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Supporting Information for

Fire presence affects patterns and controls on asymbiotic nitrogen fixation in seasonally flooded forests of southern Amazonia

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Additional Supporting Information (Files uploaded separately)

Introduction: This file contains details on the implications of using $^{15}N_2$ to measure nitrogen fixation in the soil and the logistics constraints related to this study, in addition to details on statistical modeling. Figures S1 to S3 include the results obtained for soil C:N, P and N across forest stands. Tables S1 to S9 include all soil and plant data used in this study and statistical model outputs.

Text S1. Implications of the use of $^{15}N_2$ labeling to measure rates of asymbiotic nitrogen fixation (ANF) in the soil

Logistic constraints

The soil samples analyzed in this study were transported to the laboratory at the University of California, Davis by airplane right after field sampling was completed (7 days) while, ideally, they would have been processed as close to collection as possible. However, simple logistics prevented immediate analysis, as more than 80 samples had to be collected during a week. Furthermore, the amount of $^{15}N_2$ gas needed for 240 samples could not easily be transported by airplane or obtained in Brazil. The measured fixation rates could have been changed to an unknown degree by the unavoidable delay and disturbance, but we presumed that at least relative comparisons between burned and unburned soil samples across forest stands, the primary focus of the study, would remain useful.

$^{15}N_2$ Contamination

Awareness of a potentially serious issue in $^{15}N_2$-based measurements of dinitrogen fixation has been recently renewed (Dabundo et al., 2014), namely, trace amounts of species like NH_3 and NO_x that can become part of the samples analyzed and therefore lead to overestimates of the true amount of N_2 fixed. Aware of the chemical process by which $^{15}N_2$ is produced, gave a similar warning was given by (Burris, 1972). In this study, 24% of the soils measured did not respond to incubation with $^{15}N_2$, i.e. the ^{15}N content of the soils after incubation was not significantly higher than the natural ^{15}N content of the same soils. If contamination had occurred, the data would have shown a systematic error across all soils. This is indirect evidence that any coincidental "apparent fixation" due to trace contaminants in the $^{15}N_2$ gas was negligible in our study. In other words, an insignificant number of contaminants was absorbed by the soil during the incubation period; any significant increases in soil ^{15}N content after incubation were indeed due to $^{15}N_2$ fixation. Similarly, (Knapp, Casciotti, Berelson, Prokopenko, & Capone, 2016) observed many instances of N_2 fixation rates at or near their limit of detection, and also concluded that contaminants in the $^{15}N_2$ gas used were inconsequential during the course of their assays. In another study, water samples were analyzed using four distinct batches of $^{15}N_2$ gas (from two different suppliers) and found no significant differences in fixation rates (Böttjer et al., 2017). In our study, the presence of clear effects of soil properties on fixation is further evidence of a biologically driven process. Similar to (Snow et al., 2015) , the fixation rates observed in our study spanned two orders of magnitude, and apparent fixation due to contamination can therefore be discounted as having any impact on our conclusions, which are based on observed relationships between variables in the dataset. To date, $^{15}N_2$ has been applied to soil assays in only a few studies (the great bulk of the work being done in aquatic systems), and so it would be prudent to keep this potential problem in mind during future studies.

Text S2.

The GAMs incorporated smooth (i.e. unspecified but estimable) functions (Wood, 2017) of one or more covariates (i.e. soil continuous and categorical variables, and the related categorical variables stand, fire presence and frequency) to model non-linear relationships between covariate and response (soil ANF) (Rose, Yang, Turner, & Simpson, 2012). Interaction models were fitted as follows: $y_{ij} = \alpha_0 + \alpha_{1j} f_{ij} + s(x_{1i}, x_{2i}, by = f) + \mathcal{E}_i$, $\mathcal{E}_i \sim N(0, \sigma^2)$, where α_0 is the model intercept (the mean value of the response, y_{ij}, in the reference level of each categorical variable f analyzed), α_1 is the difference between the mean response for the jth f and α_0, $s()$ is the smooth effect of soil variables x_{1i}, x_{2i}, etc. on the level of factor f, *by* represents the means for implementing smooth-factor interactions (Wood, 2017) and $\mathcal{E}_i$ are the model residuals, assuming Gaussian distribution with mean 0 and variance σ^2. In these fitted GAMs, a smooth function (Wood, 2017) of a soil variable (or two interacting soil variables) was created for each categorical variable mentioned above. Considering that the categorical variable fire frequency did not significantly affect soil ANF, factor-smooth functions were fit considering fire presence, site and soil depth.

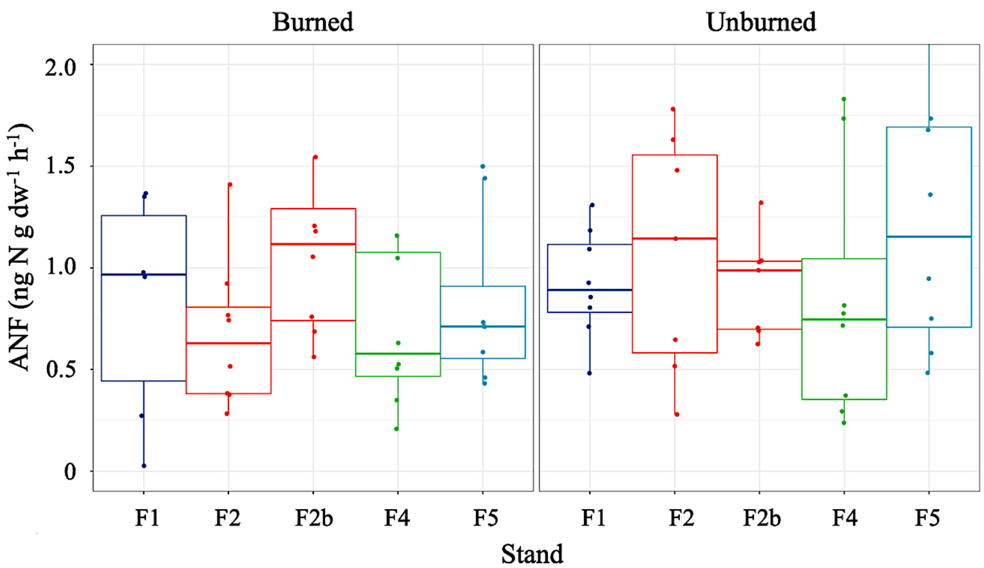

Figure S1. Asymbiotic nitrogen fixation rates (ANF, ng N g dry weight^{-1} h^{-1}) measured in burned and unburned soils collected in five seasonally flooded (SF) forest stands at Araguaia State Park, Mato Grosso State, Brazil. In each box, the middle band is the median ANF rate, and the top and bottom of the box are the first and third quartiles, respectively. Whiskers indicate the maximum and minimum values. No significant difference was found across all soils at a 95% significance level (least squares means adjusted for Tukey's test).

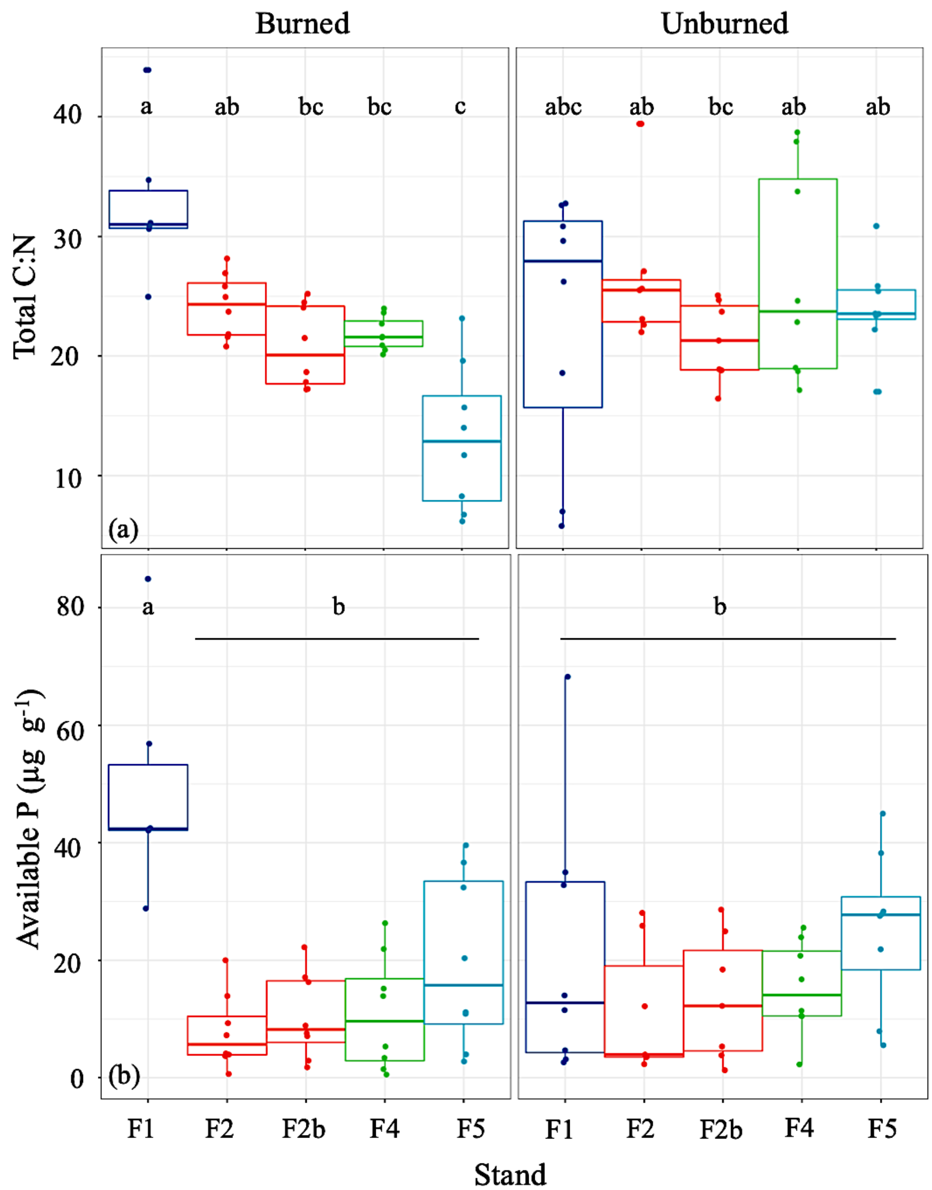

Figure S2. Total soil C:N ratio (a) and available P (b) in burned (B) and the unburned (UB) soils collected from seasonally flooded (SF) forest stands affected by recurrent fires at Araguaia State Park, Mato Grosso State, Brazil. In each box, the middle band is the median ANF rate, and the top and bottom of the box are the first and third quartiles, respectively. Whiskers indicate the maximum and minimum values. Different letters indicate significant differences across all soils at a 95% significance level (least squares means adjusted for Tukey's test).

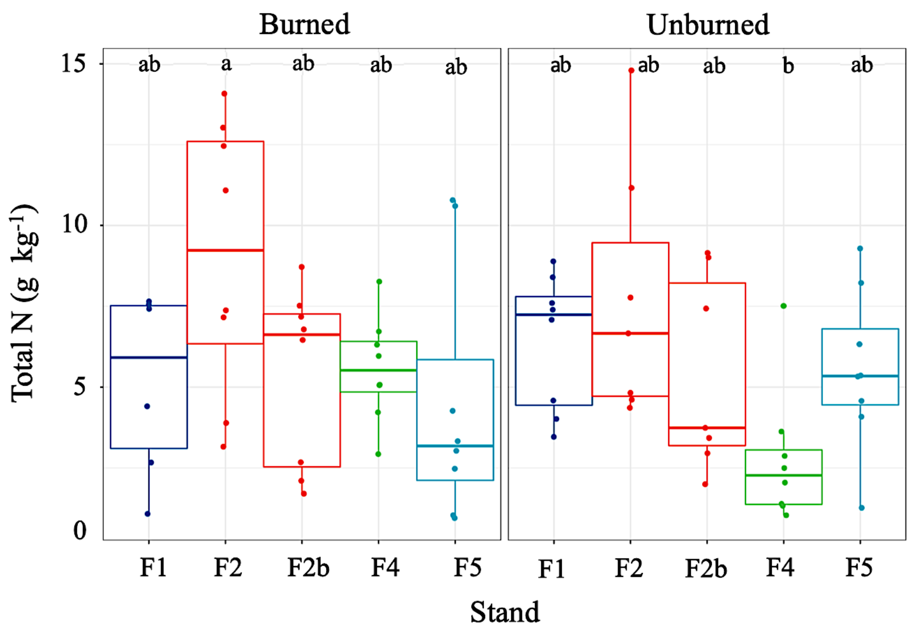

Figure S3. Total soil nitrogen-N (g kg^{-1}, 0-30 cm) measured in burned (B) and unburned (UB) soils collected in the studied seasonally flooded (SF) forest stands at Araguaia State Park, Mato Grosso State, Brazil. In each box, the middle band is the median ANF rate, and the top and bottom of the box are the first and third quartiles, respectively. Whiskers indicate the maximum and minimum values. Different letters indicate significant differences across all soils at a 95% significance level (least squares means adjusted for Tukey's test).

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Table S1. Matrix of tree species abundance (number of individuals ha^{-1} with diameter at breast height-DBH ≥ 10) surveyed in 2014/2016 at the five SF forest stands studied at Araguaia State Park, Mato Grosso, Brazil.

Stand	F2	F2b	F1	F4
F2b	0.69			
F1	0.58	0.22		
F4	0.81	0.33	0.4	
F5	0.8	0.48	0.56	0.38

Table S2. Beta diversity matrix (Bray-Curtis index) based on the abundance of trees (number of trees with DBH ≥ 10 cm ha^{-1}) surveyed in each of the studied seasonally flooded forest stands at Araguaia State Park, Mato Grosso State, Brazil.

Stand	Tree density	Basal area (BA)	Relative BA (%)
F1	49	1.05	3.13
F2	157	6.28	35.21
F2b	37	1.59	7.49
F4	46	2.84	9.90
F5	15	0.95	6.18

Table S3. Tree density (stems ha^{-1}), basal area (m^2 ha^{-1}) and basal area relative to stand total (%) of individuals of the Fabaceae family surveyed (DBH ≥ 10) in each of the five seasonally flooded (SF) forest stands studied in the Araguaia State Park, Mato Grosso, Brazil.

Stand	Fire presence	Soil depth (cm)	Replicate	n	Mean ^{15}N (atom-%)	SD ^{15}N (atom-%)	Mean excess ^{15}N (atom-%)	SD excess ^{15}N (atom-%)
F1	UB	0 – 10	I - III	12	0.367199736	0.000258318	7.25519E-05	5.64266E-05
			Controls	8	0.367133979	0.000252894		
		10 – 30	I - III	12	0.367365583	0.000384976	0.000106456	6.64926E-05
			Controls	8	0.367268818	0.000388175		
	B	0 – 10	I - III	12	0.367279239	0.000246253	6.14208E-05	5.38843E-05
			Controls	8	0.367293023	0.000254268		
		10 – 30	I - III	12	0.368029553	0.000115252	7.63416E-05	6.41253E-05
			Controls	8	0.367963687	9.59915E-05		
F2	UB	0 – 10	I - III	12	0.366415058	0.000230044	0.000124275	5.84958E-05
			Controls	8	0.366472289	0.000313802		
		10 – 30	I - III	12	0.3670293	0.000133194	5.39821E-05	3.69671E-05
			Controls	8	0.367007587	0.000130632		
	B	0 – 10	I - III	12	0.366609364	0.000597331	0.000221246	0.00053924
			Controls	8	0.366486233	0.000330816		
		10 – 30	I - III	12	0.367052408	0.000122765	8.93435E-05	4.24689E-05
			Controls	8	0.366975961	0.000165195		
F2b	UB	0 – 10	I - III	12	0.367316973	0.000115129	4.93066E-05	3.38772E-05
			Controls	8	0.367296088	0.000117579		
		10 – 30	I - III	12	0.36830748	0.000324613	0.00016867	7.91003E-05
			Controls	8	0.368162476	0.000341095		
	B	0 – 10	I - III	12	0.36772801	0.000318823	0.030747239	0.110779087
			Controls	8	0.367595149	2.80585E-04		
		10 – 30	I - III	12	0.368595844	0.000402626	0.000208986	0.000196239
			Controls	8	0.368414703	0.000281891		
F4	UB	0 – 10	I - III	12	0.367449202	0.000188302	0.000199447	1.19E-04
			Controls	8	0.367310139	0.000119296		
		10 – 30	I - III	12	0.368721661	0.000203957	0.000130079	7.50635E-05
			Controls	8	0.368645944	0.000128609		
	B	0 – 10	I - III	12	0.367254036	0.000149727	5.9968E-05	4.84168E-05
			Controls	8	0.367235044	0.000118821		
		10 – 30	I - III	12	0.368176803	0.000276307	9.5865E-05	5.71861E-05
			Controls	8	0.368080938	0.000313539		
F5	UB	0 – 10	I - III	12	0.367553871	0.000158561	0.000115963	5.06116E-05
			Controls	8	0.367472547	1.41E-04		
		10 – 30	I - III	12	0.368626755	0.000571468	0.000111499	8.65772E-05
			Controls	8	0.368537277	0.000509557		
	B	0 – 10	I - III	12	0.367929085	0.000152512	0.000132868	5.83093E-05
			Controls	8	0.367796217	0.000136757		
		10 – 30	I - III	12	0.369345219	2.41E-04	0.000233157	0.000147512
			Controls	8	0.369146902	0.000140778		

Table S4. Nitrogen isotopic values (15N atom-%) for enriched soil samples and controls used to calculate soil asymbiotic nitrogen fixation (ANF) across forest stands and depths. SD is the standard deviation.

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Table S5. Physicochemical properties (mean ± standard deviation) of soils collected in the studied seasonally flooded (SF) forest stands located at Araguaia State Park, Mato Grosso State, Brazil.

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Table S6. Asymbiotic nitrogen fixation data used to prepare Figure 2.

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Table S7. Soil data used to prepare Figure 3.

	pH (H_2O)	Total C:N	PMN (mg g^{-1})	pH (KCl)	Moisture (%)	Total C (g kg^{-1})	Total N (g kg^{-1})	M3-Fe (mg g^{-1})	M3-P (mg g^{-1})
pH (H_2O)	1	-0.32^{ns}	-0.25	0.47**	0	-0.32^{ns}	-0.23^{ns}	0.06^{ns}	-0.06^{ns}
Total C:N	-0.32**	1	0.39	-0.26^{ns}	0.34*	0.69**	0.34*	0.11^{ns}	0.47**
PMN	-0.25*	0.39**	1	-0.37*	0.23^{ns}	0.68**	0.63*	0.34*	0.41**
pH (KCl)	0.47***	-0.26**	-0.37**	1	0.24^{ns}	-0.33^{ns}	-0.24^{ns}	-0.18^{ns}	-0.23^{ns}
Moisture	0^{ns}	0.34***	0.23*	0.24*	1	0.43**	0.34*	-0.12^{ns}	0.08^{ns}
Total C	-0.32**	0.69***	0.68**	-0.33**	0.43**	1	0.87*	0.25^{ns}	0.37**
Total N	-0.23*	0.34***	0.63**	-0.24*	0.34**	0.87**	1	0.3^{ns}	0.27^{ns}
M3-Fe	0.06^{ns}	0.11^{ns}	0.34**	-0.18^{ns}	-0.12^{ns}	0.25**	0.3*	1	0.43**
M3-P	-0.06^{ns}	0.47***	0.41**	-0.23*	0.08^{ns}	0.37***	0.27*	0.43**	1

Asterisks indicate correlations are significant at the following levels: '***' 0.01, '**' 0.05, '*' 0.1, 'ns' > 0.1

Table S8. Spearman's correlations between major soil physicochemical variables across all five seasonally flooded (SF) forest stands studied at Araguaia State Park, Mato Grosso, Brazil.

Factors and Interactions	**df**	**F**	***p***
Depth	1	0.003	0.96^{ns}
Site	4	1.63	0.18^{ns}
Fire presence	1	5.34	0.03**
Fire frequency	2	2.25	0.12^{ns}
Depth * Stand	4	1.05	0.39^{ns}
Depth * Fire presence	1	3.96	0.05*
Stand * Fire presence	4	1.16	0.34^{ns}
Depth * Fire frequency	2	0.66	0.52^{ns}
Stand * Fire frequency	2	2.2	0.12^{ns}
Fire presence * Fire frequency	2	2.94	0.06*
Depth * Stand * Fire presence	4	0.96	0.44^{ns}
Depth * Stand * Fire frequency	2	0.69	0.51^{ns}
Depth * Fire presence * Fire frequency	2	2.21	0.12^{ns}
Stand * Fire presence * Fire frequency	2	2.23	0.12^{ns}

Table S9. Analysis of variance of the full factorial linear model fitted for ANF (log ng N g dw^{-1} h^{-1}) in the studied forest soils as a function of the categorical variables soil depth (0-10 and 10-30 cm), forest stand (F1, F2, F2b, F4 and F5), fire presence (burned and unburned), fire frequency (1, 2, 4 and 5), and interactions.

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