

Manuscript title: Native Amazonian *canga* grasses show distinct nitrogen growth responses in iron mining substrates

Authors: Cecilio F. Caldeira, Madson O. Lima, Silvio J. Ramos, Markus Gastauer

Author affiliations: Instituto Tecnológico Vale, Rua Boaventura da Silva 955, CEP 66055-090, Belém, Pará, Brazil

***Corresponding author:** cecilio.caldeira@itv.org, Instituto Tecnológico Vale. Boaventura da Silva, 955. Belém/PA, Brazil.

TABLES

Table S1. Chemical and physical properties of the red mining waste substrate collected from a representative location of the S11D Eliezer Batista Complex, Canaã dos Carajás, Pará/Brazil.

pH	OC	N	P	K	S	B	Zn	Fe	Mn	Cu	Ca	Mg	Al	Sand	Clay	Silt
	%	mg dm ⁻³									cmolc dm ⁻³			%		
5.1	0.4	<0.1	14.8	8.1	92.5	0.2	1.7	76.2	5.5	0.4	0.20	0.10	<0.1	39.3	16.7	44.0

Table S2. The regression parameters of 37 variables measured in the plants of *Paspalum cinerascens* cultivated in mining waste substrate in response to nitrogen addition.

Species	Variable	Intercept	x	x ^{0.5}	r ²	p.value
<i>P. cinerascens</i>	Tillering rate (tiller plant ⁻¹)	3.8410	-0.0375	1.2333	0.6601	6.1E-08
	Plant dry mass (g)	7.2975	-0.0855	2.1523	0.7829	9.0E-11
	Root: shoot ratio (g g ⁻¹)	1.0289	0.0041	-0.0859	0.3563	6.4E-04
	A - carbon assimilation (μmolCO ₂ m ⁻² s ⁻¹)	10.5460	-0.0737	1.8827	0.7246	2.2E-16
	gs - stomatal conductance (molH ₂ O m ⁻² s ⁻¹)	0.1232	-0.0007	0.0171	0.7475	2.2E-16
	WUE - water use efficiency (μmolCO ₂ mmolH ₂ O ⁻¹)	4.1605	-0.0101	0.2291	0.3824	2.2E-16
	Chlorophyl a (mg g ⁻¹ FW)	4.0679	0.0177	-	0.5017	6.5E-05
	Chlorophyl b (mg g ⁻¹ FW)	2.5033	0.0135	-	0.6777	4.7E-07
	Carotenoids (mg g ⁻¹ FW)	1.5037	0.0048	-	0.4446	2.2E-04
	N shoot (g kg ⁻¹)	4.7112	-0.0334	0.5969	0.4166	2.2E-16
	N root (g kg ⁻¹)	4.4092	-0.0265	0.4059	0.2923	6.3E-04
	P shoot (g kg ⁻¹)	0.6358	-0.0001	-	-0.0054	6.2E-01
	P root (g kg ⁻¹)	0.4890	0.0003	-	0.0088	2.5E-01
	K shoot (g kg ⁻¹)	10.0548	-0.0195	-	0.5369	2.2E-16
	K root (g kg ⁻¹)	8.0553	-0.0138	-	0.2257	1.1E-03
	Ca shoot (g kg ⁻¹)	2.2360	-0.0022	-	0.0974	1.1E-04
	Ca root (g kg ⁻¹)	1.5192	-0.0069	0.0857	0.1607	1.5E-02
	Mg shoot (g kg ⁻¹)	1.0245	-0.0083	0.1723	0.2843	4.9E-11
	Mg root (g kg ⁻¹)	1.2799	-0.0094	0.1562	0.2561	1.6E-03
	S shoot (g kg ⁻¹)	3.5299	0.0096	-0.2952	0.7768	2.2E-16
	S root (g kg ⁻¹)	2.1386	0.0040	-	0.3406	4.6E-05
	B shoot (mg kg ⁻¹)	11.0089	0.0082	-0.3289	0.1548	4.0E-06
	B root (mg kg ⁻¹)	8.1800	0.0464	0.9599	0.5088	7.3E-07
	Cu shoot (mg kg ⁻¹)	7.0579	0.0977	-0.7792	0.5511	2.2E-16
	Cu root (mg kg ⁻¹)	9.3160	0.0274	-	0.1838	3.4E-03
	Fe shoot (mg kg ⁻¹)	0.2550	0.0001	-0.0090	0.1550	3.9E-06
	Fe root (mg kg ⁻¹)	18.1	0.1024	-1.4347	0.3303	2.3E-04
	Mn shoot (mg kg ⁻¹)	0.1595	0.0002	-0.0059	0.1555	3.8E-06
	Mn root (mg kg ⁻¹)	0.0947	0.0006	-0.0087	0.1696	1.2E-02
	Zn shoot (mg kg ⁻¹)	29.0025	-0.0869	0.7495	0.0983	3.2E-04
	Zn root (mg kg ⁻¹)	22.8319	-0.1196	1.8137	0.1080	4.6E-02
	Stomatal frequence - adaxial	10.0924	0.0088	-	-0.0407	6.5E-01
	Stomatal frequence - abaxial	106.2524	-0.2065	-	0.3673	1.7E-03
	Stomatal length - adaxial	29.8621	0.0271	-	0.3787	0.0014
	Stomatal width - adaxial	23.2206	0.0231	-	0.5230	8.6E-05
	Stomatal length - abaxial	23.6545	0.0495	-	0.4457	4.1E-04
	Stomatal width - abaxial	16.4847	0.0116	-	0.2854	6.1E-03

Table S3. The regression parameters of 26 variables measured in the plants of *Axonopus longispicus* cultivated in mining waste substrate in response to nitrogen addition.

Species	Variable	Intercept	x	x ^{0.5}	r ²	p.value
A. longispicus	Tillering rate (tiller plant ⁻¹)	5.4200	0.0399	-	0.3025	6.5E-04
	Plant dry mass (g)	2.4204	0.0013	-	0.0239	6.0E-01
	Root: shoot ratio (g g ⁻¹)	0.2663	-0.0005	-	-0.0327	8.9E-01
	A - carbon assimilation (μmolCO ₂ m ⁻² s ⁻¹)	9.9732	0.0055	-	0.0174	4.3E-02
	gs - stomatal conductance (molH ₂ O m ⁻² s ⁻¹)	0.1009	0.0001	-	0.0604	5.1E-04
	WUE - water use efficiency (μmolCO ₂ mmolH ₂ O ⁻¹)	4.7480	-0.0013	-	0.0017	2.6E-01
	Chlorophyl a (mg g ⁻¹ FW)	2.3185	0.0052	-	-0.0074	3.7E-01
	Chlorophyl b (mg g ⁻¹ FW)	1.8453	0.0023	-	-0.0334	5.8E-01
	Carotenoids (mg g ⁻¹ FW)	0.7991	0.0051	-	0.1264	5.8E-02
	N shoot (g kg ⁻¹)	8.9435	0.0177	-	0.2746	2.2E-11
	P shoot (g kg ⁻¹)	0.4882	0.0014	-	0.1666	3.6E-07
	K shoot (g kg ⁻¹)	8.4459	0.0141	-	0.1564	8.6E-07
	Ca shoot (g kg ⁻¹)	1.8117	0.0021	-	0.2557	1.3E-10
	Mg shoot (g kg ⁻¹)	2.0938	0.0015	-	0.0822	3.7E-04
	S shoot (g kg ⁻¹)	2.7010	0.0051	-	0.1509	1.4E-09
	B shoot (mg kg ⁻¹)	8.8717	0.0095	-	0.0817	3.8E-03
	Cu shoot (mg kg ⁻¹)	10.0545	0.1828	-1.6700	0.7677	2.2E-16
	Fe shoot (mg kg ⁻¹)	0.4973	-0.0008	-	0.0641	1.5E-03
	Mn shoot (mg kg ⁻¹)	0.2946	-0.0022	0.0376	0.3139	2.8E-12
	Zn shoot (mg kg ⁻¹)	29.3572	-0.0722	1.4001	0.2658	2.8E-10
	Stomatal frequence - adaxial	88.2948	0.0179	-	-0.0505	7.7E-01
	Stomatal frequence - abaxial	91.9995	-0.1171	-	0.4954	3.2E-04
	Stomatal length - adaxial	32.059	-0.007	-	0.022	2.5E-01
	Stomatal width - adaxial	18.121	-0.004	-	0.016	2.7E-01
	Stomatal length - abaxial	34.043	0.001	-	-0.053	8.5E-01
	Stomatal width - abaxial	19.250	0.000	-	-0.055	9.4E-01

FIGURES

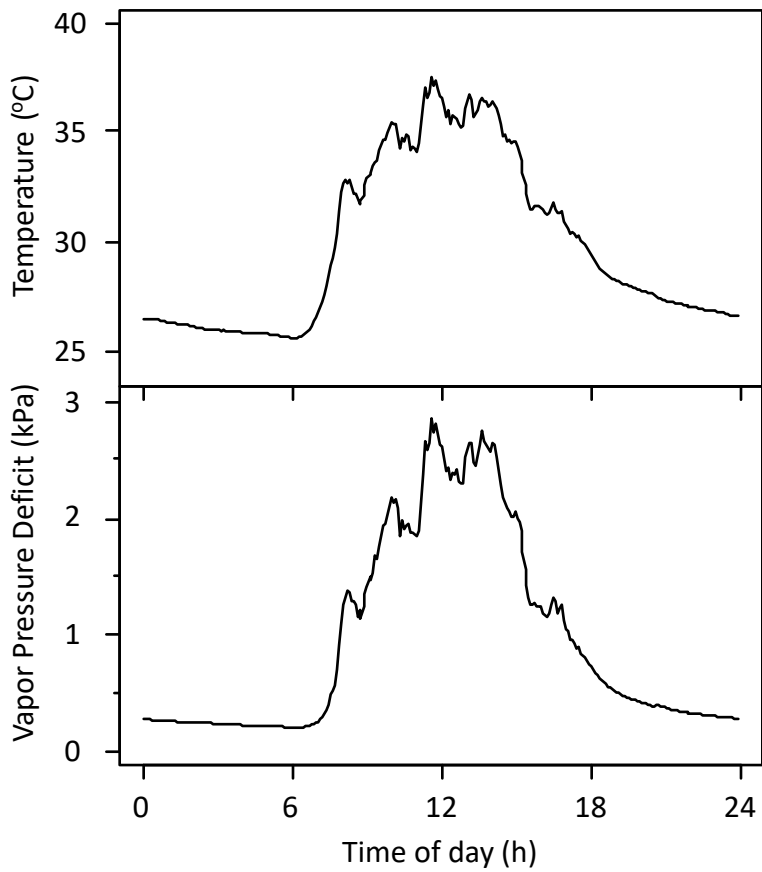


Figure S1. Mean air temperature and vapor pressure deficit inside the greenhouse during the 110 days of plants growing.

FIGURES

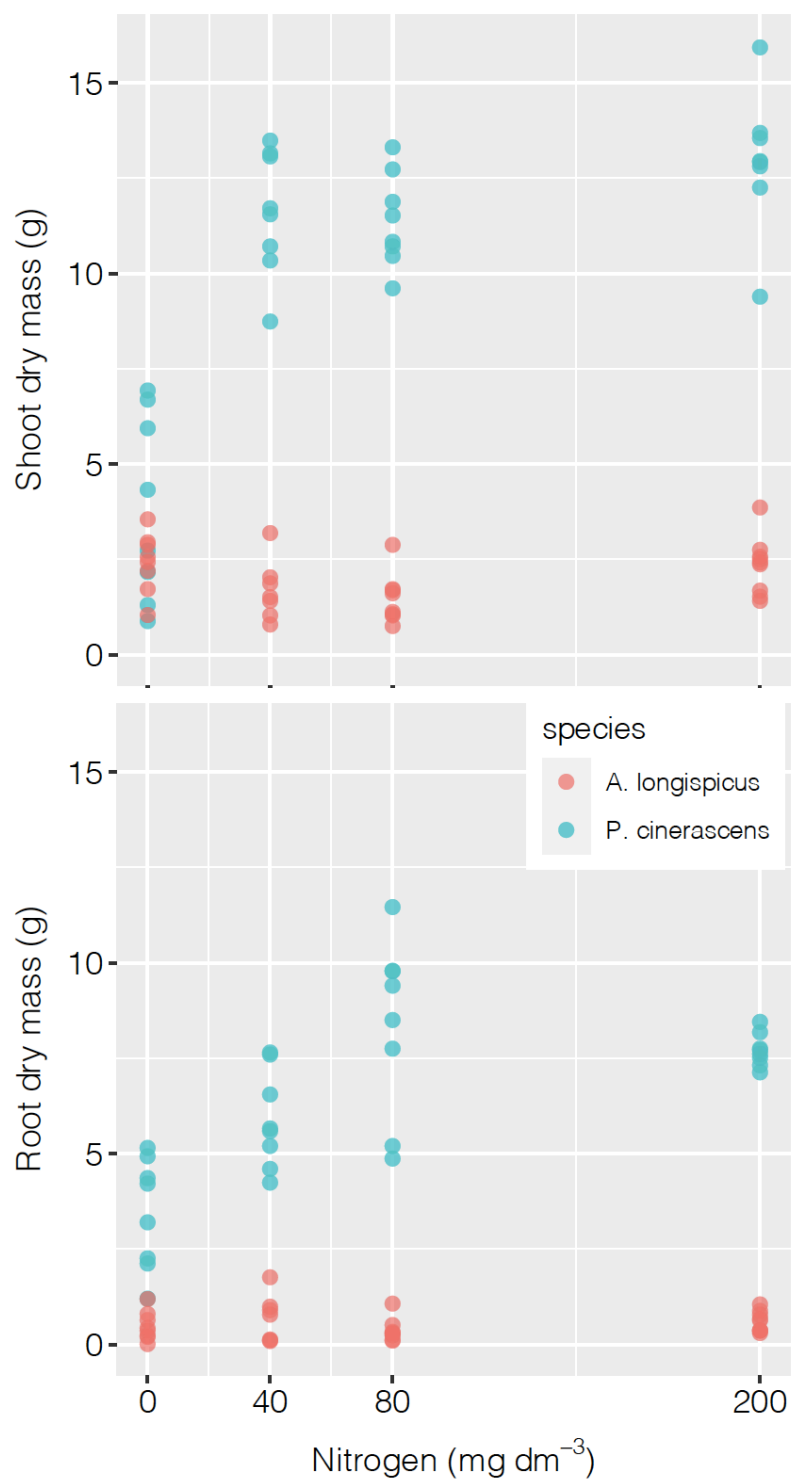


Figure S2. Nitrogen growth response of two native grass species from *canga* in Serra dos Carajás (eastern Amazon) grown in mining waste substrate. The shoot and root plant dry mass of *Paspalum cinerascens* (blue) and *Axonopus longispicus* (red) are shown.

FIGURES

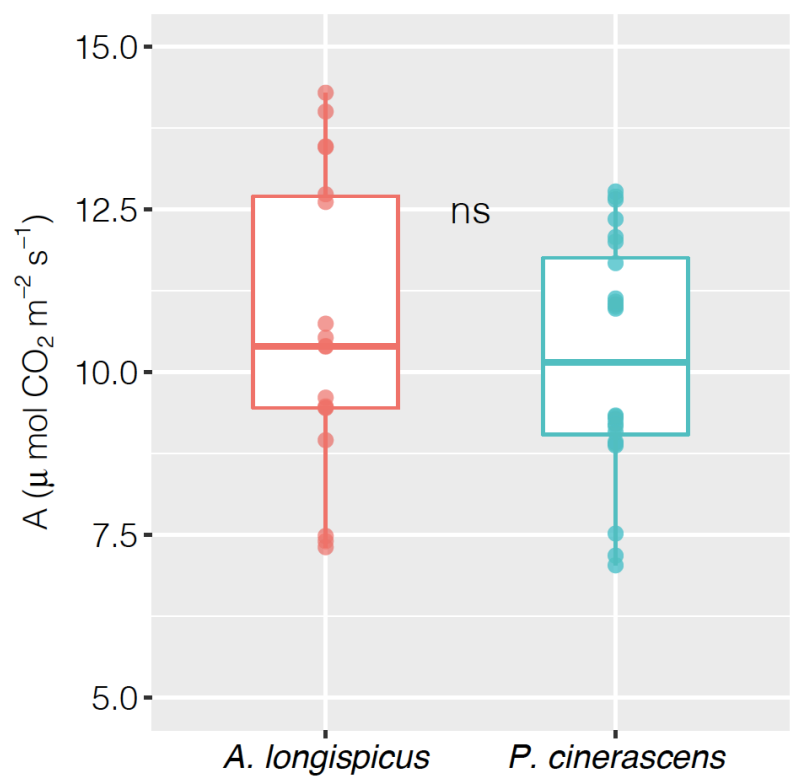


Figure S3. Boxplots of the carbon assimilation rates of two native grass species (*Paspalum cinerascens* (blue) and *Axonopus longispicus* (red)) from *canga* in Serra dos Carajás (eastern Amazon) growing in the mining waste substrates without nitrogen addition (control treatment).

FIGURES

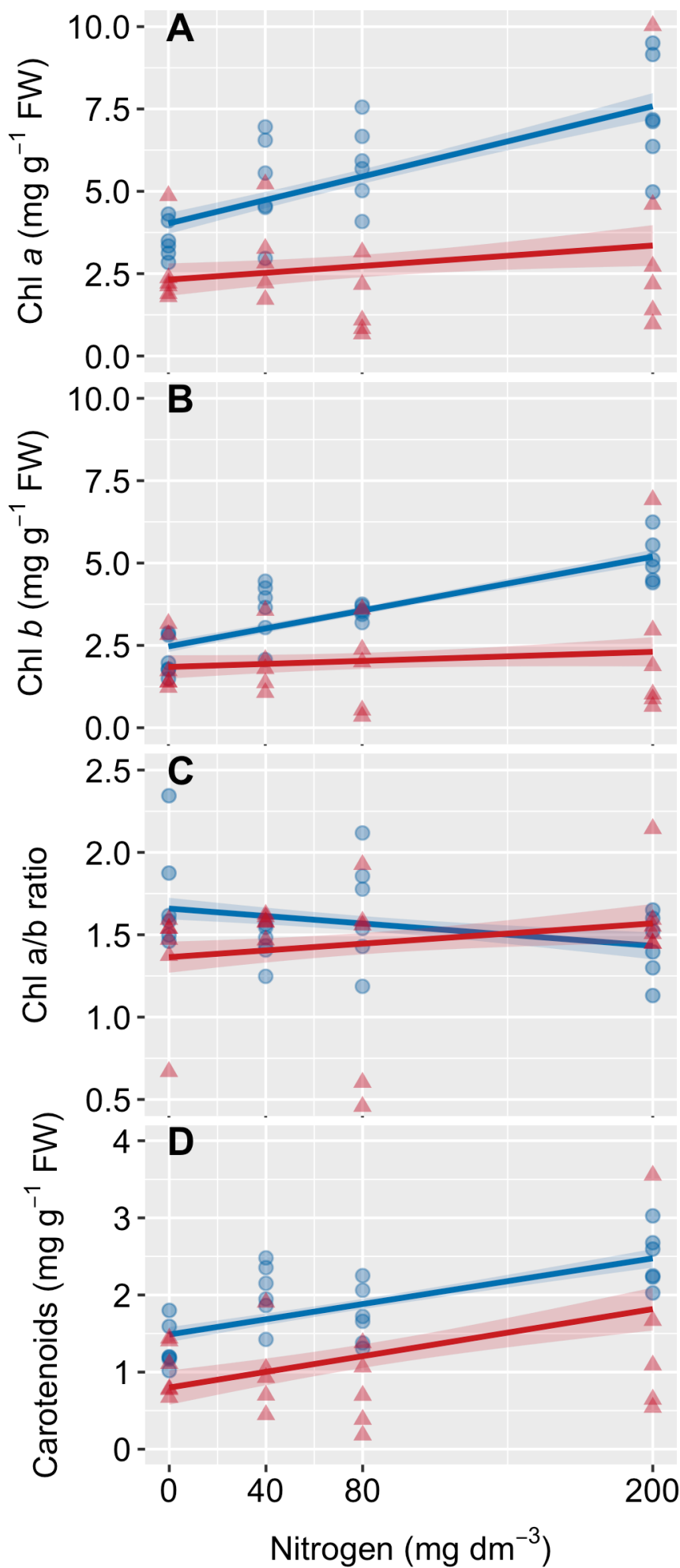


Figure S4. Effects of nitrogen increment to mining waste substrate in the pigment contents of two native grass species from canga of Carajás (eastern Amazon). Chlorophyll *a* (A), chlorophyll *b* (B), the ratio between chlorophyll *a/b* (C), and carotenoids (D) from fresh leaf tissue of *Paspalum cinerascens* (circles, blue) and *Axonopus longispicus* (triangles, red). Lines correspond to best fitted model and shaded area to its confidence intervals. Regression parameters are shown in the Supplemental Tables S2 and S3.

FIGURES



Figure S5. Plants of *Paspalum cinerascens* cultivated in a nutrient-rich substrate (a mixture of loam soil, organic matter, sand and fertilizer) under greenhouse conditions. Images captured from (A) plants three month-old, and (B) five month-old.