Frugivory underpins the nitrogen cycle

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Abstract

1. Tropical rainforests are populated by large frugivores that feed upon fruit-producing woody species, yet their role in regulating the cycle of globally important biogeochemical elements such as nitrogen is still unknown. This is particularly relevant because tropical forests play a prominent role in the nitrogen cycle and are becoming rapidly defaunated. Furthermore, frugivory is not considered in current plant-large herbivore-nutrient cycling frameworks exclusively focused on grazers and browsers.

2. Here we used a long-term replicated paired control-exclusion experiment in the Atlantic Forest of Brazil, where peccaries and tapirs are the largest native frugivores, to examine the impact of large ground-dwelling frugivores on modulating soil nitrogen cycling, considering their effects across a gradient of abundance of a hyper-dominant palm.

3. We found that both large frugivores and dominant palms play a substantial role in modulating ammonium availability and nitrification rates. Large frugivores increased ammonium by 95%, which also increased additively with palm abundance. Nitrification rates increased with palm abundance in the presence of large frugivores, but not on exclosure plots. Large frugivores also stimulated the regulation of the functions of soil-nitrifying microorganisms, and modulated the landscape-scale variance in nitrogen availability. Such joint effects of large frugivores and palms are consistent with the notion of ‘fruiting lawns’.

4. Our study indicates that frugivory plays a pivotal role in zoogeochemistry in tropical forests by regulating and structuring the nitrogen cycle, urging to accommodate frugivory in plant-large herbivore-nutrient cycling frameworks. It also indicates that defaunation, deforestation and illegal palm and timber harvesting seriously affect nitrogen cycling in tropical forests, that play a prominent role in the global cycle of this nutrient.

KEYWORDS
defaunation, ecosystem function, Euterpe edulis, foundation species, frugivores, fruiting lawns, nutrient cycling, zoogeochemistry

1 | INTRODUCTION

Nitrogen (N) is one of the most important and primary nutrients for life since it is an essential component of many biomolecules and regulates primary productivity in many ecosystems. Tropical forests play an important role in the N cycle, accounting for nearly ~70% of terrestrial N fixation and ~50% of terrestrial nitrous oxide (N₂O) emissions (Townsend et al., 2011). As a consequence, there is an
urgent need to understand how the N cycle is regulated by soil microorganisms in tropical forests, and the abiotic factors underpinning those microbial communities (Pajares & Bohannan, 2016). A missing element of this increasing body of evidence is the impact of the large vertebrates, particularly large herbivores, which in other ecosystems have been shown to exert a strong regulating role of biogeochemical cycles and other critical ecosystem functions (Dirzo et al., 2014; Malhi et al., 2016; Sinclair, 2003).

Tropical rainforests are populated by large frugivores that capitalize on the large amounts of fruits produced by tropical trees (Beck, 2006; Bodmer, 1991; Bufalo et al., 2016; O’Farrill et al., 2013), yet their role in N cycling has not been documented (Forbes et al., 2019; Villar et al., 2020). Previous studies have primarily focused on frugivore effects on plant recruitment, diversity and functional traits (Jia et al., 2018; Kurten & Carson, 2015; Villar et al., 2020), paying less attention to other important ecological functions such as nutrient and carbon cycling and soil functions (Forbes et al., 2019; Villar et al., 2020). Tropical forests are increasingly experiencing widespread defaunation—the human-induced loss of species or populations of large vertebrates (Benítez-López et al., 2019; Dirzo et al., 2014), and this important knowledge gap precludes understanding the impact of defaunation on important functions and services provided by these forest ecosystems.

Large herbivores increase soil available N through dung and urine deposition, but they also affect N cycling through a myriad of direct and indirect pathways: their effects on plant composition, physiognomy and biomass, soil moisture, water retention and evapotranspiration, compaction, temperature and stoichiometry, soil microbial communities and mesofauna, amongst others, have positive and negative effects on N cycling rates, leading to highly context-dependent effects (Augustine & McNaughton, 1998; Frank et al., 1998; Schrama et al., 2013; Sitters & Olde Venterink, 2015; Veldhuis et al., 2014). Early studies on grassland and forest ecosystems suggested that herbivory might accelerate N cycling in nutrient-rich systems and decelerate it in nutrient-poor ones (Augustine & McNaughton, 1998; Bakker et al., 2009; Frank & Groffman, 1998; Frank et al., 1998; Ritchie et al., 1998). This early body of work also suggested that in many grassland ecosystems, large herbivores accelerate the N cycle and induce a positive feedback on N cycling rates at moderate grazing intensities (Frank & Groffman, 1998; Frank et al., 1998; Schrama et al., 2013), whilst on temperate forests browsers had a decelerating effect on N cycling rates (Harrison & Bardgett, 2003; Pastor et al., 1993; Ritchie et al., 1998). However, there’s mounting evidence suggesting a strong departure from that model, suggesting that a more complex integrative model of large herbivore effects across environmental gradients might be necessary (Ellis & Leroux, 2017; Kolstad et al., 2018; Schrama et al., 2013; Sitters & Olde Venterink, 2015). So far the only contribution to that body of work from tropical forests comes from a correlational study which concluded that the folivorous howler monkeys Alouatta seniculus might reduce nutrient cycling by promoting the dominance of non-preferred, nutrient-poor woody species (Feeley & Terborgh, 2005).

Whilst most of the debate pertains to differences in the underlying abiotic determinants of large herbivore effects (temperature, precipitation, soil type and nutrients) between ecosystem types or locations, more cryptic are large herbivore effects on nutrient cycling across (local and biotic) gradients of resource availability. Studies in grassland and temperate forest ecosystems suggest that in ecosystems with large spatial heterogeneity in resource availability where herbivores show strong resource selection responses, sustained promotion of nutrient cycling and plant productivity only occurs under a restricted number of conditions (Augustine & McNaughton, 1998). At the core of this framework lies the ability of plants to tolerate foliar depletion and regrow after different herbivore defoliation regimes, which might itself be constrained by abiotic conditions. However, many tropical rainforests do not fit well within this framework because they are dominated by fruit-producing woody species and large frugivore consumers, and therefore frugivore feedbacks on nutrient cycling are most likely linked to fruit consumption and not strictly to defoliation and regrowth of foliar tissues. A model that might help to extend this paradigm to frugivore-dominated tropical forests would account for feedbacks between fruit production and frugivore consumption through nutrient cycling. In essence, such a model of ‘fruiting lawns’ (Figure 1) would be conceptually equivalent to the model of ‘grazing lawns’ operating in many grassland ecosystems (Hempson et al., 2015).

Foraging theory predicts that selective consumer effects on their resources should vary across gradients of resource availability (Stephens & Krebs, 1986), and therefore the magnitude and feedbacks of large herbivore functions should also vary across gradients of key resources. Palms (family Arecaceae) are both hyper-abundant and key resources for many vertebrate species, including mammals and birds of all sizes, which feed on their fruits across different neotropical biomes (Galetti et al., 2001; Staggemeier et al., 2017; ter Steege et al., 2013; Tucker et al., 2018). In the neotropics, the largest and the most abundant large ground-dwelling herbivore mammals—herding peccaries (family Tayassuidae) and solitary tapirs (genus Tapirus)—feed on and disperse large numbers of palm fruits, and show strong preference for habitats where palms are abundant (Akkawi et al., 2020; Beck, 2006; Bodmer, 1990; Fragoso, 1997; Keuroghlian & Eaton, 2009). Thus, according to a fruiting lawn model, nutrient cycling should be structured across gradients of palm abundance on many neotropical forests.

In this work, we used a long-term exclusion experiment in the Atlantic Forest of Brazil to test whether frugivory underpins the soil N cycle, exploring the potential impacts of large frugivore defaunation for the N cycle on tropical forests. By comparing the N cycle in paired replicates of open control versus experimentally fenced enclosure plots we examine the impact of large ground-dwelling mammalian frugivores on soil N cycling whilst controlling for other confounding factors. First we consider the mean effects of large frugivores and how they vary across gradients in abundance of a key fruiting palm resource, so as to structure N availability (ammonium (NH₄⁺), nitrate (NO₃⁻) and total available nitrogen—hereafter referred as ‘TotN’), and N cycling rates (nitrification and mineralization) across such gradients in this frugivore-dominated ecosystem. The key resource in this ecosystem is the hyper-dominant palm *Euterpe edulis* which provides...
abundant fruit resources during the subtropical dry season, when fruit availability is limited, hence attracting a large variety of vertebrate consumers (Akkawi et al., 2020; Castro et al., 2007; Galetti & Aleixo, 1998; Peres, 1994; Staggemeier et al., 2017). Second, we explore if large frugivores affected the strength of the regulation of soil N availability by N cycling rates, an indirect method to assess the performance of soil N cycling microorganisms (see Section 2. Grazers and browsers have been shown to alter the performance of N cycling microorganisms in other ecosystem types (Frank et al., 1998; Pastor et al., 1993; Singer & Schoenecker, 2003). Third, given that large frugivores can transport nutrients across large tracts of tropical forests (Doughty et al., 2013), and might have differential impacts across gradients of resource availability (e.g. palms), we assess if they might redistribute nutrients and affect landscape-scale modulation of N and N cycling rates.

2 | MATERIALS AND METHODS

2.1 | Study site and field experimental design

The study was carried out on the Serra do Mar State Park (Núcleo Santa Virgínia, Itamambuca Research Station, 45°5′16″W/23°19′29″S), the largest continuous reserve area of Atlantic Forest in Brazil, where white-lipped peccaries Tayassu pecari, and lowland tapirs Tapirus terrestris, the largest ground-dwelling native frugivores across the neotropics, are abundant (Galetti et al., 2017; Rocha-Mendes et al., 2015). Agoutis Dasyprocta spp., pacas Cuniculus paca, brocket deer Mazama spp. and collared peccaries Pecari tajacu, amongst other frugivorous species, are also present at the site. Arboreal primates present at the site, such as the folivore howler monkey Alouatta guariba or the frugivore woolly spider monkey Brachyteles arachnoides (Galetti et al., 2017), might have had an impact on nitrogen cycling (Feeley & Terborgh, 2005), but they are not the focus of this study.

In 2010, a large terrestrial herbivore exclusion experiment including paired fenced exclosures and open control plots was established (5 m long x 3 m wide = 15 m² each, 1.6-m-high metal fence, 5 cm wire mesh). Within every pair, open and control plots were separated 2–7 m from each other, and replicated pairs were located at least 200 m from each other. This experimental design allowed us to unequivocally isolate the effects of large herbivores, without affecting the impacts of arboreal species (primates, birds, small mammals, bats, invertebrates) or other small ground-dwelling groups (mammals, birds and invertebrates) that could roam plots from both treatments at ease. Records from camera traps deployed on control
and enclosure plots show that the white-lipped peccary, a voracious frugivore, is the overwhelmingly dominant species, and the guild of large frugivorous mammals dominates the wider community of ground-dwelling mammals using the plots (Table S1). Small frugivorous and omnivorous mammals were also common, and camera trap records show they used slightly more exclosures than open plots, but were clearly outweighed in numbers and biomass by large frugivorous mammals.

In our site, *E. edulis* palms dominate the canopy, with up to 300 individuals per hectare in undisturbed areas (de Souza & Prevedello, 2019; Joly et al., 2012). This palm is widely distributed across a large latitudinal gradient of the biome (15°S to 29°S, de Souza & Prevedello, 2019) and can be found in most of the remaining forests of the Atlantic forest across its distribution (Siqueira, 1994). In order to characterize the effect of large frugivores across gradients of palm abundance, in August 2017 we counted the number of adult *E. edulis* (GBH ≥ 30 cm at 1.3 m) within 10 m radius of every plot, yielding a wide range of palm densities in the vicinity of the plots (0–255 adults/ha). Plot locations were not purportedly stratified according to palm densities, so that palm densities measured reflect the range of different densities existing on the forest. More information on the site and experimental design can be found in Villar et al. (2020).

### 2.2 Soil collection, nitrogen extraction and sample analyses

In August 2017 and May 2018, we collected three replicate soil samples (0–15 cm depth) from eight large herbivore exclusion plots and their paired open controls using 10 cm diameter cores. All soils had a sandy clay loam texture, and there were no differences between treatments in the proportion of sand (66% on open plots vs. 68% on exclosures), clay (28% vs. 27%) or silt (6% vs. 5%). Upon collection, samples were transported to the field station and processed within a few hours. The three subsamples from each plot were mixed and homogenized into a single plot sample, removing any roots or litter from the soil. A first batch of 10 g samples from every individual plot homogenized were mixed with 50 ml of 2 mol/L KCl solution for nitrogen extraction, and let sit for 24 hr to allow proper extraction. After that period we filtered the supernatant with Whatman no. 1 paper, and the filtered solution was immediately frozen at −20°C until analysis of available nitrogen in the laboratory. A second batch of homogenized soil samples from each of the plots was incubated during 7 days under dark conditions, and on the 7th day nitrogen was extracted with KCl following the same protocol described above. This allowed us to estimate potential nitrification and mineralization rates. Determination of ammonium and nitrate concentrations from extracts (mg/kg) in the laboratory followed the method of Bremner (1965). Total available nitrogen was the sum of ammonium plus nitrate concentrations (Ritchie et al., 1998). Net nitrification and mineralization rates (mg kg⁻¹ week⁻¹) were calculated as the difference in nitrate concentrations and ammonium plus nitrate concentrations, respectively, between the incubated and not-incubated soil samples (Pastor et al., 1993; Zak & Grigal, 1991). Ex-situ incubation is a standard procedure to estimate potential nitrification and mineralization rates in many large herbivore experiments (Frank & Groffman, 1998; Harrison & Bardgett, 2003; Pastor et al., 1993).

### 2.3 Statistical analyses

We tested the effect of excluding large frugivores on ammonium, nitrate, TotN concentrations and nitrification and mineralization rates across gradients of abundance of adult *E. edulis* palms by fitting generalized mixed effect models (GLMMs) with the interaction between experimental treatment and the log number of adult palms as explanatory variables, and plot pair within date as a random effect. Exploratory analyses suggested that models with the log number of adult palms conformed better to underlying model assumptions (Zuur et al., 2010) than models without transformation. Exploratory analyses also suggested standard Gaussian and non-gaussian distributions did not fit well raw values of NH₄⁺, but that a Gaussian distribution on log-transformed values of NH₄⁺ was a good fit. We used ANCOVA to assess the level of significance of fixed effects in the models.

We explored whether large frugivores affected the performance of soil nitrifying microorganisms indirectly, by focusing on the effect of experimental treatment on the relationship between nitrate and nitrification rates, ammonium and nitrification rates and TotN and mineralization rates. This indirect method does not measure the microbial activity of different functional groups per se, but nevertheless allows to infer indirectly the performance of nitrifying bacteria by assessing how the strength of the regulation of soil nitrogen content by nitrification and mineralization rates are affected by experimental exclusion. Exposing soil samples to the incubation period allowed us to indirectly evaluate the effect of microbial activity in the absence of losses from leakage and plant assimilation. In this context, a strong positive relationship between nitrate or TotN and nitrogen cycling rates after the incubation period would indicate a tight regulation of nitrogen availability in the soil by nitrification processes, whereas a neutral or negative relationship would indicate interference from denitrifying bacteria, assimilation by soil microorganisms or other losses linked to anaerobic transformation of N. In addition, a strong decline of ammonium with nitrification rates would indicate that ammonium availability limits nitrogen cycling rates.

In order to examine these dynamic relationships, we modelled soil nitrogen concentrations after the incubation period as the function of the interaction between experimental treatment and nitrogen cycling rates as fixed effects, and plot pair within date as random effects. Values of ammonium were log-transformed to better fit a Gaussian frequency distribution. We used ANCOVA to assess the level of significance of the fixed effect in the models.

Large frugivores might be able to compensate for low background levels of N availability in some sites through inputs from urine and faeces and through stimulation of N cycling rates. To test this hypothesis, we
evaluated how the effects of large frugivore exclusion on nitrogen and nitrogen cycling rates changed across gradients of nitrogen availability. First, we estimated effect sizes of excluding large frugivores on nitrogen and nitrogen cycling rates as percentage change according to the following formula: 

$$\text{Effect Size} = \left( \frac{N_{ci} - N_{ei}}{N_{ei}} \right) \times 100$$

where $N_{ci}$ and $N_{ei}$ are the concentration or rate of interest on the control and exclosure plots of plot pair $i$. In this way positive effect sizes denote percent increases in the control plot respective to the exclosure, whilst negative values indicate the converse. The extra unit ($+1$) in the numerator and denominator was included to avoid infinity in the quotient, as this was the minimum possible detectable value of nitrogen concentration. Effect sizes for every plot pair were calculated at both sampling dates independently.

Using GLMMs, we modelled effect sizes of large frugivore exclusion on ammonium concentration (calculated as above) as a function of TotN on the exclosure plots as fixed effect, and plot pair within date as random effects. We used the same GLMM structure to model effect sizes of large frugivore exclusion in nitrate and TotN as a function of the TotN in the exclosure plots, and effect sizes of large frugivore exclusion on nitrification and mineralization rates as a function of mineralization rates in exclosure plots. Upon inspection, we detected strong nonlinearities in some of these relationships, and we fitted generalized additive mixed models (henceforth GAMMs) to those, preserving the same model structure. We used ANOVA to assess the level of significance of the fixed effect in the models. Outliers representing extreme values of nitrification and mineralization were excluded in these last analyses, but their inclusion did not affect the significance of the relationship (Figure S1).

Additionally, we examined if large frugivores regulated landscape-scale variability in N availability and N cycling rates and induced shifts in the frequency distributions of ammonium, nitrate, TotN, nitrification, and mineralization rates. We hypothesized a higher spatial regulation and redistribution of N and N cycling rates by frugivores, which would be reflected in lower coefficients of variation and lower variances in control plots. We used $F$ tests to compare variances of control versus exclosure plots for each of the variables, and log-transformed values of ammonium and TotN to better fit a Gaussian frequency distribution. We used $r$ (R Core Team, 2019) for all statistical analyses, and packages `nlme` and `mgcv` (Pinheiro et al., 2019; Wood, 2011) to fit GLMMs and GAMMs with a Gaussian frequency distribution to data. All data are deposited in the Dryad Digital Repository https://doi.org/10.5061/dryad.fbg79cns (Villar, Paz, et al., 2014).

### 3 | RESULTS

#### 3.1 | Effects of large frugivores on soil nitrogen and nitrogen cycling rates across gradients in palm abundance

We found evidence of a strong impact of large frugivores on N availability and of differential impacts of large frugivores on nitrification rates across gradients of palm abundance. Ammonium concentration in the soil was about 95% higher in the presence of large frugivores (Figure 2b; Table S2; open ($M \pm SE$, mg/kg, in log scale) = 2.78 ± 0.33), exclosure = 2.11 ± 0.33, equivalent to concentrations of 16.12 and

![Figure 2](image-url)
8.25 mg/kg respectively). Ammonium also increased substantially with the log number of adult palms, in an additive way across control and exclosure plots (slope across treatments = 0.49 ± 0.20; Figure 2b; Table S2). In contrast, nitrate concentration did not change with frugivore treatment or palm abundance (Table S2). As a result, TotN increased from 57.19 mg/kg ± 6.81 in exclosures to 77.75 ± 6.81 in open plots, whereas no effect of palms was apparent in TotN. As indicated by a significant frugivore exclusion treatment x palm abundance interaction (Table S2), nitrification rates in open plots increased substantially with log palm abundance (mg kg⁻¹ week⁻¹, intercept: −30.10 ± 18.74; slope: 32.95 ± 11.21, Figure 2c), whilst in exclosures there was a net subtle decline with increasing abundance of palms (intercept: 23.05 ± 18.74; slope: −36.97 ± 15.85). However, neither experimental exclosure treatment nor palm abundance had a significant effect on net mineralization rates (Table S2).

3.2 | Effects of large frugivores on the regulation of N availability by N cycling rates

Inspection of the dynamic relationship between N availability and N cycling rates suggested a strong impact of large frugivores on the regulation of N availability by nitrifying microorganisms. Nitrate strongly increased with nitrification rates in incubated samples from the control treatment (1.01 ± 0.14, \( R^2 = 0.75 \)) but not in the exclosures (0.32 ± 0.18, Figure 3), as indicated by a significant interaction (\( F_{1,13} = 9.4, p = 0.009 \)). For TotN patterns were similar: a significant interaction (\( F_{1,13} = 9.3, p = 0.009 \)) indicated that TotN strongly increased with mineralization rates in the control treatment (0.91 ± 0.09, \( R^2 = 0.91 \)) but not in the exclosures (0.23 ± 0.20, Figure 3). Thus, concentrations of nitrate and TotN were strongly regulated by nitrification and mineralization rates on incubated soils from plots where large frugivores had access, but not on soils from exclosures. Ammonium concentration in contrast did not show any relationship with nitrification rates in any of the frugivore treatments, suggesting that ammonium supply was not limiting N cycling rates.

3.3 | Redistribution and landscape-scale regulation of nitrogen and nitrogen cycling rates by large frugivores

Results suggested that large herbivores had a strong impact in modulating landscape-scale N availability. The effect of large frugivore exclusion on percent changes in ammonium, nitrate and TotN changed in sign and magnitude across a gradient of available N in exclosure plots. At locations where exclosure plots showed low values of TotN, paired controls showed much higher levels of ammonium, nitrate and TotN, whereas at exclosure plots where TotN was high, the effect of large frugivores was the opposite (Figure 4b; ammonium: \( F_{1,13} = 11.452, p < 0.005 \); nitrate: \( F_{1,13} = 9.809, p = 0.008 \); TotN: \( F_{1,13} = 14.381, p < 0.005 \)). For nitrate and TotN the relationship was strongly nonlinear, which was supported by a posteriori GAMMs (Figure 4b, ammonium: estimated degrees of freedom (edf) = 1, \( F = 12.27, p = 0.003 \); nitrate: edf = 8.99, \( F = 12,946, p < 0.005 \); TotN:...
edf = 7.39, F = 58.9, p < 0.005). On the other hand, analyses suggested that the effects of large frugivores on changes in net nitrification and mineralization rates were uncorrelated with background levels of mineralization (F_{1,12} = 2.689, p = 0.127; F_{1,11} = 0.464, p = 0.510). Control plots showed much lower coefficients of variation and sample variances of log ammonia, nitrate and log TotN than exclosures (and Figure 4c; Table S3), hence lower variance at the landscape scale. In contrast, nitrification and especially total mineralization rates showed higher coefficients of variation and sample variances on control plots than on exclosures.

**4 | DISCUSSION**

Our results indicate that the interaction between large frugivores and key food resources have a substantial impact on nutrient cycling in the Atlantic Forest. The palm *E. edulis* plays a prominent role in directly structuring N availability (in the form of ammonium) and, through its frugivory interaction with large frugivores, nitrification rates. Given the hyper-dominance of the species, its high fruit production and the nutritional quality (fiber, fatty acids and anthocyanins, Santamarina et al., 2019) of the fruits produced it is likely that such effects might be mediated by its consumers. Fruits of this palm attract many frugivores, so that an increase in *E. edulis* densities and a concomitant increase in the number of consumers might lead to increasing inputs of ammonium through excretion of droppings, urine and faeces. A variety of arboreal species (birds and bats) and ground-dwelling small mammals with access to both experimental treatments feeding on palm fruits (Galetti & Aleixo, 1998; Galetti et al., 1999, 2013) might provide a substantial source of this nutrient with increasing palm abundance. Camera trap records indeed show that small mammals, not excluded by the fenced treatment, were present in both treatments with similar numbers of records, suggesting an equivalent contribution to soil nitrogen availability in both treatments. Arboreal primates though, might have had an impact on nitrogen cycling across treatments,
but the absence of records of primates feeding on the fruits of *E. edulis* here or elsewhere across the Atlantic Forests (Bufalo et al., 2016) clearly discards any link to an increase in available nitrogen in palm hotspots. Higher concentrations of ammonium on open plots than on exclosures, in turn, might be the result of the additional excreta from large ground-dwelling frugivores, specially from the white-lipped peccary (the dominant large frugivore in our plots), which are also strongly attracted to palms (Akkawi et al., 2020; Beck, 2006; Keuroghlian & Eaton, 2009). A previous study showed that densities of *E. edulis* also structure alpha and beta diversity of seedlings, and affects their recruitment (Villard et al., 2020). Analogous structuring effects have been found for several foundation tree species in temperate forests that control ecosystem structure and dynamics (Ellison et al., 2005), and suggest a foundational role for *E. edulis* in the Atlantic Forest.

An intuitive proximate mechanism that can lead to elevated levels of nitrification with increasing palm density on plots where large frugivores had access might be related to the mechanical impact of large frugivores. With their trampling and feeding, large frugivores alter the physical conditions of the soil, which in turn may affect porosity, water retention and nitrification and denitrification rates (Schrama et al., 2013). In tropical grasslands of southern Africa for example, large herbivores increase soil compaction and evaporation rates (Veldhuis et al., 2014). In our site, as in many undisturbed areas across the Atlantic Forest, the white-lipped peccary is the dominant large terrestrial herbivore, accounting for about 80%-85% of the whole mammal biomass (Galetti et al., 2017). Peccaries are generalist consumers feeding on a variety of fruits, roots, fungi and other food resources occurring in the understorey of tropical forests, yet they are strongly attracted to palms (Akkawi et al., 2020; Beck, 2006; Keuroghlian & Eaton, 2009). Through their feeding behaviour, peccaries overturn large amounts of soil (Beck, 2006; Keuroghlian & Eaton, 2009), which might decrease soil compaction and increase the porosity of soils. They also forage in large herds, often in excess of the hundreds, so that their physical impact on soil and simultaneous N input through excretion is likely to be substantial. Furthermore, by overturning large amounts of soil, peccaries might mobilize phosphorous towards the upper layers of the soil, stimulating nitrification rates, which are themselves limited by phosphorous availability (Purchase, 1974). Thus, through elevated ammonium inputs from excretion and high mechanical impacts on the soil, peccaries might simultaneously stimulate nutrient cycling through alternative pathways (Schrama et al., 2013; Veldhuis et al., 2014).

Results indicated that on plots where large frugivores had access, nitrification processes strongly regulate available N, suggesting that available N losses from denitrification by anaerobic bacteria or immobilization during the incubation period were largely outweighed by the performance of nitrifying bacteria but only on plots where large herbivores have access. For example, on open plots, mineralization rates predicted TotN availability with high accuracy, but this was not the case in exclosure plots. Tropical rainforests are subject to elevated rainfall that can eventually lead to occasional waterlogging of soils depending on the soil texture, promoting hypoxic conditions associated to anaerobic metabolism and larger denitrification rates (Pajares & Bohannan, 2016). It is possible that through their mechanical action, large ground-dwelling frugivores might increase soil evapotranspiration, hence driving wet soils to dryer and more aerobic conditions, promoting the performance of nitrifying bacteria and stimulating decomposition rates, as suggested elsewhere (Schrama et al., 2013). It is also remarkable that despite temporal variance in N cycling rates in open plots where large frugivores had access, the efficiency of nitrification rates (i.e. the slope of the positive correlation between TotN and nitrate, and N cycling rates after the incubation period) on these plots was very consistent across dates (Figure S2). Such observation reinforces the view that soil microorganisms play an important role in mediating the effects of large frugivores on N cycling.

Whilst primary productivity in many tropical forests is assumed to be phosphorous limited, there is growing evidence of limitation and co-limitation by other nutrients, such as nitrogen and potassium (Marklein & Houlton, 2012; Pajares & Bohannan, 2016). In our site, the amount of N available to plants varied by two orders of magnitude among plots, contradicting the monolithic view that tropical forest soils are homogenously N-saturated. Our results support the notion that N availability is indeed highly spatially heterogenous in these forests, and that large frugivores alter substantially the spatial distribution of N availability and N cycling through (a) local-scale positive N cycling feedback on soils where available N is high, and (b) landscape-scale redistribution of available N amongst forest patches.

At the local scales, our results indicate that large frugivores caused a positive in-situ feedback on soil N cycling in forest patches with high amount of soil ammonium. In these patches, where palms dominate, large frugivores increased nitrification rates and therefore stimulated the conversion of ammonium into nitrate. This was evident in incubated samples with high nitrification rates, which were clearly enriched with nitrate. However, we could not find such trend in on-field samples before the incubation period (Figure S2), neither was nitrate aligned with palm density. This suggests that in those palm-rich patches nitrate is limiting, so that despite high ammonium availability and high nitrification rates, most of the nitrate produced might be uptaken by plants. We suggest that by increasing nitrification rates on areas of high palm fruit production, improving the performance of nitrifying microorganisms and decreasing losses through denitrification (that results in the release of N₂O, an important greenhouse gas) and microorganism assimilation, large frugivores might streamline N uptake by plants. In some grassland ecosystems, large grazers maintain ‘grazing lawns’ (Hempson et al., 2015), where higher available N and stimulation of N cycling rates by large herbivores are linked to increased nutrient transfer to plants leading to increased primary productivity (though the converse is not necessarily true, Schrama et al., 2013). Our results from the below-ground section of the system strongly support the notion of ‘fruiting lawns’ maintained by large frugivores in the Atlantic Forest, yet cannot resolve whether large frugivores may actually accelerate N cycling in the above-ground section of the ecosystem.
In addition, our study also provides strong evidence of the impact of large frugivores in modulating the spatial variance in nutrient availability at the landscape scale. Large frugivores appeared to reduce landscape-level variability in N availability to plants but simultaneously increase variability in N cycling rates. Highly mobile peccary herds have large home ranges and move across large transects of neotropical forests on a daily basis. Thus, their excretion, foraging and mechanical impacts on the understory are widely distributed across the forest, and so is their contribution to the pool of ammonium and, indirectly, to nitrate by stimulating nitrification rates. This would explain how large frugivores shifted some of the less fertile soil spots towards higher values of available N through increases in ammonium and nitrate, some of these being of orders of magnitude larger on controls than on exclosures. Our results show that this process shifts the overall frequency distribution of these nutrients towards more positive values. Furthermore, the lower variance and coefficient of variation in the frequency distribution of ammonium, nitrate and TotN in control plots suggests that large frugivores modulate the levels of available N to plants at the landscape scale, leading to a more homogeneous nutrient supply at large spatial scales. In contrast, frugivores increased variance in N cycling rates, a pattern consistent with evidence from ungulate exclusion experiments in some grassland ecosystems where grazers accelerate nitrogen cycling (Frank & Groffman, 1998).

Diffusion models at macro-ecological scales suggest that extant megafauna might have had an important role in the transport of nutrients at large spatial scales in neotropical forests (Doughty et al., 2013, 2016). Yet, empirical studies directed at measuring the effects of nutrient transport within a single ecosystem are scarce. On savanna ecosystems, recent evidence suggests that whilst megaherbivores concentrated nutrient accumulation on low-risk open areas, megaherbivores such as the white rhino Ceratotherium simum distributed nutrients more homogeneously across the landscape (Le Roux et al., 2018). Furthermore, wildebeest Connochaetes taurinus and hippopotami Hippopotamus amphibius subsidize aquatic food webs in African savannas (Pringle, 2017). Our results suggest that large ground-dwelling tropical frugivores can also redistribute large amounts of nutrients within tropical rainforests. Thus, large frugivores might have a relevant role in mobilizing and transporting large amounts of nutrients among forest patches with differential nutrient loads, hence contributing to regulate primary productivity in tropical forests.

Our study addresses a key missing piece of evidence from the rich literature involving the impact of large herbivores on ecosystem functions. Yet, the generality of our findings should be validated through research on other ecosystems with different frugivory systems, and the relevance of palm–frugivore interactions on other neotropical systems. The dominance, key role as food resource and wide geographical distribution of E. edulis strongly suggest that it may act as a foundation species across the Atlantic Forest. Another member of the Euterpe genera, E. precatoria, is the most abundant tree in the Amazon (ter Steege et al., 2013), and, on a broader geographical scale, palms are dominant and play key roles as resources for many large frugivores. Thus, it is likely that palm-large frugivore interactions have a prominent role structuring nutrient cycling in other neotropical ecosystems. Future detailed studies should also address some of the detailed mechanisms behind the patterns investigated (here inferred from naturalistic observations and the available literature involving grazers and browsers in the absence of studies involving frugivores) and quantify shifts in soil microbial processes and functional groups.

The temporal and spatial scale of the experiment might have partially influenced our results. For example, the small size of the exclosures might prevent the compensatory buildup of large densities of small sized consumers, such as invertebrates or small mammals. Camera trap data show little differences in small mammal abundance between plot types, suggesting that small mammal densities in the exclosures were similar to those outside of them. Yet, in the Atlantic Forest, like in many other ecosystems (Dirzo et al., 2014; Villar, Cornulier, et al., 2014), small mammals thrive in the absence of large vertebrates (Galetti et al., 2015; Galetti, et al., 2015), and might potentially compensate for the loss of large frugivores in defaunated forests. However, the evidence from the few large scale replicated exclusion experiments in other ecosystems suggest that compensatory effects of small mammals or any other small-sized consumers are unlikely (Austrheim et al., 2014; Bakker et al., 2006; Dirzo et al., 2014; Evans et al., 2015). In addition, it might be argued that nutrients from the surrounding forest where frugivores had access might have leached to the interior of the exclosures, thus reducing differences between experimental treatments. In relation to temporal scales, it is likely that full effects of N cycling on forest dynamics might take more than decades to appear, and this might not be visible within the period of exclusion (8 years). However, the ‘fruiting lawns’ model proposed here does not require growth of saplings and forest regeneration, and we expect that large herbivore effects on N uptake and fruit production on existing adult trees might be almost immediate. Lastly, whilst our replicated experimental paired open versus exclusion plot design is robust against local heterogeneity in confounding variables, it is possible that the magnitude of our results might be contingent on the specific biotic and abiotic conditions found on our site. Thus, we hope that our study inspires future work evaluating the generality of our findings and the mechanisms involved.

This work demonstrates that large frugivores have a substantial role not only in redistributing nutrients, but also in modulating nutrient cycling rates across gradients in key resource availability. The combined effect of both processes has the potential to drive a wealth of other related ecosystem processes, including decomposition rates, primary productivity, carbon sequestration and greenhouse gas emissions (Pajares & Bohannan, 2016; Townsend et al., 2011). Preserving the key roles of large frugivores and their key resources will require strong conservation measures aiming at halting the relentless pace of defaunation in the neotropics and concomitant illegal harvesting of palms and deforestation in these forests.
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AUTHORS' CONTRIBUTIONS
N.V., M.G., C.P., V.Z. and S.N. designed the research; N.V., M.G. and E.S.B. conceived the manuscript; N.V., C.P., L.B. and S.N. collected the data; N.V. analysed the data and drafted the manuscript; N.V., E.S.B., C.P., M.G. and L.B. contributed towards the final version of the manuscript.

DATA AVAILABILITY STATEMENT
Data deposited in the Dryad Digital Repository https://doi.org/10.5061/dryad.fbg79cns (Villar, Paz, et al., 2014).

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

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