biocontrol, School of Ecology, Sun Yat-sen University, Shenzhen, 518107, China
[b] Freie Universität Berlin, Institut für Biologie, Plant Ecology, D-14195, Berlin, Germany
[c] Berlin-Brandenburg Institute of Advanced Biodiversity Research (BBIB), D-14195, Berlin, Germany
[d] CSIC, Global Ecology Unit CREAF-CSIC-UAB, Bellaterra, Catalonia, 08193, Barcelona, Spain
[e] CREAF, Cerdanyola del Valles, Catalonia, 08193, Barcelona, Spain
[f] Laboratory of Ecology and Environmental Sciences, Department of Crop Science, Agricultural University of Athens, Iera Odos 75, 11855, Athens, Greece

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ABSTRACT

Agriculture is a major emitter of greenhouse gases (GHG), requiring mitigation through optimized sustainable practices. Although sustainable management practices show promise, especially for significant sources, such as rice paddies, their comparative effectiveness remains unclear due to a lack of multi-treatment experiments conducted under consistent conditions. To address this issue, we conducted a large-scale field study over four growing seasons (i.e. two years) in two rice paddies approximately 500 m apart. The study evaluated the impact of five management practices, halting fertilization, no pesticides, no-tillage, mycorrhizal inoculations, and alternative cultivars, on N_2O, CO_2, and CH_4 emissions. We monitored twenty parallel bifactorial experiments, comprising a total of 640 field plots, and collected data on gas emissions exceeding 23,000 records. Our analysis focused on both fitted coefficients (slopes) and effect sizes across the 40 experiments. Mycorrhizal inoculation decreased equivalent CO_2 emissions by 667 mg m^{-2} h^{-1}, while halting pesticide use increased them by 779 mg m^{-2} h^{-1}. None of the other four sustainable agricultural practices reduced equivalent CO_2 emissions. Halting fertilization showed the greatest reduction in N_2O emissions ($F = 8.07$, $P < 0.001$), though this effect diminished over time. Variations in emission types were observed, assessed through the CH_4:CO_2 ratio, with no-tillage yielding the highest and mycorrhizal inoculum additions the lowest ratios ($F = 3.1$, $P = 0.02$). Our findings highlight the context-dependent nature of sustainable management practices' effects on greenhouse gas emissions, emphasizing the importance of AMF in mitigating climate change and the need for *in situ* greenhouse-gas-emission measurements when assessing agricultural management strategies.

1. Introduction

Agriculture is a major contributor of greenhouse gas emissions (Laborde et al., 2021; Syakila and Kroeze, 2011; Li et al., 2025), responsible for about 60% of global N_2O emissions (Reay et al., 2012) and over 25% of CH_4 emissions (Smith et al., 2021). While sustainable intensification of agriculture could reduce food shortages (Tilman et al., 2011), its efficacy in reducing GHG emissions remains uncertain (Sánchez et al., 2016; Linquist et al., 2018; Roe et al., 2019).

The directional effects of individual sustainable practices are often understood in isolation. For example, reduced fertilization typically lowers N_2O emissions (Yangjin et al., 2021; Chen et al., 2024), while minimizing pesticide applications normally reduces GHG emissions (Jezierska-Tus et al., 2021), reducing tillage typically lowers greenhouse gas emissions (Yue et al., 2023) and applying indigenous arbuscular mycorrhizal fungal (AMF) propagules most likely reduces N_2O emissions (Bender et al., 2015; Storer et al., 2018; but see Okiobe et al., 2023). However, a critical gap exists in comparing their relative efficacy simultaneously under identical field conditions. Meta-analyses are confounded by geographic biases, varying study durations (e.g. Si et al., 2021 on short vs long term effects on GHGs), while process models lack robust multi-practice validation.

* Corresponding author. State Key Laboratory of Biocontrol, School of Ecology, Sun Yat-sen University, Shenzhen, 518107, China.
E-mail address: stavrosv@mail.sysu.edu.cn (S.D. Veresoglou).

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To address this critical comparative gap, we required a model system with high emission intensity. Rice paddies have among the highest emission rates per unit arable area across crops (Zhang et al., 2017, 2024; Laborde et al., 2021) and account for over 90% of grain yield in Southern China (National Bureau of Statistics of China, 2022). To enable direct comparisons, we implemented here an experimental framework that specifically enables direct comparison of different management practices (Veresoglou et al., 2020). This approach allows for an unambiguous ranking of practices based on their GHG mitigation potential under consistent environmental and temporal settings—a comparison rarely achieved in agronomic research.

We selected five sustainable management practices for simultaneous comparison: no fertilization, no tillage, no pesticide application, addition of mycorrhizal propagules, and the use of alternative cultivars. These represent field scale practices that maintain a high potential to promote sustainability and that presented differences to the degree that they became integrated in today's agriculture (Wezel et al., 2014). Our first objective was to quantify how these practices modify CO_2, N_2O, and CH_4 emissions and how their relative importance changes over time. Based on literature showing the high mitigation potential of practices like reduced fertilization (e.g. Langeroodi et al., 2019; Chiriacò et al., 2019; Yangjin et al., 2021; but see Six et al., 2004; Hou et al., 2012), we hypothesized that all four sustainable management practices will result in a statistically significant reduction in at least one greenhouse gas emission type (*Hypothesis One*). The mechanisms by which these practices reduce emissions are grounded in soil biogeochemistry. For example, the anticipated reduction in N_2O emissions under no fertilization is directly linked to limiting the substrate (inorganic nitrogen) available for microbial denitrification and nitrification (van der Krift and Berendse, 2001). Reduced pesticide use and no-tillage are expected to lower microbial community turnover and N mineralization rates, cascading to decreased N_2O production (Prommer et al., 2020). Similarly, AMF addition may reduce N_2O emissions by enhancing plant nitrogen use efficiency and by competing with free-living nitrifiers for soil ammonium (Bender et al., 2015). We excluded alternative cultivars from this hypothesis due to a lack of prior evidence for their sustainability benefits (but see Qian et al., 2023).

Beyond their individual effects, we expected that practices manipulating agrochemical inputs would have a disproportionate and more persistent influence on greenhouse gas emissions. Worldwide increases in the application of agrochemicals currently pose major risks to human health (Devi et al., 2022; Elumalai et al., 2025). Sustainable management practices (Priya et al., 2023) offer the means to mitigate such risks through reducing inputs or replacing them with eco-friendly alternatives (Carvalho, 2006). We hence grouped together for the next objective the two practices in our experimental design that involved the administration of agrochemicals, fertilization, and pesticide usage and asked how they fared compared to the two practices unrelated to agrochemical manipulations. We are aware that management practices that do not involve agrochemicals are also responsible for massive emission rates of greenhouse gases (e.g. Mooney and Sjogersten, 2022 but see Pelster et al., 2011). We nonetheless anticipated that the persistent nature of agrochemicals in soils would result in their application (i.e. reducing fertilizer application and minimizing pesticide emissions) having higher relative importance in mitigating greenhouse gas emissions than the other manipulations and that their relative importance would decrease less over time due to the persistence of chemical substrates in the soil. We hypothesize that manipulations of agrochemicals (fertilization and pesticide use) will have significantly greater effects on greenhouse gas emissions (as measured by F-statistics from ANOVAs) than other manipulations, and that the relative importance of these manipulations will not decrease significantly over time (*Hypothesis Two*).

We also investigated the ratio between CH_4 and CO_2 emissions as a parameter to capture CH_4 oxidation rates, anticipating that this ratio would reflect the adaptation of methanotroph communities to agricultural settings (Pratt et al., 2013). Methanotrophic communities respond strongly to soil water availability (Zhang et al., 2019), which is high in rice paddies, but also compete for resources with other microbes in soil (Ho et al., 2011), potentially slowing down community shifts across methanotrophs and adaptation to new environmental settings. A high CH_4 over CO_2 ratio could thus indicate that methanotrophs have not caught up with changes in environmental settings. In addition to providing an indication of the rate of methane reduction in soils, the ratio is also instructive regarding the time scale over which we should expect to see climatic effects: CH_4 has a much shorter lifetime in the atmosphere than CO_2 (Mar et al., 2022), meaning that even after expressing the two emission rates in equivalent global warming potential units, the two gases pose distinct environmental concerns. Because the recovery of the CH4-oxidizing community after disturbance can take considerable time (Tate, 2015), reducing environmental disturbance through sustainable management practices should also reduce the CH_4/CO_2 ratio in soils, with the decline becoming more pronounced over time. Specifically, we expected that manipulations of agrochemicals would produce the lowest CH_4/CO_2 emission ratios, and that these would further decline over time. We thus hypothesize that manipulations of agrochemicals will result in a significantly lower CH4/CO2 emission ratio compared to other manipulations, and that this ratio will decline significantly over time (*Hypothesis Three*).

2. Materials and methods

2.1. Experimental design

We present here experimental work that was carried out over the period 23rd April 2023 to 27th November 2024, corresponding to four growing seasons for rice. We conducted a series of ten replicated bifactorial experiments in each of the two experimental fields per season (i.e. 10×4 growth seasons x 2 fields = 80 experiments in total). Both experimental sites were located near the Shenzhen campus at Sun Yat-sen University (Guangming District, Shenzhen, Guangdong, 22.82° N, 113.94° E). Shenzhen experiences a subtropical monsoon climate characterized by long summers and short winters. The average annual temperature is 23.3 °C, with January recording the lowest average temperature at 15.7 °C and July the highest at 29.0 °C. The region receives an average annual sunshine duration of 1853 h and about 86% of its 1932.9 mm annual rainfall occurs between April and September. Field one had a pH of 6.07, with 6.9 g kg^{-1} soil organic carbon, 1.1 g kg^{-1} total nitrogen, and 3.2 mg kg^{-1} available phosphorus (Olsen P). Field two had a pH of 5.4, with 9.6 g kg^{-1} soil organic carbon, 1.1 g kg^{-1} total nitrogen, and 1.3 mg kg^{-1} available phosphorus. Field one (Fig. S1) comprised clay loam soil (35% sand, 29% clay, and 36% silt), while field two (Fig. S2) consisted of loam soil (24% sand, 33% clay, and 42% silt).

In each of the two fields, we ran the full combinatorial – $\binom{5}{2} = 10$ combinations – set of bifactorial experiments with the five manipulation parameters being no fertilizer additions, no tillage, no pesticide usage, addition of mycorrhizal propagules and experimenting with four different cultivars. Control settings across all experiments mirror conventional agricultural practices (i.e. fertilization, use of pesticides, tillage and no additions of mycorrhizal propagules). Each bifactorial experiment followed a randomized complete block design with eight replicates, except when cultivars were considered as experimental factor, where four replicates were used due to the four levels of cultivars (i.e. in both cases there were 32 experimental plots). Plot size was 2m $\times$ 2 m, separated by a 1 m wide field ridge. The total number of experimental units was thus 32×10 (experiments) x 2 (sites) x 4 (seasons) = 2,560.

2.2. Experimental settings

The control rice cultivar utilized in all experiments was the *Maoming* local variety, with additional cultivars "Zhongyou445", "Xiangliangyou900", and "Fenghuazhan" included in cultivar manipulation experiments. Mycorrhizal treatment utilized a commercially available spore inoculum from Nanjing Cuijingyuan Biotechnology Co., Ltd., containing a mixture of four types of arbuscular mycorrhizal fungi (*Rhizophagus clarus*, *Rhizophagus intraradices*, *Funneliformis mosseae*, *Claroideoglomus etunicatum*) and kaolin, with over 70 g of spores per g of active ingredient. We immersed the seeds into an aqueous solution of 60 g (direct seeding in the first harvest of field one) or 100 g (transplanting with an additional inoculation step in AMF powder later on over the transplanting stage based on manufacturer guidelines) of mycorrhizal inoculum. Tillage was performed using a tractor or a small tiller, with soil flattened using a rake in no tillage treatments to control water depth in paddies. Fertilizer application comprised 220-300 kg ha^{-1} urea fertilizer (>46% content) and 220-250 compound NPK fertilizer, applied in two doses at the beginning of the growth season and 40–60 days later upon temporary water drainage to reduce leaching. Over the first growth season in field one we directly seeded rice at a density of 3.9 g m^{-2}. We deemed this approach suboptimal and in the other three cases we transplanted seedlings through spacing holes at approximately 30 cm intervals, with 2-3 rice seedlings per hole.

The first growing season in Field-1 was initiated via sowing on 23 April 2023 and the harvest was on 13 August, while the second season was initiated via transplanted seedlings on 5 September and harvested on 30 November. There was a wheat season over the winter and the third growing season lasted between the 26th of March 2024 and the 26th of July 2024, whereas the respective dates for the fourth season were 27th of July 2024 till 24th of November 2024. In Field-2, the first season was initiated via transplanting on 15 June 2023 and harvest was on 16 August, and the second season was initiated via transplanting on 9 September and harvest was on 1 December. Third and fourth seasons lasted between 26th of March 2024 – 27th July 2024 and 27th July 2024 till 27th of November 204, respectively. Standard local agricultural practices were followed for irrigation, fertilization, and pesticide application during rice growth. After harvesting, root stubble remained in the field, and stalks were not returned.

2.3. Greenhouse gas assays

Greenhouse gases (CH_4, CO_2, and N_2O) were measured using the static chamber method. Ventilated static chambers (20 cm internal diameter and 30 cm internal height - Chadwick et al. (2014) out of PVC; the headspace volume of the static champers was about 4.7 l, depending on the soil depth that the base reached. We monitored effective height for each observation) into soil two weeks prior to assaying greenhouse gases. We calibrated measurements in a subset of units and determined that a two-point sampling approach would be sufficient given that headspace greenhouse concentrations over the first 20 min increased linearly. Gas samples (10 ml) were collected at the beginning and 10 min after chamber sealing, with temperature monitored simultaneously. Gas samples were collected every fifteen days during the rice growing season, totaling 320 samples. We carried out this way 12 measurements (i.e. each static chamber was assayed six times) over the duration of the two growth seasons, May to July 2023 and September to December 2023. Collected gas samples were transferred to the laboratory in headspace vials and were analyzed within two weeks using a gas chromatograph (GC 9790plus, Zhejiang Fuli Analytical Instruments Inc, China). Gas emission flux was calculated using the equation:

$$F = H \times \frac{273}{273 + T} \times \frac{P}{P_0} \times \rho \times \frac{d_c}{d_t}$$

Where F is the emission flux of gas, H is the chamber headspace height, T is the average chamber temperature, P is the local atmospheric pressure, P_0 is the atmospheric pressure at standard conditions, ρ is the gas density under standard conditions, and dc/dt is the slope of the gas concentration curve variation. Negative flux values represent the absorption of CH_4, CO_2, N_2O from the atmosphere, and positive flux values represent the emission of CH_4, CO_2, N_2O to the atmosphere. Gas samples were collected in the dark (i.e. the static champers stopped the light from entering), meaning that net respiration (i.e. CO_2 fluxes) estimates did not include photosynthesis.

2.4. Analytical approaches

Over two growth seasons, 23,040 records of greenhouse gas emission rates were derived from 7,680 gas samples for N_2O, CO_2, and CH_4. F values and fitted coefficients from ANOVA tests were analyzed to assess the predictive power of a predictor in an ANOVA, with higher F values indicating a higher predictive power, and the changes that the predictors induce are usually significant ($P < 0.05$) when the F values exceed 3 (Wang et al., 2024). In those fixed effects tests we used emission rates as a response variable and the two manipulations as categorical predictors without an interaction term. We fitted this way 10 (experiments) x 2 (fields) x 6 (harvests) = 120 ANOVAs and a total of 240 (i.e. 120 × 2 – two manipulations per experiment) effect sizes/coefficients. Each manipulation was represented in 48 of those effect sizes, which represented the unit of our analyses. We also extracted the coefficients per factor for the bifactorial ANOVAs. These coefficients capture the direction and magnitude of change that each of the two manipulation practices induced in the respective greenhouse gas emission rates. Each of our statistical tests (fixed effects ANOVAs) had a relatively low statistical power because we did not replicate the measurements extensively in those experiments (n = 8 – or n = 4 when cultivars were tested) and because our gas measurements contained a high measurement error.

To test whether sustainable management practices reduce greenhouse gas emissions (*Hypothesis One*), we first plotted the fitted coefficients (which represent the change caused by the treatment) against their corresponding effect sizes, expressed as F values. This allowed us to examine the observed changes in experiments with high explanatory power (high F values). These coefficients indicate the magnitude of change in greenhouse gas emissions resulting from sustainable management practices. We then used general additive models to fit a smoothing function, helping assess the direction of the effects. For large effect sizes (high F values), coefficients are more informative about overall changes. Accordingly, we compared smoothed values across manipulations with comparably large F values. Next, we converted CO_2, N_2O, and CH_4 emissions into equivalent CO_2 emissions using conversion coefficients of 14/12, 265 × 44/28, and 28 × 16/12, respectively (Tian et al., 2016). To minimize the influence of stochastic localized disturbances, we applied stringent filtering to exclude experiments with small effect sizes, retaining only high-quality observations. Using this subset, we summarized the impact of sustainable management practices on equivalent CO_2 emissions with one-sample t-tests. We further filtered results based on P-values for the three greenhouse gases. This was not to select statistically significant outcomes (which would use $P < 0.05$), but to remove experiments where external factors like defoliation might have produced unrealistic emissions. Our default stringent criterion required at least one gas to have a P-value <0.1, which excluded over half of the observations.

To test *Hypothesis Two*, that agrochemical manipulations are the most effective practice for mitigating greenhouse gas emissions and maintain the highest relative importance over time, we performed the following analyses: First, we used an analysis of variance to compare the F values (representing explanatory power) across the four sustainable management practices for each of the three greenhouse gases. This initial step aimed to demonstrate whether the practices differed in their influence on emissions. To formally test for these differences, we fitted mixed-effects linear models with F values as the response variable (separately for each gas) and the type of manipulation as the predictor. Field (a two-level categorical variable) was included as a random effect to account for differences between the two fields. Because the response variable was an effect size (F value), we did not correct for other potential dependencies (e.g., plot location or growth season), as is standard in effect-size studies such as meta-analyses.

When the treatment factor was significant, we simplified the model to a fixed-effects ANOVA (which produced identical results) and conducted Tukey post-hoc tests to identify which specific manipulations differed from each other. To evaluate the direction of change induced by each manipulation, we then created plots for each greenhouse gas showing the relationship between F values and their corresponding fitted coefficients. Finally, to assess how the explanatory power of each practice changed over time, we fitted linear models with F values as the response and two predictors: the categorical sustainable practice and a continuous harvest variable (ordinal, 1–6). We also ran an alternative version in which the six harvests were grouped into three growth-stage categories: early, middle, and late season.

To test *Hypothesis Three,* that manipulations on agrochemicals would present the lowest CH_4/CO_2 emission ratios, and that these would further decline over time we replicated the analysis we carried out for the three greenhouse gases in relation to *Hypothesis Two* but this time we used as a response variable the ratio of the two gases.

3. Results

3.1. Sustainable management practices reduce greenhouse gas emissions

The median N_2O emissions rate in our dataset was 26.9 μg m^{-2} h^{-1} with quartiles of −8.2 and 98 (Fig. 1; S3). For CO_2, the median emission rate was 130 mg m^{-2} h^{-1} with quartiles of −92 and 479, while for CH_4, it was 9.21 mg m^{-2} h^{-1} with quartiles of 0.04 and 5.55 (Fig. S3). We present N_2O emissions in μg m^{-2} h^{-1} (compared to CO_2, and CH_4 emissions which are presented in mg m^{-2} h^{-1}) to signal that while the fluxes are typically much lower, the sensitivity of the electron capture detectors is much greater (i.e. despite the estimates differing by several orders of magnitude, their measurement error is comparable). Ceasing fertilization reduced by 87% N_2O and increased CH_4 and CO_2 emissions by 10% and 60%, respectively. No tillage reduced N_2O, CH_4 and CO_2 emissions by 66%, 55% and 13%, respectively. Surprisingly, abstaining from pesticide use increased N_2O emissions by 83%, while adding arbuscular mycorrhizal fungi (AMF) propagules reduced CO_2 emissions by 14% without affecting N_2O (+0.1%) and CH_4 (+7%) emissions. Equivalent CO_2 emissions declined upon additions of AMF propagules ($t = -2.21$, $P = 0.039$; Fig. 2). Changing the fertilization, pesticide and tillage regimes did not modify the greenhouse gas emissions rates. There were only subtle differences in greenhouse gas emission patterns across the four cultivars (Fig. S4).

3.2. Manipulations of agrochemicals rank first in mitigating greenhouse gas emissions

The frequency of significant effects varied across greenhouse gases and management practices. For N_2O emissions, 35 out of 384 tested effects were significant (F-tests). Of these, 21 were associated with the cessation of fertilization. For CO_2 emissions, 20 effects were significant, with 8 linked to stopping pesticide use. For CH_4, 19 effects were significant, 13 of which concerned the tillage manipulation. The overall rate of significance was close to the 5% alpha level, which could indicate Type I errors. However, if this were the case, we would not expect the significant effects to be disproportionately clustered within specific manipulations, such as the 21 significant reductions in N_2O from ceased fertilization. Therefore, to further explore these patterns, we treated the F-statistics as continuous variables to test for differences in explanatory power across manipulations.

Analysis of these F-statistics revealed that stopping fertilizer and pesticide application tended to lower emissions most effectively. While there were no significant differences among treatments for CO_2 emissions ($F = 1.02$, $P = 0.39$), significant differences were detected for both N_2O ($F = 13.8$, $P < 0.001$) and CH_4 ($F = 5.5$, $P < 0.001$). Post-hoc comparisons showed that for N_2O emissions, ceasing fertilization produced significantly higher F values than manipulations involving alternative cultivars, pesticide cessation, and AMF inoculation ($P < 0.001$). For CH_4 emissions, the no-tillage practice resulted in significantly greater emission suppression compared to no pesticides ($P = 0.001$), alternative cultivars ($P = 0.003$), and AMF additions ($P = 0.003$). Regarding the direction of effects (Fig. 3), AMF inoculum additions

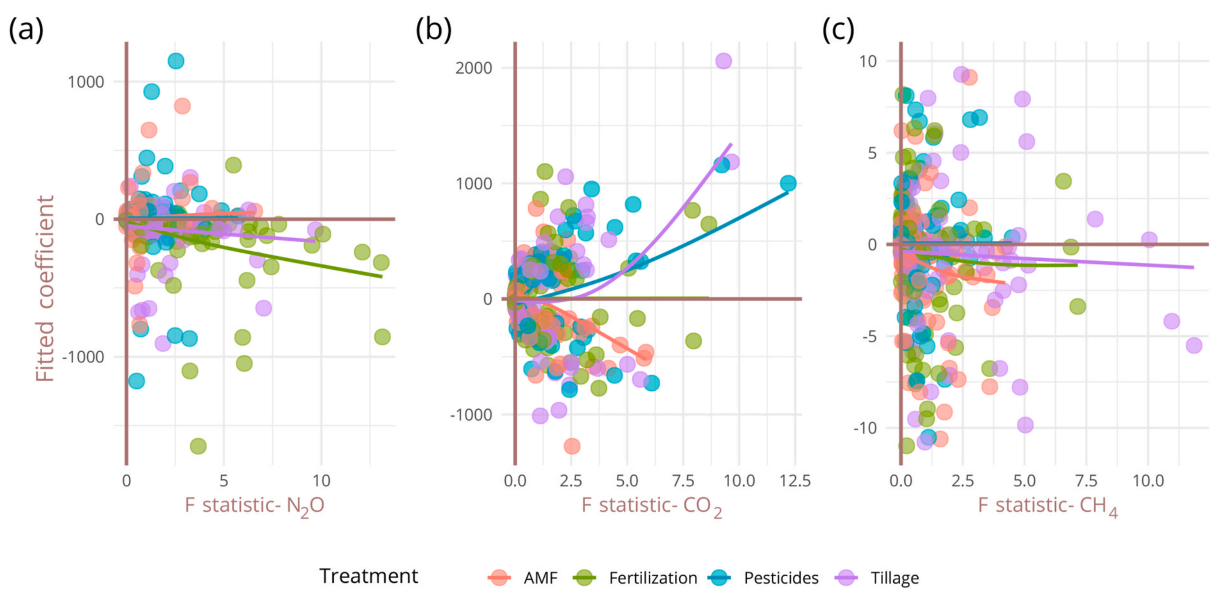

Fig. 1. Relationships between coefficients (*y*-axis; essentially describing the direction and the magnitude of the changes that the respective sustainable management practices induced to greenhouse gas emissions in the respective experiment) and F statistics (*x*-axis) of the four (out of five) effects across the 240 ANOVA tests for the three greenhouse gases in our study: (a) N2O; (b) CO2; (c) CH4. The idea of plotting the coefficients against the effect size of each test, was that coefficients should asymptotically reach a value when the statistical power of the test is high (i.e. high effect sizes). We overlayed the distribution of the points with general additive model fits (continuous lines) to highlight the convergence of the points for high F values (i.e. the take home message of the display item). F values above 3 usually describe significant effect sizes. Note that ceasing pesticide usage resulted into positive coefficients in relation to N_2O and CO_2 emissions, meaning that there was an increase of the respective greenhouse gases.

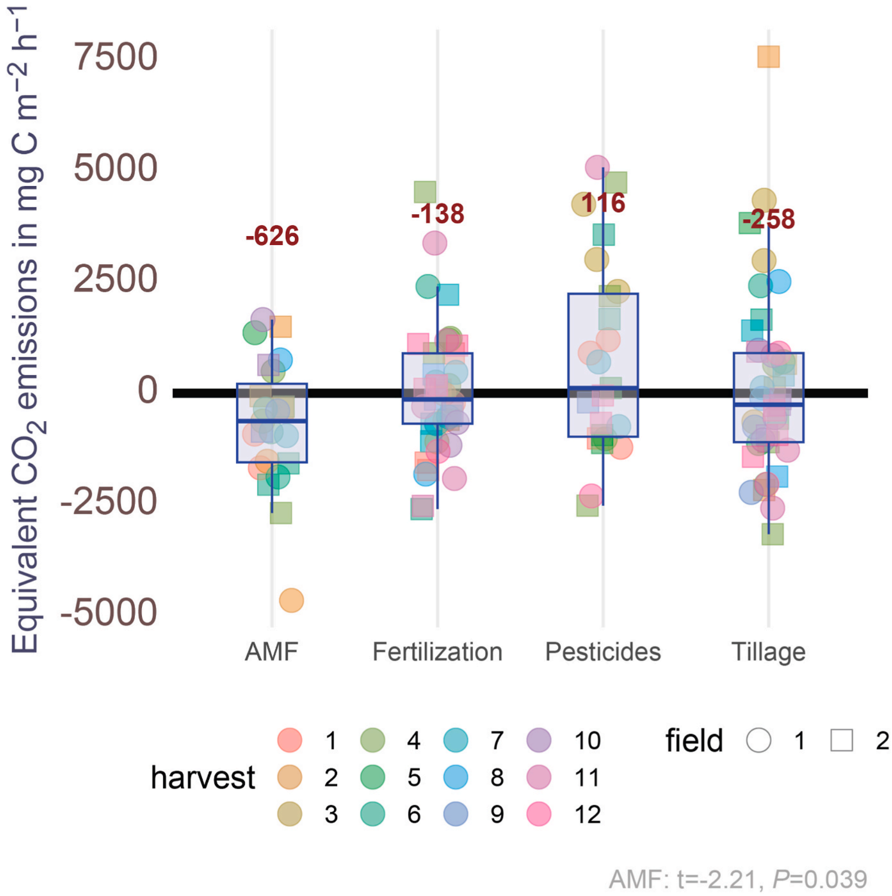

Fig. 2. Boxplot depicting the changes that the four sustainable practices induced on equivalent CO_2 emissions. For this analysis, we only considered effects if the sustainable practice had induced a change at $P < 0.1$ to a minimum one of the three (i.e. CO_2, N_2O and CH_4) greenhouse gases. This way reduced the risk of including unusual observations and as a result the total number of observations went down from 384 to 128. AMF reduced (i.e. one sample *t*-tests) equivalent CO_2 emissions (t = 2.21, P = 0.039) The effects of stopping fertilization and tillage were indistinguishable from zero.

yielded the most negative coefficients per F value for CO_2 emissions. In contrast, no-tillage and stopping pesticide use were associated with comparable increases in CO_2 emissions. For CH_4, AMF reduced emissions whereas ceasing fertilization increased them.

Temporal dynamics were also evident (Fig. 4). The relative importance of pesticide manipulation declined over time. Significant differences across harvests were observed only for pesticide cessation, both for CO_2 ($F = 5.7$, $P = 0.02$) and CH_4 emissions ($F = 100.4$, $P < 0.001$). AMF propagule additions showed a non-significant trend toward stronger effect sizes over time ($P_{N2O} = 0.14$, $P_{CO2} = 0.06$, $P_{CH4} = 0.09$). Seasonal changes in emission rates were observed for specific practice-gas pairs. The effect of fertilization on N_2O emissions peaked early in the season and then declined. Conversely, the effect of no-tillage on CO_2 emissions increased over the growing season, peaking at the final harvest.

3.3. Manipulations on agrochemicals present the lowest CH_4/CO_2 emission ratios

The ratio of CH_4 over CO_2 emissions differs across treatments and declines most over time for fertilization and pesticide manipulations. Adding AMF or stopping the use of pesticides slightly increased the emission ratio of CH_4 over CO_2 whereas no tillage slightly reduced it, but these changes were independent of F values (Fig. 1) (overall test: F = 3.13, P = 0.016; Fig. 5) in the contrast between No Tillage (higher F values with a median of 0.9) and AMF (lower F values with a median of 0.64, P = 0.02). There were no changes in F values over time for any of the manipulations (Fig. S5).

4. Discussion

4.1. Study overview

In our study, we conducted a thorough assessment of how five sustainable management practices influenced the emissions of three greenhouse gases over multiple growing seasons in two rice paddies. Our findings revealed differential effects of these practices on N_2O and CH_4 emissions, but not on CO_2 emissions. The high predictive power of coefficients per management practice allowed us to simultaneously compare the consistency and magnitude of changes induced. Ceasing fertilization resulted in significant reductions in N_2O emissions early over the growth seasons, although this effect diminished over time. Conversely, adding arbuscular mycorrhizal fungi (AMF) propagules substantially decreased equivalent CO_2 (i.e. total greenhouse gas) emissions (Fig. 2). Surprisingly, abstaining from pesticide use increased both N_2O and CO_2 emissions. The significant reduction in N_2O emissions coupled to the expected lower soil nitrate levels in the no-fertilizer plots supports the substrate-limitation mechanism. Conversely, the unexpected rise in CH_4 emissions under no-tillage could be explained by increased soil moisture retention creating more persistent anaerobic microsites, favoring methanogenesis.

4.2. Sustainable management practices reduce greenhouse gas emissions

Only 143 out of 384 observations (or 37%) on effect sizes met the criterion that at minimum for one greenhouse gas the P value associated with the effect size was below 0.1. The addition of AMF propagules in

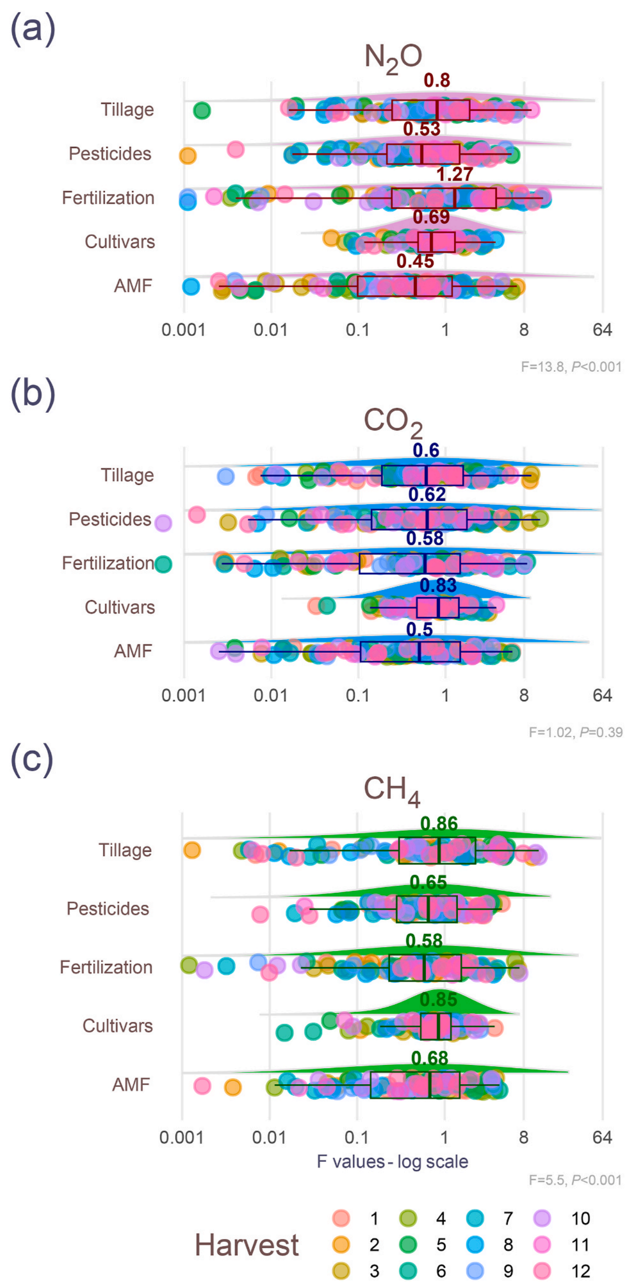

Fig. 3. Distribution (boxplots) of effect sizes (F values) across the five manipulations in relation to (a) N_2O (b) CO_2 and (c) CH_4 emission rates. We used filled symbols only for the subset of tests which yielded significance and present the F values log-transformed for a better visualization. We highlight the median F values per manipulation and use different symbols for the two fields. The take home message of this display item was that we observed a considerable variation in our effect sizes. We could nonetheless, in the cases of N_2O and CH_4 – statistics in the captions – find differences across the manipulations in relation to the F values.

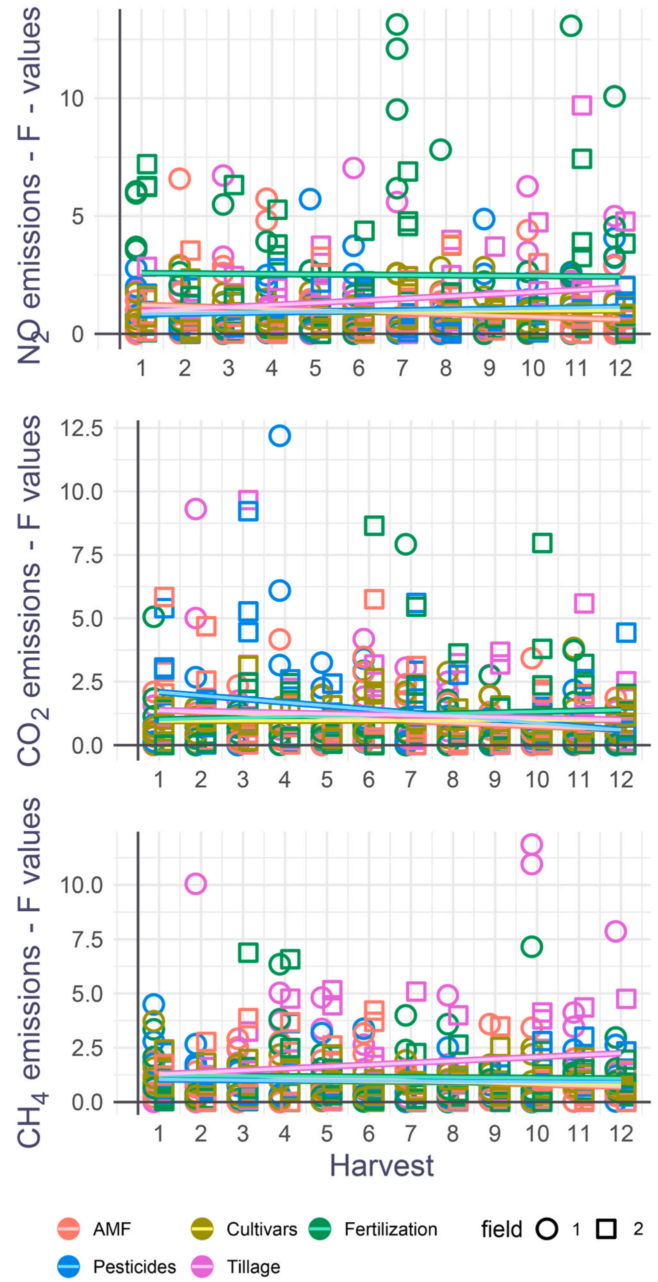

Fig. 4. F statistics (*y*-axis) across the six harvests (*x*-axis) for the three greenhouse gases in (a) N_2O (b) CO_2 and (c) CH_4 emission rates. We overlayed best-fit regression lines. Through this display item we wanted to communicate the small temporal differences we observed in emission rates. The only significant slope was that of pesticides in relation to CO_2 emissions ($F = 5.7$, $P = 0.019$), whereas we additionally observed differences across the three growth stages over the growth season in relation to N_2O emissions following manipulating fertilization ($F = 4.68$, $P = 0.012$) and in relation to CO_2 emissions following manipulating tillage ($F = 4.09$, $P = 0.02$).

this dataset reduced equivalent CO_2 emissions (Fig. 2), which was the only component of the hypothesis that was supported. F values associated with mycorrhiza (Fig. 3c but also the fitted coefficients in Fig. 1) ranked first in relation to CH_4 emissions whereas there was a large mitigating potential in relation to CO_2 emissions (Fig. 3b). Managing systems for conserving and amplifying mycorrhiza holds much promise for sustainably managing agriculture but for the time being commercial use of mycorrhizal products remains low (Veresoglou and Agathokleous, 2023). Unsurprisingly the body of literature that currently assesses mycorrhiza in relation to greenhouse gas emissions is quite limited (Veresoglou and Chen, 2024).

In our experimental system adding mycorrhiza increased N_2O emissions (Fig. 3a). As we state earlier there has been an anticipation that mycorrhiza offsets N_2O emissions as well (e.g. Bender et al., 2015; Storer et al., 2018) even though most of the studies supporting this idea originate from controlled studies where available N stocks have been heavily depleted and the state of the nitrifying community remains uncertain (Okiobe et al., 2023). Under more realistic conditions, we have observed instead that the N_2O emission potential of the soil increases (Okiobe et al., 2019, 2020), and in fertile systems that favor

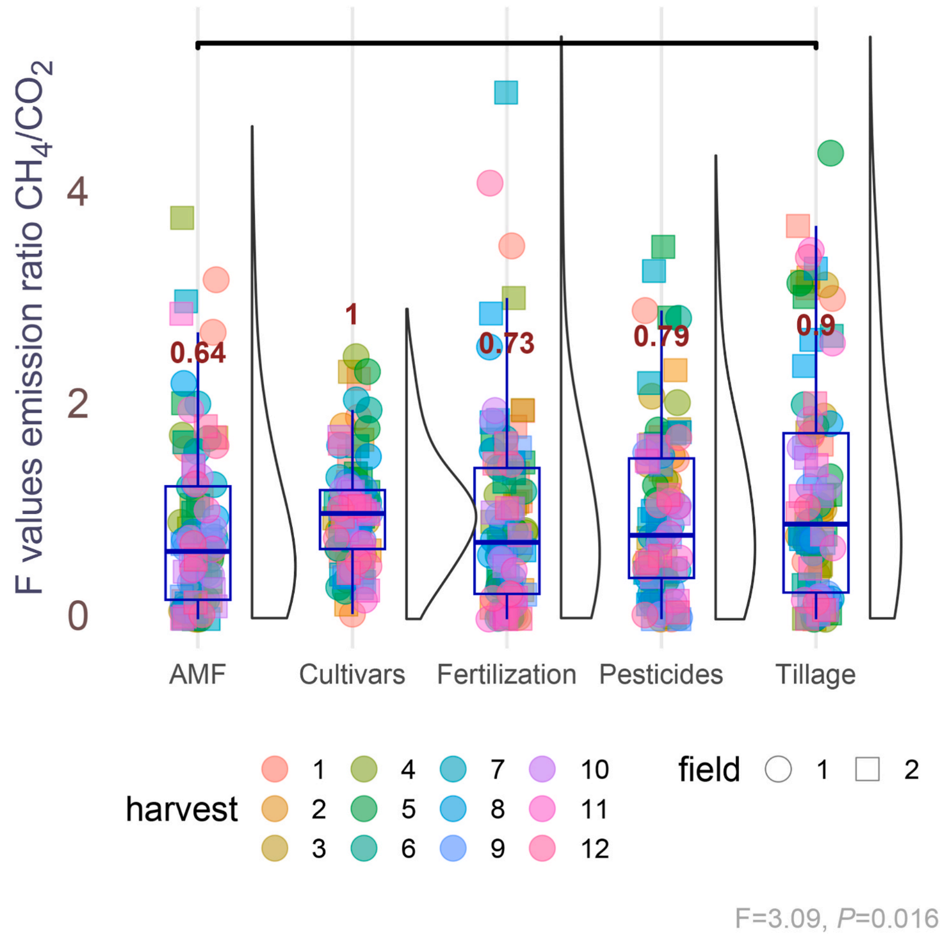

Fig. 5. Differences in F values in the emission ratio of CH_4 over CO_2 across the five manipulations. The effect size for tillage was higher than that for AMF (Tukey posthoc test), which we highlight with the black bar on top.

denitrification such as rice paddies we expect that mycorrhiza increases N_2O emissions. In this regard, the observation on N_2O emissions matches our expectations. There are several reports in the agronomy and horticulture literature that raise concerns about the management of mycorrhizal propagules to enhance crop yield (e.g. Ryan and Graham, 2018; Buil et al., 2023). Concerns could emanate from the inconsistency with which mycorrhiza enhances crop growth. Although there has been great progress in understanding why mycorrhiza sometimes reduces crop yield (e.g. Lutz et al., 2023) and although these reports rarely consider non-crop growth benefits such as soil structure (Wang and Kuzyakov, 2024), we uncover here yet another great reason why agricultural policy should foster the commercial use of mycorrhizal technologies. The changes that mycorrhiza induced to greenhouse gas emissions were manifested mainly as lower respiration rates (Fig. 1). The literature reports inconsistent effects of mycorrhiza on soil respiration (e.g. Teutscherova et al., 2019; Boyno et al., 2024; but see Lazcano et al., 2014; Ma et al., 2015) but is congruent that most likely such changes are induced through improved aeration and water relations in plants that have been colonized with mycorrhiza. Those two mechanisms have been particularly important in our system, given that rice was submerged in water.

It was remarkable that no manipulation other than mycorrhiza reduced equivalent CO_2 emissions. Pesticide use actually increased equivalent CO_2 emissions (Fig. 2; Fig. 3). We think that in our analysis we captured many short-term alterations which agricultural management practices induce. We are aware for example that plant diversity increases soil respiration (Chen et al., 2019). In the absence of pesticides, we had many weeds establishing in the plots, which we could observe macroscopically and which increased plant diversity and most likely soil respiration. We could think of comparable arguments in relation to tillage, where the decomposing soil roots in the absence of tillage partially promoted soil respiration. Unsurprisingly, most of the changes in equivalent CO_2 emissions mirrored changes in soil respiration. In relation to N_2O and CH_4 emissions, for example, stopping tillage reduced emissions (Fig. 3). This is in agreement with several recent studies investigating the implications of conservation tillage treatments on rice in relation to greenhouse gas emissions (Guo et al., 2021; Zhang et al., 2024b; Wang et al., 2025). It is unclear whether and to what degree those differences persist for long-term agricultural management practices. From an environmental point of view, it is, nonetheless, important to discriminate between CO_2 and CH_4/N_2O emissions (Mar et al., 2022), which is something we did with *Hypothesis Three.*

4.3. Ranking of agrochemicals in mitigating greenhouse gas emissions and changes over time

We found differences in F values only in relation to N_2O and CH_4 emissions. For N_2O, ceasing fertilization produced higher F values than AMF inoculation, stopping pesticide use, or experimenting with cultivars (Fig. 4). This is in agreement with Wang et al. (2024) showing that reducing N fertilizer dose massively modifies nutrient cycling processes in rice paddies. For CH_4, no-tillage had higher effect sizes (F values) than changing cultivars, ceasing pesticides, or AMF inoculation (Fig. 4). In contrast, differences among treatments for CO_2 emissions were non-significant ($F = 1.02$, $P = 0.40$). These detected differences confirmed that our analysis had sufficient resolution to distinguish among practices, though ranking practices proved less straightforward than anticipated, preventing a clear test of *Hypothesis Two*.

The median F values generally ranged between 0.5 and 1 (Fig. 4), representing relatively small but meaningful effect sizes. However, the range of F values was wide. We did not observe any consistent patterns in bifactorial experiments that drove systematically high or low F values. This high variability may be attributed to several factors: greenhouse gas emissions are sensitive to diurnal timing and environmental conditions like temperature and soil anoxia (Savage et al., 2014; Wu et al., 2021), which likely differed across sampling events. Although samples were taken sequentially, this could still have affected the experiments' descriptive power. Additionally, the timing of agricultural interventions may have varied across the twenty parallel experiments. Still, these factors likely explain only a small portion of the observed variance.

While agricultural manipulations differentially affected greenhouse gas emissions, temporal changes in these effects over the study period were weak. Pesticide abstention reduced CO_2 and CH_4 emissions less over the longer term (Fig. 5a), while N_2O emission differences after stopping fertilization peaked early in the growing season. The timing of emission peaks can vary considerably with management practices and can inform mitigation strategies (Qian et al., 2023), underscoring the need to evaluate both seasonal and multi-seasonal trajectories. Management practices often exert distinct short- and long-term effects; for example, defoliation causes a short-term surge in rhizodeposition and microbial respiration, followed by a long-term decline (e.g., Henry et al., 2008). We had expected fertilization to become less important later in the season for N_2O emissions—contrary to *Hypothesis Two*, highlighting the uncertainty in these systems. Unfertilized systems may deplete existing inorganic fertilizer stocks in the short term but transition toward higher nitrogen-use efficiency over time (You et al., 2023). The absence of other temporal shifts may reflect an insufficient observation window, as many changes require longer time spans to manifest, or because our tests were underpowered due to high variance in F values per harvest. Under these conditions, only exceptionally pronounced changes would be detectable.

4.4. Manipulations on agrochemicals, present the lowest CH_4/CO_2 emission ratios, and further decline over time

We further considered the ratio of CH_4 over CO_2 emissions. To meet the targets of the Paris agreement, there is the expectation that we will collectively lower non-CO_2 emissions more than CO_2 emissions, meaning that we should aim for emissions ratios below one. Gaseous CH_4 is reduced to CO_2 in soil by methanotrophs, with CO_2 being considerably less deleterious for climate warming potential. It has been proposed that in systems with high CH_4 emissions this emission ratio would be reflective of the activity of methanotrophs (Pratt et al., 2013). No-tillage had a greater impact on the ratio compared to adding mycorrhizal inoculum. However, we couldn't predict the direction of these changes. For tillage, the mean change in the ratio was −0.02, with a one-sample *t*-test statistic of 0.9 ($P = 0.38$), compared to a slightly negative median. The respective statistics for AMF were a mean of −0.07, $t = -0.43$, $P=0.68$. No-tillage practices have been shown to induce significant increases in CH_4 emissions from agricultural soils (e.g., Mooney and Sjogersten, 2022), and this has been observed in rice paddies as well (Gangopadhyay et al., 2023). Hence, we were surprised that the overall effect in our system was indistinguishable from zero. One possibility is that no-tillage alters the CH_4:CO_2 ratio differently when combined with other treatments that have substantial effects on CO_2 emissions, such as pesticide. It is also noteworthy that the ratio did not change systematically over time, and we observed no differences in relation to practices involving agrochemicals. (i.e. evidence against *Hypothesis Three*). We were hoping that studying the ratio would have further uncovered interesting insights of how agricultural soils mitigate greenhouse gas emissions but all our estimates contained a high uncertainty (Fig. S3).

4.5. Further steps to investigate sustainable management practices

We have collated data from four growing seasons, so the trajectories on which we have relied are relatively short. Much of the uncertainty in relation to global change emissions comes from interannual differences, describing drivers such as interannual variation in precipitation and periodic phenomena such as the El Niño (e.g. Rowlings et al., 2015; Zhu et al., 2017; Cheng and Redfern, 2022), which we were unable to capture with our experimental duration. Interannual variation in emission rates can be massive, but it is extremely unusual for emission rates to be assayed *in situ* in long-term experiments (e.g. Lauk et al., 2024). For example, Li et al. (2024) reported that for China the upper bound estimate of greenhouse gas emissions is about 25 times higher than the lower estimate. An obvious gap in the literature is the long-term monitoring of the effects of sustainable management practices on the climate and distinguishing between short- and long-term effects and how consistent they are across different systems. While we could clearly see the benefit of focusing on rice here, it is clear that similar studies need to be carried out for other crops. We have shown in a recent meta-analysis that manipulations such as reducing water availability affect rice paddies in a different way than other crops in relation to N_2O emissions and this is likely the case for CH_4 as well (Chen et al., 2024). Our study demonstrates that unreplicated observations on greenhouse gas emissions from agriculture can be considerably idiosyncratic and thus uninformative for policy.

5. Conclusion

We implemented a novel multi-treatment experimental framework to directly compare the efficacy of five sustainable management practices—no fertilization, no tillage, no pesticide, AMF inoculation, and alternative cultivars—on GHG emissions in rice paddies. We found that practices reducing agrochemical inputs (no fertilizer and no pesticide) consistently led to the strongest mitigation of N_2O and CO_2 emissions, supporting our hypothesis that agrochemical manipulations exert a dominant and persistent effect on the GHG budget. Notably, the no-fertilizer practice emerged as the most effective single intervention for reducing N_2O emissions. In contrast, the effects of no-tillage and AMF addition were more variable, while alternative cultivars showed limited direct mitigation potential. The CH_4/CO_2 ratio proved to be a sensitive indicator of soil methanotroph activity, declining most significantly under reduced agrochemical treatments.

CRediT authorship contribution statement

Min Wang: Writing – original draft, Formal analysis, Data curation. **Junjiang Chen:** Writing – review & editing. **Matthias C. Rillig:** Writing – original draft. **Josep Peñuelas:** Writing – original draft. **Stavros D. Veresoglou:** Writing – original draft, Supervision, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Generative AI and figures, images and artwork

We did not use AI tools to generate text or display items.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Stavros D Veresoglou reports financial support was provided by National Natural Science Foundation of China. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2026.129557.

Data availability

Data are made available via zenodo: https://doi.org/10.5281/zenodo.19126150

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