### A peer-reviewed version of this preprint was published in PeerJ on 29 November 2018.

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Padgurschi MCG, Vieira SA, Stefani EJF, Nardoto GB, Joly CA. 2018. Nitrogen input by bamboos in neotropical forest: a new perspective. PeerJ 6:e6024 <u>https://doi.org/10.7717/peerj.6024</u>

# Bamboos and a new perspective on nitrogen input in tropical forests

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**Background.** Bamboos are typical plants in tropical forests acting as resource for different animals and influencing forest structure and dynamics, but our knowledge about their influence on ecosystem functioning remains in its infancy. Nitrogen (N) is an important macronutrient that exerts control over productivity of ecosystems. Biological Nitrogen Fixation (BNF) is one of the main N input to terrestrial systems, more specifically by free-living BNF in tropical forests. In these forests, the dominant presence of bamboo and the occurrence of free-living N-fixers in its leaf surfaces appear to play a relevant role in N cycling. Here, we explore the importance of a native bamboo to N cycle in a Neotropical forest to provide insights on bamboo's role on ecosystem functioning.

**Methods.** The study was conducted in a pristine montane Atlantic Forest, Brazil. We select 100 sample units (100 m<sup>2</sup> each) and counted all bamboo clumps and live culms, estimated the leaf area, the litter production and its N content. We estimated the potential N input mediated by bamboo based on available data of free-living BNF rates for this species and then we contextualized it with information about N cycling components in the study area.

**Results.** We counted 4000 live culms ha<sup>-1</sup> and estimated  $4.3 \times 10^4 \text{ m}^2 \text{ ha}^{-1}$  of bamboo leaf area for freeliving N-fixers colonization. Considering the free-living BNF rates quantified for the same study area, the native bamboo may contribute up to 61.6 kg N ha<sup>-1</sup> y<sup>-1</sup> which represents a reduction in the N deficit of at least 25% in the Neotropical forest area we studied. Through its litter, the bamboo adds 9 kg N ha<sup>-1</sup> y<sup>-1</sup> to the system (15% of the total fixed on its leaf surface).

**Discussion.** The bamboo's contribution for N input we found may be explained by free-living community composition on its leaf as previously indicated by other studies, as well as by the bamboo overabundance, and thus habitat availability for colonization. Such N input represents a reduction in N deficit in the study area where the rates of symbiotic BNF (0.2 kg N ha<sup>-1</sup>y<sup>-1</sup>) are lower than values in Amazon Forest (3 kg N ha<sup>-1</sup>y<sup>-1</sup>). This native bamboo may supply N to the system probably through the decomposition of its leaves which represents a return of 15% of the total N fixed. Although it is still necessary to evaluate the characteristics of bamboo that will further elucidate its role in the functioning of the forests, our findings suggest that it closely regulates N inputs and may better explain high diversity and carbon stocks of the area.

#### 1 TITLE: BAMBOOS AND A NEW PERSPECTIVE ON NITROGEN INPUT IN

#### 2 TROPICAL FORESTS

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#### 14 Abstract

Background. Bamboos are typical plants in tropical forests acting as resource for different 15 16 animals and influencing forest structure and dynamics, but our knowledge about their influence on ecosystem functioning remains in its infancy. Nitrogen (N) is an important macronutrient that 17 exerts control over productivity of ecosystems. Biological Nitrogen Fixation (BNF) is one of the 18 19 main N input to terrestrial systems, more specifically by free-living BNF in tropical forests. In these forests, the dominant presence of bamboo and the occurrence of free-living N-fixers in its 20 leaf surfaces appear to play a relevant role in N cycling. Here, we explore the importance of a 21 native bamboo to N cycle in a Neotropical forest to provide insights on bamboo's role on 22 ecosystem functioning. **Methods.** The study was conducted in a pristine montane Atlantic Forest. 23 Brazil. We select 100 sample units (100 m<sup>2</sup> each) and counted all bamboo clumps and live culms, 24 estimated the leaf area, the litter production and its N content. We estimated the potential N input 25 mediated by bamboo based on available data of free-living BNF rates for this species and then we 26 contextualized it with information about N cycling components in the study area. **Results.** We 27 counted 4000 live culms ha<sup>-1</sup> and estimated 4.3 x 10<sup>4</sup> m<sup>2</sup> ha<sup>-1</sup> of bamboo leaf area for free-living 28 N-fixers colonization. Considering the free-living BNF rates quantified for the same study area, 29 the native bamboo may contribute up to 61.6 kg N ha<sup>-1</sup> y<sup>-1</sup> which represents a reduction in the N 30 deficit of at least 25% in the Neotropical forest area we studied. Through its litter, the bamboo 31 adds 9 kg N ha<sup>-1</sup> y<sup>-1</sup> to the system (15% of the total fixed on its leaf surface). **Discussion.** The 32 bamboo's contribution for N input we found may be explained by free-living community 33 composition on its leaf as previously indicated by other studies, as well as by the bamboo 34 overabundance, and thus habitat availability for colonization. Such N input represents a reduction 35 in N deficit in the study area where the rates of symbiotic BNF (0.2 kg N ha<sup>-1</sup>y<sup>-1</sup>) are lower than 36 values in Amazon Forest (3 kg N ha<sup>-1</sup>y<sup>-1</sup>). This native bamboo may supply N to the system 37 38 probably through the decomposition of its leaves which represents a return of 15% of the total N

- 39 fixed. Although it is still necessary to evaluate the characteristics of bamboo that will further
- 40 elucidate its role in the functioning of the forests, our findings suggest that it closely regulates N
- 41 inputs and may better explain high diversity and carbon stocks of the area.
- 42 Keywords: Merostachys neesii, Neotropical bamboo, Atlantic forest, N cycling, free-living
- 43 Biological Nitrogen Fixation

44 45	Introduction Woody bamboos are typical plants in many tropical forests (Humboldt & Bonpland 1907;
46	Judziewicz et al. 1999). They have a prolonged life cycle during which the rhizomes give origin
47	to the clones (culms) that remain connected in a clump (Judziewicz et al. 1999). The clump
48	structure and the rapid colonization in a greater availability of light (Widmer 1998; Yang et al.
49	2014), soil nutrients (Cirtain et al. 2009) or root colonizing fungi (Jiang et al. 2013) make the
50	group occur at high densities inside forests (Judziewicz et al. 1999). Such density influences the
51	structure (Tabarelli & Mantovani 2000; Griscom & Ashton 2003; Lima et al. 2012) and the
52	dynamic of forest, opening an opportunity for tree regeneration after gregarious flowering
53	(Giordano et al. 2009) or limiting it during the clones' phase (Rother et al. 2009; Grombone-
54	Guaratini et al. 2014); it also acts as a resource for different animals (Reid et al. 2004; Areta et al.
55	2009; Hilário & Ferrari 2010; Cestari & Bernardi 2011). These bamboos' roles are relatively well
56 57	known, but do they have any influence on ecosystem functioning? Nitrogen (N) exerts strong control over composition, diversity and productivity of
58	ecosystems (Townsend et al. 2011). Biological nitrogen fixation (BNF) and N deposition are the
59	two dominant pathways of N input to terrestrial ecosystems (Hedin et al. 2009, Sullivan et al.
60	2014). In mature tropical forests, the contribution of symbiotic BNF associated with legume tree
61	species do not explain the high nitrogen availability (Gehring et al. 2005; Nardoto et al. 2008;
62	Hedin et al. 2009; Cleveland et al. 2010) and may not be as important as believed (Sullivan et al.
63 64	2014) occurring only in cases of transient N limitation (Barron et al. 2011; Nardoto et al. 2014). Conversely, free-living BNF in litter and associated with aerial parts of plants can
65	contribute to N inputs, notably in tropical forests (Bentley 1987; Benner et al. 2007; Reed et al.
66	2008) where free-living BNF often exceeds symbiotic N inputs (Reed et al. 2011). The leaf
67	surfaces (phyllosphere) of tropical tree species harbor a great diversity of bacteria, including
68	putative diazotrophs (Lambais et al. 2006; Fürnkranz et al. 2008; Lambais et al. 2014). The N

cycling through litter is important in the nutrition budgeting once vegetation depends on the 69 recycling of nutrients contained in plant debris (Kuruvilla et al. 2014). 70 71 Studies about bamboo's influences on N cycling has been increasing mostly because it plays a role on the recuperation of soil fertility (Christanty et al. 1997, Singh & Singh 1999, 72 Embave et al. 2005, Shiau et al. 2017). In an agroforestry at Indonesia, the N content in bamboo 73 74 litterfall varied between 28.2 and 45.2 kg ha<sup>-1</sup> (Mailly et al. 1997); in India varied from 33.2 (Kuruvilla et al. 2014, Kuruvilla et al. 2016) to 79 kg N ha<sup>-1</sup> (Singh & Singh 1999), but nothing 75 compared to 115 kg N ha<sup>-1</sup> added by Yushania alpina in Ethiopia (Embaye et al. 2005). These 76 works explicit the importance of bamboos on N cycling through litterfall, but we do not know 77 how these N enters the system. In the Brazilian Atlantic Forest, a threatened hotspot and the place 78 79 of the highest bamboo diversity in the Neotropics (Judziewicz et al. 1999), Gómez (2012) found prominent level of bacterial diversity in the phyllosphere of *Merostachys neesii* Rupr. (Poaceae: 80 Bambusoideae), including several groups of putative free-living diazotrophs. Rigonato et al. 81 (2016) recorded the greatest abundance of cyanobacteria affiliated to the diazotrophic order 82 83 Nostacales on the *M. neesii*'s phyllosphere. 84 Studying in this pristine Atlantic Forest, we want to provide insights on bamboo's role in neotropical forest functioning addressing the questions: how much N does the M. neesii may 85 contribute by free-living diazotrophs communities on its phyllosphere? How much litter and N 86 through the litter does the *M. neesii* add to the system? Is this addition stable at different seasons? 87 We calculated the *M. neesii* density, leaf area, litter production and its N content. Using available 88 data on free-living BNF rates (Gómez 2012), we estimated the potential bamboo N input and 89 contextualized it with information about N cycling components in the study area. The presence of 90 M. neesii with some evidence of free-living diazotrophs suggests that they have efficient 91

mechanisms to cope with potential nutrient limitations in such acidic, dystrophic soils (Martins et

93 al. 2015).

#### 94 Materials and Methods

The study was conducted in the northeastern of São Paulo State, Brazil, in the Serra do
Mar State Park (PESM in Portuguese), Santa Virginia administrative nucleus (Fig. 1). We select
100 sample units (100 m <sup>2</sup> each) inside the previously established permanent plots (Joly et al.
2012). The physiognomy is pristine montane Atlantic Forest (1000 meters a.s.l.) with humid
subtropical climate (Cfa and Cfb), an average annual temperature of 21°C and an average annual
rainfall of 2,180 mm without a dry season (Salemi et al. 2013). A dense fog covers the region
almost daily, especially in the winter. The soil order is Inceptisol (United States Department of
Agriculture taxonomy) with very low pH ( $\approx$ 3.8), low fertility and elevated levels of aluminum
saturation (Martins et al. 2015). The aboveground biomass (283.2 Mg ha <sup>-1</sup> ) (Alves et al. 2010)
and the floristic diversity ( $\approx 200$ tree species/ha) (Padgurschi et al. 2011) are both high (Joly et al.
2014). Arecaceae, Myrtaceae, Lauraceae and Sapotaceae are among the most abundant families
(Padgurschi et al. 2011). <b>Bamboo species: density, leaf area and litter</b> Merostachys neesii Rupr. (Poaceae: Bambusoideae) is a native and endemic species of the
Brazilian Atlantic Forest (Fig. 2). We estimated its total leaf area $(LA_i)$ based on the culms'
density, on previously estimated leaf biomass per culm ( $L_b$ ) (551.4 ± 362.8 g, Padgurschi 2010),
on the average leaf dry weight ( $L_w$ ) (n=50; at 65°C until constant weight) and on the average leaf
area ( $LA_a$ ) (n=50) (LI-3100 area meter - LI-COR, Lincoln, Nebraska, USA). We counted all clumps and live culms (culms' density) within the sample units. To
contrast the number of clumps of <i>M. neesii</i> , we compared it to the highest density species in the
area (Euterpe edulis Mart. Arecaceae - Padgurschi et al. 2011). To obtain the number of leaves
per culm ( $L_c$ ), we divided estimated leaf biomass per culm ( $L_b$ ) by the average leaf dry weight
$(L_w)$ . For the leaf area per culm $(LA_c)$ we multiplied $L_c$ by the average leaf area $(LA_a)$ . Finally, we
estimated the total bamboo leaf area $(LA_t)$ (m <sup>2</sup> ha <sup>-1</sup> ) by: $LA_t = \frac{LA_c * \text{culms}}{10000}$ Equation (1)

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Among the 100 sample units, we randomly selected 40 to install litter collectors  $(0.22 \text{ m}^2)$ 

121 each) which were analyzed every 15 days for 20 months. The material was dried (at 65°C until

- 122 constant weight) and weighted to obtain the dry mass; all bamboo leaves were separated. We
- 123 calculated the production of bamboo litter from Sylvestre & Rosa (2002):

$$LP = \frac{\left(\frac{\sum MA * 10,000}{CA}\right)}{1000}$$
 Equation (2)

where LP = annual litter production (kg ha<sup>-1</sup> y<sup>-1</sup>); MA = month average of litter production (kg ha<sup>-1</sup>);

125 CA= collector's area  $(m^2)$ . For N chemical analysis, we grinded the leaves and prepared a composite

sample of which we random selected 3 subsamples for each season (January, May, July and October)

127 during the months of collection (results expressed in kg N ha<sup>-1</sup>). The research was performed with

#### 128 permits COTEC/IF 010.323/2013, 002.766/2013 and 010.631/2013 and IBAMA/SISBIO #33217.

129 Estimation of N input by free-living BNF rates in M. neesii's phyllosphere

130 To estimate the N input, we applied available data on free-living BNF rates in *M. neesii's* 

131 phyllosphere (Gómez 2012) that were recorded at the same site we studied. The BNF rate is 64.25 ng

132 N cm<sup>-2</sup> h<sup>-1</sup> (summer) and 34.78 ng N cm<sup>-2</sup> h<sup>-1</sup> (winter) (Gómez 2012). Considering 8 hours of light per

133 day (365 days) and the mean value (  $BNF_m \dot{c}$  of 49.52 ng N cm<sup>-2</sup> h<sup>-1</sup>, we estimated the N potential

134 fixing ( 
$$N_f$$
 expressed in kg N ha<sup>-1</sup>y<sup>-1</sup>) by:

$$N_f = \frac{(BNF_m * LA_t) * 2920}{10^{12}}$$
 Equation (3)

135 where 2920 are the hours of light in a year.

136 N cycling

137 To contextualize the estimation of N input mediated by *M. neesii*, we set up a table of N

138 cycling components in the study area. For N input, we considered the two dominant pathways in

139 most terrestrial ecosystems (Hedin et al. 2009): symbiotic BNF (Manarin 2012) and the total

140 atmospheric N deposition (Groppo 2010). In terms of N required by the system (demand), we

- 141 considered 1.5 mm y<sup>-1</sup> of tree growth (Scaranello 2015) and the biomass of the life forms (trees,
- 142 palms and ferns Alves et al. 2010) from an Atlantic Forest database (<u>www.forestplost.net</u>) to

143	calculate the annual increment of biomass. We then multiplied the leaf and stem biomass by its
144	respective N concentration (leaves = $2.61\%$ ; stems = $0.52\%$ - Vieira et al. 2011) to obtain the N
145	demand for tree growth. In the same way, we estimated the annual N for bamboo growth based
146	on the average biomass for each compartment (leaves = $0.5 \text{ kg}$ , culms = $2.9$ , branches = $0.9$ ) of
147	M. neesii (Padgurschi 2010), on net change of culms density (1.82% per year) of another
148	Merostachys species also from Atlantic Forest (M. riedeliana Rupr. ex Döll - Guilherme et al.
149	2004), on the number of live culms we counted and on N concentration of a native Guadua
150	species (leaves = $2.7\%$ ; culms and branches = $0.8\%$ - Galvão et al. 2012). From data on litter
151	biomass (5.5 Mg ha <sup>-1</sup> y <sup>-1</sup> - Sousa Neto et al. 2011) and on N content of the litter layer (1.72% -
152 153	Vieira et al. 2011), we estimated the N demand (for bamboo litter please check methods above). Finally, we considered as outputs the N via riverine transport and the losses to the
154	atmosphere of $N_2O$ and NO by soil emissions (Groppo 2010; Sousa Neto et al. 2011; Ghehi et al.
155	2013). Usually, there is considerable spatial and temporal variability of $NO_x$ emissions due to
156	environmental conditions (Ghehi et al. 2013). The $N_2O$ emission we present here were measured at
157	the same plot we studied, but NO emission is based on models developed for Tropical Highland
158	Forest (Ghehi et al. 2013). However, both areas are similar: (i) pristine montane forest; (ii) 1000 m
159	a.s.l.; (iii) 2000 mm y <sup>-1</sup> of rainfall; (iv) presence of bamboo; and (v) pH 3.8 (Ghehi et al. 2013;
160	Martins et al. 2015). Therefore, we consider valid the use of NO emission only as a reference for
161	the cycling of N in the Atlantic Forest. All analysis and graphs were performed at R environment
162	(R Core Team 2014).
163 164	<b>Results</b> By free-living diazotrophs on its phyllosphere, <i>M. neesii</i> may contribute up to 61.6 kg N
165	ha <sup>-1</sup> y <sup>-1</sup> . Its annual litter production is 540 kg ha <sup>-1</sup> y <sup>-1</sup> with significantly higher values during
166	summer/spring when compared to fall/winter (p<0.001) (Fig. 3). Through its litter, M.neesii adds
167	9 kg N ha <sup>-1</sup> y <sup>-1</sup> to the system, which represents 15% of the total fixed on its phyllosphere, with the
168	highest value in spring (3 kg N ha <sup>-1</sup> y <sup>-1</sup> ). Considering the inputs, demands and outputs of N in the

169	Atlantic Forest system we studied, such bamboo contribution may represent a reduction in the
170	nutrient deficit of at least 25% (Table 1), although the final balance remains negative (-170 kg N
171 172	ha <sup>-1</sup> y <sup>-1</sup> ). The bamboo density is 579 clumps ha <sup>-1</sup> , a value as high as the highest density tree species
173	in the study area: <i>Euterpe edulis</i> (574 trees ha <sup>-1</sup> ). With an average of 7.5 ( $\pm$ 2.5) live culms per
174	clump, we counted 4000 culms ha <sup>-1</sup> . Based on this and on available data on bamboo biomass, we
175	estimated 10.66 m <sup>2</sup> of leaf area in each of these culms and 4.3 x $10^4$ m <sup>2</sup> ha <sup>-1</sup> of bamboo leaf area
176	$(LA_i)$ for microbial colonization. These and other information on <i>M. neesii</i> traits are available in
177	Table 2.
178 179	<b>Discussion</b> The phyllosphere of <i>M. neesii</i> growing in a pristine montane Neotropical forest harbored
180	prominent levels of BNF rates (61.6 kg N ha <sup>-1</sup> y <sup>-1</sup> ), which are greater than other values of our
181	knowledge. Some tropical tree species - Spathacanthus hoffmannii Lindau (Acanthaceae),
182	Myriocarpa longipes Liebm. (Urticaceae), Chamaedorea tepejilote Liebm. (Arecaceae) and
183	Cyclanthus bipartitus Poit. (Cyclanthaceae) - contribute up to 5 kg N ha <sup>-1</sup> y <sup>-1</sup> by free-living BNF
184	(Freiberg 1998). These rates are even considerably less for Brosimum utile (Moraceae), Caryocar
185	costaricense (Caryocaraceae), Staminodella manilkara (Sapotaceae), Qualea paraensis
186	(Vochysiaceae), Schizolobium parahybum (Fabaceae) and Symphonia globulifera (Clusiaceae)
187 188	which contribute with 0.035 kg N ha <sup>-1</sup> y <sup>-1</sup> (Reed et al. 2008). Several factors may act and result in these rate differences: leaf age, intensity of light,
189	temperature, micro and macronutrient availability, tree species (phorophyte) and free-living
190	community composition (see Reed et al. 2011 for a review). In the same area of our study,
191	Rigonato et al. (2016) recorded the greatest abundance of cyanobacteria affiliated to the
192	diazotrophic order Nostacales on M. neesii's phyllosphere. Similarly, Gómez (2012) recorded
193	prominent level of groups of putative free-living diazotrophs on bamboo leaves, even when
194	compared to Euterpe edulis Mart. and other species in the same area. Although we did not

measure these variables, here we provide evidence of the great abundance of *M. neesii* (Table 2), 195 resulting in a large habitat availability for microbial colonization which added to the high amount 196 of N fixed on its phyllosphere, closely regulate the N input in this Atlantic Forest. 197 In mature tropical forests, the contribution of symbiotic BNF associated with legume tree 198 species do not explain the nitrogen availability (Gehring et al. 2005; Nardoto et al. 2008; Hedin et 199 al. 2009; Cleveland et al. 2010). In our study site, in addition to low occurrence of tree legumes 200 (Padgurschi et al. 2011), Manarin (2012) found rates of symbiotic BNF (0.2 kg N ha<sup>-1</sup> y<sup>-1</sup>) lower 201 than values found in Amazon Forest (3 kg N ha<sup>-1</sup> y<sup>-1</sup>) (Nardoto et al. 2014) and Costa Rica (1.2 kg 202 N ha<sup>-1</sup> y<sup>-1</sup>) (Sullivan et al. 2014). This may suggest that other forms of N input contribute to the N 203 maintenance in these pristine forests. Indeed, the *M. neesii*'s contribution increased more than 20 204 205 times the N input values in our study area (Table 1). This is particularly important since the demand for N, such as tree and bamboos growth and litter production, is at least 127.3 kg N ha<sup>-1</sup> 206 y<sup>-1</sup> (Table 1). Therefore, the association of this bamboo species with N-fixers may play a key role 207 in the functioning of the system supplying N to other plants probably through the decomposition 208 of its leaves. Such N recycling is important in the nutrition budgeting on tropical ecosystems 209 where vegetation mainly depends on the nutrients contained in plant debris (Kuruvilla et al. 210 2014). 211 Bamboo is important in the recuperation of soil fertility (Christanty et al. 1997, Embaye et 212 al. 2005), soil physiochemical properties (Shiau et al. 2017), net primary production and soil 213 redevelopment (Singh & Singh 1999). In an agroforestry at Indonesia, the "talun-bakun" system 214 success, which includes four years of bamboo plantation, is due to the recovery of nutrients 215 leached by the pumping action exerted by the bamboos (Christanty et al. 1997). In this system, 216 the N content in bamboo litterfall varied between 28.2 and 45.2 kg ha<sup>-1</sup> and the N concentration in 217 litter did not differ from live leaf (Mailly et al. 1997). In a natural forest in India, Joshi et al. 218 (1991) reported 580 kg ha<sup>-1</sup> of annual bamboo litterfall of which 5.4 kg ha<sup>-1</sup> were N that returned 219 to system. Other authors reported higher values ranging from 33.2 (Kuruvilla et al. 2014, 220

Kuruvilla et al. 2016) to79 kg N ha<sup>-1</sup> (Singh & Singh 1999) and 115 kg N ha<sup>-1</sup> (Embaye et al.
2005). Except for Joshi et al. (1991), all these N values are higher than one we found in this
study. However, the N concentrations they recorded are all similar to *M. neesii* (1.6%): 1.2%
(Joshi et al. 1991), 1.5% (Kuruvilla et al. 2014), 1.7% (Kuruvilla et al. 2016), 1.4% (Embaye et
al. 2005) and 0.7% (Singh & Singh 1999). We thus attributed the differences to the amount of
bamboo litterfall they found that varied from 2 to 10 Mg ha<sup>-1</sup> y<sup>-1</sup> (Kuruvilla et al. 2014; Singh &
Singh 1999, respectively).

#### 228 Conclusion

The development of lush tropical forests on acidic and dystrophic soils has been a puzzle 229 230 in tropical forest ecology for many decades (Hardy 1936) and the explanation has partially relied on the nutrient cycling mechanisms of these forests (Vitousek & Sanford 1986). Our findings 231 suggest that the amount of N fixed by free-living N-fixers associated with bamboo closely 232 regulates N inputs in these montane tropical forests and may better explain high diversity 233 (Padgurschi et al. 2011) and carbon stocks (Vieira et al. 2011) even with the overabundance of 234 bamboo. 235 Woody bamboos are typical plants in many tropical forests playing a vital role on forest 236 structure, dynamics and as resource for different animals, but our knowledge about their 237 influence on ecosystem functioning remains in its infancy. This is the first time to our best 238 knowledge that a work explores free-living BNF in bamboo phyllosphere making linkages to N 239 cycling in Neotropical forest. However, it is still necessary to evaluate the characteristics of 240 bamboo that will further elucidate its role in the functioning of these forests in addition to 241 features that can better explain the relationship between bamboo and its associated free-living 242 243 BNF community.

#### 244 Acknowledgments

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245	We thank C. Maguas for the valuable suggestions and critical discussion; the students and
246	technicians engaged in fieldwork; the Serra do Mar State Park, Santa Virgínia Nucleus, for
247	logistical support; and to the field technician Renato Belinelo for his empirical knowledge of
248	Atlantic Forest which helped us during all the field trips. We acknowledge helpful comments
249	from two reviewers, which have improved the manuscript.

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## Figure 1

Location of the study area (45°W 04' 34" 23°S 17' 24").

Figures (A) and (B) illustrate the geographic localization of the region; (C) indicates the position of the study region (São Paulo State, SE/Brazil) and the position of the sample units (yellow dot) compared to neighboring municipalities (São Luis do Paraitinga and Ubatuba).



## Figure 2

*Merostachys neesii* Rupr. (Poaceae: Bambusoideae), a native woody bamboo in a pristine montane forest (Atlantic Forest), Brazil.

(A) Flowers at anthesis; (B) Detail of a clump in the study area; (C) Detail of characteristic *M.neesii*'s leaf culm. Photos credit: MCG Padgurschi.



## Figure 3

*M.neesii*'s annual litter production (540 kg ha<sup>-1</sup> y<sup>-1</sup>) in the pristine montane Atlantic Forest, Brazil.

(A) Seasonal variation with significantly higher values during summer/spring when compared to fall/winter (p<0.001); (B) Annual variation.



### Table 1(on next page)

Estimates of N inputs, demand and outputs in the Atlantic Forest studied.

When local data were not available, we used information from literature. \* We considered the litter as an indirect measurement of N demand to leaves' production.

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 Table 1. Estimates of N inputs, demand and outputs in the Atlantic Forest studied. When local data were not available, we used information from literature. \* We considered the litter as an indirect measurement of N demand to leaves' production.

	Reference	Biome	Compartment	Nitrogen (kg N ha <sup>-1</sup> y <sup>-1</sup> )
Inputs	Groppo 2010	Atlantic Forest, Brazil	$N_{\text{total}}(N\text{-}N_{\text{inorg}}\text{+}N\text{-}N_{\text{org}})$	2.8
	Manarin 2012	Atlantic Forest, Brazil	BNF by legume trees	0.2
Demand			Total	3.0
	Scaranello 2015	Atlantic Forest, Brazil	Tree growth	20.0
	Galvão <i>et al.</i> 2012 Padgurschi 2010; Guilherme <i>et al.</i> 2004	Atlantic Forest, Brazil	Bamboo growth	3.3
	Sousa-Neto <i>et al.</i> 2011 Vieira <i>et al.</i> 2011	Atlantic Forest, Brazil	Litter (general) *	95.0
	This study	Atlantic Forest, Brazil	Litter (bamboo leaves) *	9
			Total	127.3
	Groppo 2010	Atlantic Forest, Brazil	Riverine transport	0.6

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	Sousa Neto et al 2011	Atlantic Forest, Brazil	N <sub>2</sub> O soil emission	0.8
	Ghahi et al. 2013	Tropical Highland Forest, Rwanda	NO soil emission	2.0
			Total	3.4
ince			Without Bamboo inputs	-231.7
Bala			With Bamboo inputs	-170.0

### Table 2(on next page)

Traits of *M. neesii* and its contribution to nitrogen input in a pristine montane Atlantic Forest, São Paulo State, Brazil.

Values in parenthesis are standard deviation (SD).  $L_w$  = Leaf dry weight;  $LA_a$  = Average leaf area;  $LA_t$  = Total bamboo's leaf area; N fixed = Total nitrogen fixed on bamboo's phyllosphere.

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**Table 2** - Traits of *M. neesii* and its contribution to nitrogen input in a pristine montane Atlantic Forest, São Paulo State, Brazil. Values in parenthesis are standard deviation (SD).  $L_w$  = Leaf dry weight;  $LA_a$  = Average leaf area;  $LA_t$  = Total bamboo's leaf area; N fixed = Total nitrogen fixed on bamboo's phyllosphere.

Merostachys neesii Traits				
Density (clumps ha <sup>-1</sup> )	579			
Culms (ha <sup>-1</sup> )	4000			
Estimated leaves per culm	4595			
$(L_w)(\mathbf{g})$	0.12 (±0.03)			
$LA_a$ (m <sup>2</sup> )	0.002 (±0.004)			
$LA_t$ (m <sup>2</sup> ha <sup>-1</sup> )	4.3 x 10 <sup>4</sup>			
N fixed (kg N ha <sup>-1</sup> y <sup>-1</sup> )	61.6			
N content in bamboo litterfall (%)	1.65			