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# Ten years of improved-fallow slash-and-mulch agroforestry in Brazilian Amazonia: Do nitrogen-fixing trees affect nitrous oxide and methane efflux?

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**Abstract** Slash-and-mulch agroforestry systems can reduce greenhouse gas emissions by mulching the vegetation instead of burning it. This mulch layer then contains greater stocks of organic material than after burning, making it a potential source of $N_2O$ and $CH_4$ efflux during decomposition. We examined $N_2O$ and $CH_4$ efflux from slash-and-mulch AFS using a two-way factorial design: with and without P + K fertilization, and with and without a nitrogen-fixing tree (*Inga edulis*). We hypothesized that inclusion of N-fixing trees would increase $N_2O$ efflux and that $CH_4$ efflux would increase due to increased soil moisture with mulching. We measured trace gas fluxes prior to the end of Rotation 1, and after mulching to begin Rotation 2. $N_2O$ efflux increased with *I. edulis* during the year prior to, but not after, mulching. No differences by treatment were detected for $CH_4$ efflux before or after mulching. Site conversion from secondary forest to Rotation 2 resulted in a 130% increase in $N_2O$ efflux and a 430% decrease in $CH_4$ efflux. The $CO_{2e}$ increase of 2,400 kg ha$^{-1}$ was an order of magnitude less than estimated releases of trace gases from burning (38,400 kg ha$^{-1}$). For both $N_2O$ and $CH_4$, land disturbance during mulching led to larger changes in trace gas fluxes than either P + K fertilization or inclusion of the N-fixer. The order-of-magnitude estimates of trace gas release as $CO_{2e}$ from mulching and the addition of N-fixers appears to be less than that from burning alone.

**Keywords** Nitrogen fixing trees · Greenhouse gas emissions · Nitrous oxide · Methane · Nitrogen

## Introduction

Agriculture and deforestation for agriculture are responsible for nearly 25% of greenhouse gas (GHG) emissions globally (Smith et al. 2014; Tubiello et al. 2015). Agroforestry systems (AFS) have been proposed to reduce the GHG emissions of agricultural practices (Campos & Nepstad 2006). Forest loss, partially for conversion to agriculture, in Brazilian Amazonia has generated at least 5% of global GHG emissions (Nepstad et al. 2009). Smallholdings (< 100 ha) in Brazilian Amazonia occupy ~ 38% of the total land area under cultivation (Brondizio et al. 2009) and are

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typically prepared for cultivation using slash-and-burn AFS, in which the standing vegetation is cut, left to dry, and then burned. The initial pulse of available nutrients via ash increases crop production. Leaching, erosion, and crop export cause fertility to decline, leading to abandonment after 2–3 years (Denich et al. 2004). Clearing forest via fire leads to pulses of GHG emissions at clearing and, if followed by conventional agriculture, continuously increased fluxes of $CO_2$, $CH_4$, and $N_2O$ (Davidson et al. 2001).

AFS vary in their potential to mitigate emissions of $CO_2$, methane ($CH_4$), and nitrous oxide ($N_2O$). Slash-and-mulch systems have been adopted in some areas to replace the use of fire in slash-and-burn systems (Kato et al. 1999). In one study comparing slash-and-burn to slash-and-mulch, the global warming potential through the first two years of the cycle was estimated at 21 and 3.6 Mg $CO_{2e}$ $ha^{-1}$, respectively (Davidson et al. 2008). This difference was largely due to the avoided release of 12 kg $N_2O$–N $ha^{-1}$ and 630 kg-$CH_4$ $ha^{-1}$ during the burn. During cropping, however, slash-and-mulch treatments resulted in $CH_4$ efflux (16 kg $CH_4$ $ha^{-1}$) compared to slash-and-burn that resulted in consumption (−5 kg $CH_4$ $ha^{-1}$). Similarly, soil $N_2O$ efflux during cropping was greater in the slash-and-mulch treatment (4.2 vs 2.9 kg $N_2O$—N $ha^{-1}$). This previous study applied 90 kg-N $ha^{-1}$ as urea in addition to including the N-fixer *Inga edulis*; therefore the role of the N-fixer could not be separated from the N fertilization effect on $N_2O$ efflux during the cropping phase. N-fixing trees have been implicated as a source of increased $N_2O$ efflux (Rosenstock et al 2014). A recent meta-analysis synthesized global agroforestry and reforestation research using N-fixing trees and found 39 papers fitting their search criteria (Kou-Giesbrecht & Menge 2021), although it included only one site from the Amazon basin, and it was from natural forest. The later work concluded that $N_2O$ efflux might double from planting N-fixing trees for reforestation, which could offset ~ 4% of the benefits of atmospheric $CO_2$ uptake derived from tree growth.

In the same locale as the Davidson et al (2008) research (Igarapé Açu, Pará, Brazil), an improved fallow study with N-fixing tree species, including *I. edulis*, but without slash-and-mulch preparation, reported low rates of $N_2O$ emissions (2 kg $N_2O$–N $ha^{-1}$) that did not differ from control treatments that lacked N fixing trees (Verchot et al. 2008). In addition, $CH_4$ was consumed in both the wet and dry season, with estimated annual consumption of −4 kg $CH_4$ $ha^{-1}$, which did not differ between treatment and control. A similar result was found in the Peruvian Amazon where, after burning, a multistrata AFS with *I. edulis* showed no increase in $N_2O$ efflux or decrease in $CH_4$ consumption compared to unburned 13-yr-old secondary forest (Palm et al. 2002).

Fertilization with inorganic N can increase production of $N_2O$ in many sites via nitrification and denitrification (Castro et al. 1994 and 1995; Basiliko et al. 2009) and can suppress $CH_4$ emissions in upland sites due to stimulation of methanotrophic bacteria (Banger et al. 2012). The use of N-fixing trees in AFS is a common substitution for mineral N fertilizer (Rosenstock et al 2014) and can increase aboveground biomass and N content (Brienza Jr, 1999; Joslin et al. 2011). This increased N content can be used to fertilize subsequent crops as a green manure after burning or mulching. N-fixers can also increase soil-N content, through root turnover or litter decomposition (Danso et al. 1992), yet increases in $N_2O$ efflux are inconsistent (Palm et al. 2002; Verchot et al. 2008).

In an AFS in the Kenyan Highlands, rates of $N_2O$ efflux were correlated with the N content of residues (i.e., green manure) applied prior to cropping (Millar et al. 2004). Rates of $N_2O$ efflux during the first 84-days were ~ 2 vs. 0.2 kg-$N_2O$–N $ha^{-1}$, respectively, when residues from improved fallows with an N-fixing tree were compared to natural fallow residues. Mulching may also enhance soil-moisture retention under the organic layer (Davidson et al. 2008), which can alter rates of N cycling, and rates of denitrification or methanogenesis due to changes in % water filled pore space (WFPS).

It is unclear how N-fixers and mulching may influence $CH_4$ and $N_2O$ fluxes but may be important given the high global warming potentials (GWP) of these gases (Davidson et al. 2004a). Upland forest soils of Brazilian Amazonia are usually sinks for atmospheric $CH_4$ (Schlesinger 1997; Verchot et al. 2008), although soils can be sources of $CH_4$ when saturated (Megonigal, 2004). This research addresses the lack of data regarding trace gas effluxes from secondary forest and agroforestry systems in Amazonia.

In the Amazonian study reported here, in the six years of improved secondary forest fallow growth that followed initial mulching and cultivation, planted *I. edulis* biomass N-content was the primary driver of increased system N-content (Joslin et al. 2016). After these 6 years, *I. edulis* was the dominant tree species where planted, comprising an estimated 82 and 71% of planted tree biomass with or without P+K fertilization, respectively. In P+K fertilized treatments *Inga* represented as much as 87.5% of N in the planted trees and held 2.6-fold more N than N in planted trees when *Inga* was not included (Joslin et al. 2016, 2019). Upland soils of secondary forests in Amazonia are generally N-limited (Jordan 1985), contributing to low $N_2O$ fluxes, but P limitation have also been observed, with productivity increase up to 25% with P-fertilization (Davidson, 2004b; Cunha et al 2022). Fertilization with P can also stimulate activity of soil microbial communities that produce $CH_4$ or $N_2O$, such as methanogenic or nitrifying bacteria (Banger et al. 2012), as can potassium (K) for soil $CH_4$ production (Conrad & Klose 2005). As such, we attempt to estimate the influence of an N-fixing tree and P+K fertilization on $N_2O$ efflux and total $CO_{2e}$ of our AFS.

Here we extend the findings on biomass and N contents to rotation age (9 years) and also test the effects of P+K fertilization and the presence of *I. edulis* on $N_2O$ and $CH_4$ fluxes. These trace gas fluxes were measured during the final year of the first crop-fallow rotation (Year 9) and during Year 1 of the second crop-fallow rotation (Year 10). We hypothesized that: 1) fluxes of $N_2O$ and $CH_4$ will be greater in the main-plot P+K fertilized treatment, as well as in subplot treatment with the presence of *I. edulis* during the final year of the first crop-fallow rotation; 2) that during Year 1 of the second crop-fallow rotation these differences would increase due to newly added mulch material; and 3) that $N_2O$ and $CH_4$ effluxes would be greater nearer (0.25 m) to *I. edulis* trees than further away (2 m).

## Methods

### Site description

The research was conducted at the Fazenda Experimental de Igarapé Açu (FEIGA) of the Universidade Federal Rural da Amazônia (UFRA) in the Municipality of Igarapé Açu (1°07′41″S 47°47′15″ W), approximately 110 km East of Belém, Pará, Brazil (Fig. S1). One of the oldest continually inhabited agricultural areas in Amazonia, the Bragantina landscape is dominated by urban areas, row-crop farms, plantation forests, cattle ranches, and secondary forests. Primary forests are < 2% of the area (Denich et al. 2004).

Upland soils in the municipality of Igarapé Açu are predominantly Kandiudults (Rego et al. 1993) with a bulk density (BD) of 1.2 g $cm^{-3}$ from 0 to 5 cm and 1.4 g $cm^{-3}$ from 5 to 10 cm (Joslin et al. 2011). Kandiudults are Ultisols in the US Department of Agriculture classification and are typically equated to Acrisols in the Food and Argiculture soil classification. These soils have a kandic horizon, or subsurface accumulation of low activity (< 16 $cmol_c$ $kg^{-1}$ clay) illuvial clays, under a udic (ud) soil moisture regime defined as no periods of 90 days dry down. Igarapé Açu has an average annual temperature of 26°C and annual rainfall of 2500 mm (IBGE, 1996; cited in: Kato et al. 2005); the driest months are August—November and the wettest months are January–May. During the years 2000–2008, the FEIGA weather station recorded mean monthly rainfall over 280 mm from January to May, peaking at 420 mm in March, and minimum rainfall occurring from September–November, reaching its low in November with 30 mm (Fig. S2).

### Species descriptions

The five planted tree species are native to forests of the Bragantina region. *Inga edulis* (Leguminosae) is the only planted known N-fixer, although other N-fixing species may be present due to natural recruitment in secondary succession. *Schizolobium amazonicum* (Leguminosae) and *Ceiba pentandra* (Bombacaceae) are rapidly growing pioneers with soft wood. *Parkia multijuga* (Leguminosae) and *Cedrela odorata* (Meliaceae) are slower growing tropical hardwoods (See Joslin et al. 2011 for more complete species description).

All five tree species were planted with the food crop manioc (*Manihot esculenta*), which was harvested after 12 and 20 months during the first crop-fallow rotation. Manioc growth response was reported in Joslin et al. (2011, 2019). After converting secondary forest via mulching, and prior to planting the second rotation, all *S. amazonicum* were harvested. *Inga edulis* were cut to stumps with residue placed in the mulch row. Larger individuals of the other species were marked and avoided as best as possible during mulching to allow for their continued growth. Within ten days of mulching via tractor in July 2014, the same tree species were planted along with manioc to begin the second rotation of the crop-fallow cycle.

## Plot establishment

In March of 2005, a one-hectare study site was created from a 7-year-old secondary forest within FEIGA by clearing via mulching tractor as described by Joslin et al. (2011). Experimental treatments were applied to the prepared site in June 2005, where four blocks (N = 4) were divided into four 24 × 24 m plots (*n* = 16). Trees and manioc were planted simultaneously, with trees planted at 4 × 1.8 m spacing, for a total of 78 trees per plot, or 1,354 trees $ha^{-1}$. The crop species *M. esculenta* was planted at 1 × 1 m spacing, for a rate of 10,000 stems $ha^{-1}$.

A factorial combination of fertilization treatment and N-fixing species additions was assigned in a split-plot randomized complete block design. The main-plot fertilizer treatment (Fert) consisted of no fertilization (PK-) and fertilization (PK+) as an application around the base of planted trees of 46 kg P $ha^{-1}$ as $P_2O_5$ (100 kg of 46% Simple-Super Phosphate) and 30 kg K $ha^{-1}$ applied as KCl (50 kg of 60% KCl). Sub-plot treatments (Nfix) consisted of planting 26 trees each of the native species *S. amazonicum, C. odorata* and *C. pentandra* together (I-), or in combination with the N-fixing species *I. edulis* as well as *P. multijuga* (I+). In I+ treatment, *I. edulis*, accounted for half of trees planed, while the remainder was divided evenly among the remaining species.

## Gas sampling

Gas sample collections, as well as soil and litter layer sampling, were performed over two years: during the final year of secondary forest growth of the first crop-fallow cycle (Rotation 1) and after conversion via mulching tractor to begin the second crop-fallow cycle (Rotation 2; See Table 1). Samples were collected in May and November of 2013, and March and late May of 2014 to complete Rotation 1. Samples were collected in early July 2014 (one week after site preparation), two weeks later in late July, November 2014, March 2015, and July 2015. To capture seasonality in rainfall samples were collected by: Rainy Season (Jan.–May), Transition Season (June–Aug.) and Dry Season (Sept.–Dec.). No soil moisture measurements or soil samples were collected during the dry seasons of 2013 and 2014.

Gas sampling was conducted using 15-cm diameter PVC ventilated static chambers (Pihlatiea et al. 2013). Eight chambers were installed per plot 2.5 cm into the soil 1-h prior to closing the chamber. After closing the chamber, a sample was drawn in 10 mL syringes and stored in 7 mL pre-evacuated vials (Exetainers, Labco Inc., UK) immediately (T = 0), and again at T = 10, 30, and 50 min. Vials were taken to the University of Georgia, Athens, GA for analysis. Samples were analyzed using a gas chromatograph with electron capture ($N_2O$) and flame ionization ($CH_4$) detectors (Shimadzu Corporation GC-2014, Kyoto, Japan). Gas efflux for $N_2O$ and $CH_4$ were calculated following Shrestha et al. (2014). Soil efflux data was not normally distributed for either $N_2O$ or $CH_4$ when analyzed as a single data set or when

**Table 1** Dates and associated sample collections in a slash-and-mulch improved-fallow agroforestry system in Igarapé Açu, Pará, Brazil

| Date | Rotation | Season | Environmental variables | | | |
|---|---|---|---|---|---|---|
| | | | Gas | VWC | Soil | Litter |
| 22-May-2013 | 1 | Transition | Yes | Yes | Plot-level | Plot-level |
| 22-Nov-2013 | 1 | Dry | Yes | | | Plot-level |
| 6-Mar-2014 | 1 | Rainy | Yes | Yes | | |
| 22-May-2014 | 1 | Rainy | Yes | Yes | Transect | Transect |
| 14-July-2014 | 2 | Transition | Yes | Yes | | Plot-level |
| 28-Jul-2014 | 2 | Transition | Yes | Yes | Transect | Transect |
| 22-Nov-2014 | 2 | Dry | Yes | | | |
| 10-Mar-2015 | 2 | Rainy | Yes | Yes | Transect | Plot-level |
| 17-Jul-2015 | 2 | Transition | Yes | | Transect | Plot-level |

analyzed by Rotation, so all gas efflux data were log-transformed for statistical analysis.

### *Gas sampling by distance from tree*

To assess the effect of the N-fixer on gas efflux by distance from the tree, sampling transects of 25, 50, 100, and 200 cm from each of two randomly selected trees were installed in all plots; in plots with *I. edulis* (I+) one of the trees sampled was *I. edulis* (I) paired with one randomly selected non-*Inga* tree of the remaining species (nI). In plots without *I. edulis* (I-), pairs of trees were randomly selected as n1 and n2.

For analysis of the flux data, means were calculated for trees in multiple ways. First, at the plot level, analysis of means for the paired trees, regardless of species, were pooled for a single mean efflux for the plot. A second level of analysis compared the paired trees within treatment. A third level of analysis pooled both trees in the I- plot and compared that mean to the mean of the sampled *I. edulis* tree (I) and non-*Inga* tree (nI) in the I+ plot.

### Soil moisture and soil sampling

Soil moisture measurements were taken with a Hydrosense II soil moisture probe (Campbell Scientific, Logan, UT) after static chamber caps were removed. During two sampling events in the dry season VWC could not be measured because the probe could not be fully inserted into the soil. Also, the soil moisture probe failed during the July 2015 sampling. Volumetric water content (VWC) data from the available measurement dates were normally distributed across the entire data set.

After gas and VWC measurements were completed, the mulch layer and 0–10 cm soil were sampled from within the static flux chambers in each plot. Soil samples were air-dried and sieved through a 2 mm screen. Subsamples were then ground to powder using a vial roller or ball mill grinder (SPEX, Metuchen, NJ). Soil and litter samples were analyzed for carbon (C) and total nitrogen (TN) concentration using a CN analyzer (CE Elantech, Lakewood, NJ). Soil samples were also extracted for analysis with 1 M KCl to assess available N and analyzed for Total Extractable N (TEN) via chemiluminescence (TNM-L, Shimadzu Corporation, Kyoto, Japan). This measure of total soil N and total extractable N was used to evaluate soil N controls on $N_2O$ efflux. Soil samples extracted with 1 M KCl were also analyzed for $NH_4$-N using flow injection analysis (OI Analytical, College Station, TX).

### Soil N availability

To capture plot-level N availability over a one-year time frame, we utilized buried resin capsules (UniBest PST-1, Bozeman, MT) at the bottom of 10 cm deep × 2 cm diameter PVC plastic tubes. Rotation 1 sampling spanned the year of pre-mulching gas sampling (May 2013–May 2014) and Rotation 2 sampling took place from site conversion in July 2014 until July 2015. A plastic tube was inserted into the soil, removed with soil, and the resin capsule was placed inside the bottom of the tube and replaced into the soil. Resin capsules were extracted with 2 M KCl solution and analyzed for Resin-captured N (RCN), as was describe above for TEN. Total RCN (mg $L^{-1}$) in the extracts was compared across treatments.

### Statistical analyses

All statistical analyses were performed using SAS (SAS, Cary, NC, USA) using $p < 0.1$ as significant. T-test analysis indicated that soil gas fluxes differed between Rotations 1 and 2 for both $N_2O$ and $CH_4$ ($P > t$: $p < 0.001$), so all analyses for soil gas fluxes were conducted within Rotation for each gas. Soil GHG fluxes were analyzed using Proc MIXED. In the factorial model the main-plot treatment was with or without P+K fertilization (Fert), and the split-plot treatment was with or without the presence of the N-fixing tree species *I. edulis* (Nfix). Fert and Nfix were fixed factors and Block was a random factor. Repeated measures analysis was used to test Date and Treatment*Date effects with plot as the repeated measure subject.

Regression analysis was used to test the effects of environmental variables, including pH, and soil and litter layer C and N concentrations, on $N_2O$ and $CH_4$ flux using Proc REG. Since not all variables were present during each sampling date, multiple regression analysis was supplemented by individual regressions of each variable against $N_2O$ and $CH_4$ flux to take advantage of all available data. Statistical nomenclature for Trees in the transect sampling is as follows:

*Inga edulis* trees = I, non-*Inga* trees in the I+ plot = nI trees in the I- plot = nN.

## Results

### Nitrous oxide

*Treatment-level responses*

At the end of Rotation 1, 9 years after study initiation, mean soil $N_2O$ fluxes from May 2013 to May 2014 ranged from 8.1 to 13.7 µg $m^{-2}$ $h^{-1}$, with the lowest flux coming from plots without *I. edulis* (I-) and the greatest mean flux coming from the full factorial treatment (PK+I+). Soil efflux of $N_2O$ differed in the presence of *I. edulis* (I+), with a mean of 12.5 ± 5.4 compared to 8.2 ± 5.4 µg $m^{-2}$ $h^{-1}$ in the I- treatment (Fig. 1). However, $N_2O$ efflux did not differ in the main-plot (Fert), with no Fert*Nfix interaction during Rotation 1 (Table S1).

Soil $N_2O$ efflux differed by Date during Rotation 1 ($p < 0.001$; Table S1) and was greatest in May 2013 and again in May 2014, and lowest during November 2013 (Fig. 2). Neither the Fert*Date nor Fert*Nfix*Date interaction was significant, but

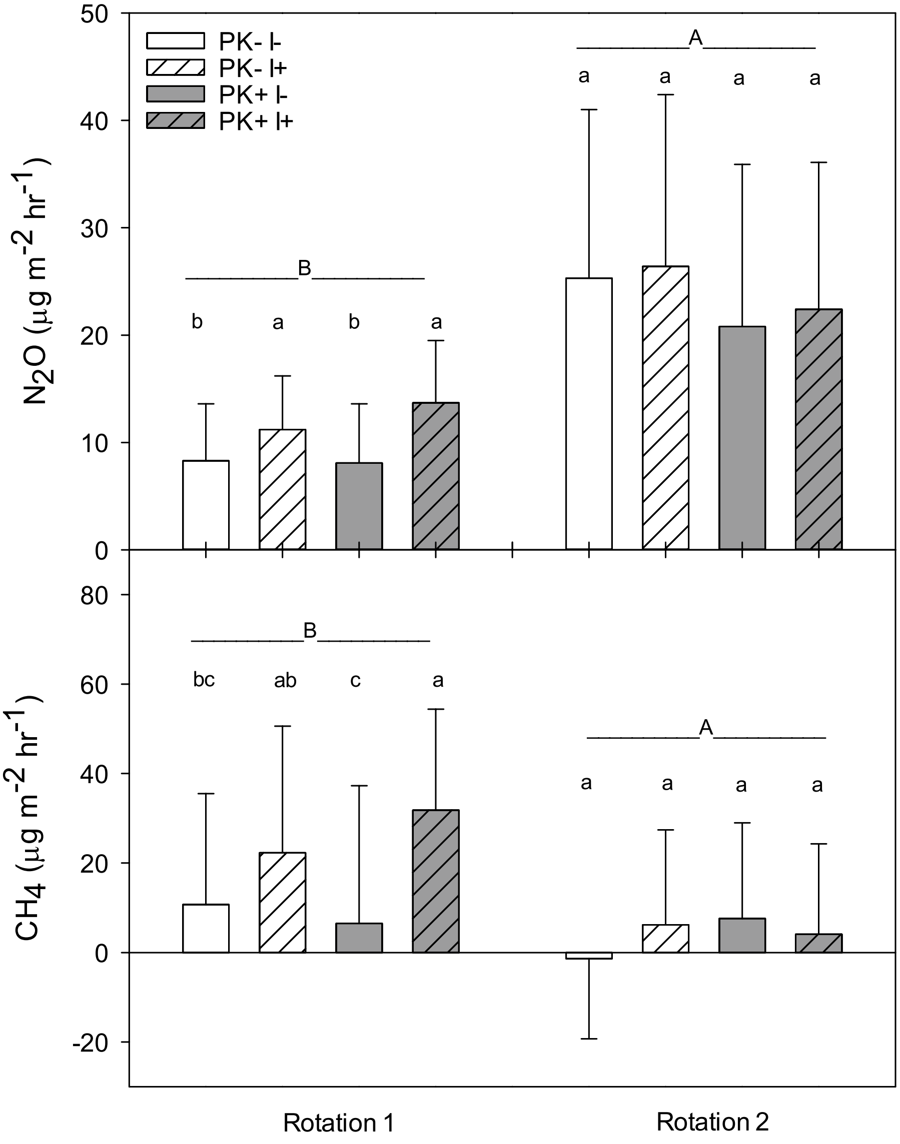

**Fig. 1** Soil $CH_4$ and $N_2O$ efflux in a slash-and-mulch improved-fallow agroforestry system in eastern Amazonia of Brazil. Main plot treatment (Fert) with P+K fertilization (PK+) or without (PK-), and sub-plot treatment (Nfix) planted with *I. edulis* (I+) or without (I-). Sampling was performed for 1-year at the end of Rotation 1 and during the year following site preparation with a mulching tractor at the beginning of Rotation 2. Capital letters indicate effect of Rotation, lower case letter indicate differences between treatments within a rotation

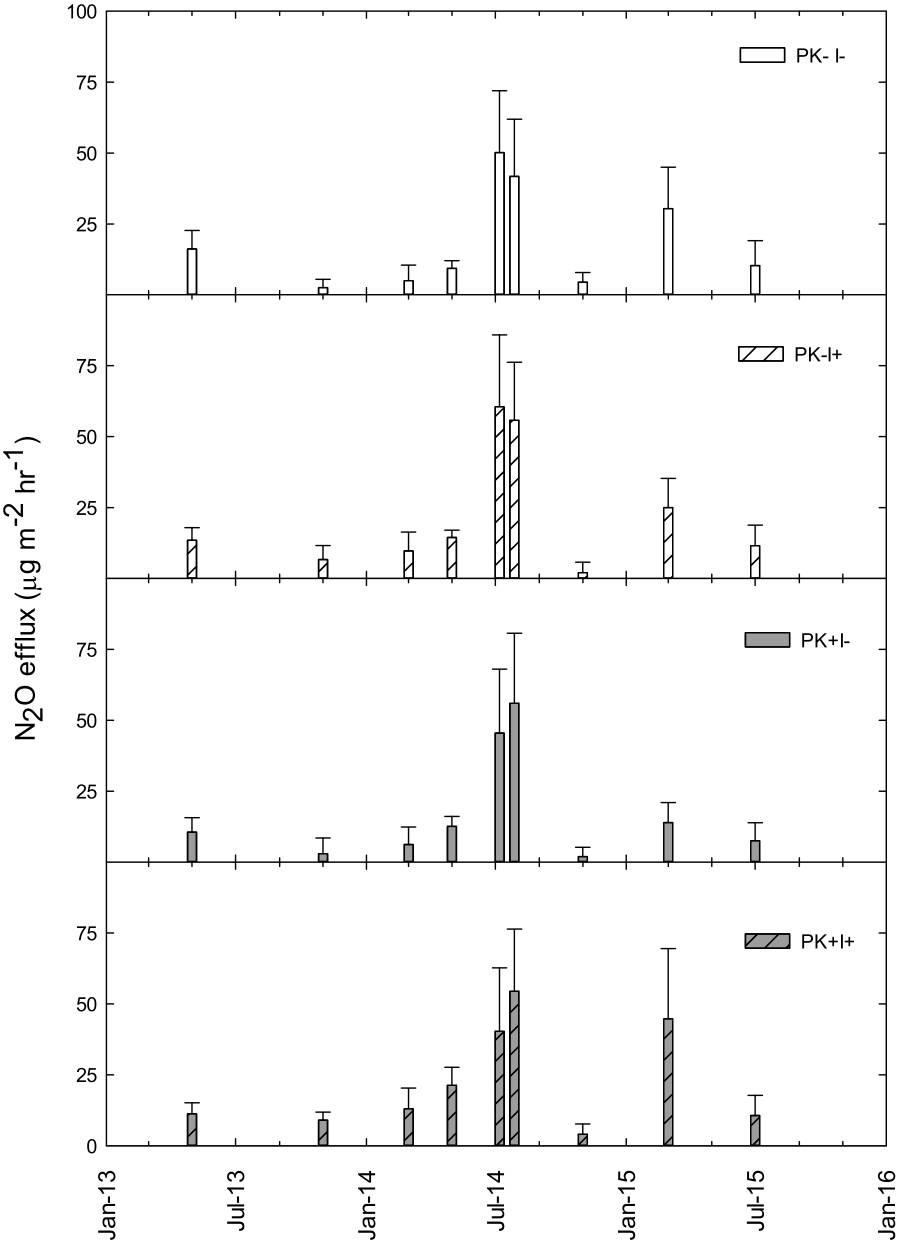

**Fig. 2** Soil $N_2O$ efflux (mean ± 1SE) in a slash-and-mulch improved-fallow agroforestry system in eastern Amazonia of Brazil. Main plot treatment ($N = 4$) with P+K fertilization (PK+) or without (PK-), and sub plot treatment planted with *I. edulis* (I+) or without (I-). Sampling was performed for 1-year at the end of Rotation 1 and during the year following site preparation with a mulching tractor at the beginning of Rotation 2

the Nfix*Date interaction was significant ($p < 0.01$; Table S1), with the flux lowest in November 2013 in the I- treatment (2.7 ± 4.4 µg m$^{-2}$ h$^{-1}$) and highest during May 2014 in the I+ treatment (17.9 ± 5.0 µg m$^{-2}$ h$^{-1}$; Fig. 2).

After slash-and-mulch conversion of the 9-yr-old secondary forest, Rotation 2 measurements (July 2014 to July 2015) of soil $N_2O$ fluxes ranged from 20.8 to 26.4 µg m$^{-2}$ h$^{-1}$ (Fig. 2), but did not differ in main-plot or sub-plot treatments, or in the Fert*Nfix interaction (Tables 2 and S1). Soil $N_2O$ efflux differed by Date during Rotation 2 ($p < 0.0001$; Table S1) and was greatest during the month after secondary forest conversion (July 7 and 28, 2014; Fig. 2), but other interactions -by Date did not differ.

### *Tree-level responses*

Soil N by distance from sampled trees did not differ across sampled dates (Tables S2 and S2).

**Table 2** Soil $CH_4$ and $N_2O$ efflux (μg $m^{-2}$ $h^{-1}$) in a slash-and-mulch improved-fallow agroforestry system in eastern Amazonia of Brazil during pre-site preparation (Rotation 1) and post-site preparation (Rotation 2) and by Tree Type

| Rotation | Tree type[a] | $CH_4$ | $N_20$ |
|---|---|---|---|
| | | μg $m^{-2}$ $h^{-1}$ (SE) | |
| 1 | I | 25.5 (31.9) | 12.1 (4.8) a[b] |
| | n | 28.6 (30.2) | 12.8 (6.1) a |
| | N | 9.2 (28.3) | 8.2 (5.4) b |
| 2 | I | 10.3 (21.2) | 25.5 (15.6) |
| | n | 0.5 (20.0) | 23.8 (14.4) |
| | N | 3.0 (19.8) | 22.9 (15.3) |

[a] Tree Type indicates: the N-fixing tree species *Inga edulis* (I), any of four non-N fixing tree species in plots with *I. edulis* (n), or any of three non-N fixing tree species in plots without *I. edulis* (N)

[b] Lower-case letters indicate significant differences ($p < 0.05$) within Rotation, gas ($N_2O$), among Tree Type

However, in these transect samples, P+K fertilization increased soil N (i = 0.09), but *I. edulis* did not. Further, in May 2014, in the final month of secondary succession, soils in transects from *Inga* trees had higher N than non-*Inga* trees in I+ plots ($p = 0.02$), but *Inga* trees did not differ from trees in I- plots. Soil-N in transect sampled soils did not differ in Rotation 2.

For measurements with distance from tree, soil $N_2O$ efflux measured by Tree type ranged from 8.2 to 12.8 μg $N_2O$–N $m^{-2}$ $h^{-1}$, producing a trend of increased flux in the presence of *I. edulis* and I+ trees, compared to trees in the I- treatment ($p = 0.10$; Table 3). The Tree*Date interaction ($p < 0.01$) revealed that soil $N_2O$ efflux was lowest in May 2013 for trees in I- plots and was highest in March 2014 near non-*Inga* trees in the I+ plots. Soil $N_2O$ efflux did not differ by distance from tree during Rotation 1.

After mulching, soil $N_2O$ efflux measured with distance from trees ranged from 22.9 – 25.5 μg $N_2O$–N $m^{-2}$ $h^{-1}$ but did not differ between Tree types (Table 3). There was a trend of decreased flux after the initial post-mulching sampling in the Tree*Date interaction ($p = 0.07$; Fig. 2), with $N_2O$ flux highest in association with *I. edulis* in July 2014 and lowest in November 2014 for all Tree types. Distance from planted trees did not affect soil $N_2O$ efflux during Rotation 2 (Tables 3 and S3).

**Table 3** Mean (SE) soil $CH_4$ and $N_2O$ efflux (μg $m^{-2}$ $h^{-1}$) in a slash-and-mulch improved-fallow agroforestry system in eastern Amazonia of Brazil during pre-site preparation (Rotation 1) and post-site preparation (Rotation 2) and by Tree Type

| Rotation | Tree type | Distance (m) | $CH_4$ | $N_2O$ |
|---|---|---|---|---|
| | | | μg $m^{-2}$ $h^{-1}$ (SE) | |
| 1 | I | 0.25 | 12.9 (25.4) a[2] | 11.5 (4.7) ab |
| | | 0.5 | 35.7 (36.9) a | 12.8 (4.9) ab |
| | | 1.0 | 25.2 (34.8) a | 14.6 (5.1) a |
| | | 2.0 | 27.5 (29.1) a | 9.6 (4.4) ab |
| | n | 0.25 | 30.5 (32.2) a | 14.2 (5.9) ab |
| | | 0.5 | 31.8 (30.3) a | 13.7 (6.2) ab |
| | | 1.0 | 23.8 (22.0) a | 12.8 (6.3) ab |
| | | 2.0 | 27.9 (35.6) a | 10.7 (6.0) ab |
| | N | 0.25 | 8.4 (28.3) a | 6.8 (4.7) b |
| | | 0.5 | 14.8 (28.2) a | 8.6 (5.5) ab |
| | | 1.0 | 5.4 (26.6) a | 8.6 (5.3) ab |
| | | 2.0 | 8.5 (30.3) a | 8.9 (6.1) ab |
| 2 | I | 0.25 | 6.9 (20.6) AB | 32.7 (18.5) A |
| | | 0.5 | $^{-}$1.8 (17.7) AB | 28.4 (18.9) A |
| | | 1.0 | 26.0 (21.4)A | 20.4 (10.0) A |
| | | 2.0 | 9.5 (23.1) AB | 21.2 (13.8) A |
| | n | 0.25 | 2.9 (18.6) AB | 18.5 (11.8) A |
| | | 0.5 | 0.7 (20.3) AB | 22.4 (13.9) A |
| | | 1.0 | $^{-}$4.0 (22.9) AB | 26.3 (16.3) A |
| | | 2.0 | 1.9 (19.1) AB | 28.7 (15.5) A |
| | N | 0.25 | 5.2 (16.0) AB | 22.1 (14.8) A |
| | | 0.5 | 4.5 (18.6) AB | 25.4 (17.7) A |
| | | 1.0 | $^{-}$5.5 (16.6) B | 19.5 (12.3) A |
| | | 2.0 | 7.5 (26.0) AB | 24.4 (16.1) A |

[a] Tree Type indicates: the N-fixing tree species *Inga edulis* (I), any of four non-N fixing tree species in plots with *I. edulis* (n), or any of three non-N fixing tree species in plots without *I. edulis* (N) are indicated

[b] Lower-case and upper-case letters indicate significant differences ($p < 0.05$) within Rotation, within gas ($CH_4$ or $N_2O$), among Tree Type per distance

## Methane

### *Treatment-level responses*

At the end of Rotation 1, 9 years after initial mulching, soil $CH_4$ fluxes ranged from 6.5 – 31.8 µg $m^{-2}$ $h^{-1}$ between May 2013 and May 2014 (Fig. 1), with the lowest flux in the control treatment (PK-I-) and highest in the full factorial treatment (PK+I+). Soil efflux of $CH_4$ did not differ in the main-plot or in the sub-plot treatments during Rotation 1. Soil $CH_4$ efflux varied by Date during Rotation 1 ($p < 0.01$) and was lowest in May 2014 (Fig. 3), but no other interactions -by Date were significant (Table S1).

For Rotation 2 measurements, July 2014–July 2015, soil $CH_4$ efflux ranged from −1.4 to 7.6 µg $m^{-2}$ $h^{-1}$ (Fig. 1) but did not differ in the main-plot or the sub-plot treatments (Table S1). The only consumption of $CH_4$ reported here was in the control treatment, $-1.4 \pm 17.9$ µg $m^{-2}$ $h^{-1}$. Soil $CH_4$

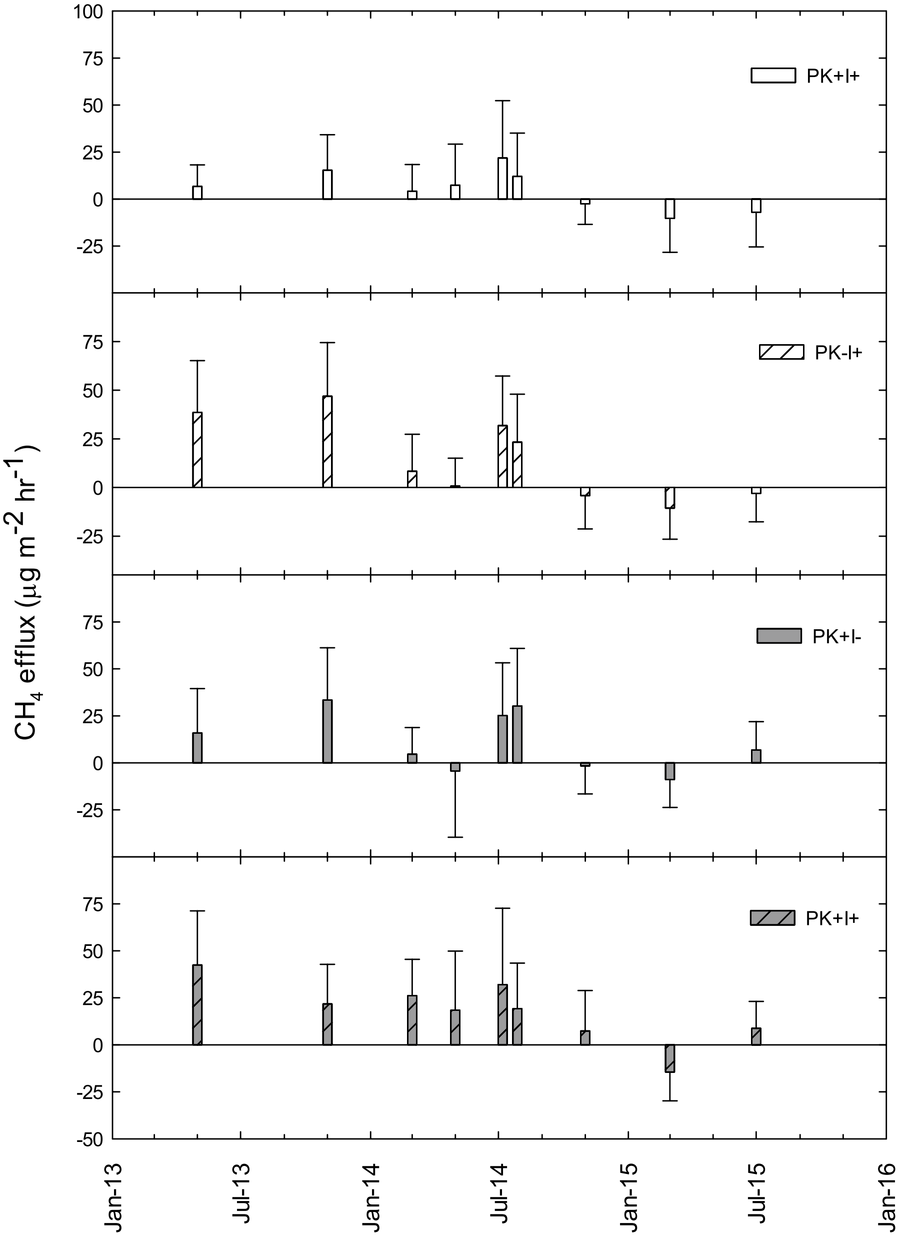

**Fig. 3** Soil $CH_4$ efflux (mean ± 1SE) in a slash-and-mulch improved-fallow agroforestry system in eastern Amazonia of Brazil. Main-plot treatment (N = 4) with P+K fertilization (PK+) or without (PK-), and sub-plot treatment planted with *I. edulis* (I+) or without (I-). Sampling was performed for 1-year at the end of Rotation 1 and during the year following site preparation with a mulching tractor at the beginning of Rotation 2

efflux differed by Date during Rotation 2 ($p < 0.01$; Table S1) and was greatest in the four weeks after mulching (July 7 and 28, 2014) and lowest during March 2015 (Fig. 3), but no other interactions -by Date were significant (Table S1).

### *Tree-level responses*

Soil-C by distance from sampled trees did not differ (Tables S2 and S3). However, in these transect samples, P+K fertilization increased soil-C ($p = 0.07$). The Fert*Tree interaction ($p = 0.10$) revealed that *Inga* with P+K fertilization had greater soil-C than unfertilized *Inga* trees ($p = 0.06$) and P+K fertilized trees in the I- plot had higher soil-C than unfertilized *Inga* ($p = 0.02$). In the final measurement of secondary succession, soils in transects from *Inga* trees had higher C than non-*Inga* trees in I+plots ($p < 0.01$), but *Inga* trees did were not differ from trees in I- plots. The Tree*Distance interaction was significant in May 2014 ($p < 0.001$), in which soil-C near I trees was greater at all distances than soil-C at 0.5 m distant from nI trees.

For measurements by distance from tree, soil $CH_4$ efflux ranged from 9.2 to 28.6 μg $m^{-2}$ $h^{-1}$ at the individual tree level during Rotation 1 (Table 2), but no differences were detected for other variables or interactions. After mulching to initiate Rotation 2, soil $CH_4$ efflux with distance from planted trees generated between 0.5—10.3 μg $m^{-2}$ $h^{-1}$, in which there was a Tree*Distance interaction ($p = 0.02$; Table 3) as $CH_4$ efflux associated with *I. edulis* was lower than I- trees at 1.0 m. No differences were detected for other variables.

## Environmental responses

### *Soil attributes*

Soil $pH_{H2O}$ and $pH_{CaCl2}$ (Table S4) did not differ in the main or sub-plot. Neither surface soil (0-10 cm) $pH_{H2O}$ nor $pH_{CaCl2}$ predicted $CH_4$ nor $N_2O$ efflux in Rotations 1, 2, or overall. Mean soil volumetric water content (VWC) was 28.2 ± 1.2% among measured dates, although for three of the driest sampling dates VWC could not be collected. The lowest VWC (%) was in the rainy season (March 2015) sampling of Rotation 2 (23.3% ± 2.3). The highest soil VWC readings (31.3 ± 1.6 and 31.2 ± 1.4%, respectively) were recorded in the final sampling prior to, and the first sampling after, mulching for Rotation 2 (May 2014 and July 7, 2014). VWC was second highest in May 2013 and July 28, 2014 (28.3% ± 2.0 and 28.1% ± 1.3, respectively), while the median VWC value (24.8% ± 1.3) was recorded in March of 2014. VWC did not differ by Fert or Nfix and there was no Fert*Nfix interaction. Rotation 1 and 2 VWC did not differ, but soil VWC differed by Date ($p < 0.001$) and Fert*Date interaction ($p < 0.0001$), with highest VWC during the first sampling event after mulching on July 7, 2014 in the PK + treatment, and the lowest in PK+ treatment in March 2014 prior to mulching.

### *Environmental controls on $N_2O$ and $CH_4$ efflux*

Stepwise regression for $N_2O$ efflux did not yield significant predictors in either Rotation. Regression for $CH_4$ efflux found that VWC and total N recovered with resin capsules (RCN; Table 4) were significant regressors across both Rotations (Table 5). RCN was negatively correlated to $CH_4$ efflux (p.e. = −0.04), and was a weak predictor in the multivariate regression (Partial $R^2 = 0.08$), but logVWC was positively correlated with $CH_4$ efflux (p.e. = 0.8) and was a strong predictor ($R^2 = 0.7$). During Rotation 1, stepwise regression showed that logVWC, TN, and Litter-C

**Table 4** Resin capsule extractions (mean ± SE) with 2 M KCl analyzed for total nitrogen to estimate Resin Captured Nitrogen (RCN) in a slash-and-mulch, improved-fallow agroforestry system in Eastern Amazonia of Brazil

| Fert[a] | Nfix[b] | Rotation[c] | |
|---|---|---|---|
| | | 1 | 2 |
| | | mg $L^{-1}$ (SE) | |
| N | N | 8.7 (3.1) b[d] | 7.2 (2.4) |
| | Y | 10.8 (4.3) a | 7.9 (3.3) |
| Y | N | 6.9 (2.7) b | 7.7 (3.0) |
| | Y | 13.9 (4.7) a | 7.2 (3.0) |

[a] Main-plot (Fert) treatment with (Y) or without (N) P+K fertilization

[b] Sub-plot (Nfix) treatment with (Y) or without (N) N-fixing *I. edulis*

[c] Rotation 1 sampling from May 2013—May 2014 prior to site conversion via mulching tractor and Rotation 2 sampling from July 2014—July 2015

[d] Letters indicate significant differences ($p < 0.10$) for the sub-plot treatment (Nfix)

**Table 5** Regression output for soil $CH_4$ and $N_2O$ efflux in a slash-and-mulch, improved-fallow agroforestry system in Eastern Amazonia, Brazil

| Gas | Rotation | Variable | Parameter estimate | Partial $R^2$ | $R^2$ | C(p) | F-value | Pr > F |
|---|---|---|---|---|---|---|---|---|
| $CH_4$ | 1 | logVWC | 1.290 | 0.581 | 0.581 | 8.35 | 20.8 | < 0.001 |
| | | Soil N (%) | 3.040 | 0.082 | 0.663 | 6.19 | 3.4 | 0.09 |
| | | Litter C | 0.003 | 0.065 | 0.728 | 4.86 | 3.1 | 0.10 |
| | 2 | logNH | 0.032 | 0.268 | 0.268 | 5.36 | 5.5 | 0.03 |
| | | Soil C/N | 0.012 | 0.093 | 0.361 | 5.03 | 2.0 | 0.18 |
| $N_2O$ | 1 | logVWC | 0.095 | 0.205 | 0.205 | 12.03 | 3.9 | 0.07 |
| | | Soil C/N | −0.001 | 0.121 | 0.326 | 10.22 | 2.5 | 0.14 |
| | 2 | Litter N | −0.043 | 0.205 | 0.205 | −2.68 | 3.9 | 0.07 |
| $CH_4$ | Both | logVWC | 0.769 | 0.743 | 0.743 | −2.31 | 43.4 | < .0001 |
| | | logTN | −0.040 | 0.079 | 0.822 | −3.60 | 6.2 | 0.03 |
| | | Soil N (%) | 0.428 | 0.018 | 0.841 | −2.37 | 1.5 | 0.20 |
| $N_2O$ | | logVWC | 0.044 | 0.147 | 0.147 | −0.76 | 2.6 | 0.13 |

concentration were the best predictors of soil $CH_4$ efflux, explaining 58% of the variation (Table 5).

Since all variables were not available during each sampling event for multivariate regression, univariate regression analyses were performed for each variable (Table 6). Despite the larger sample size for the univariate regressions, there were no significant regressors for $N_2O$ efflux in either Rotation. In contrast, logVWC and TN concentration were significant during Rotation 1 for $CH_4$ efflux ($p = 0.001$ and 0.02, respectively) while soil-C:N and log$NH_4$ were significant for $CH_4$ efflux during Rotation 2 ($p = 0.05$ and 0.04, respectively).

## Discussion

The environmental benefits of AFS with N-fixers has been questioned due to the potential for increased GHG efflux (Rosenstock et al 2014; Kou-Giesbrecht & Menge 2019, 2021). In this research we created a novel system for the simultaneous cultivation of the staple crop manioc with native trees for eventual timber sale. We also incorporated the N-fixer *I. edulis* for improved fallow and green manure. To evaluate the impact of this novel, slash-and-mulch system we measured the seasonal soil effluxes of $N_2O$ and $CH_4$ during the year prior to, and the year following, site conversion by mulching tractor. We expected that use of P+K fertilizer (PK+) and the inclusion of the N-fixing tree *Inga edulis* (I+) would increase soil fluxes of both $CH_4$ and $N_2O$, that the newly deposited mulched biomass layer would increase fluxes of both, and that fluxes of both would be greater nearer to individual *I. edulis* trees.

### Environmental responses

Slash-and-mulch research from the Bragantina region has shown increased soil water retention, by

**Table 6** Univariate regression statistics of environmental variables for soil $CH_4$ and $N_2O$ efflux in a slash-and-mulch, improved-fallow agroforestry system in Eastern Amazonia of Brazil

| Rotation | Variable | $CH_4$ | | | $N_2O$ | | |
|---|---|---|---|---|---|---|---|
| | | Parameter estimate | $R^2$ | Pr > \|t\| | Parameter estimate | $R^2$ | Pr > \|t\| |
| 1 | logVWC | 1.29 | 0.551 | 0.001* | 0.095 | 0.205 | 0.08 + |
| | Soil N (%) | 3.04 | 0.267 | 0.02* | 0.135 | 0.047 | 0.4 |
| 2 | SoilC/N | 0.012 | 0.240 | 0.05* | 0.001 | − 0.050 | 0.6 |
| | Litter N (%) | 0.000 | 0.000 | 1.00 | − 0.043 | 0.149 | 0.08 + |
| | Litter C/N | 0.000 | 0.012 | 0.7 | 0.000 | 0.139 | 0.09 + |
| | NH4 | 0.032 | 0.268 | 0.04* | -0.006 | 0.028 | 0.3 |

up to 22%, compared to slash-and-burn (Comte et al. 2012). Fluxes of both $CH_4$ and $N_2O$ are affected by VWC; anaerobic soils increase flux of both gases, while aerobic soils often result in $CH_4$ consumption (Segers 1998; Butterbach-Bahl et al. 2013). In this research, methane was consumed in control plots during the dry season but consumption was reduced or production increased during the rainy season, which is consistent with previous findings (Vasconcelos et al. 2004). Davidson et al. (2008) reported consumption of $CH_4$ in secondary forests in Eastern Amazonia, and emissions of $CH_4$ immediately after mulching of secondary forests, but did not correlate these findings to VWC.

Soil moisture was a significant predictor during both Rotations for $CH_4$, but only marginally important for $N_2O$ ($p = 0.13$). Within each Rotation the influence of VWC was less clear, as it was a good predictor of $CH_4$ and $N_2O$ efflux in Rotation 1 but not for either gas during Rotation 2. Nine years of secondary forest growth and mulch decomposition may have stabilized soil N and C concentrations, and soil moisture distribution, reducing soil nutrient and moisture variability. However, the large pulse of woody material put onto the soil surface during site preparation likely caused heterogeneous distribution of N and C resources, and soil moisture conditions leading to inconsistent responses to VWC (Millar et al. 2004; Kravchenko et al. 2017). The lack of dry season soil moisture data, and thus a limited distribution of VWC values, likely also impacted regression relationships. Previous research found better relationships for $N_2O$ efflux with %WFPS across broad (20–100%) ranges (Davidson et al. 2001; Reich et al. 1997).

Other environmental variables measured were inconsistent in predicting soil $CH_4$ and $N_2O$ efflux. Soil pH, for instance, lowers soil methanogenic and nitrifying bacterial activity at low pH (Oertel et al. 2016), yet we observed no effects of soil pH on efflux of either gas. Production of $CH_4$ and $N_2O$ are dependent on C and N resources in the soil on which bacteria act (Hütsch 1998; Bodelier & Laanbroek 2004), yet the data presented here showed variable responses. For example, when analyzed across both Rotations, $CH_4$ was correlated with extractable TN (i.e., TEN) and total soil N (i.e., TN), but not with any C variables. Alternatively, $N_2O$ efflux was not related to any N variables during either Rotation, but was correlated to litter layer mass and litter layer C content. It is difficult to draw strong conclusions about environmental variables controlling efflux rates from these data.

## Tree level responses

We hypothesized that plots with the N fixing tree *I. edulis* would have greater $N_2O$ efflux and measured rates of $N_2O$ efflux would decrease with increasing distance from the stem of *I. edulis* due to soil N changes from root and leaf litter inputs (Tobita et al 2010). By the end of Rotation 1, the I- treatment generated less $N_2O$ than the I+ treatment, but within the I+ treatment tree types did not differ. These findings suggest that the influence of *I. edulis* on N cycling and $N_2O$ efflux was not constrained to the canopy cover or dripline of the tree but had an influence on $N_2O$ efflux at the plot level. This may be due to the high density of *Inga* trees in that treatment (50% of all trees in I+), as well as biotic factors (such as monkeys, coatimundi, and ants, which were all frequently observed during Rotation 1) above and below ground that have the potential to distribute N-rich *Inga* components (such as fruits and leaves).

## Treatment level responses

### *Methane*

We hypothesized that both fertilization with P+K and inclusion of the N-fixing *I. edulis* would enhance soil $CH_4$ efflux. Despite large $CH_4$ efflux percentage differences these effects were not significant. In a controlled setting, effects of P+K fertilization on $CH_4$ efflux were observed (Conrad & Klose 2005) but the large variance in this field experiment indicates that other factors obscured contributions to higher $CH_4$ efflux.

Mean $CH_4$ efflux was generally greater in Rotation 1 than 2, with the PK+I- treatment as the exception. Enhanced porosity in the surface soil due to the input of mulched material likely played a role in reducing $CH_4$ efflux in Rotation 2 via increased aeration. Soil $CH_4$ efflux increased immediately after mulching and decreased over the following year as the mulch layer decomposed. This pattern is consistent with other slash-and-mulch studies from Eastern Amazonia, in which soil $CH_4$ efflux peaked after mulching and planting, but then declined soon after, and remained relatively stable over the next year (Davidson et al. 2008). Conversion of tropical forests to agriculture has also shown either reduced rates of consumption of $CH_4$ (Keller et al. 1990), or production of $CH_4$ (Keller & Reiners 1994).

Relatively large rates of $CH_4$ production immediately after mulching and sustained consumption as soon as four months afterward indicate that site disturbance itself is a primary influence on $CH_4$ efflux. The most intense agricultural activity in this research took place at and directly after mulching (via tree and crop planting), with virtually no disturbance activities during the following year of gas efflux measurement, which is consistent with research that showed a positive correlation between intensity of agricultural activity in AFS and $CH_4$ efflux (Palm et al. 2002).

### *Nitrous Oxide—$N_2O$*

By the end of Rotation 1 inclusion of the N-fixing tree *I. edulis* increased $N_2O$ efflux by 52%. In other research from Eastern Amazonia, inclusion of the N-fixer *I. edulis* increased $N_2O$ efflux during secondary succession between 0.25 – 0.47 kg N $ha^{-1}$ $yr^{-1}$ (38 – 76%), and in improved-fallow increased ($N_2O + NO$)-N by 0.17 kg N $ha^{-1}$ $yr^{-1}$ (8.5%; Verchot et al. 2008). However, in the present study, P+K fertilization did not cause an increase in $N_2O$ production in either Rotation.

An increase in $N_2O$ emissions during the first month after site preparation and deposition of mulched material returned to pre-mulch levels 1 year after mulching, which is consistent with other research showing large pulses of $N_2O$ emissions after inorganic N fertilization (Signor et al. 2013) and after green manure application (Millar et al. 2004). Incorporating green manure from N-fixing trees of an improved-fallow AFS increased $N_2O$ soil efflux by 620—1,725% over 84 days, and as much as 90% of $N_2O$-N loss occurred during the first 30 days (Millar et al. 2004), where the highest rates of $N_2O$-N loss accompanied the lowest C:N mulch (113—560 g-N $ha^{-1}$ $ton^{-1}$ of leguminous mulch vs. 30 g-N $ha^{-1}$ $ton^{-1}$ with unimproved fallow mulch; Table S5). Data presented here indicate that 515 g-N $ha^{-1}$ were lost as $N_2O$ over the 28 days from the date of mulching until the end of July, equivalent to 24% of total emissions during the first year after mulching (Table S6), or 0.14% of newly added mulch layer N. Incorporation of organic residue in the Millar et al. (2004) study led to ~ 1.25% of applied N lost as $N_2O$. Inorganic N fertilizers can cause greater losses, as found in Brazil, where 1–12% of N applied as urea and ammonium nitrate was lost as $N_2O$ (Signor et al. 2013). Results here indicate that mulched secondary vegetation is more conservative of agroecosystem N than biomass burning or applying mineral-N fertilizers.

Plant residue can create “hot spots” of $N_2O$ emissions by absorbing water, creating conditions for nitrification and/or denitrification (Kravchenko et al. 2017). Mulch layer additions at site preparation for Rotation 2 added 106 Mg $ha^{-1}$ of plant residue and 360 kg N $ha^{-1}$, contributing to increased $N_2O$ flux compared to Rotation 1. Soil $N_2O$ efflux increased immediately after mulching, decreasing over time as the forest floor litter layer decomposed. However, the spatial distribution of soil $N_2O$ efflux was not due to the proximity of planted N-fixing tree species. These data suggest that N-fixing tree species planted in improved-fallow systems contributed to enhanced soil $N_2O$ efflux by enhancing N content of the agroecosystem at the plot scale. Other research from tropical forests indicates that N-fixing trees do not facilitate growth or N acquisition of neighboring trees (Taylor et al. 2017) or increase biomass recovery of secondary forests (Lai et al. 2018). In a meta-analysis (Kou-Giesbrecht & Menge 2021), reporting mostly on temperate forests, increased $N_2O$ efflux at the stand level was also evident. Results reported here, however, agree most closely with Verchot et al. (2008) that inclusion of *I. edulis* in an improved-fallow mix increased soil $N_2O$ fluxes.

### Annualized trace gas fluxes

The above ANOVA analyses for gas fluxes across time and four replicate blocks are robust for assessing the treatment responses. To assess the potential year-long GHG contribution, however, we annualized the soil $CH_4$ flux rates, which adds uncertainty but follows previously published research (Verchot et al. 1999). We extrapolated the measured flux rate (μg $hr^{-1}$) by 24 h $day^{-1}$ by the number of days in each season for the available data to provide an order of magnitude estimate. Annualized soil $CH_4$ flux rates were higher in each treatment during Rotation 1 than for any treatment during Rotation 2 (Table S6), with fluxes ranging from 2.7 to -0.3 kg $CH_4$ $ha^{-1}$ $yr^{-1}$

during Rotations 1 and 2, respectively. Annualized soil $CH_4$ flux rates estimated here fall within the range of flux rates reported in other studies from Amazonia (Vasconcelos et al. 2004; Davidson et al. 2004a and 2008; Verchot et al. 2000; Cattânio et al. 2002; Fernandes et al. 2002; Nepstad et al. 2002). However, following mulch treatment the annualized flux rates estimated here for $CH_4$ are one order of magnitude lower (0.3 vs. 10.2 kg $CH_4$ ha$^{-1}$ yr$^{-1}$) than reported by Davidson et al. (2008). In contrast, our estimates are very similar during fallow (2.7 vs. 2.4 kg $CH_4$ ha$^{-1}$ yr$^{-1}$) to those from a slash-and-mulch study in close proximity (<1 km) to the current research location. In Davidson et al. (2008) mulching increased $CH_4$ efflux, while in the current study, as soon as four months after site conversion via mulching tractor, soils became a sink for $CH_4$, although fertilized plots consumed less $CH_4$ than unfertilized plots.

Estimates of annualized soil $N_2O$ flux rates were generated in the same way as for $CH_4$, and, in contrast to patterns of $CH_4$ emissions, decreased in each treatment during Rotation 2 compared to any treatment during Rotation 1 (Table S6), with fluxes ranging from 0.6 to 2.9 kg $N_2O$–N ha$^{-1}$ year$^{-1}$. The range of soil $N_2O$-N flux reported here is similar to other research in Amazonia (0.33–6.9 kg N ha$^{-1}$ year$^{-1}$; Vasconcelos et al. 2004; Davidson et al. 2001; Verchot et al. 1999, 2000, and 2008; Cattânio et al. 2002; Fernandes et al. 2002; Nepstad et al. 2002; Palm et al. 2002; Table S5). Compared to Davidson et al. (2008) emissions reported here for $N_2O$ are lower during fallow and following mulch treatment. At the end of the fallow during Rotation 1, the unfertilized treatment with *I. edulis* (PK-I+) generated 0.25 kg N ha$^{-1}$ yr$^{-1}$ (38%) more $N_2O$ than control (PK-I-), while the full factorial treatment of fertilization with *I. edulis* (PK+I+) produced 0.47 kg N ha$^{-1}$ yr$^{-1}$ (76%) more $N_2O$ than fertilization without *I. edulis* (PK+I-). After mulching, total emissions of $N_2O$ increased such that during Rotation 2 there were 1.36 kg N ha$^{-1}$ year$^{-1}$ (89%) greater emissions from PK+I+ than from PK+I-.

## $CO_2$ Equivalents of $N_2O$ and $CH_4$ emissions

We used the methodology in Davidson et al. (2008) to estimate the global warming potential (GWP) of the slash-and-mulch treatments when compared to slash-and-burn. Again, the limited number of sampling dates creates uncertainty but the annualized estimates can provide a sense of changes in magnitude. Since we did not perform a burn treatment, we used the estimates from Davidson et al. (2004a) to estimate the $N_2O$ and $CH_4$ emissions that would have been created by burning of the mulched biomass at site conversion, relative to the 99.6 Mg ha$^{-1}$ of biomass from that study, and compared those values to the observed fluxes during the first year after mulching for Rotation 2. In addition to the fluxes of $N_2O$ and $CH_4$, we added $CO_2$ generated by the mulching tractor, 780 kg $CO_2$ ha$^{-1}$, to all mulching treatments, and 12.5 and 11 kg ha$^{-1}$ $CO_2$ equivalents for P and K fertilizer. Release of $CO_2$ during burning and during mulch decomposition were not considered.

Emissions of $N_2O$ and $CH_4$ during burning alone were up to one order of magnitude greater than emissions Year 1 post-mulching, so regardless of whether N-fixers were included in the planting mix, the improved-fallow slash-and-mulch system had far lower estimated emissions than burning (Table 7). Concerns about the release of $N_2O$ due to the inclusion of N-fixers in forests or AFS should be balanced against these potentially greater impacts of burning or mineral N fertilization (Rosenstock et al. 2014; Kou-Giesbrecht & Menge 2021). However, burning of the improved-fallow, full-factorial treatment (PK+I+) is estimated to generate nearly twice the $CO_2$ equivalents as the control (PK-I-) largely due to greater

**Table 7** Estimates of $CO_2$ equivalent emissions of $N_2O$ and $CH_4$ during burning of 9-yr old improved-fallow vs Year 1 fluxes of a slash-and-mulch agroforestry system in Eastern Amazonia, Brazil

| Treatment[a,b] | $CO_{2e}$ by Burn[c] | | $CO_{2e}$ Mulch[d] | |
|---|---|---|---|---|
| | $N_2O$ | $CH_4$ | $N_2O$ | $CH_4$ |
| | kg ha$^{-1}$ | | | |
| PK-I- | 5700 | 14,900 | 1400 | 800 |
| PK-I+ | 6300 | 16,600 | 1400 | 800 |
| PK+I- | 7600 | 20,000 | 1200 | 800 |
| PK+I+ | 10,800 | 28,600 | 1600 | 800 |

[a] Main plot treatment with P+K fertilization (PK+) or without (PK-)

[b] Sub-plot treatment planted with (I+) *I. edulis* or without (I-)

[c] $CO_2$ equivalents of mulch mass if burned, method:Davidson et al. (2008)

[d] $CO_2$ equivalents of mulch layer measured during Year 1 post-mulch

biomass. Inclusion of *I. edulis* in the planting mix had no effect on $CH_4$ efflux, although $CH_4$ efflux declined in all treatments after mulching reducing GWP. For $N_2O$ emissions, the incorporation of the N-fixing tree had no effect on GWP after mulching without fertilization but increased GWP by 33% with P + K fertilization.

## Conclusions

Inclusion of the N-fixing tree species *I. edulis* in this improved-fallow slash-and-mulch agroforestry system significantly increased soil flux of $N_2O$ but only after site preparation via mulching tractor. *I. edulis* did not enhance $CH_4$ emissions. Mean values for $CH_4$ and $N_2O$ were higher in the presence of the N-fixing tree, but high variance negated the statistical significance of these observations. The effects of the N-fixing tree were detectable at the plot level, but not at the individual tree level or by distance from the tree, suggesting that the mechanism of flux enhancement is not restricted to interactions with individual trees but rather at a broader spatial scale. Fertilization with P+K did not significantly impact soil $CH_4$ or $N_2O$ flux. Substitution of fire with mulching for site preparation will benefit agricultural GHG budgets by reducing $N_2O$ and $CH_4$ emissions. Incorporation of the N-fixer *I. edulis* in the planting design of the AFS had a minimal impact on GWP. Use of slash-and-mulch technology reduced total GHG emissions and the inclusion of N-fixing species in improved-fallow practices did not eliminate those benefits.

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**Author's contribution** AJ—Wrote the manuscript text, analyzed the data, assisted in creating the research design, implemented the research study, and conducted the fieldwork. FAO—Assisted in creating the research design, coordinated research activities and facilities, and provided intellectual contributions to the research. OK—Assisted in creating the research design, directed overall research project under which this research was conducted, provided material support to the research, and provided intellecutal contributions to the research. SSV—Provided material support to the research, and provided intellectual contributions to the research. LM—Assisted in creating the research design, provided intellectual contibutions to the research, performed editing of the manuscript, served on the dissertation committee of AJ, and conducted fieldwork. DM—Assisted in creating the research design, provided intellectual contibutions to the research, performed editing of the manuscript, served as dissertation committee chair to AJ, provided material support to the research and analysis, and conducted fieldwork.

**Data availability** Data is available at: https://docs.google.com/spreadsheets/d/1alM9k1_lUMk3Xo_urTlsXbfF6foZbgyG/edit#gid = 156011827.

### Declarations

**Conflict of interest** The authors declare no competing interests.

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