



Nitrogen dynamics during ecosystem development in tropical forest restoration

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ABSTRACT

We considered whether ecological restoration using high diversity of native tree species serves to restore nitrogen dynamics in the Brazilian Atlantic Forest. We measured $\delta^{15}\text{N}$ and N content in green foliage and soil; vegetation N:P ratio; and soil N mineralization in a preserved natural forest and restored forests of ages 21 and 52 years. Green foliage $\delta^{15}\text{N}$ values, N content, N:P ratio, inorganic N and net mineralization and nitrification rates were all higher, the older the forest. Our findings indicate that the recuperation of N cycling has not been achieved yet in the restored forests even after 52 years, but show that they are following a trajectory of development that is characterized by their N cycling intensity becoming similar to a natural mature forest of the same original forest formation. This study demonstrated that some young restored forests are more limited by N compared to mature natural forests. We document that the recuperation of N cycling in tropical forests can be achieved through ecological restoration actions.

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1. Introduction

It is well known that nitrogen (N) and phosphorus (P) are the most limiting nutrients to plant growth in young secondary tropical forests (Davidson et al., 2004). From an ecosystem perspective, this proposition is especially valid for young forests that have high metabolic rate potentials growing on degraded soils, such as areas undergoing restoration after many years of intensive use for agriculture in the tropics. The development of these forests ultimately depends on changes of ecosystem functioning and processes.

In abandoned agricultural areas, secondary succession often starts with low N availability (Vitousek et al., 1989). Re-accumulation of N during secondary succession has been demonstrated for a fairly wide range of regenerating communities (Hooker and Compton, 2003; Davidson et al., 2007; Knops and Bradley, 2009; Leduc and Rothstein, 2010). N cycling properties in secondary tropical forests regenerating after agricultural abandonment recover over time with succession, and P can become even more limiting than N at a certain point of ecosystem development (Davidson et al., 2007).

Although the patterns of change in nutrient dynamics during secondary succession of abandoned agricultural areas through

natural regeneration have been explored for some tropical ecosystems (Vitousek et al., 1989; Davidson et al., 2007), it remains unclear how nutrient dynamics change during tropical forest restoration, and the few studies that have been done focus on relatively recently restored forests (<10 years). Some recent advances in this field of knowledge have highlighted interesting traits, such as the importance of relative compositions of species in determining nutrient ratios, especially regarding the role played by N-fixing trees (Macedo et al., 2008; Siddique et al., 2008). It seems reasonable to assume that, for forests undergoing restoration, changes in N dynamics occur through similar processes as known for regenerating forests. However, such changes have not been demonstrated yet.

Due to the relatively new character of ecological restoration as a science, and especially to the scarcity of old restored forests worldwide, very little is known about how nutrient dynamics change over secondary succession of tropical forests that have undergone restoration, compared to what has already been investigated for natural regeneration. We examined how N and P dynamics differ along secondary succession of restored tropical forests in 21- and 52-year old highly diverse plantings in the Brazilian Atlantic Forest. We hypothesized that, as restored tropical forests age, the N dynamics in the ecosystem would become more similar to values found in native mature forests. To evaluate forests, we used parameters that indicate the maturity of N cycling and the increase of the N pools in the system (adapted from Davidson et al., 2007). Such parameters include N concentration and composition, $\delta^{15}\text{N}$, vegetation N:P ratio, and N mineralization

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in soil. All parameters are derived from chemical analysis of green foliage, litter and soil.

2. Material and methods

2.1. Study sites

We selected two restored forests, 21- and 52-years old, and a well-preserved natural forest to evaluate parameters related to nitrogen dynamics. All three forests are classified as Seasonal Semideciduous Forest, in the Atlantic Forest biome (Veloso, 1992). The forests are located in different municipalities in São Paulo state, Brazil, with a maximum distance between sites of ca. 65 km. The 21-year old forest is located in Iracemápolis (between 22°35'S and 47°31'W; 605 m above the mean sea level); the 52-year old in Cosmópolis (between 22°39'S and 47°12'W; 560 m above the mean sea level); and the natural forest is located in Campinas (between 22°50'S and 46°55'W; 650 m above the mean sea level). The two restored sites were deforested and used for agricultural purposes for many years (exact dates of deforestation are unknown) before being reforested with high diversity of tree species, and a predominance of regional native trees. The 21-year old forest was reforested with about 140 tree species (Rodrigues et al., 1987) and the 52-year old forest with approximately 70 different species of trees (Nogueira, 1977). All three forests have a well defined closed canopy. Average canopy height is 7.9 ± 0.9 m in the 21-year old forest, 10 ± 3 m in the 52-year old forest (L.G. Couto, personal communication). The natural forest has a dense vegetation and average canopy height is 20 ± 5 with emergent trees reaching up to over 30 m (Santos and Kinoshita, 2003). A floristic survey by Santos and Kinoshita (2003) in the natural forest, taken as a reference here, listed 150 species of trees. In the younger forest, understory is composed mostly by young trees resulting from reproduction of planted older trees. The intermediate aged forest has a better defined structure with some understory characteristic species, and also many young trees from reproduction of planted older trees. The natural forest, in turn, has a structure common of a natural preserved tropical forest, with a well defined understory layer. After randomly selecting trees for sampling, respecting diameter classes proportions of each forest, we sampled from 30 different species in the 21-year old forest, 34 in the 52-year old forest and 38 in the natural forest (Table 1). Climate is classified as Cwa (Köppen, 1948), with a rainy season during the summer and a dry season during the winter. Mean annual temperature is 21.5 °C, and mean annual precipitation is approximately 1400 mm (CEPAGRI, 2009a, b, c). Forests are under the same climate, but different soil types (Latosols for the restored forests and Podzolic soil for the natural forest) (Nogueira, 1977; Rodrigues et al., 1987; Santos and Kinoshita, 2003). A brief characterization of soils of the studied forests was performed to better understand differences between the sites (Tables 2 and 3). We randomly selected five sampling spots in each forest and collected soil from three layers (0–10 cm, 10–30 cm, and 30–50 cm). In each study site, samples from a given layer composed one composite sample. Methods for soil analysis are described in Rajj et al. (2001).

2.2. Experimental design and sampling

We used indicators, calculated from laboratory analysis of samples of soil, foliage and litter, to compare important indicators related to N dynamics in the forests studied. This indicators-based approach (adapted from Davidson et al., 2007) allows us to compare different forests, in a community scale, by simple trustable values that represent important processes related to N dynamics

and their variation in space (different individuals/plots/depth) and time (different seasons).

Each of our three study sites comprised a 2.5-hectare portion of riparian forest. At each site, we established 30 vegetation plots (10 × 10 m) for green foliage sampling, randomly allocated at a maximum distance of 70 m from the water edge; five transects equally distributed with 10 collection plots for litter sampling; four plots for sampling the soil profile in five layers (0–2.5 cm, 2.5–5 cm, 5–10 cm, 10–30 cm, 30–50 cm) for $\delta^{15}\text{N}$ analysis; and 20 plots for surface soil sampling (0–5 cm) randomly located inside 20 of 30 vegetation plots for N mineralization and water content analysis. Samples for all indicators were collected during the peak of the dry season (July 2008) and the rainy season (February 2009). To determine N mineralization related parameters, besides sampling surface soil during the peaks of the dry and the rainy seasons, we also sampled it in the transition to the rainy season (October 2008) and in the transition to the dry season (April 2009).

2.3. Nitrogen mineralization

We estimated N mineralization parameters using methods adapted from Picollo et al. (1994). During the two seasons and transitions, samples of approximately 500 cm³ (10 × 10 × 5 cm) were collected, as described in the above section, and maintained refrigerated until processed. All the material was homogenized (2 mm mesh) and had roots removed. Each sample was divided into three parts: (1) 10 g for determining water content; (2) 10 g for extraction in “Time zero” (T0); (3) the remaining material for incubation and extraction at “Time seven” (T7). Water content was determined by the gravimetric method, with samples weighted, dried out at 105 °C for three days, and weighed again. Samples were incubated at room temperature inside wrapped plastic bags. Extractions were made using KCl 1 M (mol L⁻¹) (Merck®) before (T0) and after the incubation period of seven days (T7). The material was filtered using quantitative filter paper 42 125 mm (Whatman®), preserved with the addition of Phenylmercuric acetate (PMA) (0.5 mg L⁻¹) and refrigerated. We used an automated flow injection system (Ruzicka and Hansen, 1981) to determine N-NH₄⁺ and N-NO₃⁻ + N-NO₂⁻ concentrations. N-NH₄⁺ was determined by conductimetry, after the Nessler reaction; N-NO₃⁻ + N-NO₂⁻ were determined, by spectrophotometry, as N-NO₂⁻, after reduction by a Cadmium catalyst. Data are presented in $\mu\text{g N g}^{-1} \text{ DS}$ (Dry Soil). A net mineralization rate was calculated from the initial minus the final concentration of N-NH₄⁺ + N-NO₃⁻, divided by 7 days of incubation. Net nitrification rate was calculated from the initial minus the final concentration of N-NO₃⁻, also divided by seven days of incubation. Results are presented based on soil mass dried out at 105 °C. N-NH₄⁺ and N-NO₃⁻ concentrations were calculated from values in $\mu\text{g g}^{-1}$ measured for the extraction before incubation, divided by the mass of dry soil and multiplied by the volume of KCl.

For isotopic analysis, we dried soil profile samples at room temperature, passed them through a 2-mm mesh, removed rocks and dead organic particulates, grounded and sieved them to fine powder (0.250-mm mesh). We then determined $\delta^{15}\text{N}$ and N concentrations using a Combustion Elementary Analyzer (EA-Carlo Erba) aligned with a Mass Spectrometer (Delta Plus, Finnigan Mat, San José, CA, USA). Atmospheric air was used as standard, and accepted analytical errors for N concentration and ¹⁵N were 0.03% and 0.30‰, respectively.

2.4. Green foliage and litter

The first step for sampling green foliage of forest communities involved a phytosociological study for means of distributing trees in diameter classes. In all three forests, trees of DBH equal to or

Table 1

Tree species sampled for foliar analysis in the studied forests (21-year old and 52-year old restored forests, and natural forest). Number of trees per species sampled in each forest are given along with information of ecological group (N = native; E = exotic; NRN = non-regional native).^a

Scientific name	Author	Family	Group	21-year old	52-year old	Natural forest
<i>Acacia cf riparia</i>	Kunth	Fabaceae	N			1
<i>Actinostemon communis</i>	(Müll. Arg.) Pax	Euphorbiaceae	N			6
<i>Actinostemon concolor</i>	(Spreng.) Müll. Arg.	Euphorbiaceae	N			1
<i>Aegiphila sellowiana</i>	Cham.	Verbenaceae	N	1		
<i>Alchornea glandulosa</i>	Poepp.	Euphorbiaceae	N			1
<i>Aleurites moluccana</i>	Willd.	Euphorbiaceae	E		2	
<i>Allophylus edulis</i>	(A. St.-Hil., Cambess. & A. Juss.) Radlk.	Sapindaceae	N		1	
<i>Almeidea caerulea</i>	A. St.-Hil. ex G. Don	Rutaceae	N			4
<i>Amaioua intermedia</i>	Mart.	Rubiaceae	N			1
<i>Aspidosperma polyneuron</i>	Müll. Arg.	Apocynaceae	N			3
<i>Aspidosperma ramiflorum</i>	Müll. Arg.	Apocynaceae	N		1	1
<i>Astronium graveolens</i>	Jacq.	Anacardiaceae	N			4
<i>Brosimum glaziovii</i>	Taub.	Moraceae	N			1
<i>Caesalpinia peltophoroides</i>	Benth.	Fabaceae	N	1		
<i>Cariniana estrellensis</i>	(Raddi) Kuntze	Lecythidaceae	N	3	4	
<i>Cariniana legalis</i>	(Mart.) Kuntze	Lecythidaceae	N	4		1
<i>Casearia sylvestris</i>	Sw.	Salicaceae	N		1	
<i>Centrolobium tomentosum</i>	Guillemin ex Benth.	Fabaceae	N	16	2	
<i>Chionanthus filiformis</i>	(Vell.) P.S. Green	Oleaceae	N			1
<i>Clausena excavata</i>	Burm. f.	Rutaceae	E	8		
<i>Colubrina glandulosa</i>	Perkins	Rhamnaceae	N		5	
<i>Copaifera langsdorffii</i>	Desf.	Fabaceae	N	1		
<i>Cordia americana</i>	(L.) Gottschling & J.S. Mill.	Boraginaceae	N		1	
<i>Cordia ecalyculata</i>	Vell.	Boraginaceae	N			1
<i>Cordia myxa</i>	L.	Boraginaceae	E	2		
<i>Cordia trichotoma</i>	(Vell.) Arráb. ex Steud.	Boraginaceae	N	1		
<i>Croton floribundus</i>	Spreng.	Euphorbiaceae	N			1
<i>Dalbergia nigra</i>	(Vell.) Allemao ex Benth.	Fabaceae	NRN		6	
<i>Dendropanax cuneatus</i>	(DC.) Decne. & Planch.	Araliaceae	N		1	
<i>Dillenia indica</i>	L.	Dilleniaceae	E	1		
<i>Esenbeckia leiocarpa</i>	Engl.	Rutaceae	N	1		8
<i>Eugenia uniflora</i>	L.	Myrtaceae	N		3	
<i>Ficus citrifolia</i>	Mill.	Moraceae	N		1	
<i>Ficus guaranitica</i>	Chodat	Moraceae	E	1		
<i>Galipea multiflora</i>	Schult.	Rutaceae	N			2
<i>Guapira opposita</i>	(Vell.) Reitz	Nyctaginaceae	N			1
<i>Guarea guidonia</i>	(L.) Sleumer	Meliaceae	N	3		
<i>Guarea sp.</i>		Meliaceae	N		5	
<i>Guazuma ulmifolia</i>	Lam.	Malvaceae	N		2	
<i>Handroanthus chrysotrichus</i>	(Mart. ex A. DC.) Mattos	Bigniniaceae	N	1	1	
<i>Holocalyx balansae</i>	Micheli	Fabaceae	N			2
<i>Hymenaea courbaril</i>	L.	Fabaceae	N		3	1
<i>Ixora gardneriana</i>	Benth.	Rubiaceae	N			1
<i>Joannesia princeps</i>	Vell.	Euphorbiaceae	NRN		3	
<i>Lafoensia glyptocarpa</i>	Koehne	Lythraceae	N	1		
<i>Licania tomentosa</i>	(Benth.) Fritsch	Chrysobalanaceae	NRN	9		
<i>Lonchocarpus muehlbergianus</i>	Hassl.	Fabaceae	N		6	
<i>Luehea divaricata</i>	Mart.	Malvaceae	N		1	
<i>Machaerium nyctitans</i>	(Vell.) Benth.	Fabaceae	N		1	
<i>Machaerium vestitum</i>	Vogel	Fabaceae	N		2	
<i>Mangifera indica</i>	L.	Anacardiaceae	E		3	
<i>Matayba cf. elaeagnoides</i>	Radlk.	Sapindaceae	N			1
<i>Metrodorea nigra</i>	A. St.-Hil.	Rutaceae	N			7
<i>Myrciaria floribunda</i>	(H. West ex Willd.) O. Berg	Myrtaceae	N			1
<i>Myroxylon peruiferum</i>	L.f.	Fabaceae	N	1		
<i>Nectandra megapotamica</i>	(Spreng.) Mez	Lauraceae	N	3	6	
<i>Ocotea beulahiae</i>	Baitello	Lauraceae	N			3
<i>Ocotea elegans</i>	Mez	Lauraceae	N			1
<i>Ocotea indecora</i>	(Schott) Mez	Lauraceae	N			2
<i>Ocotea odorifera</i>	Rohwer	Lauraceae	N			1
<i>Ormosia arborea</i>	(Vell.) Harms	Fabaceae	N			2
<i>Pachira aquatica</i>	Aubl.	Malvaceae	N	1		
<i>Parapiptadenia rigida</i>	(Benth.) Brenan	Fabaceae	N	4		
<i>Parapiptadenia sp.</i>		Fabaceae	N		2	
<i>Piper amalago</i>	L.	Piperaceae	N		1	
<i>Piptadenia gonoacantha</i>	(Mart.) J.F. Macbr.	Fabaceae	N	1	1	
<i>Pittosporum undulatum</i>	Vent.	Pittosporaceae	E		1	
<i>Poecilanthe parviflora</i>	Benth.	Fabaceae	N	2		
<i>Pterocarpus violaceus</i>	Vogel	Fabaceae	N	2		
<i>Pterogyne nitens</i>	Tul.	Fabaceae	N	1	1	
<i>Rudgea jasminoides</i>	(Cham.) Müll. Arg.	Rubiaceae	N			1
<i>Sapindus saponaria</i>	L.	Sapindaceae	NRN	1		
<i>Savia dictyocarpa</i>	Müll. Arg.	Euphorbiaceae	N			8

(continued on next page)

Table 1 (continued)

Scientific name	Author	Family	Group	21-year old	52-year old	Natural forest
<i>Schinus terebinthifolia</i>	Raddi	Anacardiaceae	N	2		
<i>Sebastiania edwalliana</i>	Pax & K. Hoffm.	Euphorbiaceae	N			1
<i>Seguiera langsdorffii</i>	Moq.	Phytolaccaceae	N			1
<i>Senna macranthera</i>	(DC. ex Collad.) H.S. Irwin & Barneby	Fabaceae	N	1		
<i>Solanum argenteum</i>	Dunal	Solanaceae	N			1
<i>Syzygium cumini</i>	(L.) Skeels	Myrtaceae	E	4	2	
<i>Syzygium jambos</i>	(L.) Alston	Myrtaceae	E	1		
<i>Tabebuia impetiginosa</i>	(Mart. ex DC.) Standl.	Bigniniaceae	N		1	
<i>Tabebuia roseoalba</i>	(Ridl.) Sandwith	Bigniniaceae	N		1	
<i>Tipuana tipu</i>	(Benth.) Kuntze	Fabaceae	E		1	
<i>Trichilia catigua</i>	A. Juss.	Meliaceae	N			3
<i>Trichilia clausenii</i>	C. DC.	Meliaceae	N		7	2
<i>Trichilia elegans</i>	A. Juss.	Meliaceae	N			1
<i>Trichilia sp.</i>	P. Browne	Meliaceae	N		1	
<i>Triplaris americana</i>	L.	Polygonaceae	NRN	1		
<i>Zanthoxylum hyemale</i>	A. St.-Hil.	Rutaceae	N			1
<i>Zanthoxylum minutiflorum</i>	Tul.	Rutaceae	N			1

^a Total number of trees sampled (*n*) is 79 for the 21-year old forest, 80 for the 52-year old forest, and 80 for the natural forest.

Table 2

Soil physical characteristics of the studied forests (21-year old and 52 year old restored forests, and natural forest).^{a,b}

Forest	Layer (cm)	Total sand (g/kg)	Gravel (2.00–0.210 mm) (g/kg)	Sand (0.210–0.053 mm) (g/kg)	Silt (0.053–0.002 mm) (g/kg)	Clay (<0.002 mm) (g/kg)
21-year old	0–10	340	150	190	179	481
21-year old	10–30	349	140	209	207	444
21-year old	30–50	350	160	190	227	423
52-year old	0–10	390	200	190	402	208
52-year old	10–30	399	209	190	282	319
52-year old	30–50	340	180	160	344	316
Nat. For.	0–10	590	450	140	270	140
Nat. For.	10–30	560	400	160	239	201
Nat. For.	30–50	490	340	150	306	204

^a Mean values ± SE are given for the parameters listed. Values were calculated from laboratory analysis of composite samples composed of 5 subsamples.

^b Soils were sampled in three layers (0–10 cm, 10–30 cm, 30–50 cm) in June 2008.

Table 3

Soil characteristics of the studied forests (21-year old and 52 year old restored forests, and natural forest).^{a,b}

Forest	Layer (cm)	pH (CaCl ₂)	N (mg/kg)	P resin (mg/dm ³)	O.M. (g/dm ³)	Cation exchange capacity (mmolc/dm ³)					S.B. (V%)	Sat. bases (V%)	Sat. Al (m%)	S (SO ₄) (mg/dm ³)	
						K	Ca	Mg	H + Al	Al					
21-year old	0–10	4.6	700	12	32	0.8	19	9	58	4	29	87	33	12	15
21-year old	10–30	4.5	560	9	23	0.5	12	6	58	5	19	77	24	21	17
21-year old	30–50	4.7	560	5	21	0.3	13	5	47	3	18	65	28	14	25
52-year old	0–10	5.1	1680	17	44	1.5	79	32	47	3	113	160	71	3	12
52-year old	10–30	4.6	840	7	25	0.6	49	24	58	2	74	132	56	3	30
52-year old	30–50	4.7	630	14	20	0.5	55	24	52	3	80	132	60	4	37
Nat. For.	0–10	4.7	2380	16	53	3.0	41	12	52	2	56	108	52	3	10
Nat. For.	10–30	4.4	1400	11	36	2.5	24	9	52	4	36	88	41	10	10
Nat. For.	30–50	4.1	700	7	17	1.8	14	7	58	11	23	81	28	33	15

^a Mean values ± SE are given for the parameters listed. Values were calculated from laboratory analysis of composite samples composed of five subsamples.

^b Soils were sampled in three layers (0–10 cm, 10–30 cm, 30–50 cm) in June 2008.

higher than 15 cm were measured and identified. A random stratified sampling was performed based on distribution of the trees into diameter classes. In each forest, 80 trees were sampled in the dry season and re-sampled in the rainy season. Samples were obtained from branches in the outer part at the middle third of tree crowns. For litter sampling, five transects were systematically distributed in each forest, perpendicular to the water-forest edge. Along each transect, we distributed 10 plots of 0.25 m² and collected all “fine litter”, *sensu* Vitousek et al. (1982), accumulated on forest floor. In laboratory, we removed eventual soil particles and dead animal remnants from litter samples in order to process mainly vegetal material, which was in different stages of decomposition. Samples of green foliage and litter were dried at 60 °C and

ground to fine powder. Nitrogen concentration and isotope ratios were determined using a Combustion Elementary Analyzer (EA-Carlo Erba) aligned with a Mass Spectrometer (Delta Plus, Finnigan Mat, San José, CA, USA). Atmospheric air was used as standard, and accepted analytical errors for N concentration and ¹⁵N were 0.03% and 0.30‰, respectively. Phosphorus concentration was determined by the Ammonium Metavanadate method (Malavolta et al., 1989).

2.5. Vegetation N:P ratio

The stoichiometric relation between N and P was evaluated for green foliage and litter at the community scale for all forests for

both dry and rainy seasons. We developed a related indicator, the N/P Economy Index, to analyze which nutrient is more limiting to each forest, derived from the indicator proposed by Koerselman and Meuleman (1996). The N/P Economy Index is calculated using a foliar N:P ratio divided by litter N:P ratio, and differences are based on proportionately different variations in foliar and litter N:P ratios. This index indicates resorption of one nutrient relative to the other. N/P Economy Index values greater than 1 indicate a higher tendency to resorb N in relation to P, whereas values lower than 1 indicate higher tendency to resorb P in relation to N. For the litter N:P ratio, each transect corresponded to a composite sample. For green foliage, five composite samples were composed of the trees within our vegetation plots around each litter transect.

2.6. Statistical analysis

All data were tested for normality and homogeneity of variance. Data transformations were used for some variables (Box and Cox, 1964): soil concentration of water, $N-NH_4^+$ and Inorganic N, and Net nitrification rate (logarithm transformation); soil concentration of $N-NO_3^-$ (multiplication by a factor of 0.2), soil $\delta^{15}N$ (multiplication by a factor of 1.5); soil N concentration (inverse square root); foliar N and P, and litter P concentrations (logarithm transformation); N/P Economy Index (inverse transformation). Statistical analyses were done using a group comparison: one-way ANOVA, Tukey's test ($\alpha = 0.05$), and Kruskal–Wallis' test ($\alpha = 0.05$). All statistical analysis was performed using the software SAS 9.1.

3. Results

Comparisons of annual means showed differences between the forests as surface soil (0–5 cm) concentration of $N-NH_4^+$ was significantly higher in the natural forest than in either of the restored forests. Concentration of $N-NO_3^-$ was similarly higher in the reference and the 52-year old forests, than in the 21-year old site. Inorganic N in the natural forest was higher than in the restored forests. The net mineralization rate was higher the older the restored forest, and was highest in the natural forest. The net nitrification rate was higher in the reference than in the two restored sites (Table 4). Surface soil (0–5 cm) moisture was lowest in the natural forest, intermediate in the 21-year old forest, and highest in the 52-year old forest.

Nitrogen was less concentrated in the soil of restored forests than in the natural forest. In contrast, soil $\delta^{15}N$ was lowest in the 52-year old site than in the 21-year old site and the reference site (Table 5).

Table 4

Mean values for surface soil (0–5 cm) concentrations of Ammonium, Nitrate, total inorganic N and net mineralization and nitrification rates in the studied forests (21-year old and 52-year old restored forests, and natural forest).^{a,b}

	21-year old forest	52-year old forest	Natural forest
$N-NH_4^+$ ($\mu g N g^{-1} DS$)	8.37 ^B ± 4.84	7.13 ^B ± 3.40	11.14 ^A ± 4.67
$N-NO_3^-$ ($\mu g N g^{-1} DS$)	4.97 ^B ± 2.92	7.07 ^A ± 3.82	7.76 ^A ± 4.80
Inorganic N ($\mu g N g^{-1} DS$)	13.34 ^B ± 6.74	14.20 ^B ± 5.68	18.89 ^A ± 7.62
Net mineralization rate	0.95 ^C ± 0.69	1.39 ^B ± 0.63	1.91 ^A ± 0.68
Net nitrification rate	1.42 ^B ± 0.77	1.56 ^B ± 0.63	2.29 ^A ± 0.92

^a Mean values ± SE are given along with grouping from Tukey's test. Significant differences ($p < 0.01$, $\alpha = 0.05$) are indicated in uppercase letters. Values followed by the same letters are not statistically different. Number of samples (n) is 78 for restored forests and 76 for the natural forest.

^b Mean values were calculated by grouping data from plots sampled in the dry and the rainy seasons, and in the two transitions in between.

Table 5

Mean values for N concentration and $\delta^{15}N$ in soils of the in the studied forests (21-year old and 52 year old restored forests, and natural forest).^{a,b}

	21-year old forest	52-year old forest	Natural forest
Soil N concentration (%)	0.13 ^A ± 0.08 (40)	0.12 ^A ± 0.07 (39)	0.17 ^B ± 0.11 (35)
Soil $\delta^{15}N$ (%)	6.99 ^A ± 1.68 (40)	6.40 ^B ± 2.01 (40)	6.93 ^A ± 1.60 (40)

^a Mean values ± SE are given along with grouping from Tukey's test. Significant differences ($p < 0.01$, $\alpha = 0.05$) are indicated in uppercase letters. Values followed by the same letters are not statistically different. Number of samples (n) is reported for each mean value.

^b Mean values were calculated by grouping samples from four soil profiles in each forest (0–2.5 cm, 2.5–5 cm, 5–10 cm, 10–30 cm, 30–50 cm) sampled in the dry season and re-sampled in the rainy season.

A total of 239 trees distributed in 91 species were sampled (Table 1). Foliar $\delta^{15}N$ was higher, the older the forest. The natural forest is 3.5 times more enriched than the 52-year old forest and five times more enriched with foliar $\delta^{15}N$ than in the 21-year old forest. Foliar N concentration was lower in the 21-year old forest than in the others. Foliar P concentration was lower in the natural forest than in restored forests (Table 6).

We found differences in foliar and litter N:P ratios between the sites with different values for all forests that were higher, the older the forest. The N/P Economy Index also varied systematically, being smaller with increasing forest age, showing significant difference between the restored sites and the reference site (Table 6).

4. Discussion

Our findings demonstrate that N dynamics are different according to the site age. We observed higher percentages of N in soil and plants, and higher mineralization rates at older ages. Systematic variation of N cycling indicators among the forests suggests recovery of N cycling processes and mechanisms with time. However, we also have to consider that these changes are due to differences in soil characteristics. Especially the youngest site (21-years old) has higher clay content than the older sites, and a lower content of basic cations (Tables 2 and 3). These differences may cause the same changes in N dynamics that we observed, independently of the forest age (Silver et al., 2000). Therefore, we can't rule out

Table 6

Mean values of foliar $\delta^{15}N$, foliar N and P concentration, foliar and litter N:P ratios, and N/P Economy Index in the studied forests (21-year old and 52-year old restored forests, and natural forest).^{a,b,c}

	21-year old forest	52-year old forest	Natural forest
Foliar $\delta^{15}N$ (%)	0.69 ^C ± 1.52 (160)	1.08 ^B ± 1.89 (160)	3.47 ^A ± 1.11 (160)
Foliar N concentration (%)	2.57 ^B ± 0.66 (160)	2.87 ^A ± 0.80 (160)	2.99 ^A ± 0.59 (160)
Foliar P concentration (%)	0.17 ^A ± 0.02 (9)	0.16 ^A ± 0.02 (10)	0.13 ^B ± 0.01 (10)
Foliar N:P	15.62 ^C ± 1.62 (9)	18.53 ^B ± 2.48 (10)	22.38 ^A ± 1.13 (10)
Litter N:P	15.51 ^C ± 2.37 (9)	21.07 ^B ± 6.02 (10)	30.25 ^A ± 2.10 (10)
N/P Economy Index	1.02 ^B ± 0.11 (9)	0.93 ^B ± 0.22 (10)	0.74 ^A ± 0.06 (10)

^a Mean values ± SE are given along with grouping from Tukey's test (Foliar N and P concentrations, Foliar and Litter N:P, and N/P Economy Index) and from Kruskal–Wallis' test (Foliar $\delta^{15}N$). Significant differences ($p < 0.01$; $\alpha = 0.05$) are indicated in uppercase letters. Values followed by the same letters are not statistically different. Number of samples (n) is reported for each mean value.

^b Foliar P concentration was determined for composite samples.

^c Mean values were calculated by grouping samples from 80 individuals sampled in each forest in the dry season and re-sampled in the rainy season.

the possibility that changes that we observed are attributed solely to stand development. Despite the fact our study reveals interesting information, we should acknowledge the experimental design we used does not represent true replications and imposes a limitation for inferential statistics interpretation of our data.

On the other hand, the differences in soil N concentrations with forest age observed in this research are in accordance with other studies of forests in secondary succession (Vitousek et al., 1989; Davidson et al., 2007). The values registered for net mineralization and net nitrification rates in the mid successional restored forest are low in comparison to the values in the later successional forest and to the even higher values in the natural forest. These values also agree with what is described in other studies for the secondary succession of abandoned agricultural areas (Vitousek et al., 1989; Davidson et al., 2007). This suggests that changes that we observed in our age transect may be due to an increase in N availability in soil as an ecosystem develops. These differences observed for N dynamics in soils suggest a gradually higher potential for NO_3^- losses both through leaching and gaseous emissions (Davidson et al., 2000) as tropical restored forests age. In the forests we studied, N dynamics in soil seem to be changing towards values common to natural mature forests.

Changes in soil N dynamics through time are related to changes in vegetation (Davidson et al., 2007; Siddique et al., 2008). Our results show that values of green foliage-derived parameters also are greater with forest age. Differences in the concentration of foliar N in the studied forests are indicative of accumulation of N during ecosystem development in restored forests. Additionally, significant differences in foliar ^{15}N natural abundance were observed in this research. As pointed out in a review by Craine et al. (2009), these patterns of change of foliar $\delta^{15}\text{N}$ and N concentration found in this study suggest that N is accumulated in the system with forest aging, and as N availability increases and forests become less limited by this nutrient, N losses also increase once the systems can afford losing N. These changes ultimately result in ^{15}N enrichment in the substrate (Vitousek et al., 1989; Martinelli et al., 1999). In contrast to what we found for foliar N concentration, foliar P concentration was significantly smaller with higher forest age in secondary succession, and was lower in forests with higher foliar $\delta^{15}\text{N}$, which was also a pattern highlighted by Craine et al. (2009).

The systematic variation observed for foliar N:P ratio and litter N:P ratio, larger with higher forest age, reflects the trajectory towards the shift from an N to a P economy. Foliar N:P ratio found for the restored 21-year old forest indicates that community may be limited by N as well as by P, whereas the values found for the 52-year old forest and for the natural forest indicate that both are more limited by P than by N (Koerselman and Meuleman, 1996). In our results, higher values of foliar N:P ratio were followed by higher values of foliar $\delta^{15}\text{N}$, consistent with Craine et al. (2009). The litter N:P ratio registered for the natural forest was significantly below the value reported for tropical forests (62) (McGroddy et al., 2004), below the value found for the Brazilian Amazon (50–68) (Davidson et al., 2007), and close to the global range reported for temperate broadleaf forests (29) (McGroddy et al., 2004). Further investigation is necessary to understand these findings.

The N:P Economy Index revealed that restored forests showed a pattern of N and P resorption similar to that reported for temperate forests (McGroddy et al., 2004), with small differences between foliar and litter N:P ratios. As expected, values for the natural forest fell within the range typical for tropical forests (McGroddy et al., 2004), with greater differences between foliar and litter N:P ratios. These results provide evidence that even the 52-year old forest does not possess N or P cycling characteristic of mature tropical forests, which show a strong tendency of resorption of P compared to N (McGroddy et al., 2004). Furthermore, the fact that N:P ratio was higher in green foliage than in litter in the 21-year old forest

indicates more resorption of N than P. The 52-year old forest, in turn, presented an index lower than 1, indicating more resorption of P, however, in a proportion still not similar to that found for the natural forest. These results support the hypothesis that younger forests are less limited by P and more limited by N compared to older forests (Davidson et al., 2007), since forests in more advanced successional stages and mature forests retranslocate more P compared to N before leaf abscission. We estimate that the shift from the N to P economy may occur in restored forests, when reforested with high species diversity and a predominance of native regional tree species, in about 50 years of forest growing the Seasonal Semi-deciduous Forest, in the Atlantic Forest biome.

Although other studies also have documented differences in N dynamics in reforested areas of the Atlantic Forest (e.g. Macedo et al., 2008; Siddique et al., 2008), this is the first time N differences have been evaluated in areas reforested using such a high species diversity. Additionally, we found interesting insights about the shift from N to P economy during ecosystem development of restored forests. Nitrogen re-accumulation in the system necessarily implies inputs of this element being greater than outputs. This balance certainly involves ecosystem mechanisms that promote increases in N inputs in the ecosystem, as well as more control over N outputs, resulting in longer residence time for this element in the forest. The role of organic forms of N in the dynamics of this element (not examined here) may also provide interesting insights about differences in N dynamics throughout secondary succession (Leduc and Rothstein, 2010). Organic matter accumulation in soils may be another important factor promoting an increase of N in the system (Kaye et al., 2003), and may comprise an important mechanism when degradation history resulted in significant losses of organic matter in soils. Moreover, plants may act on increasing N residence time in the ecosystem through changes in retranslocation rates of N before leaf abscission. N/P Economy Index provided evidence of differences in this mechanism of control among the studied forests, with higher N resorption rates, the younger the forest. There may be many other mechanisms acting on N re-accumulation in the ecosystems, including atmospheric N deposition, N fixation by symbiotic and free-living organisms in soil, leaves, tree trunks, and mycorrhizal associations.

5. Conclusion

Our findings indicate that the recuperation of N cycling has not yet been achieved in the studied restored forests, but show that they are following a trajectory of development characterized by their N cycling intensity becoming similar to a natural mature forest of the same original forest formation. The use of high species diversity with predominance of native trees to restore the studied forests potentially promotes the recuperation of N cycling as restored communities develop. This process includes gradual re-accumulation of nitrogen in the system, N availability increase, and a clear tendency of shifting from an N to a P economy, which is typical of mature natural tropical forests. However, in order to confirm these findings future studies have to control any other variable that may influence N dynamics. In our case, difference in soil characteristics, especially in the young site, allow us to only speculate about stand development and N enrichment, but not to reach a definite conclusion.

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