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REGULAR ARTICLE

Nitrogen isotopic patterns in tropical forests along a rainfall gradient in Northeast Brazil

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Abstract
Background and aims The interpretation of ecosystem nitrogen isotopic patterns is subject to controversies since there are few surveys of signals along climate gradients within the same region. We determined the effects of a rainfall gradient, under high temperatures, on the soil and plant $\delta^{15}N$ signals in Northeast Brazil.
Methods Nitrogen, carbon and $\delta^{15}N$ signals were determined from coastal perennial to subhumid and semiarid deciduous forests to savanna – deciduous forest transition.
Results Soil C and N concentrations were linearly related and both related quadratically with mean annual rainfall while $\delta^{15}N$ concentrations decreased with higher rainfall (16 to 4‰). Plant $\delta^{15}N$ was 1–3‰ lower than soil $\delta^{15}N$ ($\Delta\delta^{15}N$) in all areas. Only in the deciduous forests, leaves of target species had significantly lower ^{15}N signals than those of non-fixing species, allowing estimation of fixation.
Conclusions In the perennial forests the low signals indicated that the ^{15}N natural abundance method was not suitable to estimate fixation, while in the savanna – deciduous forest transition, high signals indicated that the legume species were not fixing. The smaller $\Delta\delta^{15}N$ than those reported in cooler areas and the absence of rainfall effect indicates that, when high, temperature overrides the rainfall effect.

Keywords N-15 depletion · Soil and plant $\delta^{15}N$ · High temperature · Symbiotic nitrogen fixation

Introduction

The biogeochemical cycle of nitrogen in land ecosystems involves several isotopic fractionation processes whose balance determines the $\delta^{15}N$ signals of their soil and plant components (Robinson 2001; Hedin et al. 2009). Inputs from symbiotic and free-living biological fixation have $\delta^{15}N$ signals close to 0‰ (Högberg 1997; Robinson 2001), while atmospheric depositions have a wide range, but usually positive, $\delta^{15}N$ signals (Agnihotri et al. 2011). Generally biological fixation prevails over deposition (Cleveland et al. 1999). Losses, from volatilization and leaching, have depleted $\delta^{15}N$ signals leaving the soil with positive signals (Shearer

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and Kohl 1986; Högberg 1997; Handley et al. 1999; Robinson 2001). Plants have more negative signals than soil and the difference depends on climatic conditions (Amundson et al. 2003) and on the preferential absorption of NO_3^- or NH_4^+ (Kahmen et al. 2008). Thus, promising applications of the signals are the possibility to determine N fixation and to estimate soil – plant system N dynamics (Craine et al. 2009).

The patterns revealed by several studies are that plant and soil $\delta^{15}N$ signals tend to decrease towards zero as the annual rainfall increases (Austin and Vitousek 1988; Handley et al. 1999; Amundson et al. 2003; Nardoto et al. 2008; Craine et al. 2009) and the signals are higher in tropical than in temperate forests (Martinelli et al. 1999; Amundson et al. 2003). The differences of plant and soil signals also decrease with increasing temperature and with decreasing rainfall and latitude (Amundson et al. 2003). This occurs because in more humid places the $\delta^{15}N$ results mainly from the interaction of the internal C cycling between dead and live organic compartments, with little N losses. This almost close cycle is interrupted in the tropics and especially in the dry tropics, where proportionally more N flows from the organic to the inorganic compartments, the latter being more subjected to losses, leading to the ^{15}N enrichment of soil and vegetation components (Handley and Scrimgeour 1997; Austin and Vitousek 1988; Handley et al. 1999; Martinelli et al. 1999; Aranibar et al. 2004; Swap et al. 2004; Freitas et al. 2010b). Therefore, dry places would have higher relative N availability to the plants than humid places, a hypothesis that is supported by the positive correlation between $\delta^{15}N$ signals and total N concentrations in plants (Craine et al. 2009; Viani et al. 2011). However, relatively high N losses have also been reported in tropical rainforests which would be compensated by fixation, and fixation would occur in spite of high relative N availability to the fixing plants, creating an apparent paradox to whose solution Hedin et al. (2009) proposed a few mechanisms.

The $\delta^{15}N$ signal also allows identifying plants with symbiotic N_2 fixation if the soil signal is distinctly different from zero, which occurs in some places (Freitas et al. 2010a; Andrews et al. 2011; Souza et al. 2012) but not in others (Roggy et al. 1999a; Gehring and Vlek 2004; Nardoto et al. 2008; Nardoto et al. 2014). If the plant biomass is known, the isotopic technique also allows determining the amount of N_2 biologically fixed (BFN).

In spite of these potential applications, the interpretation of patterns of differences of $\delta^{15}N$ among places and between soil and plants is still subject to controversies. Several exceptions to the general trends have been reported (Amundson et al. 2003; Swap et al. 2004; Freitas et al. 2010b) and the relation between plant $\delta^{15}N$ and climate is not completely clear (Craine et al. 2009). The isotopic signals of most of the tropical forests are unknown and there are few systematic surveys of signals along rainfall and soil gradients occurring at short distances, mainly at high mean annual temperatures (Craine et al. 2009; Handley et al. 1999). At mean annual temperatures above 23 °C, data on the comparison of soil and plant $\delta^{15}N$ were absent until few years ago (Amundson et al. 2003) and no more recent report on the subject was found.

The Northeast region of Brazil, with its diversity of rainfall and soil conditions and similarity of temperatures, offers a good opportunity for to explore the relationship between climatic variables, the isotopic composition of tropical plants and soil, and the implications these relationships hold for ecosystem scale patterns of N cycling. In this article we describe the ^{15}N signals of plants and soils along a rainfall gradient under similar high mean annual temperature in three parallel transects about 1 latitude degree apart, in Northeast Brazil, to gain insights into the N cycling in the soil – plant system. We also evaluated the possibility to estimate biological N_2 fixation from the signals of fixing and non-fixing plants. While we acknowledge the complexity of C, N and N isotope dynamics, we propose the following null hypotheses: 1) *soil $\delta^{15}N$ signals decrease with increasing rainfall but are high enough to allow determination of symbiotic N_2 fixation in legume plants*. Plant ^{15}N signals in Brazilian Northeastern deciduous forests are high ($\delta^{15}N$ of 7–9‰; Freitas et al. 2010b, Souza et al. 2012), as those of other dry places in the world (Handley et al. 1999, Aranibar et al. 2004, Viani et al. 2011), possibly because mineralization peaks earlier than plant absorption in the onset of the rainy season and mineral N is subject to losses. Signals in more humid forests of Northeast are not available but some tropical rainforests have signals higher than temperate forests and in the range of 4 to 6 ‰ (Martinelli et al. 1999, Roggy et al. 1999b, Nardoto et al. 2014), sufficiently higher than the signal of biologically fixed N and indicating that the N cycle is not completely close; 2) *since the soil signals are more positive with higher relative N losses they become enriched in the heavier isotope with lower total*

C:N ratios. N losses are higher when soil organic matter has mineralized beyond the point of N immobilization and the C:N ratio decreases towards the limit of composition of its chemical compounds (about 10:1), justifying the negative relationship of ^{15}N signals and C:N ratios described by Amundson et al. (2003) and by Stevenson et al. (2010); 3) *the differences of plant and soil* $\delta^{15}N$ ($\Delta\delta^{15}N$) *in the hot and dry areas of Northeast are lower than those in dry and colder regions of the world but these differences increase in Northeast areas of higher rainfall*. The patterns of decreasing $\Delta\delta^{15}$N with higher temperature and higher rainfall, in a global scale, were reviewed by Amundson et al. (2003) and are probably linked to differences in the chemical form of the N absorbed by the plants; and 4) *symbiotic fixation is higher in areas of high soil C:N ratio*, since fixation can be inhibited by high N availability and under this condition the ecological advantage of fixing plants is lost (Menge and Hedin 2009).

Material and methods

Study sites

The Brazilian Northeast region has an Atlantic coastal line roughly following a north – south direction. Annual rainfall is abundant in the coastal area (>1800 mm) and decreases westward to a semiarid core area, with low and erratic rainfall (about 500 mm). West of this core area, rainfall increases again to 1000–1500 mm, more regularly distributed in a 5–6 months rainy season. Interspersed in the semiarid area there are a few mountains (about 1000 m a.s.l.), where rainfall is higher than in the surrounding area. Soils in the coastal zone are deep, low fertility Latosols in the Brazilian classification (Embrapa 2006) or Ferrasols or Oxisosl in the FAO and American classifications, respectively; in the semiarid zone are shallow, high fertility Luvisols (Luvisols or Alfisols); and in the western portion are similar to those of the coastal zone. The coastal area was covered by tropical rain forest, a few remnants of which still dot the landscape. Half of the semiarid area is covered by a low dense shrub and tree vegetation, locally called caatinga. Few fragments remain of the semideciduous tall forest that covered the mountain tops in the semi-arid area. The western portion vegetation is a mixture of caatinga and savanna (cerrado) vegetation.

Fourteen areas with native vegetation without any major disturbance were selected following roughly three parallel east – west transects (see Fig. 1 for site locations), along the states of Paraíba (PB), Pernambuco (PE) and Alagoas (AL). The areas cover a large variation of rainfall (from 400 to more than 2000 mm y^{-1}) and water availability, conditioned by different rainfall distribution and evapotranspiration rates along the year, and a short variation of temperatures (23 to 26 °C). This variation translates into six vegetation types (Table 1): 1) coastal perennial rainforest, in João Pessoa (PB), Recife (PE) and Rio Largo (AL) municipalities; 2) coastal semideciduous forest in Aliança (PE) and São Vicente (PE) municipalities; 3) subhumid deciduous forest (Agreste caatinga), in Caruaru (PE) and Remígio (PB); 4) semiarid deciduous forest (Sertão caatinga), in Santa Teresinha (PB), Serra Talhada (PE) and Pão de Açúcar (AL) municipalities; 5) semideciduous montane forest in Areia (PB), Caruaru (PE) and Mata Grande (AL); and 6) savanna – deciduous forest transition in Araripina (PE).

Soil and plant sampling and analysis

In each site, five to six circular plots of about 20 m radius each and at least 50 m apart were marked in native mature vegetation patches. In each plot, one plant of the species previously selected was sampled. The selected species included all the legumes in the plot and also those non-legume species with the most abundant and largest plants, based on previous phytosociological studies. In Araripina, legume plants of species of known nodulating potential were also sampled in an experimental site adjacent to the native vegetation site. One composite sample of 10 to 20 fully expanded healthy leaves was collected from each plant, at about the same canopy height (1.5 to 2 m). Sampling was done in the peak of the rainy season in all the semideciduous and deciduous vegetation but in different seasons in the rainforests, always avoiding the period of leaf flushing. Part of the data for the subhumid deciduous forest, in Caruaru (PE) and Remígio (PB), and for the semiarid deciduous forest, in Santa Teresinha (PB) and Serra Talhada (PE) was already published by Freitas et al. (2010a, b)).

The plants were classified as non-legumes, non-nodulating legumes and target species. Non-nodulation potential was based on Allen and Allen (1981), Faria et al. (1984, 1987, 1989, 1994, 2010), Faria and Lima

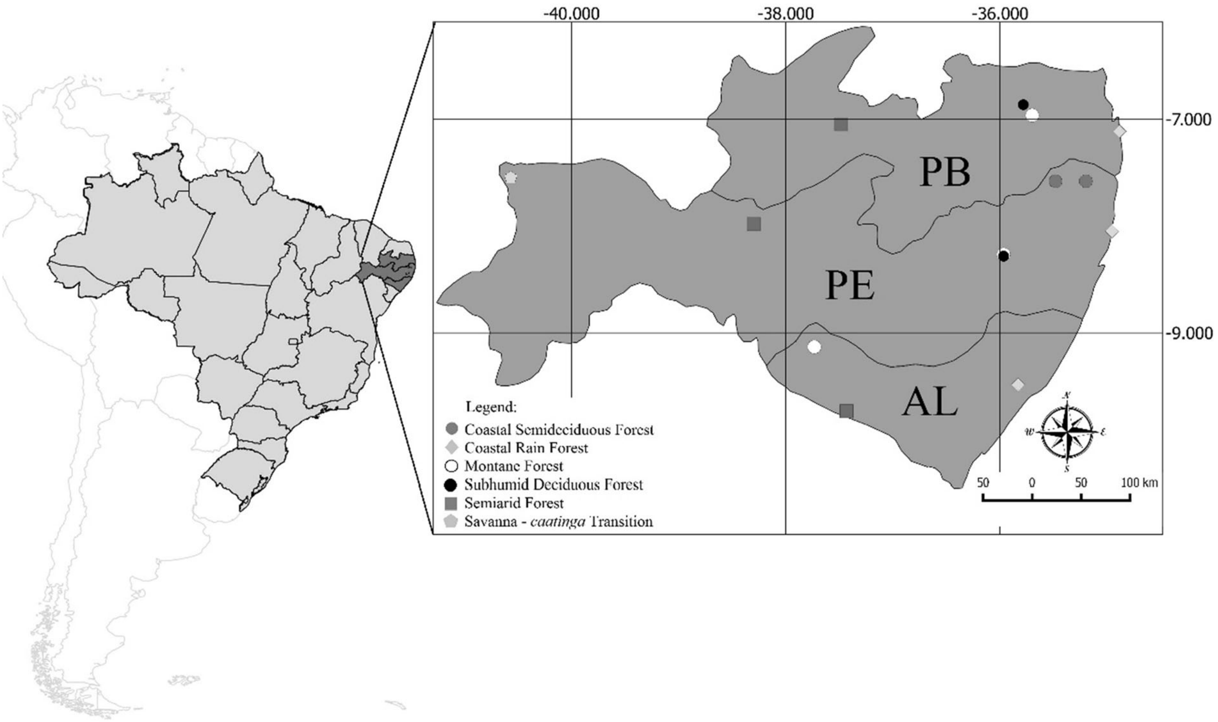

Fig. 1 Map of the studied areas in Paraiba (*PB*), Pernambuco (*PE*) and Alagoas (*AL*) states, Brazil

(1998), Sprent (2009) and Freitas et al. (2010a)) and these non nodulating legumes and the non-legumes were considered as reference species, to estimate symbiotic N_2 fixation of the target species. Target species were all those with a known nodulation potential and also all legume species without complete botanical identification or for which the nodulating potential was unknown. These later species could not be safely considered as reference species and their $\delta^{15}N$ values were compared to the values of the reference species. In case their $\delta^{15}N$ values were significantly and more than 2 ‰ lower (Högberg 1997) they could be considered as potentially fixing species. In case their $\delta^{15}N$ values were not significantly different from the values of the reference species, they could either be non-nodulating species or be potentially nodulating but not fixing sufficiently N to lower the $\delta^{15}N$ signals.

Soil samples were collected from the top 20 cm layer of each plot, air-dried, sieved and cleaned of any plant debris. Those of the coastal semideciduous forest in Aliança, PE, were lost. Subsamples were ground to finer powder. The plant samples were dried and also ground to fine powder. A sub-sample of leaf or soil was placed in a capsule and loaded into a ThermoQuest-Finnigan Delta Plus isotope ratio mass spectrometer (Finnigan-MAT; CA, USA) interfaced with an Elemental Analyzer (Carlo Erba model 1110; Milan, Italy) at the Laboratory of Isotope Ecology (CENA-USP, Brazil) to obtain the nitrogen isotope ratio and the total nitrogen content of these samples. Stable isotope ratios of nitrogen were measured relative to internationally recognized standards. Internal reference materials (atropine, yeast and soil standard no. 502–308 from LECO Corporation) were included in every analytical run. The concentrations of ^{15}N were expressed in δ units in relation to the international standard (atmospheric N_2), based on the equation $\delta=(R_{sample}/R_{standard}-1)\times 1000$.

The difference between leaf and soil $\delta^{15}N$, designated here as $\Delta\delta^{15}N$ (Amundson et al. 2003), was calculated for each plant. Foliar $\Delta\delta^{15}N$ values represent the ^{15}N depletion of a plant leaf compared to the soil $\delta^{15}N$ background (Kahmen et al. 2008).

Data analysis and statistics

Soil organic C and total N values were regressed against each other and against rainfall and soil $\delta^{15}N$ signature were regressed against soil C:N ratio and leaf $\delta^{15}N$ signature of reference species. Leaf $\delta^{15}N$ signature of reference species were regressed against rainfall and

Table 1 Coordinates and mean annual rainfall of the municipalities where the studied areas were located along three parallel transects in Paraíba (PB, transect 1), Pernambuco (PE, transect 2) and Alagoas (AL, transect 3) states, Northeast Brazil

Vegetation type /Municipality (state)	Coordinates	Altitude (ma.s.l.)	Rainfall (mm)
Coastal rainforest			
Rio Largo (AL)	09°29′S 35°50′W	62	1932
Recife (PE)	8°03′S 34°55′W	4	1774
João Pessoa (PB)	07°07′S 34°53′W	5	1852
Coastal semideciduous forest			
Aliança (PE)	07°35′S 35°12′W	60	1131
São Vicente Ferrer (PE)	07°35′S 35°29′W	419	1006
Montane forest			
Mata Grande (AL)	09°08′S 37°44′W	633	1073
Caruaru (PE)	08°16′S 35°58″W	950	1200
Areia (PB)	6°58′S 35°42′W	623	1430
Subhumid deciduous forest			
Caruaru (PE)	08°17′S 35°58′W	545	565
Remígio (PB)	6°52′S 35°47′W	596	700
Semiarid deciduous forest			
Pão-de-Açúcar (AL)	9°44′S 37°26′W	45	573
Serra Talhada (PE)	07°59′S 38°18′W	500	716
Santa Teresinha (PB)	07°03′S 37°29′W	380	693
Savanna – caatinga transition			
Araripina (PE)	07°33′S 40°34′W	620	728

against the coefficient of variation of the $\delta^{15}N$ signals of all reference plants in the site. Only the values of reference species were used to avoid the interference of atmospheric N_2 fixation of the nodulating legume species. All regressions were tested for homocedasticity.

Soil data (C, N and $\delta^{15}N$) were submitted to analysis of variance and the averages of the different sites were compared using the Tukey test at the 0.05 probability level. Plant data of the different sites did not have homogeneous variation and were not submitted to analysis of variance. Within each site, $\delta^{15}N$ values of target legume species were compared with the average $\delta^{15}N$ values of reference species, using a *t* test at the 0.05 probability level. Only the comparison of the reference average value with average value of the target species with the lower $\delta^{15}N$ average is presented in the text, to identify if any species in the site was fixing atmospheric N_2.

Results

Total carbon and nitrogen concentrations in the superficial soil layer (0 to 20 cm) varied significantly among areas (Table 2) and related quadratically with mean annual rainfall (Fig. 2), the highest values in areas with intermediate rainfall (1000 to 1500 mm). C and N were linearly related (Fig. 3), and the slope of the equation translates into a C:N ratio of 14.9:1. The abundance of the ^{15}N isotope ($\delta^{15}N$) also varied significantly among areas (Table 2) and it was not significantly related to total C or N concentrations but it was negatively related to the C:N ratio, if the data from the savanna caatinga transition area is excluded (Fig. 4).

Concentrations of ^{15}N of reference species varied considerably among areas (Table 3), in general areas with higher rainfall (>1000 mm) having lower values than areas with lower rainfall (<1000 mm), with a negative significant relationship (Fig. 5). Leaves of reference species and soil had significantly related $\delta^{15}N$ signals (Fig. 6) and leaves were depleted of ^{15}N in relation to the soil ($\Delta\delta^{15}N$), except those of one montane forest (Fig. 7). In spite of the good correlation of the averages, the differences of individual plant value to that of the soil in the same area varied considerably, typically in a range of 1 to 2 $\delta^{15}N$‰ (Fig. 7). There were no significant effects of rainfall, soil C, N and C:N ratio, and latitude on $\Delta\delta^{15}N$ values of the different areas.

Table 2 Soil carbon and nitrogen concentrations in different vegetation types in Northeast Brazil

Vegetation/municipality	n[a]	C (%)[b]	N (%)[b]	δ^{15}N (‰)[b]
Coastal rainforest				
Rio Largo	5	1.26±0.38 e	0.067±0.01 e	5.60±0.77 gh
Recife	5	0.96±0.25 e	0.053±0.02 e	4.85±0.52 hi
João Pessoa	5	1.53±0.55 de	0.088±0.03 de	3.25±0.29 j
Coastal semideciduous forest				
São Vicente Ferrer	5	3.96±1.07 ab	0.260±0.06 ab	5.95±0.97 gh
Montane forest				
Mata Grande	5	4.62±1.85 a	0.327±0.03 a	6.72±0.78 fg
Caruaru	5	3.42±0.90 abc	0.227±0.05 b	5.38±0.71 ghi
Areia	5	2.99±0.47 bcd	0.191±0.02 bc	4.03±0.59 ij
Subhumid deciduous forest				
Remígio	5	0.96±0.28 e	0.084±0.02 de	12.96±0.66 b
Caruaru	5	2.13±0.69 cde	0.169±0.04 bcd	9.04±0.80 de
Semiarid deciduous forest				
Pão de Açúcar	5	0.91±0.27 e	0.074±0.02 de	9.66±0.83 cd
Serra Talhada	5	1.08±0.15 e	0.098±0.01 cde	10.95±0.33 c
Santa Terezinha	5	0.73±0.09 e	0.067±0.01 e	7.85±0.30 ef
Savanna – caatinga transition				
Araripina	5	0.69±0.12 e	0.038±0.01 e	16.20±0.31 a

Averages in the column followed by the same letter are not significantly different by the Tukey test at 0.05 level

[a] Number of samples

[b] Average ± standard deviation

Only in the deciduous forests and in one montane forest, leaves of target species had significantly lower ^{15}N signals than those of reference species (Table 3. Additional data in Table ESM 1, presented as electronic supplementary material). Fixation of atmospheric N in these deciduous forests accounted for percentages of the N in the fixing legume species generally above 50 % (Freitas et al. 2010a). In the montane forest, the signals of two target species were significantly lower than those of the reference species but the difference was lower than 2 ‰. In all other sites, the signals of target species and reference species were not significantly different.

In the coastal rainforests the signals of both species groups were 1 to 3 δ^{15}N‰ units above atmosphere, indicating the natural abundance method was not suitable to estimate fixation. In the savanna caatinga transition, both signals were high, indicating absence of fixation of all target species.

Leaf N concentrations of non-legume species varied within a narrow range among areas and were lower than concentrations of legume species but the differences were only statistically significant in the montane and in two deciduous forests (Table 4).

Discussion

Soil C (7 to 46 g kg^{-1}) and N concentrations (0.7 to 3.3 g kg^{-1}) were relatively low (Table 2) but within the range usually reported for the surface horizons of tropical soils (Marin-Spiotta et al. 2009; Kirkby et al. 2011; Marín-Spiotta and Sharma 2013; Xu et al. 2013). The highest values were found in the coastal semideciduous and montane forests, located in areas with intermediate annual rainfall (1000 to 1500 mm), indicating that the balance of organic matter deposition and mineralization favored accumulation to a higher degree than in the other areas. The size of trees and consequently the aboveground biomass in these coastal semideciduous and montane forests are similar to or somewhat lower than in the coastal rainforests and higher than in the

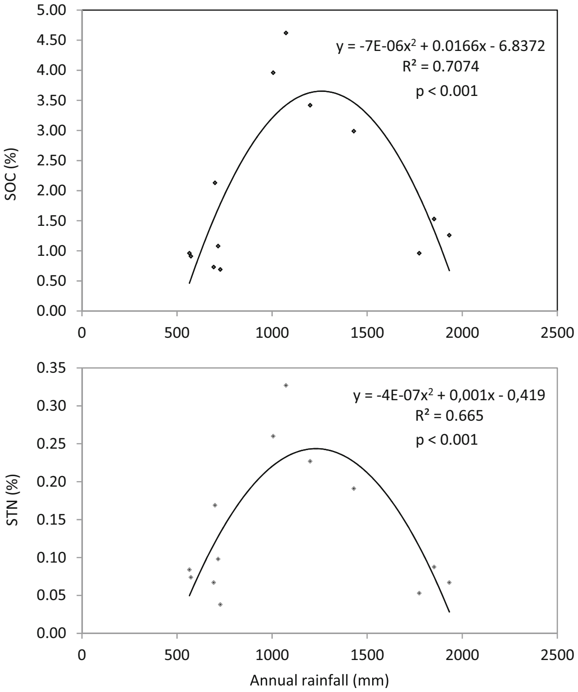

Fig. 2 Soil organic carbon (*SOC*) and total nitrogen (*STN*) concentrations as a function of mean annual rainfall in different vegetation types in Northeast Brazil

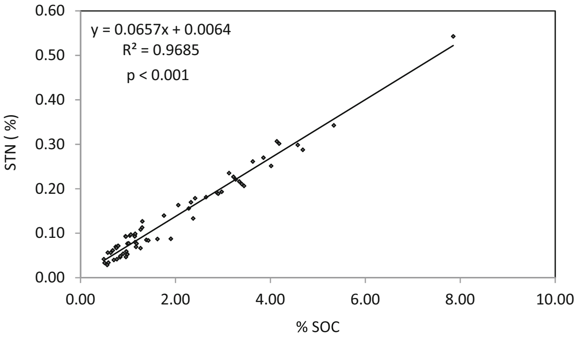

Fig. 3 Soil N as a function of soil C in different vegetation types in Northeast Brazil

subhumid and semiarid deciduous forest and in the savanna – caatinga transition vegetation (Sampaio 1996). Assuming that the organic deposition is proportional to the aboveground biomass (Batjes 1996; Marín-Spiotta and Sharma 2013), the litter mineralization rates in these drier semideciduous and montane forests must be lower than in the costal rainforests. In the even drier subhumid and semiarid deciduous forest and in the savanna – deciduous forest transition vegetation, the lower soil C and N accumulations probably result from lower litter depositions. The C:N ratios had an average close to 15 and a lower relative variation than the variation of the C and N concentrations. The range and the average are similar to those reported for soils in the world (Batjes 1996) and indicate that the C and N cycles in these soils are approximately equilibrated.

The accumulation of ^{15}N has been appointed as a measure of the openness of the N cycle (Austin and Vitousek 1988; Handley et al. 1999; Roggy et al. 1999b; Eshetu and Högberg 2000; Aranibar et al. 2004; Swap et al. 2004; Ometto et al. 2006; Coletta et al. 2009; Freitas et al. 2010b; Viani et al. 2011), a more open cycle having higher losses of the lighter isotope (^{14}N). The negative relation of the C:N ratio and the soil δ^{15}N values (Fig. 4) confirms our second hypothesis and suggests that soils with lower C:N ratios would have more open N cycles, that is, they would have greater N losses. However, considering the lower C:N ratios in these soils, losses of C are even proportionally higher than those of N. Negative relation of total C:N ratio and soil δ^{15}N values was reported in the comprehensive review of Amundson et al. (2003) and by Stevenson et al. (2010). Therefore, the subhumid and semiarid deciduous forests, with their highest δ^{15}N values (excluding the savanna – caatinga transition vegetation), would have the greatest relative N and C losses. Losses have not been determined in any of these areas but temperate forests with higher C:N ratios had lower nitrate losses through leaching (Gundersen et al. 1998). The losses may be related to seasonal variations in litter deposition and decomposition. Deposition in the deciduous forests is concentrated after the end of the rainy period and decomposition may start with photodegradation while the vegetation is denuded of foliage (Parton et al. 2007) and must accelerate with the beginning of the next rainy season, with abundant water availability. In the other less seasonal vegetations, particularly the rainforests, the almost constant litter deposition (Sampaio et al. 1993) with high C:N ratio would result in an equilibrium with higher soil C:N ratio.

The case of the savanna – caatinga transition vegetation, with both high C:N ratio and high δ^{15}N values, requires a different explanation. The extremely low soil P availability (Salcedo et al. 1997) may be implicated in higher relative N losses than C losses. The vegetation is short and quite open with many bare spots and probably has a large root:shoot ratio, as other savanna (Mokany et al. 2006) and caatinga (Costa et al. 2014) areas. Therefore the biomass input to the soil is probably low

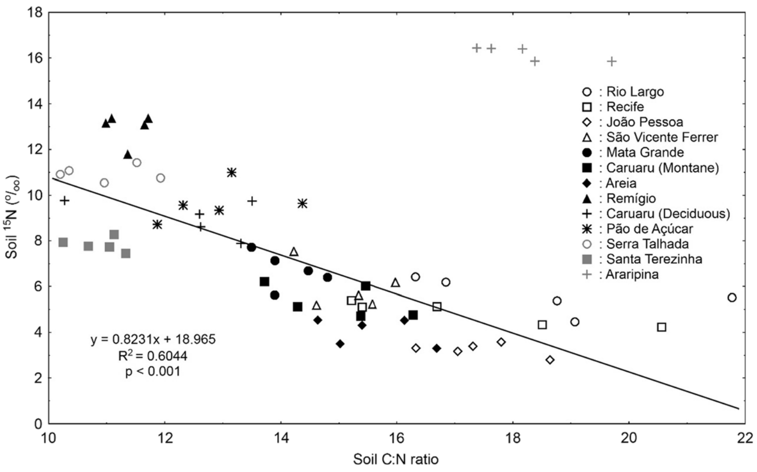

Fig. 4 Soil ^{15}N abundance as a function of soil C:N ratio in different vegetation types in Northeast Brazil (Araripina data were not included in the regression)

and composed of hard and leathery leaves, which may take longer to decompose than in other areas, and a large part of underground deposition where the biomass is not subjected to photodegradation (Parton et al. 2007). On the other hand, they have high N concentrations indicating that N is not a limiting nutrient, the mineral pool may not be readily depleted and some mineral N may be lost through leaching. The openness of the system and the absence of symbiotic fixation (discussed below), which would incorporate N with $\delta^{15}N$ values close to zero, turn the N balance towards high relative N losses and, therefore, high $\delta^{15}N$ signals.

The pattern of leaf ^{15}N of reference species followed closely that of soil ^{15}N, as expected considering that the soil is their sole source of N. In all cases except one, the plant signals are impoverished in ^{15}N in relation to the soil where they grow, with an average $\Delta\delta^{15}N$ signal of −2.34‰, corresponding to the absorption of mineralized forms of N which are depleted in the heavier isotope. The values are lower than all those listed by Amundson et al. (2003) for areas with lower mean annual temperatures, confirming the trend they reported of lower values with increasing temperatures, as also stated in our third hypothesis. However, contrary to their finding and our hypothesis, differences in rainfall had no significant effect on the $\Delta\delta^{15}N$ values. Apparently, when the temperature is high its influence overrides that of the water availability. Even in the colder sites reviewed by Amundson et al. (2003), the influence of temperature was stronger than that of rainfall. There was no effect of latitude on the $\Delta\delta^{15}N$ values (or in any other measured variable) but the 1° difference in our sites is too small and the latitude effect is probably mediated through temperature variation, which was not relevant among our sites. In all Brazilian Northeastern forests more N could be absorbed as NO_3^-, with its lower depletion factor, than as NH_4^+, as a result of faster soil organic matter turnover (Tiessen et al. 1994) and nitrification due to higher temperatures but the subject is still controversial and may involve enrichment of mineral N due to denitrification and less absorption through mycorrhizal fungi/plant interactions (Amundson et al. 2003; Kahmen et al. 2008). For all these processes there is no available information for forests in Northeast Brazil.

The low $\delta^{15}N$ signals in the more humid coastal and montane forests and the high $\delta^{15}N$ signals in the subhumid and semiarid vegetations partially confirm our first hypothesis of decreasing signals with increasing rainfall. The pattern of higher $\delta^{15}N$ signals as water availability decreases has not been described within many regions but many dry area vegetations in Africa have also high plant $\delta^{15}N$ signals (Handley et al. 1999; Aranibar et al. 2004). The savanna – deciduous forest transition $\delta^{15}N$ signal is also much higher than those found in savanna areas in Central Brazil (Bustamante et al. 2004; Coletta et al. 2009; Viani et al. 2011) where rainfall is also much higher. In contrast, the signals of the coastal rainforests are lower than those reported for Amazonian forests (Roggy et al. 1999b; Ometto et al. 2006) although even in these last forests low values have also been reported (Nardoto et al. 2014). Therefore, the signals of our rainforest do not support the statement that tropical forests have higher signals than temperate forests (Martinelli et al. 1999) and they were so few units above 0 ‰ that left little margin to detect significant decreases in the signals of fixing species. Therefore, the second

Table 3 Leaf $\delta^{15}N$ (‰) from reference (non-N_2-fixing species) and target (potentially N_2-fixing species or species without information on their nodulation) tree species growing in different ecosystems in Northeast Brazil

Ecosystem / Municipality	Reference species			Target species	
	n[a]	$\delta^{15}N$ (‰)	n[a]	Species with lower $\delta^{15}N$ average	$\delta^{15}N$ (‰)
Coastal rainforest					
Rio Largo	25	1.91±1.38[b]	7	*Inga* sp. 1 (w.i.+)[c] (2)[d]	0.17±0.68 ns[b, e]
Dois Irmãos	61	3.46±2.30	20	*Inga* sp. 3 (w.i.+) (5)	2.37±0.76 ns
João Pessoa	31	1.53±1.66	11	*Tachigali densiflora* (w.i.+) (3)	2.38±1.23 ns
Coastal semideciduous forest					
Aliança	14	5.10±0.98	8	*Swartzia pickelii* (w.i.+) (3)	3.53±2.72 ns
São Vicente	38	2.71±1.86	9	*Inga capitata* (+) (4)	3.62±0.70 ns
Montane forest					
Mata Grande	12	3.76±1.16	22	*Machaerium hirtum* (+) (3)	2.12±1.24 ns
Caruaru	24	1.57±1.31	36	*Machaerium hirtum* (+) (3)	0.67±0.69 ns
Areia	24	4.39±1.53	17	*Albizia polycephala* (+) (4)	2.68±1.96 *
Subhumid deciduous forest					
Remígio[f]	57	10.17±1.15	33	*Mimosa tenuiflora* (+) (3)	3.47±1.02 *
Caruaru[f]	14	9.43±1.49	24	*Mimosa arenosa* (+) (6)	1.04±0.74 *
Semiarid deciduous forest					
Pão de Açúcar	10	8.61±2.00	3	*Senegalia bahiensis* (w.i.−) (3)	7.23±2.45 ns
Serra Talhada[f]	32	6.34±1.40	24	*Mimosa* sp. 4 (w.i.+) (4)	2.05±0.64 *
Santa Teresinha[f]	15	6.95±1.27	12	*Mimosa tenuiflora* (+) (5)	4.22±1.68 *
Savanna – caatinga transition					
Araripina	31	12.69±1.49	29	*Senna* sp. 4 (w.i.−) (3)	13.01±0.43 ns
Experimental site	15	11.20±1.63	20	*Mimosa caesalpiniifolia* (+) (5)	11.31±1.53 ns

[a] Total number of sampled plants

[b] Average ± standard deviation

[c] Literature information on nodulation occurrence: w.i. = without information, + = nodulating legume

[d] Number of sampled plants of the species with lower $\delta^{15}N$ average

[e] Averages of target species marked with * are significantly different and those marked with ns are not significantly different from values of the reference species in the same area, according to the *t* test at 0.05 level

[f] Data on reference species was already published by Freitas et al. (2010a), separating non fixing legume and non-legume. Data on target species was already published by Freitas et al. (2010b)

part of our first hypothesis was not confirmed and the technique of natural abundance would only be adequate to measure fixation in our rainforests if the plants were acquiring very high percentages of their N through fixation.

Certainly, water and latitude are not the only drivers of the N cycle and plant and soil ^{15}N signals are not only influenced by lower losses of the heavy isotope but also by fixation either by plant symbiosis or by soil free-living microorganisms, with their influx of 0 ‰ $\delta^{15}N$ (Högberg 1997; Robinson 2001), and by atmospheric deposition, usually with positive $\delta^{15}N$ values (Agnihotri et al. 2011). Therefore, areas with low $\delta^{15}N$ values may have closed cycles with fast immobilization or absorption of mineralized N or, alternatively, high losses compensated by high fixation and low deposition. The uncertainty about fixation in our rainforests prevents determining which of the two patterns they follow. In some of these forests (Sampaio 1996), the low number of potentially fixing legume plants would limit the fixation input even if the plants fixed high proportions of their N but not all of the forests had the floristic composition determined. The C and N cycles are also affected by the availability of other nutrients, especially P (Brenner

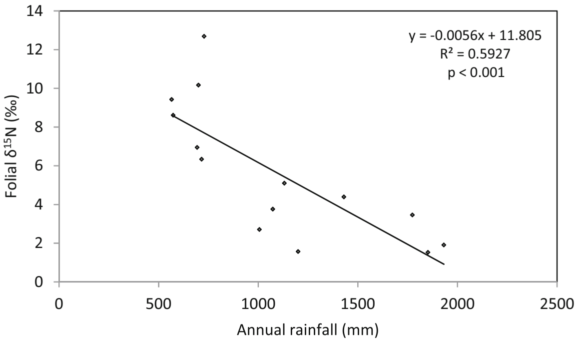

Fig. 5 Nitrogen-15 abundance ($\delta^{15}N$) of leaves of non-N_2-fixing species as a function of rainfall in different vegetation types in Northeast Brazil

et al. 2001). The low soil P availability in the savanna – deciduous forest transition area (Salcedo et al. 1997) could result in high N losses and explain part of its high $\delta^{15}N$ signal.

The $\delta^{15}N$ signals of target species were only significantly lower than averages of reference species and more than 2 ‰ $\delta^{15}N$ in the four deciduous forests which were the subject of a previous publication (Freitas et al. 2010b). Since the value of 2 ‰ $\delta^{15}N$ was established by Högberg (1997) as a safe limit to estimate biological fixation, we only estimated fixation in these forests. In these forests, symbiotic fixation accounted for high proportions of the plant N but the amounts fixed were low because of the low densities of the populations of the fixing legume species. The proportions of fixed N were usually above 50 % of the total leaf N but the annual contribution of fixed N was below 10 kg ha^{-1} (Freitas et al. 2010a). In one montane forest the $\delta^{15}N$ averages of two species were significantly lower than the reference average (Table ESM 1, presented as electronic supplementary material) but the differences were lower than 2 ‰. These two species, *Bowdichia virgilioides* Kunth and *Albizia polycephala* (Benth.) Killip, are known to nodulate and could be fixing small proportions of their N.

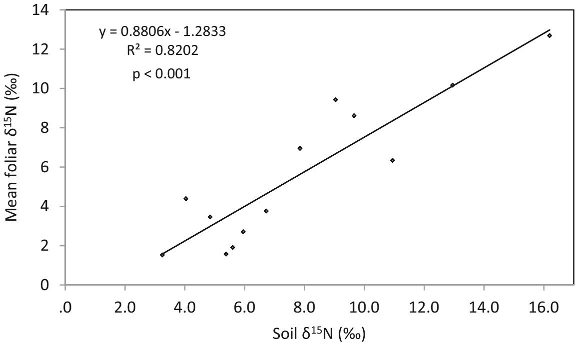

Fig. 6 Nitrogen-15 abundance ($\delta^{15}N$) of leaves of non-fixing species as a function of nitrogen-15 abundance in the soil in different vegetation types in Northeast Brazil

In all other areas there was no apparent contribution of symbiotically fixed N to the target species. In the rainforests the lack of significant differences resulted from signals of reference species with low values and high variances. In fact, the variances of signals in all areas were not much different, with standard deviations from 0.98 to 2.30‰ (Table 3), in a way that their coefficient of variation decreased with the increase in the average value (Fig. 8). The consequence is that it is more difficult to detect the decrease in signal caused by fixation in areas with low reference values. It is the case of all coastal perennial forests and one of two of the coastal semideciduous and two of the three montane forests, with signals below 4 ‰, in which the technique of natural abundance could not be used to estimate fixation. Therefore, we cannot determine if their relatively low soil and plant values originate from a large contribution of biological fixation. No data are available for fixation by soil free-living microorganisms in these forests but in other regions (Hedin et al. 2009) this fixation can be of similar magnitude as those we found in the four deciduous forests (Freitas et al. 2010a).

In the other coastal semideciduous and one montane forests, and especially in the savanna – deciduous forest transition, the signals of the reference species were sufficiently higher than atmosphere N, theoretically allowing estimation of fixation if the signals of the fixing species were significantly lower. However, the differences between the average signals of these reference species and those of target species were too small to indicate any dilution of the ^{15}N coming from the soil by ^{15}N from the atmosphere (Table ESM 1, presented as electronic supplementary material). This is particularly clear in the savanna – caatinga transition area, where the signals of the reference species were very high, one of the highest in the literature (Handley et al. 1999; Amundson et al. 2003; Craine et al. 2009). The absence of fixation of the native legume species was confirmed by the analysis of well-known potentially fixing species from the growth experiment in the same site. The causes of this absence are not known but a similar fact was registered for regenerating caatinga in one of the semideciduous forests (Freitas et al. 2012) and has been noticed in other regions (Ndiaye and Ganry 1997; Faye

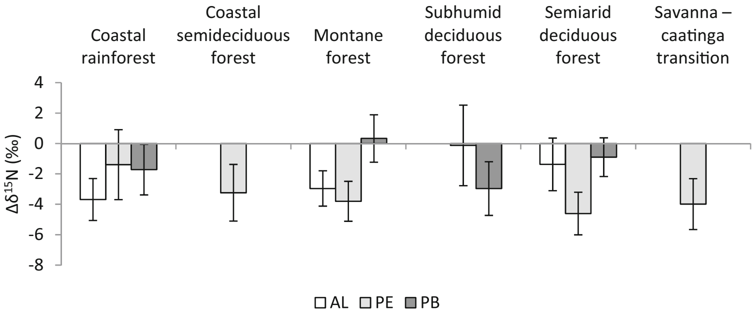

Fig. 7 Difference between leaf and soil $\delta^{15}N$ ($\Delta\delta^{15}N$) in different vegetation types in Northeast Brazil. *PB* Paraiba state (transect 1); *PE* Pernambuco state (transect 2); *AL* Alagoas state (transect 3)

et al. 2007). It is interesting to note that in the coastal semideciduous and montane forests, the signals of the target species had larger variations than those of the reference species and frequently higher absolute averages (Table 3). This could indicate not only absence of fixation but also a preference for absorption of soil NH_4^+ for NO_3^- (Kahmen et al. 2008), but there is not enough information on the subject to reach any firm conclusion.

One consequence of the absence of fixation in the savanna – caatinga transition area and the absence or undefinition of fixation in the montane and rainforests, the areas with higher soil C:N ratios, is that our fourth hypothesis of higher fixation in areas with high C:N ratios was not validated. In fact, fixation was only determined in the deciduous forests, which had the lowest C:N ratios. As discussed above, these areas had

Table 4 Leaf nitrogen concentration in legume and non-legume species in different vegetation types in Northeast Brazil

Ecosystem / Municipality	Nitrogen concentration (%) (average ± standard deviation)			
	n[a]	Non legume	n	Legume
Coastal rainforest				
Rio Largo	17	1.84±0.25 A	15	2.60±0.38 A
Dois Irmãos	50	2.50±0.88 A	31	2.84±0.56 A
João Pessoa	31	1.80±0.36 A	11	2.38±0.54 A
Coastal semideciduous forest				
Aliança	9	1.75±0.30 A	13	2.84±0.64 A
São Vicente	35	2.18±1.07 A	12	2.73±0.86 A
Montane forest				
Mata Grande	12	2.42±0.59 B	22	3.62±0.84 A
Caruaru	22	1.86±0.51 B	38	3.85±0.89 A
Areia	20	1.93±0.28 B	21	3.53±1.13 A
Subhumid deciduous forest				
Remígio	27	2.29±0.30 B	63	3.36±0.69 A
Caruaru	5	1.81±0.20 A	33	2.77±0.60 A
Semiarid deciduous forest				
Pão de Açúcar	10	1.66±0.54 B	3	3.75±0.71 A
Serra Talhada	12	1.77±0.24 A	44	2.46±0.66 A
Santa Teresinha	5	1.62±0.15 A	22	2.06±0.44 A
Savanna – caatinga transition				
Araripina	31	2.35±0.59 A	29	2.59±0.57 A
Experimental site	15	1.60±0.52 B	20	3.80±0.59 A

Averages followed by the same letter in the line are not significantly different, according to the *t* test at 0.05 level

[a] Number of sampled plants

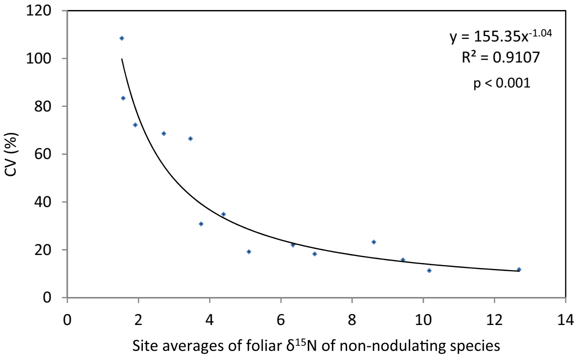

Fig. 8 Coefficient of variation of leaf ^{15}N signals as a function of average leaf ^{15}N signals in different vegetation types in Northeast Brazil

great losses of N, reflected in their high δ^{15}N, but even greater losses of C, reflected in the low C:N ratios.

Fixation has an influence in the ^{15}N signal of the soil, proportional to the amount fixed in relation to the general recycling of N in the area. Soil δ^{15}N in the deciduous forests would be lowered by the contribution of organic matter coming from the fixing species, but the effect would be small because this contribution is small and, in fact, the soil signals in these forests are among the highest of all areas.

Conclusions

Total soil carbon and nitrogen concentrations were higher in areas with intermediate (1000 to 1500 mm) than with lower or higher rainfall, where biomass productions are low and high, respectively, indicating high losses of organic matter in the rainier forests. The abundance of the ^{15}N isotope (δ^{15}N) was negatively related to the mean annual precipitation and had a large span, from some of the highest values in the world (>10 ‰) to values slightly above atmosphere abundance. Therefore, the dry deciduous forests had higher relative N losses and the rainier forests a more closed N cycling in spite of high organic matter losses. The δ^{15}N values were also negatively correlated to the soil C:N ratio indicating higher N losses where soil organic matter had mineralized to a greater degree, the dry deciduous forests.

In general, plants were depleted of ^{15}N in relation to the soil ($\Delta\delta^{15}$N) and the differences were lower than those reported for colder places of the world, confirming the tendency of decreasing values in higher mean annual temperatures. Contrary to the world trend, rainfall had no significant influence on the $\Delta\delta^{15}$N values, indicating that when the temperature is high its effect overrides that of water availability. The similarity of $\Delta\delta^{15}$N in all northeastern forests suggests that they are absorbing the same forms of soil N, possibly more NO_3^-.

Except in the deciduous forests, the low signals of reference plants and/or the small depletion of ^{15}N of target species did not allow estimation of fixation. In the rainforests the low signals of reference species indicate that the ^{15}N natural abundance technique is not suitable to estimate fixation. In the savanna – deciduous forest transition fixation was not occurring.

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