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Effects of seasonality, fertilization and species on the chlorophyll *a* fluorescence as related with
photosynthesis and leguminous tree growth during Amazonian forest restoration

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1 **Abstract**

2 The ability of species to adjust their light energy uptake is determined during plant
3 establishment and development. Changes in resource availability may impact energy fluxes and
4 photosynthesis. General and specific variations in chlorophyll *a* fluorescence under high vs. low
5 water and nutrient conditions have been studied. N₂-fixing leguminous trees, which are
6 commonly used in tropical forest restoration, seem to be very well adapted to degraded
7 ecosystems. To understand the effects of biological nitrogen fixation on Chl *a* fluorescence
8 variables, three of the six Fabaceae species selected for this study were N₂-fixing species.
9 Additionally, the correlation among Chl *a* fluorescence and growth, photosynthesis and nutrient
10 levels was evaluated. A 24-month forest restoration experiment was established, and data on
11 dark-adapted Chl *a* fluorescence, photosynthesis, diameter growth and foliar nutrients were
12 collected. Multivariate analysis was performed to detect the effects of seasonality and
13 fertilization. Under high water- and nutrient-availability conditions, plants exhibited enhanced
14 performance index values that were correlated with electron transport fluxes. Under drought and
15 nutrient-poor conditions, most species exhibited increased energy dissipation as a method of
16 photoprotection. Great interspecific variation was found; therefore, species-specific responses to
17 the test conditions should be considered in future studies. N₂-fixing species showed increased
18 performance index and maximum fluorescence values, indicating their ability to colonize high-
19 light environments. Negative correlations were found between photosynthesis and trapped
20 fluxes and between diameter growth and initial fluorescence. Electron transport fluxes were
21 positively correlated with growth. Given the different responses identified among species, Chl *a*
22 fluorescence is considered a cost-effective technique to screen for seasonality, nutrient and N₂-
23 fixing species effects and should be considered for use during forest restoration. Finally,
24 including N₂-fixing species and multiple fertilization treatments in related studies may greatly
25 facilitate the restoration of biogeochemical cycles in the tropics.

26

27 **Introduction**

28 Plant species use light from the sun as their primary source of energy for
29 photosynthesis. The excessive light energy in degraded tropical forest ecosystems (above 3000
30 $\mu\text{mol m}^{-2} \text{s}^{-1}$) may cause photoinhibition in poorly adapted species, affecting their
31 photosynthesis and growth (1,2). Some species may exhibit enhanced light uptake and plastic
32 responses under high-light environments, and these species are considered fundamental for the

33 establishment of forest restoration vegetation (3,4). N₂-fixing tree species may enhance light
34 uptake, particularly in nutrient-limited soils (5,6).

35 Dark-adapted chlorophyll *a* fluorescence (ChF) measurements based on dissipative
36 (*DI*), absorbed (*ABS*), trapped (*TR*) and transport (*ET*) energy fluxes are considered effective
37 indicators of the effects of stress on photosynthetic performance (7,8). The OJIP curve (shape)
38 has also been applied in the early detection of different abiotic stresses (9). Various effects on
39 the quantum yield of PSII photochemistry (F_V/F_M) and the performance index have been
40 reported under drought depending on the water deficit severity and on the tested species (10).

41 As drought progresses, plants may downregulate electron transport and increase
42 dissipation fluxes through the inactivation of PSII *RCs* (11,12). Degraded areas with multiple
43 nutrient deficiencies and decreased soil organic matter in the Amazon Basin can influence plant
44 photochemical activities, thus restricting photosynthesis and plant growth (13,14). Increased
45 drought resistance after fertilization has been reported to be due to the enhanced quantum yield
46 and photosynthesis (15,16). Studies on tropical environments have demonstrated the synergistic
47 effects of multiple-nutrient fertilization on biomass growth during forest restoration with
48 leguminous trees (17,18). However, only a few studies have evaluated the effects of combined
49 drought and nutrient stresses on growth and photosynthetic performance.

50 There has been increased interest in ChF responses to environmental cues and their
51 correlation to photosynthesis and growth traits (19–21). ChF has been demonstrated to be an
52 effective technique to detect different environmental cues, although the large number of
53 variables based on *ABS*, reaction centers (*RCs*) and cross-sections (*CSs*) may confuse
54 researchers (22). Multivariate analyses such as principal component analyses (PCAs) may
55 reduce the noise associated with large ChF datasets, allowing researchers to understand how
56 different species and ecological groups adjust energy fluxes (23). Likewise, in addition to being
57 less expensive than photosynthesis measurements, ChF can also detect effects such as
58 seasonality, which are harder to notice during growth analysis under field conditions.

59 Understanding ChF responses in ecological restoration species may increase forest
60 restoration success. While a substantial number of ChF studies exist, some knowledge gaps still

61 remain. For instance, no ecophysiological studies on important *Clitoria* or *Cenostigma* species
62 have been published. Studies of the effects of drought on ChF traits in tropical leguminous trees
63 are also lacking. Moreover, few studies have demonstrated the correlations between ChF
64 variables and growth, photosynthesis and nutrient traits. Therefore, three main hypotheses were
65 tested: 1. Leguminous trees adjust their photochemical energy fluxes under different water and
66 nutrient availability conditions; 2. Evolutionary N₂-fixing species will exhibit enhanced
67 transport fluxes and plant performance regardless of nutritional status; and 3. ChF variables are
68 correlated with growth, photosynthesis and nutrient traits.

69 **Materials and methods**

70 **Experimental trial, species and treatments**

71 Substantial variability in precipitation, temperature and soil properties can be found
72 across Amazonian ecosystems. Central Amazonia is characterized by low natural soil fertility
73 and high irradiance, temperatures, and monthly precipitation. Without delving into a
74 geopolitical and scientific discussion, large- and small-scale degradation is widespread
75 throughout the basin. The experimental trial was established in a typical homogeneous area of
76 the Forest Restoration Program of the Balbina Hydropower Dam in Amazonas state, Brazil.
77 Located two hours by car from Manaus, the 3 ha area was degraded and abandoned 30 years
78 ago by the complete removal without burning of the nonflooded and dense *terra firme* forest.
79 The area is surrounded by a natural nonflooded and dense *terra firme* forest. Despite
80 maintaining its good physical characteristics of the soil, nutrient deficiencies for nitrogen (N)
81 (0.16 g kg⁻¹), phosphorus (P) (0.14 mg dm⁻³), calcium (Ca) and magnesium (Mg) (0.3 cmol_c dm⁻³)
82 ³), were found. Natural regeneration was not observed before intervention supporting the
83 planting seedlings choice. Nine blocks (n = 9) measuring 6 m x 72 m (432 m² area) each were
84 placed across the area containing all 12 treatments in a combination of six tree legumes species
85 under low- (unfertilized) and high-nutrient (fertilized) treatments (Fig 1). The 108 studied plants
86 were selected from a total of 432 individuals planted. The high-nutrient treatment received four

87 applications of macro- and micronutrients in the beginning of dry and wet season, while the
88 low-nutrient treatment received no fertilization throughout the 24-month experiment.

89

90 **Fig 1. Graphical depiction with the location of the “Alalau” degraded area, the**
91 **experimental design, and the apparent conditions before intervention and 2 years after the**
92 **tropical forest restoration.** Experimental design with 12 treatments (fertilization x species)
93 randomly allocated in nine blocks (n = 9). Lost parcels (n) due to the mortality of four replicates
94 are represented by the crossed squares (⊠). T1 and T7, *C. tocaninum*; T2 and T8, *S. reticulata*;
95 T3 and T9, *D. odorata*, T4 and T10, *C. fairchildiana*; T5 and T11, *I. edulis*; T6 and T12, *Acacia*
96 sp.; T1 to T6, low-nutrient treatments; and T7 to T12, high-nutrient. For example, the four
97 replicates of T3 treatment (low-nutrient x *D. odorata*) on block eight, are defined with the cross
98 symbol (+) in the enlarged parcel, and the circulate-cross (⊕) illustrate the selected plant for
99 data sampling. The map was accomplished with scribblemaps.com free version.

100

101 Under normal conditions precipitation around the area is well distributed with less rainy
102 months (around 150 mm month⁻¹) from June to November. During the experimental course the
103 strong 2015/16 El Niño-Southern Oscillation (ENSO) event caused a 60-day period with no
104 precipitation when first measurements were performed (dry season). Second measurements
105 were accomplished 6 months later (rainy season), after recovery. The inclusion of inoculated
106 N₂-fixing species was expected to improve resource use efficiency by lowering the necessary
107 external inputs and the time required to rebuild C stocks (24). The six species selected from
108 Fabaceae for planting were three non-N₂-fixing: *Cenostigma tocaninum* Ducke, *Senna*
109 *reticulata* (Willd.) H.S. Irwin & Barneby, *Dipteryx odorata* (Aubl.) Willd.; and three N₂-fixing:
110 *Clitoria fairchildiana* R.A. Howard., *Inga edulis* Mart.—and one alien *Acacia* sp. The alien
111 *Acacia* sp. is frequently planted as capable of growing even in highly degraded areas.

112 The relative growth rate of the diameter (RGR_D) of each tree, hereafter the diameter
113 growth, was calculated 24 months after plantating (17). Detailed information on the species

114 used, fertilization methods, experimental trials and monthly precipitation during the
115 experimental period can be found in (25).

116 **Photosynthesis measurements**

117 Photosynthesis was measured between 8:00 and 11:30 h in nine selected plants per
118 treatment in the dry and wet seasons. Healthy, sun-exposed and completely expanded leaves on
119 the east side of the plants were selected from the middle third of each plant. The net
120 photosynthetic rate (P_n), hereafter referred to as photosynthesis, was measured using a portable
121 photosynthesis system (Li-6400, Li-Cor Inc., Lincoln, NE, USA) as described by (26). Each
122 measurement was performed at photosynthetic photon flux densities of 0 and 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$
123 with the foliar chamber adjusted to a CO_2 concentration, temperature and water vapor
124 concentration of $400 \pm 4 \mu\text{mol mol}^{-1}$, $31 \text{ }^\circ\text{C} \pm 1 \text{ }^\circ\text{C}$ and $21 \pm 1 \text{ mmol mol}^{-1}$, respectively.

125 **Chlorophyll *a* fluorescence**

126 The dark-adapted ChF measurements were performed with a portable fluorometer (Handy
127 PEA, MK2-9600-Hansatech, Norfolk, UK) on the same individuals and leaves used for the
128 photosynthesis measurements between 8:30 and 11:00 h. The selected leaves were dark-adapted
129 for a period of 30 min and then exposed to a 1 s excitation pulse of intensely saturating light
130 ($3000 \mu\text{mol m}^{-2} \text{s}^{-1}$) at a wavelength of 650 nm. Fast fluorescence transients were calculated
131 based on the so-called “JIP-test” (27). The performance index on an absorption basis (PI_{ABS}),
132 hereafter the performance index, which combines DI , TR and ET fluxes, was calculated as
133 described by (28). The units and formulae used are provided in Table S2.

134 **Foliar nutrient concentration**

135 The macro- and micronutrient concentrations of the leaves were determined in each
136 treatment after the leaf samples were oven-dried at $65 \text{ }^\circ\text{C}$ and ground. The N concentration
137 (mass basis) was determined with a 2400 Series II CHNS/O Organic Elemental Analyzer
138 (PerkinElmer Inc., Waltham, MA, USA). The P concentration was determined by

139 spectrophotometry at 725 nm. The potassium (K), Ca and Mg, iron (Fe) and zinc (Zn)
140 concentrations were determined using atomic absorption spectrophotometry (PerkinElmer
141 1100B, Inc., Waltham, MA, USA) according to Jaqueti et al. (17).

142 **Data analysis**

143 A complete randomized block experimental design was used. The interrelationships
144 among ChF variables were assessed using the PCA ordination method, which reduces the
145 dimensionality of the original data (29). PCA was performed to evaluate the effects of the
146 seasonality and fertilization treatments. All variables were standardized by the maximum
147 relatedness (30) prior to analysis. Product-moment correlations were used to assess the
148 influences of seasonality (dry and wet seasons) and fertilization (fertilized and unfertilized
149 treatments) on the ordination axes and each original variable. At a probability level of $P < 0.05$,
150 pairwise t-tests were performed to evaluate the significance of the seasonality and fertilization
151 effects. The analyses were run initially with 21 ChF variables and were run again with the 11
152 most responsive after removing similar and nonresponsive variables (S1 Table). The effects of
153 seasonality and fertilization on the performance index and the energy dissipation flux per active
154 PSII (DI_0/RC), hereafter the energy dissipation, were compared using repeated measure two-
155 way ANOVA with seasonality (dry and wet) as the repeated measure and species and
156 fertilization treatments as factors. The relationships among ChF variables and functional traits
157 were tested using nonparametric Spearman pairwise correlation analysis in the fertilized plants
158 and during the wet season. The PCA was performed with PAST-UiO 3.0 (Hammer and Harper,
159 Oslo, NO), and the inferential tests were performed with STATISTICA 12.0 (TIBCO Software
160 Inc., CA, USA).

161

162 **Results**

163 **Seasonal effects in the high- and low-nutrient treatments**

164 Corroborating the first hypothesis, plants in the high-nutrient treatment adjust energy
165 fluxes to seasonality. Significant differences separating dry and wet seasons were found in
166 PCA axis 1 ($t = -3.12, p < 0.01$) (S1 Table). Most individuals enhanced energy dissipation
167 fluxes and apparent antenna size of an active PSII (ABS/RC), hereafter antenna size, in the dry
168 season (see S2 Table for the product-moment correlations). Enhanced efficiency of electron
169 transport after Q_A^- (ET_0/TR_0), hereafter electron transport, and performance index were found in
170 the wet season (Fig 2). Low-nutrient plants adjust poorly to seasonal effects with no clear
171 separation between dry and wet seasons. Slight differences were found in PCA axis 1 ($t = -2.05,$
172 $p < 0.05$) (S1 Table and S1 Fig), suggesting that multiple nutrient deficiencies weakened the
173 leguminous trees ChF adjustments to seasonal effects.

174

175 **Fig 2. PCA ordination and loadings graphic of seasonal effects in the high-nutrient**
176 **treatment.** PI_{ABS} , performance index; ET_0/TR_0 , efficiency of electron transport; DI_0/RC , energy
177 dissipation flux; ABS/RC , antenna size of an active PSII RC ; F_M , maximum fluorescence; F_V ,
178 maximum variable fluorescence.

179

180 **Fertilization effects during dry and wet seasons**

181 Most plants adjust energy fluxes to different nutrient regimes during the wet season. An
182 evident separation between high- and low-nutrient treatments was found in PCA axis 1 ($t = -$
183 $3.14, p < 0.01$) (S1 Table). Plants in the high-nutrient treatment exhibited higher performance
184 index values and greater electron transport, while low-nutrient plants exhibited increased energy
185 dissipation and initial Chl a fluorescence (F_0), hereafter the initial fluorescence (Fig 3). In the
186 dry season, a noticeable effect for the fertilization treatment was found in PCA axis 1 ($t = -3.61,$
187 $p < 0.001$). Low-nutrient plants contribute to increased energy dissipation fluxes, while high-
188 nutrient plants improved electron transport and the electron transport flux per active PSII
189 (ET_0/RC) (S2 Fig). These findings suggest that the drought resistance of leguminous trees
190 increased under the fertilization treatments. Low-nutrient *Acacia* sp. adjusts ChF variables

191 differently with high electron transport and performance index values independent of seasonal
192 effects (S3 Table).

193

194 **Fig 3. PCA ordination and loadings graphic of fertilization effects during the wet season.**

195 PI_{ABS} , performance index on an absorption basis; ET_0/TR_0 , efficiency of electron transport;

196 DI_0/RC , energy dissipation flux; ABS/RC , antenna size of an active PSII RC; F_M , maximum

197 fluorescence; F_v , maximum variable fluorescence.

198

199 **Seasonal, fertilization and specific effects on PI_{ABS} and DI_0/RC**

200 Considering the performance index significant effects were found for the fertilization and
201 species treatments with a significant interaction between factors. For the repeated measures

202 differences were found between dry and wet seasons with the interaction between species and

203 seasonal effects. No interaction was found between seasonal and fertilization effects (S4 Table).

204 When species were compared significant differences were found between N₂-fixing and non-N₂-
205 fixing species with highest performance values for *Acacia* sp. and *I. edulis* (Fig 4).

206

207 **Fig 4. Performance index values under low vs. high water and nutrient availability (Mean**
208 **± SD).**

209

210 Considering the energy dissipation significant differences were found for both

211 fertilization and species treatments with interaction between factors. For the seasonal effects

212 statistical differences were found among dry and wet seasons with no interactions among

213 factors. Comparing the species, the late-successional *D. odorata* increased energy dissipation,

214 while no differences were found among other species (Fig 5).

215

216 **Fig 5. Energy dissipation flux values under low vs. high water and nutrient availability**
217 **(Mean ± SD).**

218

219 **Correlation of ChF with photosynthesis, foliar nutrient and**
 220 **plant growth**

221 Under high water and nutrient availability, performance index was positively correlated
 222 with diameter growth ($r_s = 0.65$) and Zn ($r_s = 0.52$). Maximum trapped exciton flux of an active
 223 PSII (TR_0/RC), hereafter the trapped flux, was negatively correlated with photosynthesis ($r_s = -$
 224 0.66), N ($r_s = -0.58$) and Zn ($r_s = -0.53$). Negative correlations with moderate collinearity were
 225 found between diameter growth and F_0 ($r_s = -0.61$) and energy dissipation ($r_s = -0.57$) (Table 1).
 226 Electron transport was positively correlated with Zn ($r_s = 0.51$) and growth ($r_s = 0.63$) and
 227 negatively correlated with Ca ($r_s = -0.58$).

228

229 **Table 1: Pairwise Spearman correlation coefficients (r_s) among ChF variables and growth,**
 230 **photosynthesis and foliar nutrient concentrations.**

	RGR_D	P_n	N	P	K	Ca	Mg	Fe	Zn
F₀	-0.61**	-0.35**	-0.28*	0.00 ^{ns}	0.21 ^{ns}	0.11 ^{ns}	-0.14 ^{ns}	-0.28*	-0.26 ^{ns}
F_M	0.31 ^{ns}	-0.05 ^{ns}	0.08 ^{ns}	0.34*	-0.25 ^{ns}	0.41**	0.02 ^{ns}	-0.19 ^{ns}	-0.09 ^{ns}
F_V	-0.13 ^{ns}	0.05 ^{ns}	0.18 ^{ns}	0.33*	-0.30*	0.37**	0.08 ^{ns}	-0.10 ^{ns}	0.01 ^{ns}
F_V/F_M	0.51**	0.29 ^{ns}	0.28*	0.08 ^{ns}	-0.27 ^{ns}	0.03 ^{ns}	0.11 ^{ns}	0.24 ^{ns}	0.22 ^{ns}
PI_{ABS}	0.65**	0.40*	0.49**	-0.21 ^{ns}	-0.17 ^{ns}	-0.40**	-0.02 ^{ns}	0.37**	0.52**
ET₀/TR₀	0.63**	0.38*	0.46**	-0.32*	-0.17 ^{ns}	-0.50**	-0.08 ^{ns}	0.33*	0.51**
TR₀/RC	-0.44**	-0.66**	-0.58**	-0.03 ^{ns}	0.09 ^{ns}	-0.41**	0.08 ^{ns}	-0.26 ^{ns}	-0.53**
DI₀/RC	-0.57**	-0.44**	-0.42**	-0.04 ^{ns}	0.23 ^{ns}	0.11 ^{ns}	-0.08 ^{ns}	-0.26 ^{ns}	-0.35*

231 F_0 , initial fluorescence; F_M , maximum fluorescence; F_V , maximum variable fluorescence;
 232 F_V/F_M , maximum quantum yield of PSII photochemistry; PI_{ABS} , performance index on an
 233 absorption basis; ET_0/TR_0 , efficiency of electron transport; TR_0/RC , maximum trapped exciton
 234 flux; DI_0/RC , energy dissipation flux; RGR_D, relative growth rate in diameter; P_n , net
 235 photosynthetic rate; N, nitrogen, P, phosphorous, K, potassium, Ca, calcium, Mg, magnesium,
 236 Fe, iron, and Zn, zinc concentrations in leaves.

237 Bold values are highly significant ($p < 0.001$) with high ($r_s > 0.70$) and moderate ($0.50 < r_s <$
 238 0.70) collinearity between variables. * Significance at the 0.05 level; ** Significance at the 0.01
 239 level.

240

241 **Discussion**

242 **Ecofunctional aspects of seasonal and nutrient effects on ChF**

243 As general stress responses plants exposed to drought and nutrient deficiencies in this
244 study exhibited enhanced energy dissipation, antenna size and initial fluorescence conformingly
245 as energy may be lost from plants through fluorescence emissions and heat dissipation (31).

246 With high water and nutrient availability, plant energy is mainly directed to photochemistry (32)
247 and electron transport in plants increases, thus improving plant performance (Fig 6).

248

249 **Fig 6: ChF adjustments in antenna size, energy dissipation and electron transport.**

250 *ABS/RC*, antenna size of an active PSII reaction center. The dark green means increased and
251 light green decreased values of, dark red represent increased quantum yield and electron
252 transport flux. Dark green and red indicates increased values of PSII antenna size and electron
253 transport flux, and light green and red indicates decreased values.

254

255 Usually, degraded environments expose plants to excessive light energy, and early-
256 successional species are thought to be less photoinhibited than late-successional species (33,34).

257 Correspondingly, energy dissipation increased in the late-successional *D. odorata*, indicating
258 photoinhibition and oxidative stress (35). Nonetheless, the late-successional *C. tocaninum*
259 appeared to be better adapted to high-light conditions when it was fertilized. Under high-light
260 environments, adapted species such as the N₂-fixing *C. fairchildiana*, *I. edulis* and *Acacia* sp.
261 enhance their electron transport, increasing their sink strength and light use efficiency (36). The
262 novel finding of the positive correlation between growth and electron transport may have
263 important applications in developing genetic improvements that will result in higher individual
264 plant performance.

265 Extreme climatic events such as the ENSO may cause drought periods in previously
266 unaffected regions of the Amazon Basin (37). Under moderate drought conditions,
267 photosynthesis may be downregulated, which restricts CO₂ availability, thus affecting
268 photochemistry and electron transport (38,39). Corroborating the present findings through
269 metabolic adjustments, drought-resistant *Acacia* species can maintain cell membrane integrity
270 and electron transport longer than other species as the water deficit increases (40,41). The
271 increased drought tolerance under fertilization found in three species in the present study may
272 be related to the increased PSII RC aperture, electron transport efficiency and photoinhibition
273 relief (42).

274 Plants may respond to fertilization treatments with increased plant performance and
275 photosynthesis due to the increased nutrient availability in the soil (6,43). The decreased
276 performance index under drought and nutrient deficiencies suggests increased photoinhibition
277 and reduced electron transport, which negatively affect photosynthesis and growth (42).

278 The present study demonstrates the negative correlations between trapped energy flux
279 and photosynthesis and between initial fluorescence and growth. Both of these findings have
280 important potential ecological and silvicultural applications in the selection of well-adapted
281 species for degraded environments. In addition to the present results, positive correlations were
282 found among photosynthesis, quantum yield and variable fluorescence in *Populus* and
283 *Miscanthus* species (44,45). Positive correlations were previously found among Fe, the
284 performance index and electron transport (46) and between electron transport and
285 photosynthesis (47,48). In contrast, no correlation was observed between photosynthesis and
286 ChF variables in barley plants under drought and control treatments (12).

287 **Performance variables, PI_{ABS} and ET_0/TR_0**

288 The PI_{ABS} , which incorporates the density of PSII RCs, electron transport beyond Q_A , and
289 trapped fluxes, is widely used to study photosynthesis and the functionality of PSII and PSI
290 under stress (1,10). The increased performance index values found under high resource
291 availability and in N₂-fixing species may result from changes in the PSII antenna size, trapping

292 efficiency and electron transport (8,27). Here, multivariate analysis demonstrated a positive
293 correlation between plant performance and electron transport adjustments. Corroborating
294 previous findings, the performance index was a more sensitive variable for evaluating
295 fertilization and drought effects than the PSII quantum yield (28,49).

296 The electron transport fluxes variables reflect the maximum electron transport between
297 PSII and PSI and indicate changes in photosynthetic apparatus activity (50). The increased
298 electron transport found in the well-adapted and highly productive *Acacia* sp. species may
299 reflect improved recovery on the PSII acceptor side. The diminished electron transport reported
300 under nutrient-poor conditions may be partially due to the overreduction of the PSII acceptor
301 side (51).

302 **Stress variables, DI_0/RC , ABS/RC and F_0**

303 Under the stressful, high-light conditions found in degraded areas, trees may increase
304 their protective quenching activity to dissipate excessive absorbed energy. The increased
305 dissipation fluxes that occur due to the inactivation of RCs may work as strong quenchers to
306 reduce photooxidative damage (28,53). Moreover, the increased energy dissipation indicates the
307 inefficient reoxidation of the reduced Q_A^- and electron transport to quinone Q_B (8,54).
308 Corroborating previous studies, the positive correlation between energy dissipation and the
309 initial fluorescence of stressed individuals may be associated with increased antenna size and
310 PSII RC inactivation (11).

311 The negative correlation between dissipative energy and P_n suggests decreased electron
312 transport and photochemical yield. Increased energy dissipation and antenna size have been
313 previously reported as early indicators of the effects of high light, drought, and nutrient
314 deficiency (9,54). Both positive and negligible effects of drought on PSII quantum yield have
315 been previously reported; the effect depends on the nutrient status of individual plants (55,56).
316 Our results indicate negligible interactions of seasonality and fertilization treatments effects.
317 Moreover, in species of the *Acacia* genus, increasing water deficit has negligible effects on
318 quantum yield, which is related to the increased protective carotenoid content (57).

319 **OJIP transient test**

320 The OJIP transient curve is used for the early detection of drought and nutrient stress
321 (58). Multiple nutrient deficiencies reduce the slope of the OJIP curves in most species due to
322 reduced ATP production under K and P deficiencies (9,58). The reduced O-J rise found in *S.*
323 *reticulata* and *C. tocaninum* reflected the impaired electron transport from pheophytin and
324 beyond Q_A (59). The stress responses of *S. reticulata* and *D. odorata* (S3 Fig) resulted in a
325 reduced amplitude of the thermal phase (I-P rise) due to the accumulation of reduced Q_A^- and
326 the decreased activity of PSII (11,32).

327 The sharp decrease in the maximum Chl *a* fluorescence (F_M) and the increase in initial
328 fluorescence values observed in *D. odorata* under high light suggest the inactivation of RCs and
329 the inhibition of electron transport beyond Q_A (60). The enhanced photochemical efficiency and
330 productivity in *Acacia* sp. were due to the increased F_M values and the absence of the P step (S3
331 Fig). The increased I-P rise in the well-adapted and highly productive *Acacia* sp. may be related
332 to the enhanced rate of ferredoxin reduction and electron transport between PQ and PSI (36).
333

334 **Conclusions**

335 The ChF technique is valuable for understanding the photochemical phase of
336 photosynthesis and how it affects other functional traits. Nevertheless, ChF has rarely been used
337 to assess the photosynthetic performance and adaptation ability of species used in forest
338 restoration. As evidenced in the present study, adjustments in energy fluxes during light uptake
339 are determinants of the growth and establishment of different species. Additionally, plants can
340 increase energy dissipation when resources are scarce and enhance electron transport when
341 resources are abundant. N_2 -fixing species with enhanced performance appear to be highly
342 adapted to degraded, high-light environments. In particular, the increased electron transport
343 fluxes in *Acacia* sp. may explain the enhanced sink strength and growth of these species in
344 locations with multiple resource limitations. Future studies on the functional aspects of

345 leguminous trees are recommended, especially studies on N₂-fixing species that may facilitate
346 the restoration of important biogeochemical cycles.

347

348

349 **Acknowledgments**

350 The authors are grateful to the Balbina Hydroelectric Dam for enabling the collection of
351 experimental data and to the Coordination for the Improvement of Higher Education Personnel
352 (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES) and the National
353 Council for Scientific and Technological Development (Conselho Nacional de
354 Desenvolvimento Científico e Tecnológico – CNPq) for financial support. J.F.C. Gonçalves is a
355 researcher with the Brazilian Council for Research and Development (CNPq).

356

357

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560 **Supporting information**

561 **S1 Fig. PCA ordination and loadings graphic for the seasonal effects in the low-nutrient**
562 **treatment.** PI_{ABS} , performance index on an absorption basis; ET_0/TR_0 , efficiency of electron
563 transport; DI_0/RC , energy dissipation flux; ABS/RC , antenna size of an active PSII reaction
564 center; F_M , maximum fluorescence; F_V , maximum variable fluorescence.

565

566 **S2 Fig. PCA ordination and loadings graphic for the fertilization effect in the dry season.**

567 ET_0/TR_0 , efficiency of electron transport; ET_0/RC , electron transport flux; DI_0/RC , energy

568 dissipation flux; ABS/RC , antenna size of an active PSII reaction center; F_M , maximum

569 fluorescence; F_V , maximum variable fluorescence.

570

571 **S3 Fig. The OJIP curve during the wet season under low (○) and high (□) nutrient**

572 **conditions.**

573

574 **S1 Table. Effects of seasonality and fertilization with 21 and 11 ChF variables.**

575

576 **S2 Table. Product-moment correlations obtained through principal component analysis**

577 **(PCA).** F_0 , initial fluorescence; F_M , maximum fluorescence; F_V , maximum variable

578 fluorescence; F_V/F_M , maximum quantum yield of PSII photochemistry; PI_{ABS} , performance

579 index on an absorption basis; ET_0/TR_0 , efficiency of electron transport; DI_0/RC , energy

580 dissipation flux; ABS/RC , antenna size of an active PSII reaction center; ET_0/RC , electron

581 transport flux; TR_0/RC , maximum trapped exciton flux; RC/CS , density of reaction centers per

582 cross-section.

583 **S3 Table. Mean ChF variables values for the six studied species under the different water**

584 **and nutrient regimes.** F_0 , initial fluorescence; F_M , maximum fluorescence; F_V , maximum

585 variable fluorescence; F_V/F_M , maximum quantum yield of PSII photochemistry; PI_{ABS} ,

586 performance index on an absorption basis; ET_0/TR_0 , efficiency of electron transport; DI_0/RC ,

587 energy dissipation flux; ABS/RC , antenna size of an active PSII RC; ET_0/RC , electron transport

588 flux; TR_0/RC , maximum trapped exciton flux; RC/CS , density of reaction centers per cross-

589 section.

590

591 **S4 Table. Results of the ANOVA for the effects of seasonality, fertilization and species on**

592 **PI_{ABS} and DI_0/RC .** PI_{ABS} , performance index on an absorption basis; DI_0/RC , energy dissipation

593 flux. Degrees of freedom for species = 5, and fertilization = 1. * Significance at the 0.05 level;

594 ** Significance at the 0.01 level.

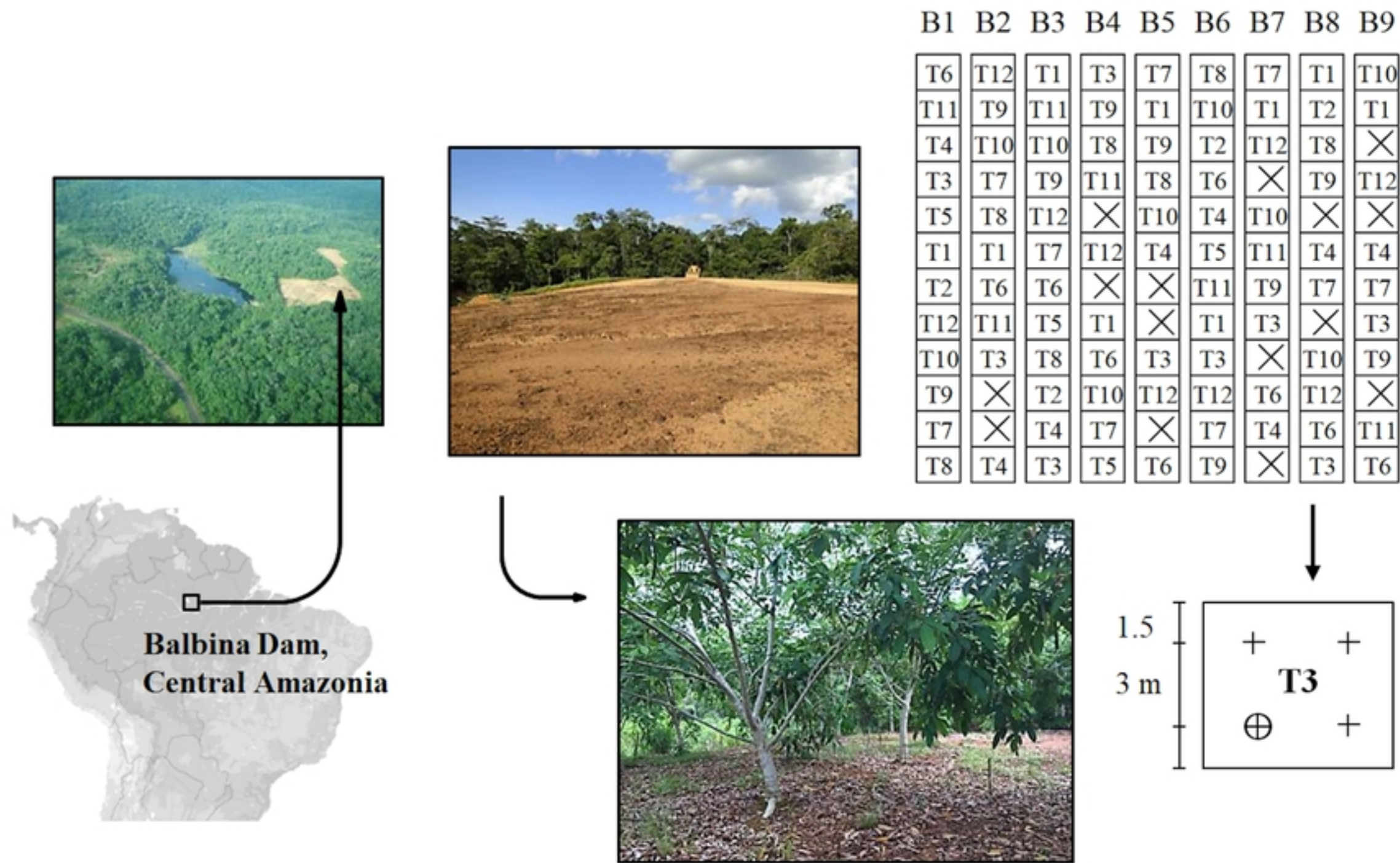


Figure 1

PCA Axis 2: (15.6%)

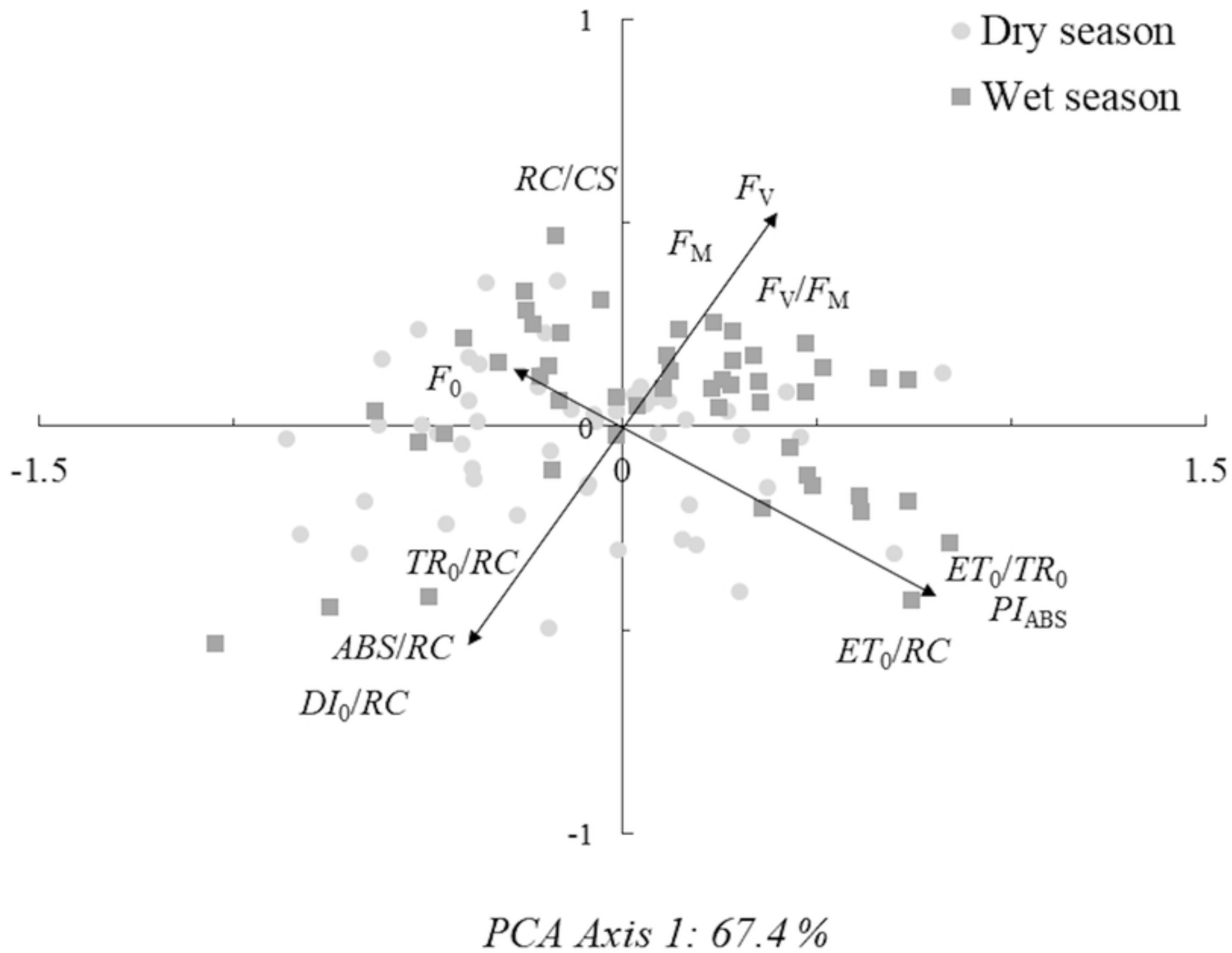


Figure 2

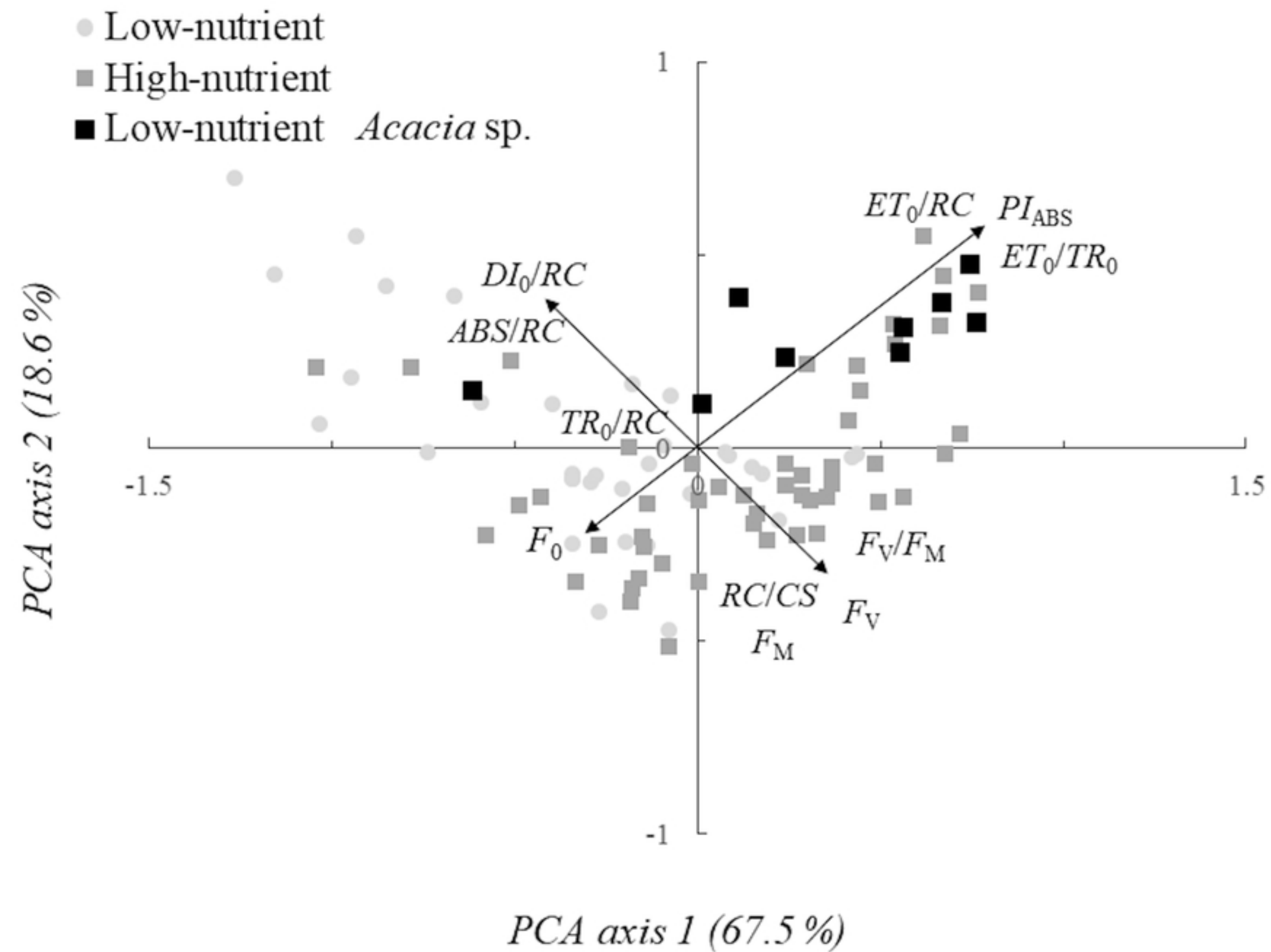


Figure 3

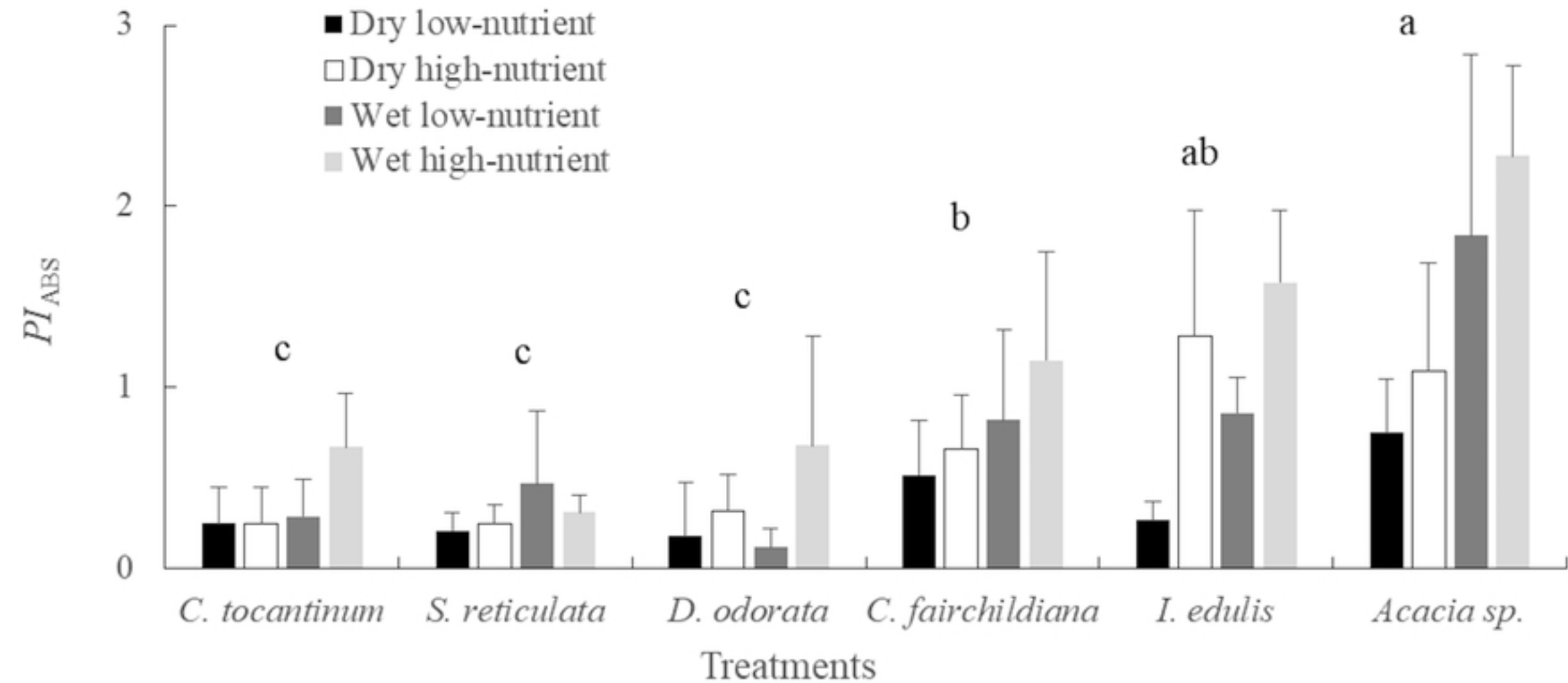


Figure 4

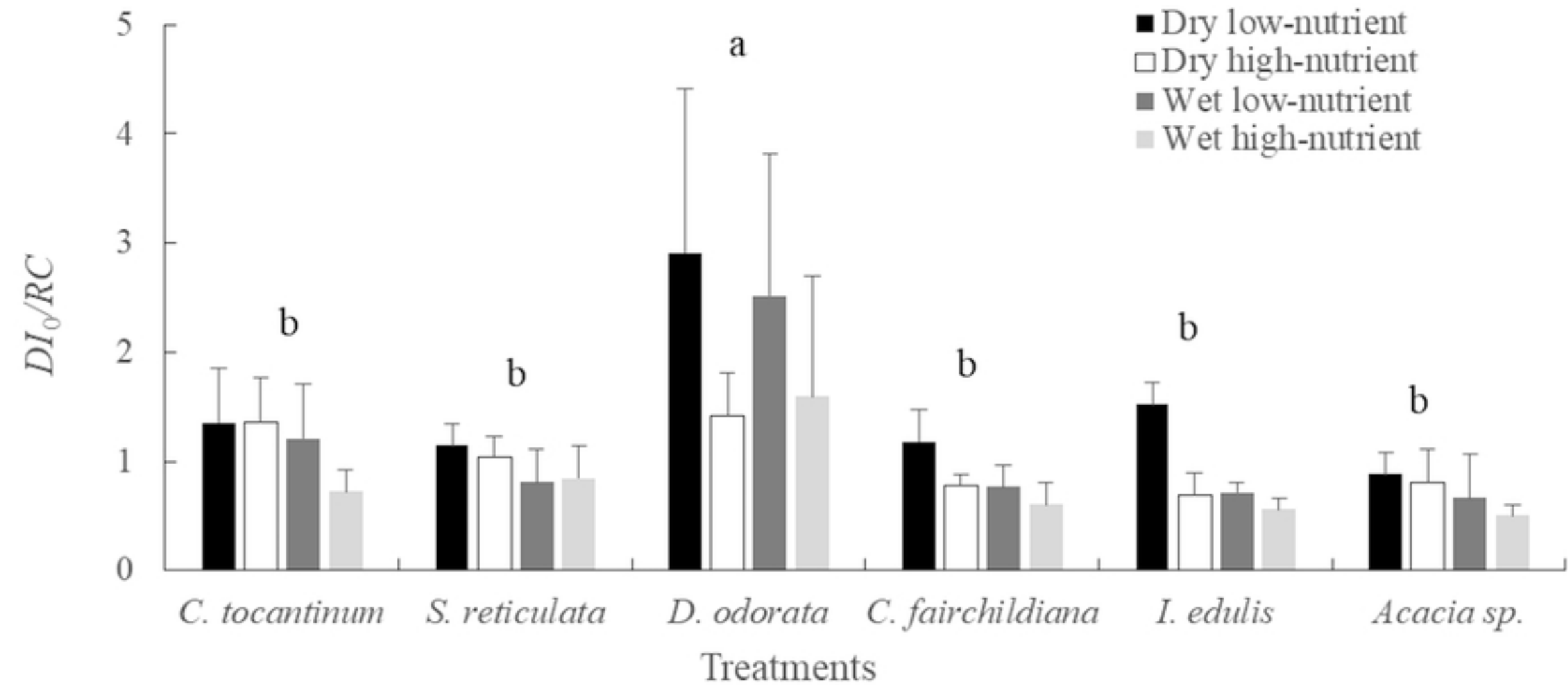
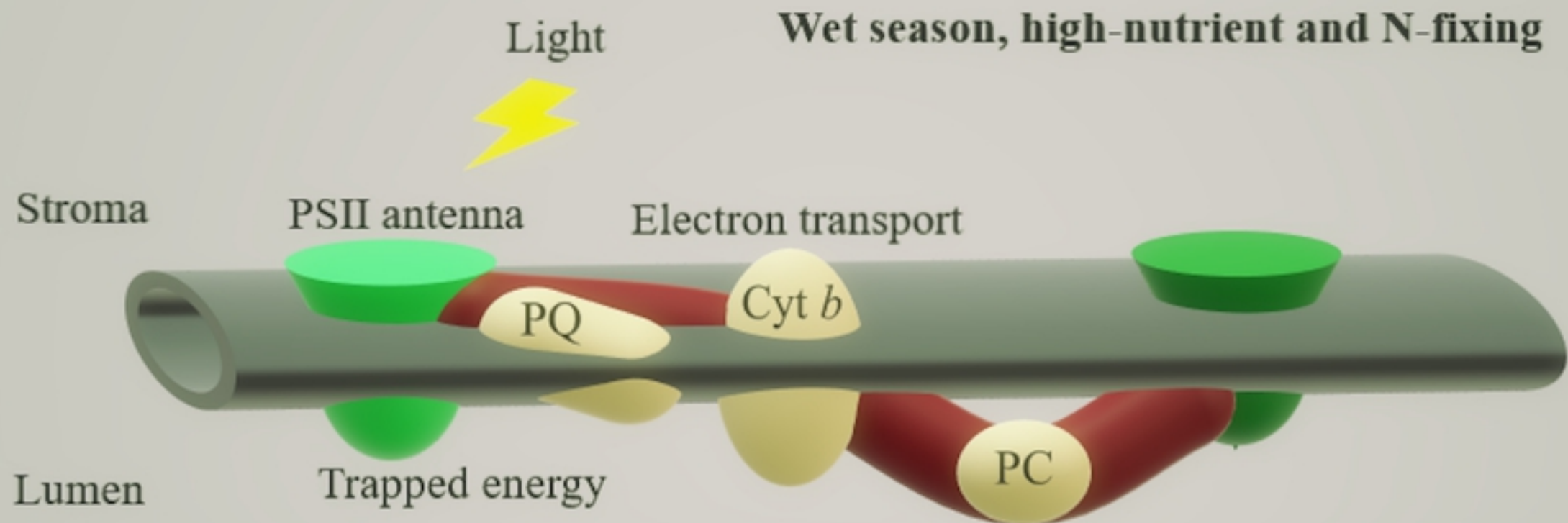


Figure 5



Energy dissipation

Dry season and low-nutrient

Figure 6