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ABUNDÂNCIA NATURAL DO ^{15}N E FIXAÇÃO BIOLÓGICA DO N_2 EM ESPÉCIES ARBÓREAS DA CAATINGA

ANA DOLORES SANTIAGO DE FREITAS

RECIFE – PERNAMBUCO - BRASIL
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ORIENTADOR: PROF. DR. EVERARDO VALADARES DE SÁ BARRETTO SAMPAIO
CO-ORIENTADOR: PROF. DR. RÕMULO SIMÕES CEZAR MENEZES

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Ana Dolores Santiago de Freitas

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ORIENTADOR: Prof. Dr. Everardo Valadares de Sá Barreto Sampaio
CO-ORIENTADOR: Prof. Dr. Rômulo Simões Cezar Menezes

COMISSÃO EXAMINADORA:

Prof. Dr. Everardo Valadares de Sá Barreto Sampaio – DEN/UFPE

Carolina Etienne Santos
Profa. Dra. Carolina Etienne de Rosália e Silva Santos – DEPA/UFRPE

Prof. Dr. Ignácio Hernan Salcedo – DEN/UFPE

Márcia do Vale Figueiredo
Profa. Dra. Márcia do Vale Barreto Figueiredo- IPA

Maria do Socorro B. Araújo
Profa. Dra. Maria do Socorro Bezerra de Araújo – DCG/UFPE

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RESUMO

Pouco se sabe sobre a fixação biológica do nitrogênio (FBN) em áreas da caatinga nordestina, apesar de sua grande riqueza em leguminosas. Para ecossistemas naturais, a metodologia mais indicada em estudos da FBN é a da abundância natural do ^{15}N (δ^{15}N), que permite a identificação de plantas diazotróficas e estimativas das quantidades de N fixadas. A precisão destas estimativas depende do padrão isotópico do N no sistema, ou seja, de um sinal de δ^{15}N adequado das plantas não fixadoras e de diferenças significativas entre os sinais de fixadoras e não fixadoras. Não existem dados sobre o padrão isotópico do N na caatinga. Acessar este padrão pode propiciar, além de um embasamento para estudos da FBN em leguminosas, um conhecimento qualitativo sobre o grau de perdas ou de reciclagem do N dentro dos sistemas e sua relação com as condições edafoclimáticas locais. Neste trabalho foram determinadas as concentrações de ^{15}N em plantas arbóreas fixadoras (leguminosas) e não fixadoras (não leguminosas e leguminosas não nodulantes) nativas da caatinga, com os objetivos de determinar se os valores de δ^{15}N de plantas não fixadora são altos e uniformes o suficiente para permitir cálculos precisos da fixação de N_2, de avaliar se existe algum padrão climático e espacial de valores de δ^{15}N e de estimar a fixação biológica associada a leguminosas. Foram escolhidos fragmentos de caatinga localizados em quatro municípios de Pernambuco e Paraíba, sendo dois no Agreste (Remígio na Paraíba e Caruaru em Pernambuco) e dois no Sertão (Santa Teresinha na Paraíba e Serra Talhada em Pernambuco), refletindo um gradiente de disponibilidade de água. Em cada local foram coletadas folhas das espécies previamente selecionadas, amostradas em 5 a 6 parcelas em cada local. As folhas de árvores não fixadoras de nitrogênio estavam bastante enriquecidas em ^{15}N e o enriquecimento foi uniforme, tanto espacialmente quanto entre espécies. Este padrão isotópico é uma condição promissora para o uso da metodologia da abundância natural do ^{15}N para estimativas da fixação biológica do nitrogênio, pois facilita a escolha de plantas referência, o esquema de amostragem e a detecção de diferença significativa entre os δ^{15}N de plantas fixadoras e não fixadoras. Os locais de Agreste, com precipitação media anual mais baixas (em torno de 700 mm) mas distribuição mais uniforme das chuvas (6 meses) tiveram valores médios de $\delta^{15}N$ foliar de plantas não fixadoras de 9,4 e 10,1‰, que estão entre os mais altos relatados na literatura. Estas médias foram significativamente maiores que as dos fragmentos localizados no Sertão (6.5 e 6.3‰), que têm maior precipitação total (em torno de 800 mm) mas distribuição mais concentrada das chuvas (3 meses). O enriquecimento isotópico das plantas não-fixadoras do Agreste pode ser resultados de maiores perdas gasosas de N empobrecido em ^{15}N ou menores perdas de materiais enriquecidos em ^{15}N em relação aos locais do Sertão. As diferenças entre sinais isotópicos de espécies alvo (fixadoras) e referência (leguminosas não nodulantes e não leguminosas) permitiram a identificação segura de indivíduos diazotróficos e cálculos razoavelmente precisos das proporções de N derivado do ar (%Ndfa). Foram identificadas espécies com grande capacidade de fixação de N_2, destacando-se *Mimosa tenuiflora*, *Mimosa arenosa* e *Piptadenia stipulacea*. Os teores de N total foram menores nas plantas não leguminosas e, entre as leguminosas, maiores nas espécies fixadoras. Nas quatro áreas avaliadas, as contribuições médias da fixação biológica do N_2 foram altas, variando de 27 a 68%. No entanto, as quantidades estimadas de N adicionadas anualmente aos sistemas foram baixas (2,5 e 11,2 kg ha^{-1} ano^{-1}), devido às baixas proporções de plantas fixadoras no conjunto geral da vegetação (2,4 a 11,8 %). Em situações de regeneração da vegetação nativa, onde a sucessão é dominada por espécies fixadoras, as estimativas poderiam chegar a 130 kg^{-1} ha^{-1} ano^{-1}.

Palavras chave: ciclo do nitrogênio, FBN, floresta seca, isótopo estável, semi-árido.

^{15}N NATURAL ABUNDANCE AND BIOLOGICAL NITROGEN FIXATION IN CAATINGA TREE SPECIES

ABSTRACT

Information on biological nitrogen fixation (BNF) in caatinga areas is scarce, in spite of its abundance of legume species. In natural ecosystems, the recommended methodology to study BNF is based on ^{15}N abundance variations (δ^{15}N), which allows identification of fixing plants and estimations of N fixed quantities. Precision of these estimations depends on the isotopic signs in the system. Non-fixing plants should have high δ^{15}N values and fixing plants signs that significantly differ from those of non-fixing plants. No data are available on the isotopic signs of caatinga plants. Determining these signs can provide the bases for BNF studies and a qualitative idea of N losses and N cycling within the system and their relationship with local soil and climate conditions. ^{15}N concentrations in caatinga native fixing (legume) and non-fixing (legume and non legume) trees were determined, in order to evaluate if the δ^{15}N of non-fixing plants were high and uniform enough to allow precise calculations of BNF, if there are any climatic and spatial patterns associated to the δ^{15}N signs and to estimate BNF of legume species. Four sites were chosen in four municipalities in Pernambuco and Paraíba, two in the Agreste region (Caruaru in Pernambuco and Remígio in Paraíba) and two in the Sertão region (Serra Talhada in Pernambuco and Santa Terezinha in Paraíba), which differ in water availability. Non-fixing tree leaves were quite enriched in ^{15}N and this enrichment was uniform, both spatially and among species. This isotopic pattern is adequate for the use of the natural abundance methodology because it facilitates the choice of reference species, the sampling procedure and the detection of significant differences between fixing and non-fixing plants. In the Agreste sites, with their lower average (about 700 mm) but more uniform annual rainfall distribution (6 months), the averages δ^{15}N of non-fixing plants were 9.4 and 10.1‰, among the highest reported in the world literature. These averages were significantly higher than those in the Sertão sites (6.5 and 6.3‰), which have higher averages but a more concentrated annual rainfall distribution (3 months). The higher isotopic enrichment of non-fixing plants in the Agreste could result from higher losses of ^{15}N depleted or lower losses of ^{15}N enriched nitrogen than in the Sertão sites. The isotopic sign differences between fixing and non-fixing species allowed a sure identification of fixing plants and reasonably precise calculations of N in the plants derived from the atmosphere. Species with a great N_2 fixing capacity were identified, especially *Mimosa tenuiflora*, *Mimosa arenosa* and *Piptadenia stipulacea*. Leaf N contents were lower in the non-legume species and, among legume species, higher in the fixing ones. Average BNF contributions to the fixing species were high in the four sites, varying from 27 to 68%. However, the N amounts added annually to the systems were low (2.5 to 11.8 kg ha^{-1} $year^{-1}$) due to the low proportions of fixing plants in the vegetation communities (2.4 to 11.8%). In regrowing vegetation, where the initial succession stage is dominated by fixing species, BNF could be as high as 130 kg ha^{-1} $year^{-1}$.

Key-words: BNF, dry forest, nitrogen cycle, semi-arid, stable isotope.

SUMÁRIO

INTRODUÇÃO

Caatinga é o tipo de vegetação que cobre a maior parte da região com clima semi-árido do Nordeste do Brasil (Rodal e Sampaio, 2002), abrangendo uma área estimada entre 6 e 9×10^5 km^2 (Sampaio, 1995). O desmatamento e a queima, para retirada da lenha e plantio no sistema de agricultura intinerante, vêm causando a perda desta vegetação em ritmo acelerado. Nas áreas abandonadas, a regeneração da vegetação nativa depende, entre outros fatores, do restabelecimento dos estoques iniciais de nutrientes, perdidos juntamente com a biomassa vegetal.

Embora o nitrogênio seja um dos elementos mais abundantes na Terra, a escassez de formas disponíveis o torna o elemento mais crítico para o crescimento da maioria das plantas (Smil, 1999; Graham e Vance, 2003), podendo ser tão limitante quanto a água em regiões áridas e semi-áridas (Hooper e Johnson, 1999). Uma compreensão integrada do ciclo do N, que requer o conhecimento das magnitudes dos principais processos envolvidos no ciclo biogeoquímico do nutriente, é fundamental para adoção de técnicas que garantam um manejo sustentável da caatinga.

A ciclagem do nitrogênio em ecossistemas terrestres (entradas, saídas e transformações do elemento) é fortemente condicionada pelas diversas características bióticas e abióticas do sistema. Além das perdas devidas às exportações de biomassa, o nitrogênio pode sair do sistema por lixiviação ou perdas gasosas (Lin et al., 2000). Em ambientes naturais, a principal forma de entrada de nitrogênio é através da fixação biológica do N_2 atmosférico (FBN) via nitrogenase (Cleveland et al, 1999). O processo de FBN em plantas vasculares desempenha um papel crucial na manutenção de estoques adequados de nitrogênio na biomassa das plantas. Provas disto são a sobrevivência de numerosos membros da família Leguminosae em solos pobres em nitrogênio, como os solos das áreas abandonadas do semi-árido nordestino, e a dominância de espécies potencialmente fixadoras (Sampaio et al., 1998, Pereira et al., 2003) durante o processo de regeneração da caatinga nestas áreas.

A FBN em leguminosas se dá através de associações simbióticas com bactérias específicas (rizóbios). Estas bactérias invadem os pelos das raízes, formando nódulos onde o nitrogênio livre no ar é convertido em nitrogênio fixado para assimilação ou estoque pela planta. Embora presente em um grande número de espécies, esta capacidade de FBN não é comum a todas as leguminosas, variando entre sub-famílias, gêneros e até mesmo entre espécies dentro de um mesmo gênero (Allen e Allen, 1981).

Como Leguminosae é a família melhor representada na caatinga, com 293 espécies, pertencentes a 77 gêneros, e com alto grau de endemismo (Queiroz, 2006), é provável que a FBN seja um processo de grande importância ecológica neste ambiente. Entretanto muito pouco se sabe sobre a capacidade de nodulação das leguminosas nativas da caatinga, não existindo informações sobre a ocorrência de fixação efetiva e nem estimativas das quantidades de N aportadas, a locais com esta vegetação, através da FBN.

Para ecossistemas naturais, a metodologia mais indicada em estudos da FBN é a da abundância natural do ^{15}N ($\delta^{15}N$), por ser um método que não perturba o ambiente (nada precisa ser adicionado ou destruído) e que integra o efeito do processo ao longo do tempo. Dependendo das características do ciclo do nitrogênio no sistema, é possível, através do sinal isotópico dos tecidos vegetais, determinar se plantas perenes de grande porte, com raízes profundas, são diazotróficas ou não e também fazer estimativas das quantidades de N fixadas anualmente (Shearer e Kohl, 1986, Högberg, 1997, Boddey et al., 2000)

Apesar destas vantagens, o sucesso da quantificação da fixação de N_2 utilizando a técnica do ^{15}N, no nível de abundância natural, depende do padrão isotópico do N no sistema, que é resultado do balanço entre entradas e perdas do elemento via processos fracionadores. Estimativas precisas da FBN dependem de um sinal adequado das plantas não fixadoras e de diferenças significativas entre os sinais de fixadoras e não fixadoras (Högberg 1997), condição freqüentemente não encontrada em florestas tropicais (Handley et al 1994; Roggy et al, 1999; Gehring e Vlek, 2004). Não existem dados sobre o padrão isotópico do N na vegetação da caatinga. Acessar este padrão pode propiciar, além de um embasamento para estudos da FBN em leguminosas, um conhecimento qualitativo sobre o grau de perdas ou de reciclagem do N dentro dos sistemas e sua relação com as condições edafoclimáticas locais, uma vez que as concentrações de ^{15}N de amostras vegetais são o resultado líquido dos processos biogeoquímicos que atuam na ciclagem do nitrogênio no local (Robinson, 2001).

Dada a inexistência de informações para a vegetação da caatinga, neste trabalho foram determinadas as concentrações de ^{15}N ($\delta^{15}N$) em plantas fixadoras e não fixadoras nativas deste ambiente, com os objetivos gerais de determinar o padrão isotópico de ^{15}N na vegetação nativa e estimar capacidade de fixação do nitrogênio em leguminosas arbóreas.

REVISÃO DE LITERATURA

O ciclo do nitrogênio em ecossistemas terrestres e sua composição isotópica

Em ecossistemas naturais, o nitrogênio é o elemento mais crítico para o crescimento da maioria das plantas, podendo ser tão limitante quanto a água (Smil, 1999; Hooper e Jonhson, 1999; Graham e Vance, 2003) sendo sua disponibilidade determinada pela decomposição da matéria orgânica. A ciclagem deste elemento em comunidades complexas de plantas envolve as entradas (deposições atmosféricas e fixação do N_2) e saídas (volatilização e lixiviação) do elemento dos sistemas e as transformações (sínteses e mineralização) que o elemento sofre ao migrar entre os diferentes compartimentos dentro dos sistemas (Lin et al., 2000). Estes processos naturais são condicionados pelas diversas características bióticas e abióticas dos ecossistemas, além de serem de difícil mensuração, o que dificulta a construção de um modelo que contemple todos os fluxos entre os diferentes compartimentos de N dos sistemas (Kirchbaun e Paul, 2002). Embora não possa ser considerado um bom traçador dos processos envolvidos na ciclagem do N, os sinais de ^{15}N do solo e da vegetação têm se revelado uma ferramenta muito útil para compreender a dinâmica do N, funcionando como integralizadores de todos os processos biogeoquímicos atuantes nos ecossistemas (Robinson, 2001).

As razões entre os dois isótopos estáveis do nitrogênio, o ^{15}N e o ^{14}N, variam na biosfera devido ao fracionamento isotópico nos processos físicos, químicos e biológicos. O N_2 atmosférico, que tem uma abundância constante de 0,3663 atom % de ^{15}N, é aceito como o padrão (Högberg, 1997). A composição isotópica de amostras de solos e tecidos de plantas são o resultado líquido de processos (mineralização, volatilização de amônia, nitrificação, denitrificação, troca de íons, difusão, absorção pelas plantas e micorrizas, fixação biológica do N_2, herbivoria,etc) que envolvem diferentes magnitudes e direções de fracionamento isotópico (Schearer e Kohl, 1989), com efeitos potenciais sobre o $\delta^{15}N$ no sistema solo/planta (Högberg, 1997). Por conveniência, a abundância natural de ^{15}N nestas amostras é expressa em unidades de δ (‰), que é o desvio, em relação ao N_2 atmosférico, da razão entre as massas de ^{15}N e ^{14}N do nitrogênio contido na amostra (Schearer e Kohl, 1989).

Um grande número de pesquisas tem utilizado o sinal de ^{15}N de amostras de folhas e solo para fazer inferências sobre o ciclo do N, em diferentes ecossistemas do globo. O grande interesse destas pesquisas é acessar, através da abundância natural de ^{15}N das amostras, o grau de perdas ou de reciclagem do N dentro dos sistemas (se o ciclo é aberto ou fechado) e sua relação com as condições edafoclimáticas dos locais (Austin e Vitousek, 1998; Martinelli et al., 1999; Roggy et al., 1999; Handley et al., 1999; Eshetu e Högberg, 2000; Arndt et al., 2004, Ometto et al., 2006, entre outros). Outra importante utilização da quantificação dos isótopos do nitrogênio em plantas são os estudos envolvendo a fixação biológica do nitrogênio (FBN) associada a plantas vasculares (entre as pesquisas publicadas mais recentemente, estão Salas et al., 2001; Gatumbi et al., 2002; Galiana et al., 2002; Spriggs et al., 2003; Koponen et al., 2003; Arndt et al., 2004; Gehring e Vlek, 2004; Raddad et al., 2005; Khadka e Tatsumi, 2006; Kreibich et al. 2006).

A importância da fixação biológica do nitrogênio em leguminosas

Na ausência de influência humana, a FBN é o principal processo de conversão do N_2 atmosférico em formas biologicamente disponíveis. Assim, esforços para avaliar os processos de ciclagem de N dentro de ecossistemas requerem a compreensão da magnitude dos “inputs” via FBN. Embora existam muitos estudos sobre a microbiologia e a fisiologia da fixação de N, bem como estimativas da FBN em determinados biomas (principalmente florestas temperadas), muito pouco se sabe sobre a magnitude do processo em florestas tropicais (Cleveland et al., 1999).

A FBN simbiótica desempenha um papel crucial na manutenção de recursos adequados de nitrogênio no compartimento das plantas. Prova disto é a sobrevivência de numerosos membros da família Leguminosae em solos bastante pobres ou destituídos de nitrogênio, devido a suas associações com bactérias específicas (rizóbios). Estas bactérias invadem os pelos radicais formando nódulos onde o nitrogênio livre no ar é convertido em nitrogênio fixado para assimilação ou estoque pela planta. Este processo é um meio eficiente de suprir as leguminosas de nitrogênio e obter uma vantagem evolutiva sobre a maioria dos demais organismos vivos, o que lhes conferiu grande sucesso ecológico (Allen e Allen, 1981).

A família das leguminosas possui mais de 20000 espécies, com distribuição global (Sprent, 2001). Em importância econômica é superada apenas por Poaceae e em tamanho apenas por Orchidaceae e Asteraceae. Compreende árvores, arbustos, ervas e lianas, com folhas geralmente alternadas e compostas, flores com estrutura variada de acordo com a sub-família e fruto característico em forma de vagem. Convencionalmente esta família é dividida em três subfamílias claramente distintas por suas estruturas florais: Mimosoideae, Caesalpinoideae e Papilionoideae, sendo que alguns taxonomistas atribuem status de família a cada uma delas (Allen e Allen, 1981).

Embora não seja uma característica comum a todas as espécies, a capacidade de fixar nitrogênio atmosférico em simbiose com bactérias do grupo dos rizóbios é a grande particularidade ecológica das leguminosas (Allen e Allen, 1981). A maioria das espécies nodulantes pertence à subfamília Papilionoideae, onde 97% das espécies examinadas são capazes de formar nódulos. Entre as Mimosoideae esta capacidade foi provada em 90% das espécies examinadas e entre as Caesalpinoideae examinadas, apenas 23% é capaz de formar nódulos. Entretanto, apenas uma fração do total de espécies leguminosas foi investigada quanto à capacidade de FBN, havendo enormes lacunas de informações sobre a maioria das espécies arbóreas da África e América Latina, devido a limitações metodológicas, como a dificuldade de identificação de nódulos em árvores adultas, a freqüente falta de nódulos em florestas e a dificuldade de obtenção de sementes nativas para estudos em vasos (Faria et al., 1984; Faria et al., 1989; Dakora e Keya, 1997).

Apesar das dificuldades, muitos esforços têm sido feitos no Brasil para identificar espécies de leguminosas capazes de formar nódulos em simbiose com rizóbios (Faria et al., 1984; 1987; 1989; 1994; Moreira et al., 1992; Souza et al., 1994), porém foram estudos que se restringiram principalmente a espécies do Sudeste e da Amazônia. Apesar da grande importância das leguminosas na caatinga (Queiroz, 2006), o conhecimento sobre o status de nodulação das espécies nativas deste bioma é bastante incompleto.

Quantificação da FBN em arbóreas

A capacidade fisiológica das leguminosas de formar nódulos não comprova a ocorrência de fixação no ambiente natural. O conhecimento sobre a importância ecológica da FBN em ambientes naturais requer que se estime a contribuição da FBN para a nutrição das espécies diretamente em campo. Por ser um método que não perturba o ambiente (nada precisa ser adicionado ou destruído) e que integra o efeito do processo de FBN ao longo do tempo, a metodologia da abundância natural do ^{15}N ($\delta^{15}N$) é o mais indicado para ecossistemas naturais, propiciando que se indique se plantas perenes de grande porte, com raízes profundas, são diazotróficas ou não (Hogberg 1997) e é o mais utilizado para estimativas da FBN em árvores, tanto em sistemas naturais quanto em agroflorestas (Shearer et al., 1983; Muofhe e Dakora, 1999; Roggy et al., 1999; Galiana et al., 2002; Gathumbi et al., 2002; Kreibich et al., 2006). O princípio deste método é que a abundância de ^{15}N de uma espécie fixadora de N, que obtém N do N_2 atmosférico em adição às fontes de N do solo, é menor que a de uma espécie referência não fixadora, que conta apenas com o N derivado do solo (Shearer e Kohl, 1986).

Apesar das vantagens em relação a outros métodos, o sucesso da quantificação da fixação de N_2 utilizando a técnica do ^{15}N a nível de abundância natural depende do padrão isotópico do N no sistema. Estimativas precisas requerem uma grande diferença (+5 ou -5‰) nos $\delta^{15}N$ das espécies referência e o N derivado da fixação. Além disto, diferenças menores que 2‰ entre espécies devem ser discutidas com cautela, mesmo que sejam estatisticamente significativas (Högberg 1997). Em determinados ambientes (principalmente florestas tropicais), estas diferenças não são detectadas (Handley et al 1994; Roggy et al, 1999; Gehring e Vlek, 2004), tornando o método inadequado para fazer estimativas da FBN.

O $\delta^{15}N$ como um integralizador dos processos da ciclagem do nitrogênio

Os solos são enriquecidos em ^{15}N, com algumas exceções em ecossistemas particulares (Schearer e Kohl, 1986). O $\delta^{15}N$ do N total do solo é determinado pelo sinal isotópico do compartimento de N orgânico, que é altamente estável e não varia durante décadas. Em contraste, os compartimentos biologicamente ativos, que são os disponíveis para as plantas, são uma fração diminuta do N total do solo e possuem uma dinâmica bastante rápida, variando em pequenos períodos de tempo (dias ou semanas). Desta forma, o $\delta^{15}N$ do N total do solo não é, em geral, uma boa aproximação do $\delta^{15}N$ do N disponível para as plantas. Por outro lado, as plantas são integradoras do $\delta^{15}N$ de todas as fontes de N disponível (N inorgânico e formas simples de N orgânico) (Högberg, 1997). Embora seja arriscado elucidar os processos de ciclagem do N com base apenas no sinal de $\delta^{15}N$ foliar (Sprent et al., 1996), a geração de grandes coleções de dados, devida à crescente disponibilidade de espectrômetros de massa automáticos, propiciou a observação de alguns padrões isotópicos reproduzíveis na vegetação de locais diferentes, que tem sido considerada uma fonte de informações valiosas sobre a ciclagem do nitrogênio em estudos biológicos e ecológicos (Handley e Scrimgeour, 1997).

Praticamente todas as transformações do nitrogênio fracionam preferencialmente para o ^{14}N, geralmente resultando em sinais isotópicos diferentes entre fontes e produtos (Handley e Raven, 1992). Devido à dinâmica extremamente rápida do N, o $\delta^{15}N$, no nível de abundância natural, não pode ser considerado um bom traçador dos fluxos do elemento entre os diferentes compartimentos de um sistema natural. As amostras tomadas de um ecossistema (solo, tecidos de planta), para as quais existem métodos analíticos de abundância natural apropriados, ocupam uma estreita faixa de valores de $\delta^{15}N$, que encobrem uma grande faixa de processos e suas interações (Handley e Scrimgeour, 1997). Os sinais totais de ^{15}N destas amostras, que integralizam os sinais de diferentes formas de N, são delineados pelo balanço entre as entradas e perdas de N no ecossistema, dando uma idéia sobre o funcionamento dos mesmos (Robinson, 2001).

Todas as entradas de N em um ecossistema natural derivam da atmosfera. A média geral destes "inputs" é empobrecida em ^{15}N, ou próxima de 0‰ (composição isotópica do N_2 atmosférico). As perdas (volatilização ou lixiviação) são de produtos da mineralização e processos subseqüentes, todos discriminadores do ^{15}N e, por conseguinte, geradores de produtos mais leves que o substrato. Quando estes compostos são perdidos, o N restante no sistema fica enriquecido em ^{15}N (Schearer e Kohl, 1986; Handley e Haven, 1992; Handley et al., 1999). Os demais processos do ciclo do N envolvem transformações do elemento dentro do sistema, gerando efeito isotópico apenas nos compartimentos menores biologicamente ativos. O balanço entre entradas e saídas é que vai delinear a extensão na qual o ecossistema ficará isotopicamente enriquecido.

Discriminações isotópicas nos processos do ciclo do N

O fracionamento isotópico, responsável por variações nas razões isotópicas dos compostos formados ao longo de uma seqüência de reações, é resultado de efeitos cinéticos e de equilíbrio. Moléculas ou íons mais pesados estão mais fortemente ligados (logo uma energia de ativação maior é requerida par dissociá-los) e reagem mais lentamente que análogos isotopicamente mais leves. Desta forma, para uma dada reação incompleta $A \leftrightarrow B$, o produto B será menos enriquecido que A em ^{15}N. O fator de fracionamento pode ser descrito pela relação entre os $\delta^{15}N$ da fonte e do produto (α) ou como $\Delta\delta$, isto é, a diferença entre o $\delta^{15}N$ do substrato (δ_A) e o $\delta^{15}N$ do produto (δ_B), comumente referido como ε (Högberg, 1997):

$$\alpha = \delta_A/\delta_B \qquad \text{e} \qquad \varepsilon(‰) = 1000(\alpha - 1)$$

Muitos valores diferentes de fracionamento são encontrados na literatura, mas não devem ser adotados para outros sistemas sem considerações apropriadas, pois dependem, além da estequiometria das reações, das condições bióticas e abióticas específicas que condicionam estas reações. Alguns valores de fracionamento isotópico (ε‰) para os principais processos do ciclo do N são: fixação do N_2 via nitrogenase (0-6), volatilização do NH_3 (40-60). nitrificação (35-60), denitrificação (28-33), amonificação (0-5) (coletados por Robinson (2001), em várias fontes referenciadas em seu trabalho).

Abertura do ciclo do N

Como as concentrações de ^{15}N das plantas e dos solos são o resultado líquido dos processos biogeoquímicos que atuam na ciclagem do nitrogênio, elas podem fornecer informações importantes sobre a ecologia e dinâmica da vegetação e suas interações com o ambiente em volta. Ao se comparar sinais isotópicos de diferentes locais, em grandes ou pequenas escalas, podem-se observar consideráveis variações no $\delta^{15}N$ de plantas não fixadoras de nitrogênio, devidas à atuação diferenciada dos processos de ciclagem do N. Os mecanismos que causam o enriquecimento de um local em comparação a outro não são claros, mas a hipótese corrente relaciona o $\delta^{15}N$ do sistema com a extensão na qual o N flui do compartimento orgânico para o inorgânico, que fica disponível para perdas por lixiviação ou volatilização (Sprent et al., 1996; Austin e Vitousek, 1998; Swap et al., 2004).

Austin e Vitousek (1998) utilizaram uma abordagem isotópica para avaliar como a variação da quantidade de precipitação afeta a ciclagem de nutrientes entre locais, comparando valores de $\delta^{15}N$ de folhas de *M. polymorpha* em 5 florestas nativas do Havaí. Com a observação de valores significativamente mais pobres com o aumento da precipitação, estes autores propuseram uma definição de "abertura" do ciclo do nitrogênio, em termos da importância da ciclagem dentro do sistema, em relação à importância de entradas e saídas do sistema solo/planta. Analisando o gradiente de precipitação dos locais estudados, eles consideraram que, nos mais secos, a demanda reduzida das plantas, resultante do stress hídrico, em conjunto com um efeito menos pronunciado da baixa disponibilidade de água na decomposição e troca de nutrientes, resultou em grandes compartimentos de nitrogênio inorgânico no solo (particularmente nitrato), que são relativamente lábeis e sujeitos a perdas por lixiviação (em conseqüência das chuvas concentradas) e por volatilização de amônia. Mesmo que a magnitude das perdas fossem pequenas, a perda relativa ao nitrogênio ciclado entre plantas e solo podia ser grande, resultando num ciclo de nitrogênio aberto em locais mais secos. Com o aumento da precipitação, o aumento da produção podia levar ao aumento da absorção pelas plantas e a um compartimento maior de carbono no solo e na serapilheira. Maior disponibilidade de carbono orgânico podia favorecer a imobilização de nutrientes, e juntamente com aumento da demanda pelas plantas, resultaria na diminuição do nitrogênio inorgânico no solo e no aumento do nitrogênio orgânico. A retenção do nitrogênio nestes locais mais úmidos podia aumentar, levando a menores perdas do sistema relativas à ciclagem entre solos e vegetação.

Handley et al. (1999) aperfeiçoaram a hipótese de "abertura" do ciclo do N desenvolvendo um modelo conceitual para explicar os valores extremamente baixos de $\delta^{15}N$ de folhas encontrados em locais úmidos e frios. Neste modelo, eles propuseram que a lixiviação de N orgânico dissolvido (proteínas solúveis, aminoácidos, peptídeos, etc), mais rico em ^{15}N que outros produtos da mineralização, pode ser um mecanismo responsável pelo empobrecimento isotópico destes ecossistemas.

Este conceito foi utilizado por diversos outros autores como uma ferramenta para interpretar aspectos do ciclo do nitrogênio (entre os mais recentes, Aranibar et al., 2004; Swap et al., 2004; Ometto et al., 2006), principalmente para avaliar efeitos da precipitação. Entretanto, qualquer fator (aridez, cultivo, pH extremo, fogo) que aumente o fluxo proporcional do compartimento orgânico para o compartimento mineral (que é mais sujeito a perdas) empurra o sistema para o enriquecimento em ^{15}N (Högberg, 1997; Handley et al., 1999). Por exemplo, Roggy et al. (1999) compararam a abertura do ciclo entre duas florestas úmidas da Guiana Francesa, sobre diferentes tipos de solos (um espodossolo e um oxissolo), atribuindo os valores de $\delta^{15}N$ mais altos das folhas da vegetação do oxissolo a um ciclo de N mais aberto que o sistema solo/planta sobre o espodossolo.

Tendências dos padrões isotópicos

Devido às fortes influências dos fatores ambientais sobre o equilíbrio entre entradas e perdas de N dos ecossistemas, algumas tendências e padrões de correlações entre condições ambientais e sinal de ^{15}N têm sido encontrados ao longo do globo (Handley e Scrimgeour, 1997). Os processos que causam o aumento do sinal de $\delta^{15}N$ de um sistema estão relacionados com a extensão na qual o nitrogênio mineral é perdido por lixiviação ou volatilização. Como o regime de água de determinado local é um dos principais condicionantes destes processos, na literatura são encontradas fortes correlações entre a composição isotópica do N do solo disponível para as plantas e a disponibilidade de água. O padrão de enriquecimento ou empobrecimento é diferente para comparações em diferentes escalas. Em escalas globais (Handley e Scrimgeour, 1997), insulares (Austin e Vitousek, 1998) ou em grandes transectos geográficos (Aranibar et al., 2004; Swap et al. 2004), a tendência é de diminuição do sinal de ^{15}N das folhas à medida que a precipitação média anual aumenta. A explicação é que, em locais úmidos, a reciclagem interna de N em forma orgânica é grande e as perdas de N mineral são pequenas. Em locais secos, proporcionalmente mais N pode permanecer em formas inorgânicas e ser perdido por volatilização ou lixiviação. Em escalas menores, no nível de paisagem, locais com maior disponibilidade de água, como depressões topográficas, em geral possuem vegetação com folhas mais enriquecidas que locais mais secos, o que é relacionado a maiores taxas de mineralização e denitrificação (Garten, 1993; Handley e Scrimgeour, 1997).

A latitude, à medida que condiciona temperatura e precipitação, também influencia os padrões (Handley et al., 1999). Esta observação foi confirmada por Martinelli et al. (1999), que demonstraram que os valores de δ^{15}N de folhas de árvores são significativamente maiores em florestas tropicais que em florestas temperadas, sugerindo que florestas tropicais têm um ciclo de nitrogênio mais aberto, com maiores perdas via processos fracionadores.

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CAPÍTULO 1

^{15}N natural abundance of non-fixing woody species in the Brazilian dry forest (caatinga).

Running head: **^{15}N natural abundance in caatinga**

Article type: research article

Title: **^{15}N natural abundance of non-fixing woody species in the Brazilian dry forest (caatinga).**

Authors: Ana Dolores Santiago de Freitas(1), Everardo Valadares de Sá Barretto Sampaio(2), Rômulo Simões Cezar Menezes(2), Holm Tiessen(3)

Affliations: (1) Universidade Federal Rural de Pernambuco (UFRPE), Departamento de Agronomia. (2) Universidade Federal de Pernambuco (UFPE), Departamento de Energia Nuclear, (3) Inter-American Institute for Global Changes (IAI).

Address for correspondence: R. Jáder de Andrade, 404/1801, CEP 52061-060, Recife (PE) Brazil. 55-81-32692660, ana.freitas@depa.ufrpe.br

Abstract

Foliar $\delta^{15}N$ values are useful to calculate N_2 fixation and N losses from ecosystems. However, a definite pattern among vegetation types throughout the world is still not recognized and no data are available for some that cover large areas, like the caatinga, in semi-arid Northeast Brazil. We sampled four sites in the caatinga, arranged in a systematic way to form two pairs, one pair one latitudinal degree apart from the other, along a water availability gradient (Agreste and Sertão regions). Previously selected non-nodulating legume and non-legume species were sampled in 5 to 6 plots at each site. Latitude had no significant effect. The Agreste sites with lower total annual rainfall (700 mm) but better distribution (6 months) had average $\delta^{15}N$ values of 9.4 and 10.1‰, which are among the highest already reported. These averages were significantly greater than those in the Sertão (6.5 and 6.3‰), which have higher total rainfall (800 mm) but worse distribution (3 months). Therefore, total rainfall explains little of the variation. There were no significant differences, at each site, among plots, among species or between the legume and the non-legume group of species, in spite of the higher N content of the first group. Therefore, they constitute ideal reference plants in estimations of legume N_2 fixation. The higher Agreste values could result from higher losses of ^{15}N depleted gases or lower losses of enriched ^{15}N material than in the Sertão sites.

Key-words: latitude, N-15, nitrogen cycle, rainfall, stable isotope, tropical vegetation.

Introduction

Measurements of plant natural $\delta^{15}N$ have two important applications: 1) estimation of atmospheric N fixation by single plants (Shearer & Kohl 1986); and 2) evaluation of the openness of the N cycle in a given site (Austin & Vitousek 1998). Both depend on the $\delta^{15}N$ signals of non-fixing plants. In the first case, they are used as reference values and, since atmospheric N has a $\delta^{15}N$ of zero, the higher they are the greater is the precision of calculation of the proportion of N fixed by leguminous plants. In the second case, higher $\delta^{15}N$ values indicate higher ^{14}N losses from the system and, therefore, a more open N cycle in the site.

In the last decade, ^{15}N natural abundance determinations have revealed considerable variation among different vegetation types (Austin & Vitousek 1998; Martinelli et al. 1999; Roggy et al. 1999; Handley et al. 1999; Eshetu & Högberg 2000; Arndt et al. 2004; Ometto et al. 2006). In a globally-derived data set for site-averaged foliar $\delta^{15}N$, Handley et al. (1999) registered values from –8.0 to +8.1‰, and even higher values (up to 12.8‰) have been reported for single species samples (Aranibar et al. 2004). In general, areas with lower mean annual rainfall (MAR) have higher $\delta^{15}N$ than areas with higher MAR (Austin & Vitousek 1998; Handley et al. 1999) and tropical forests have higher values than temperate ones (Martinelli et al. 1999). However, many exceptions of these general trends are found (Austin & Vitousek 1998, Handley et al. 1999, Swap et al. 2004), probably because both characterizations include a broad range of environmental conditions. Therefore, more site data are necessary before a clearer picture can be delineated.

No information is available on $\delta^{15}N$ values of plants in the large tropical semi-arid caatinga vegetation of Northeast Brazil. As a consequence, there are also no data on N_2 fixation of native plants in the region. Therefore, a project was established to determine the $\delta^{15}N$ of non-fixing and fixing plants in sites chosen in a systematic away to form two pairs, one pair one latitudinal degree apart from the other, along an east-west water availability gradient. The first objective was to determine if $\delta^{15}N$ values of non-fixing plants were high and similar enough to allow reasonable calculations of N_2 fixation by legume plants. The second objective was to evaluate if there was any spatial and climatic pattern in the $\delta^{15}N$ values. If the first hypothesis were validated, a third objective was to estimate N_2 fixation by leguminous species in the four sites. Results concerning the first two objectives are the subject of this paper, while those concerning the third one are being published in another paper (Freitas et al., submitted).

Material and methods

Site description

Four municipalities were selected in the states of Pernambuco and Paraíba, one in the Agreste and one in the Sertão zone of each state (Table 1). Both Agreste municipalities (Remígio in Paraíba and Caruaru in Pernambuco) are located about 100 km from the Atlantic coast in the Borborema Mountain Range (Silva et al. 1993). Remígio is approximately 150 km north of Caruaru. The two Sertão municipalities (Santa Teresinha in Paraíba and Serra Talhada in Pernambuco) are at about the same latitudes as the Agreste ones but 300 to 400 km from the coast, within the large interplanaltic depression zone (Silva et al. 1993).

The two Agreste municipalities have total mean annual rainfall (MAR) close to 700 mm while the two Sertão municipalities have MAR around 800 mm (Table 1). In spite of the relatively small difference of only 10-15% lower MAR, the Agreste sites have a higher water availability throughout the year than the Sertão sites. In fact, MAR alone is not a good measure of the rainfall regime, specially in the Sertão, because of the large interannual variation. In a 30-year time series, total annual rainfall varied from 187 to 1522 mm in the

Sertão sites but only from 465 to 1004 mm in the Agreste sites. Rainfall is concentrated mostly in three months (February to April) in the Sertão sites (average of 73% of the annual rainfall) and the month of highest rainfall represents 41% of the annual rainfall. In contrast, in the Agreste sites rainfall is reasonably well distributed over five months, from March to July (67% of the annual rainfall), and the month of highest precipitation represents only 16% of the annual rainfall. Average temperatures vary little in the region but Remígio is slightly cooler and Santa Teresinha slightly hotter than the other areas (Table 1).

The more uniform rainfall distribution in the Agreste is reflected in its vegetation. Descriptions of vegetation composition and structure have been published for the sampling sites of Caruaru (Alcoforado Filho et al. 2003), Serra Talhada (Ferraz et al. 2003) and Remígio (Pereira et al. 2002). The number of species, the height and diameter of plants is higher in the Agreste sites than in Serra Talhada. There is no published description of vegetation in the Santa Teresinha site but umpublished data from this site shows slightly lower number of species, height and diameter of plants than in Serra Talhada, most likely reflecting an even lower water availability.

Sampling procedure and analyses

One sampling site was chosen in each municipality, except in Remígio, where two sites were selected. At Remígio, site 1 was an area with a vegetation that has had little disturbance over the last 60 years while site 2 was a caatinga forest fragment about 100 m from the border of site 1 and subjected to a higher degree of human impact (Pereira et al. 2003). The pair was intended to evaluate the influence of management under the same environmental conditions. The sampling sites at Caruaru and Serra Talhada were also caatingas that have had little impact in the last decades. The Santa Teresinha site, although located in the experimental station of Universidade Federal de Campina Grande, is subject to a degree of disturbance similar to that of the forest fragment in Remígio (presence of cattle and occasional firewood gathering by the local population).

Two groups of plants were selected for sampling: non-nodulating Leguminosae species (Allen & Allen 1981; Faria et al. 1984, 1987; Faria & Lima, 1998) and species belonging to other families. The Leguminosae species were: *Acacia glomerosa* Benth, *Amburana cearensis* (Allemão) A.C. Smith, *Bauhinia cheilantha* (Bong.) Steud, *Caesalpinia ferrea* Mart. ex Tul, *Caesalpinia pyramidalis* Tul., *Senna macranthera* (Collad.) H.S.Irwin & Barneby and *Senna spectabilis* (DC.) H.S.Irwin & Barneby. The species belonging to other families were: *Aspidosperma pyrifolium* Mart., *Commiphora leptophloeos* Mart. J.B. Gillett, *Croton sonderianus* Muell. Arg. *and Tabebuia impetiginosa* (Mart. ex DC.) Standl. They are all tree or large shrub species commonly found in caatinga areas and their presence in each site, except Santa Teresinha, was known from previous studies in the same areas (Alcoforado Filho et al. 2002; Ferraz et al. 2003; Pereira et al. 2002). The two groups were selected because the legume species, in spite of being non-nodulating, tend to have higher leaf N content than plants belonging to other families (Sprent et al. 1996; Martinelli et al. 2000).

In each site, five to six sampling plots were established. The center of each plot was established in an area where the largest diversity of the previously defined sampling species was found. Plants belonging to these species were sampled usually within 10 m distance from the center point but occasionally up to 20 m if no other plant of one species had been found at a shorter distance. Plots were at least 50 m distant from each other. Even extending the plot size to include a nearby plant, not all selected species were found in each plot and for most species we did not obtain the five replicates originally intended.

All plants selected for sampling had at least 5 cm diameter at the base of the stem and 3 m of total height. Approximately 100 to 200 g of dry mass of fully expanded green leaves from each selected plant were cut, oven dried at 60 ^{o}C, ground and analyzed for their total N and ^{15}N abundance contents. Total N was analyzed by the micro kjeldhal procedure and ^{15}N abundance by mass spectrometry and expressed as "delta" notation:

$$\delta = (R_{sample}/R_{standard} - 1) \times 1000$$

where R_{sample} and $R_{standard}$ are the ratio ^{15}N:^{14}N of the sample and the standard (air), respectively.

The data were subjected to usual statistical analysis, comparing different plant groups. Using individual plant values as replicates, $\delta^{15}N$ comparisons were made of: 1) averages of all species of a same site; 2) averages of the group of all legumes versus all non-legume species of a same site; and 3) averages of legume, non-legume and all species of the two Remígio sites (little disturbed and forest fragment). Also using individual plant values as replicates, comparisons were made of site averages of total N content for legume, non-legume and all species. Considering the absence of significant differences among species of a same site, values of different species were used as replicates to compare plot averages of a same site. Significant differences were established considering the 0.05 probability level of the Tukey test.

Results

There were no significant differences between averages of $\delta^{15}N$ values of the two Agreste sites (9.4 and 10.1 ‰) nor between the two Sertão sites (6.7 and 6.3 ‰) but those of the Agreste were significantly higher than those of the Sertão (Table 2). The average of the disturbed forest fragment at Remígio (10.2 ‰) was not significantly different from that of the little disturbed caatinga at the same site (10.1 ‰).

Within each site, the similarity of $\delta^{15}N$ of all plants was remarkable. The averages of the legume group were not different from the non-legume group in any of the sites (Table 2), despite differences in the leaf N content (Table 3). The leaf N contents of the legume plants were higher than those of the non-legume group but the differences were only significant in the Agreste sites, which also tended to have higher values than the Sertão sites.

There were also no significant differences in $\delta^{15}N$ among species in any of the sites (Table 2). The largest absolute differences between averages of two species, within the same site, varied from 0.85 (Remígio) to 2.4 $\delta^{15}N$ units (Santa Teresinha). In the two Sertão sites, the lowest absolute values were for two of the legume species (*Bauhinia cheilantha* in Santa Teresinha and *Senna macranthera* in Serra Talhada) and in the two Agreste sites for a non-legume species (*Croton sonderianus*). The opposite happened with the highest values: in the two Agreste sites they were for two legume species (*Acacia glomerosa* in Remígio and *Acacia paniculata* in Caruraru) and in the two Sertão sites for non-legumes (*Croton sonderianus* in Santa Teresinha and *Aspidosperma pyrifolium* in Serra Talhada). However, in all sites there were legume and non-legume species with very similar values.

Considering the similarity in $\delta^{15}N$ averages of species, the values of all plants in each plot (data not shown) were used as replicates to test for spatial differences within each site. In any of the sites, there were no significant differences among plots. The absolute difference values varied from 1.06 (Remígio) to 2.24‰ (Serra Talhada).

Discussion

The site-averages of foliar $\delta^{15}N$ reported in this paper for the Agreste sites (9.4 to 10.1‰) were among the highest reported in the literature (Schulze et al. 1991; Handley et al. 1999; Swap et al. 2004). The highest average previously reported went up to about 11‰ (Schulze et al. 1991) but the large review of Handley et al. (1999) showed less than ten sites around the world having averages above 7.0‰. Most of these sites are in tropical dry regions, in Africa and Australia, and were the basis of the confirmation by Handley et al. (1999) of the tendency proposed by Austin & Vitousek (1998) of increasing $\delta^{15}N$ values in decreasing MAR sites. The fact that our high $\delta^{15}N$ caatinga values were obtained under semiarid conditions seem to confirm the hypothesis proposed by those authors and corroborated by some others (Aranibar et al. 2004; Swap et al. 2004).

A comparison of the Agreste and Sertão values also seems to confirm the tendency. The Sertão sites with their higher MAR had significantly lower $\delta^{15}N$ averages (6.7 and 6.3‰) than the Agreste sites (9.4 and 10.1‰). However, the differences in mean average rainfall are rather small (100 mm in averages of 700-800 mm, or less than 15%) and their large annual variations indicate that these means are not a good measure of local precipitation. A critical examination of world values shows that the general tendency is contradicted by both types of exceptions: high MAR with high $\delta^{15}N$ and low MAR with low $\delta^{15}N$. Values of $\delta^{15}N$ as high as those of the Sertão sites have been reported in Amazon sites with MAR around 2000 mm (Ghering & Vleck 2004; Ometto et al. 2006) and also in other tropical rain forests (Martinelli et al. 1999). On the other hand, there are sites with similar and even lower MAR as the Agreste ones and much lower $\delta^{15}N$ values. In the paper where this hypothesis was proposed (Austin & Vitousek 1998), the site with lowest MAR (500 mm) had a $\delta^{15}N$ value of only 0.04‰. In Africa, several sites with MAR below 700 mm had lower $\delta^{15}N$ values than those of the Agreste, down to values as low as 1.0‰ (Handley et al. 1999; Aranibar et al. 2004; Swap et al. 2004).

Exceptions to the MAR tendency have been observed not only at this global scale, comparing sites in different continents, as was done by Handley et al. (1999). They are also present at the continental level in Africa (Swap et al. 2004) and in South America, if we compare the data from our caatinga sites in the present study with those of Amazonia (Roggy et al. 1999; Ghering & Vleck 2004; Ometto et al. 2006) and cerrado sites (Sprent et al. 1996; Bustamante et al. 2004). Among these three regions, the lowest average $\delta^{15}N$ values (0,49 to 1,73‰) were reported for cerrado sites (Sprent et al. 1996) which have MAR values (around 1500 mm) intermediate between Anazonian (> 2000 mm) and caatinga sites (< 1000 mm).

In addition to these comparisons at the continental scale, differences in $\delta^{15}N$ that could not be explained by variations in MAR have also been reported at the local level (Garten 1993; Handley & Scrimgeour, 1997). At the local level, the explanation for the differences in $\delta^{15}N$ were shifted to water availability instead of MAR (Garten 1993; Handley & Scrimgeour, 1997) because MAR has less variation than other variables affecting water availability in the soil – plant system. When larger areas are considered, MAR is probably the variable responsible for the largest variation in water availability besides being the simplest to refer and most frequently available data. MAR was certainly used as an approximation to water availability in all regional and continental comparisons of $\delta^{15}N$, even if not explicitly stated (Austin & Vitousek 1998; Handley et al. 1999; Aranibar et al. 2004; Swap et al. 2004).

When comparing the two caatinga sites in the present study, MAR may not be the best estimator of water availability. Not only the mean values are not very different, but the differences in rainfall distribution indicate that water is more available, along the year, in Agreste than in Sertão, in spite of the slightly lower MAR of the former. The larger size of the vegetation and the greater number of species seem to confirm the better growing conditions in Agreste, most of which determined by water availability since other soil characteristics are more favorable for plant growth and development in the Sertão sites (Alcoforado Filho et al. 2003; Pereira et al. 2002; Ferraz et al. 2003). If we consider these indications, the sites with higher water availability had also higher $\delta^{15}N$. However, in a continental scale, comparing Amazonian, cerrado and caatinga sites, water availability has the same pattern as MAR and, in this comparison, cerrado, with its intermediate availability, has the lowest $\delta^{15}N$, while the two extreme regions have similar ^{15}N concentrations. Clearly, MAR or water availability alone cannot explain the variations in $\delta^{15}N$.

Martinelli et al. (1999) proposed that tropical forests had higher $\delta^{15}N$ values than temperate ones, implying an effect of latitude. The similarity of $\delta^{15}N$ values between both the two Agreste and the two Sertão sites indicate that they were not affected by latitude. However, the differences in latitude were small, only one degree. Besides, latitude per se has no direct influence in the soil – plant system. Its clearer indirect influence is higher temperature at lower latitudes, a factor that is present in the broad comparison of tropical and temperate forests but not at the regional scale of our caatinga sites. Differences in temperature were small (maximum of 4 ^{o}C) and more affected by local conditions than by latitude, both the lowest and the highest temperatures at the sites of the same parallel (Remígio and Santa Teresinha). Comparing this pair of sites, the one with lowest temperature had the highest $\delta^{15}N$ value, contrary to the expected tendency of hotter areas with higher ^{15}N enrichment. Temperature has a direct effect in plant and soil microorganisms metabolism and an indirect effect in water availability, higher temperatures resulting in higher potential evapotranspiration. Both higher metabolism and higher evapotranspiration may result in faster N cycling in the soil – plant system, provided other growth factors are not limiting.

The faster N cycling of tropical forests compared to temperate ones has been proposed as one of the causes of their higher $\delta^{15}N$ (Martinelli et al. 1999). The higher $\delta^{15}N$ values in the Agreste sites, which most likely have a faster cycle than the sites at Sertão, seem to confirm the tendency. However, the tendency does not hold at the regional or continental scales. Forests and other vegetation types in tropical dry areas have their N cycles limited by water availability and yet have the highest $\delta^{15}N$ signals. N cycles in cerrado areas are certainly faster than those in caatinga and their $\delta^{15}N$ values are much lower.

Ultimately, the causes of higher $\delta^{15}N$ in a soil – plant system have to be higher inputs of ^{15}N enriched nitrogen and/or higher losses of ^{15}N depleted nitrogen (Handley et al. 1999). Since, in general, the balance of N inputs tends to have a $\delta^{15}N$ signal close to zero, it is assumed that sites with higher $\delta^{15}N$ have higher relative losses of ^{15}N depleted nitrogen. Therefore, high N losses have become the standard explanation for the high $\delta^{15}N$ of any site. They are referred as the openness of the N cycle (Austin & Vitousek 1998). In most cases, the real losses are not known but hypotheses are created to justify their high relative level in any high $\delta^{15}N$ site. In the absence of data, they are theoretical speculations. For these reasons, measurements of N losses coupled with determination of the ^{15}N abundance in the different forms of N are strongly needed but they are difficult to make (Högbom et al. 2002). In a few measured cases, the losses did not justify the $\delta^{15}N$ signal (Aranibar et al. 2004).

Measurements of N losses at the caatinga sites were not made but the indirect available evidence can be used to propose hypotheses and compare situations. The Sertão sites, with their lower $\delta^{15}N$ averages than the Agreste sites, would have either higher N_2 fixation or lower ^{14}N losses. The number of potentially fixing legume species and plants (Alcoforado Filho et al. 2003; Pereira et al. 2002; Ferraz et al. 2003) and their estimated biomasses and total N content are lower in Sertão than in Agreste, therefore it is unlikely that N_2 fixation is higher in the former. Besides that, the soil in the Sertão sites remains wet for a shorter period than Agreste ones and this could result in lower N volatilization and denitrification. Since the gaseous losses are enriched in ^{14}N this could be the cause of the lower $\delta^{15}N$ in the Sertão. Data on gaseous losses and ^{15}N abundance of soil – plant system are rather scarce but Aranibar et al. (2004) could find no direct connection of the two measurements in a dry area, in Africa. On the other hand, the more intense and concentrated rains of the Sertão sites could result in higher N leaching than in the Agreste. It has been assumed that this leaching results in loss of enriched ^{14}N (Handley & Raven 1992; Högberg, 1997; Handley et al. 1999) but the general observation that the superficial soil layer has a lower $\delta^{15}N$ than subsuperficial layers (Martinelli et al. 1999; Eshetu & Högberg 2000) indicates a preferential movement of enriched ^{15}N compounds to deeper layers. In the shallow soils of the Sertão sites, the downward moving enriched ^{15}N compounds may be lost as leaching to a higher degree than in the Agreste sites. At a continental scale, the lower $\delta^{15}N$ of cerrado could be a combination of higher N_2 fixation in a system with a high temporal renewal of biomass and/or higher losses of enriched ^{15}N compounds to deeper soil layers.

The results indicate a series of variables with little effect on the ^{15}N discrimination (Table 2). Anthropic disturbance caused little alteration in the $\delta^{15}N$ of plants in the disturbed fragment compared to those of the more preserved neighbor site at Remígio. The nature of disturbance (more frequent cattle grazing and eventual cutting of wood) is less drastic than those assumed to cause higher N losses from the system, like burning and clear cutting (Neil et al. 1995; Piccolo et al. 1994, 1996).

Plot averages, within each site, were not significant different and varied in a relatively narrow range (data not shown), implying that local scale (less than 1 km distance) variations in environmental conditions had little effect in the N cycle. Similar plot comparisons were not found in the literature but comparisons of sites located at greater distances within a 1350 ha ecological reserve in the cerrado region also showed little variation, in spite of a marked difference in vegetation physiognomy (Bustamante et al. 2004). Papers relating differences among sites usually sampled places further apart than our plots or these cerrado sites (Sprent et al. 1996; Ometto et al. 2006). One important consequence of the absence of differences among plots is that it simplifies sampling of reference plants when trying to quantify N_2 fixation using ^{15}N natural abundance.

The $\delta^{15}N$ averages of the group of legume plants were not different from those of the non-legume species group and species within the same site had similar ^{15}N abundances.

Significant differences between the two groups of plants were found in cerrado sites, with the interesting fact that in some places non legumes had higher $\delta^{15}N$ than non-nodulating legumes while in other sites the opposite occurred (Sprent et al. 1996). Within the same ecological reserve (Bustamante et al. 2004), non-legumes had a slightly higher, although significant, $\delta^{15}N$ than legumes (0.52 versus –0.52‰) but the result may have been influenced by the absence of a clear separation of fixing and non-fixing legumes.

The similarity between the legume and the non-legume groups is more striking because the first group had a significantly higher N content than the second one, in the more humid Agreste sites (Table 3). This superiority has been registered elsewhere (Sprent et al. 1996; Martinelli et al. 2000; Bustamante et al. 2004). McKey (1994) suggested that, in general, Leguminoseae are nitrogen-demanding plants that maintain high levels of N in leaf tissue in order to maximize photosynthetic rates per unit leaf area. This, in turn, allows them to amortize the carbon costs of leaf construction and enables them to produce and drop leaves quickly in response to changing resource availability.

The absence of differences among species and plants within the same site contrasts sharply with results obtained in other vegetation types. Differences of up to 4-5 $\delta^{15}N$ units have been reported among species of a single site (Sprent et al. 1996; Martinelli et al. 1999; Bustamante et al. 2004) and up to 8 $\delta^{15}N$ units among plants of a same site (Ometto et al. 2006). In the present study, the limitation of species to deciduous trees and large shrubs may have contributed to this uniformity. They are probably absorbing N from the same sources, with roots distributed in all layers of the relatively shallow soils present in all sites. The number of species was also not very high and the same ones were collected in all sites, reducing the variability of the whole sample. It is possible that other caatinga plant types (vines, epiphytes, herbaceous, etc) present different $\delta^{15}N$ but no data are yet available.

Conclusions

Plants in semiarid Northeast Brazil had high δ^{15}N values, indicating that their cycles are subjected to high losses of ^{15}N depleted nitrogen. Plants in the Agreste sites, which have lower mean average but a more uniform rainfall distribution along the year than the Sertão sites, had the highest δ^{15}N values, and among the highest already reported in the world. The latitude difference of one degree had no significant effect on the δ^{15}N values.

The high δ^{15}N values of all non fixing plants within a site were very similar, both spatially and among species. This isotopic pattern is ideal for studies of biological N_2 fixation, facilitating the selection of reference plants and the sampling procedure.

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Table 1 – General characteristics of the four chosen municipalities, in the States of Paraíba (PB) and Pernambuco (PE), Brazil.

Characteristics	Municipality (state)			
	Remígio (PB)	Santa Teresinha (PB)	Caruaru (PE)	Serra Talhada (PE)
Coordinates	6°52’S 35°47’W	07º03'S 37º29'W	08º15'S 35º57'W	07º59'S 38º18'W
Altitude (m)	596	380	545	500
Soil type	Regolithic Neosol	Litholic Neosol	Yellow Argisol	Luvisol
Annual rainfall (mm)	700	824	696	768
Months with water deficit	4 - 5	9 - 10	4 - 5	6 - 7
Average temperature (°C)	22	26	24	24

Table 2: Leaf δ^{15}N of plants belonging to non N_2 fixing species of Leguminosae or of other families, collected at four different sites in the semi-arid caatinga of Paraíba and Pernambuco States, Brazil. Numbers in parentheses refer to replicates.

Species	Remígio		Caruaru	Serra Talhada	Santa Teresinha
	(undisturbed)	(disturbed)	(undisturbed)	(undisturbed)	(disturbed)
Leguminosae					
Acacia glomerosa	(1) 10.63	(2) 11.37±1.40	(4) 9.39±0.51	-	-
Amburana cearensis	(5) 10,07±0,54	-	-	(4) 5,12±0,97	-
Bauhinia cheilantha	(4) 9.92±0.72	(1) 7.63	-	(5) 6.30±1.56	(5) 5.87±0.63
Caesalpinia ferrea	-	(1) 11.58	-	(2) 6.76±0.25	-
C. pyramidalis.	(4) 10.03±0.48	(4) 10.01±1.76	(5) 9.48±1.06	(5) 6.54±1.28	(3) 6.76±0.67
Senna macranthera	(3) 10.61±050	-	-	(3) 5.59±0.44	-
S. spectabilis	-	(2) 10.29±1.98	-	(1) 8.34	-
Average	10.19±0.60	10.19±1.71	9.73±0.96	6,01±1.24	6.16±0.72
Other families					
Aapidosperma pyrifolium	(5) 9.71±0.68	(5) 11.14±1.40	-	(5) 7.75±1.62	(2) 7.20±0.97
Commiphora leptophloeos	(6) 10.65±0.65	-	-	(2) 6.10±0.61	-
Croton sonderianus	(6) 9.84±0.83	(5) 9.22±1.79	(4) 8.46±2.55	(5) 5.73±1.20	(2) 8.27±1.37
Tabebuia impetiginosa	-	-	-	-	(1) 6.08
Average	10.08±0.80	10.21±1.79	8.46±2.55	6.64±1.58	7.40±1.23
Overall Average	10.12±0.71 A	10,22±1,72 A	9.44±1.49 A	6.34±1.37 B	6.71±1.27 B

Averages followed by the letter are not significant different at the 0.05 level (Tukey test).

Table 3: Average leaf nitrogen concentrations of plants belonging to non N_2 fixing species of Leguminosae or of other families, collected at five different localities in the semi-arid caatinga of Paraíba and Pernambuco States, Brazil.

Site	Leguminosae	Other families	Average
Remígio (undisturbed area)	3.08±0.42Aa	2.29±0.34Ba	2.70±0.54a
Remígio (disturbed area)	3.09±0.52Aa	2.29±0.26Ba	2.69±0.58a
Caruaru (undisturbed area)	2.50±0.58Ab	1.81±0.20Bab	2.31±0.60ab
Serra Talhada (undisturbed area)	2.24±0.45Ab	1.75±0.24Ab	2.04±0.41b
Santa Teresinha (disturbed area)	1.99±0.54Ab	1.62±0.15Ab	1.86±0.48b
Average	2.58±0.63A	2.04±0.38B	

Averages followed by the same capital letter in the line and small letter in the column are not significant different at the 0.05 level (Tukey test).

CAPÍTULO 2

Biological Nitrogen Fixation in Legume Trees of the Brazilian Caatinga

Title: **Biological Nitrogen Fixation in Legume Trees of the Brazilian Caatinga**

Authors: Ana Dolores Santiago de Freitas [a*]; Everardo Valadares de Sá Barretto Sampaio [b]; Acácia da Rocha Fernandes [b].

Affiliations: [a] Universidade Federal Rural de Pernambuco. Departamento de Agronomia. R. Dom Manoel de Medeiros, s/n, Recife (PE), 52060-060, Brazil. [b] Universidade Federal de Pernambuco, Departamento de Energia Nuclear, Av. Prof. Luís Freire, 1000, Recife (PE), 50740-540, Brazil.

Correspondence address: * R. Jáder de Andrade, 404/1801, Recife (PE), 52061-060, Brazil. Phone: 55-81-21267979. E-mail: ana.freitas@depa.ufrpe.br

Abstract

Information on biological nitrogen fixation (BNF) in the dry forests of the northeast region of Brazil (caatinga) is scarce, in spite of its abundance of legume species. The BNF of tree legumes was estimated in four areas of the caatinga, analyzing potentially fixing species (target species) and non fixing species (reference species), using the ^{15}N natural abundance methodology. The differences between the isotopic signals of the target and reference species permit the safe identification of diazotrophic plants and reasonably precise calculations of proportions of N derived from the air (%Ndfa). Species with great N_2 fixation capacity were identified, the most remarkable being *Mimosa tenuiflora*, *Mimosa arenosa* and *Piptadenia stipulace*. In the four areas, the mean biological fixation contributions to the plants were high, varying from 27 to 68%. However, the estimated quantities of N, added annually to the systems, were low (2.5 and 11.2 kg ha^{-1} $year^{-1}$), due to the low proportions of fixing plants in the vegetation communities (2.4 to 11.8%). In situations of native vegetation regeneration, when the succession is dominated by fixing species, fixation can reach up to 130 kg ha^{-1} $year^{-1}$.

Key words: Dry forest, natural abundance, Northeast of Brazil, N-15, Semi-arid

Introduction

Biological fixation is one of the principal processes of nitrogen entry into natural ecosystems. For temperate forests there already are sufficient data for making reasonable estimations of quantities of N added annually to the systems (Cleveland et al., 1999). For tropical forests, knowledge on the importance of the biological nitrogen fixation process (BNF) associated to vascular plants is still very incipient, there being only a few estimates, principally for humid tropical forests (Roggy et al., 1999; Gehring et al., 2005). There are no data on BNF for the dry forests of Northeast Brazil, which cover an area estimated at approximately 1 million km^2. These forests, generally called caatingas, are conditioned by the predominant semi-arid climate, which has a high potential evotranspiration during the year (1500 to 2000 mm year^{-1}) and low, concentrated and erratic rainfall (300 to 1000 mm year^{-1}). The great richness and abundance of legumes in these forests (Queiroz, 2006) and the great contribution of BNF to the nutrition of legumes in arid or semi-arid environments (Shearer et al., 1983; Ndiaye and Ganry, 1997; Raddad et al., 2005) are indicative that this process can play an important ecological role in the caatingas.

The physiological capacity of forming nodules, that is only known for a small fraction of the legume species that occur in the caatinga (Allen and Allen, 1981; Faria et al., 1984, 1987, 1994; Faria and Lima, 1998), do not confirm fixation occurrence in the natural environment, since species that are able to form active symbiosis can obtain insignificant inputs of BNF if the N is well conserved in the system (Boddey et al., 2000). In this way, knowledge on the importance of BNF in natural environments requires that one estimates the atmospheric N contribution to direct nutrition of the species in the field. The natural ^{15}N abundance (δ^{15}N) is the most appropriate methodology for fixation measures of N_2 in natural ecosystems (Boddey et al., 2000; Högberg, 1997; Shearer and Kohl, 1986).

A previous work (Freitas et al., submitted) showed that non fixing nitrogen caatinga plants are very much enriched with ^{15}N and that this enrichment is uniform, as much spatially as between species. This isotopic standard is a promising condition for use of the ^{15}N natural abundance methodology for BNF estimates, because it facilitates the choice of reference plants, the sample scheme and the detection of significant differences between the δ^{15}N of diazotrophic and non diazotrophic plants (Högberg, 1997; Boddey et al., 2000).

The objective of this work was to estimate the importance of BNF in tree legumes of caatinga environment, using the ^{15}N natural abundance methodology.

Material and Methods

Studied areas

Four fragments of native caatinga were studied in four municipalities of the semi-arid that were siteized in the states of Paraiba and Pernambuco. Remígio (PB) and Caruaru (PE) are siteized in the Agreste physiographic zone and Santa Teresinha (PB) and Serra Talhada (PE) are siteized in the Sertão zone, and these zones reflect the different water availability conditions for plants (intensity and duration of water deficit period) in the fragments studied (Table 1).

Descriptions of composition and structure were published for the forest fragments of Caruaru (Alcoforado Filho et al., 2002), Serra Talhada (Ferraz et al., 2003) and Remígio (Pereira et al., 2002). The number of species, height and diameter of plants are greater in the Agreste sites than in Serra Talhada. There is no published description of the caatinga fragment of Santa Teresinha, but the number of species, height and diameter of the plants are slightly lower than those of Serra Talhada, probably reflecting the lower availability of water.

Sampling scheme

In each fragment, 5 to 6 plots were established with a 20 m radius for each and 50 m or more distant one from the other. Species potentially able to fixate N_2 (target species), or not (reference species) were sampled in each plot. The target species were legumes with a known nodulation capacity or without information on nodulation (Allen and Allen, 1981; Faria et al., 1984, 1987, 1994, Faria and Lima, 1998). Samples were collected from all the target species found in each plot and also from four reference species (Table 2). Due to variations in frequency of occurrence of the species, it was not possible to sample the same number of plants for all of the species, in all the sites. For being species of good distribution and high density in the majority of the caatinga areas, the following reference species were selected: two non legume species (*Croton sonderianus* Muell. Arg. and *Aspidosperma pyrifolium* Mart.) and two non nodulating legume species (*Bauhinia cheilanta* (Bong.) Steud and *Caesalpinia pyramidalis* Tul.).

Leaf material from different branches was collected from adult plants with diameters at breast height larger than 3cm. After collection, completely expanded health green leaves were selected, forming a composite sample of around 100 to 200g of leaves per plant. In all the areas the samples were collected during the rainy season. From all the sample species, botanical material was collected for comparison with the collections deposited in herbarium, from the floristic surveys carried out by Alcoforado Filho et al. (2002), in Caruaru, Ferraz et al. (2003), in Serra Talhada, and Pereira et al. (2002), in Remígio.

Determination and statistical analysis

The leaf material was oven dried at 60°C for 72 hours and then ground to fine powder. The total N was analyzed by micro Kjeldhal methodology and ^{15}N abundance by mass spectrometry and expressed in “delta” notation, which is the deviation per thousand (‰) of ^{15}N abundance of the sample in relation to the standard, which is atmospheric N_2:

$$\delta = (R_{sample}/R_{standard} - 1) \times 1000$$

where R_{sample} and $R_{standard}$ are the ratio ^{15}N:^{14}N of the sample and the standard (air), respectively.

The date were submitted to a statistical analysis to compare the mean isotopic signals of the target legume species with the reference species signals, in each site, using the values of each individual of the same species as replications. Significant differences were established considering the 0.01 level of probability of the Tukey test. The species whose mean were significantly impoverished in ^{15}N in relation to the reference species were considered as being capable of atmospheric N_2 fixation (fixing species).

Estimation of BNF

For the fixing species, estimates of percentage of plant nitrogen derived from the air (%Ndfa) were made, using the formula recommended by Shearer and Kohl (1986):

$$\%Ndfa = (\delta^{15}N_{(reference)} - \delta^{15}N_{(diazotrophic)}/\ \delta^{15}N_{(reference)} - B) \times 100$$

Where $\delta^{15}N_{(reference)}$ is the mean value of the $\delta^{15}N$ of the reference species of each site, $\delta^{15}{}_{(diazotrophic)}$ is the mean $\delta^{15}N$ value for the plants of each specie identified as diazotrophic and B is the $\delta^{15}N$ value for fixing plants cultivated in the absence of a mineral N supply. Due to the high $\delta^{15}N$ values found for non fixing plants of the caatinga and methodological complications for estimating this value in arboreal species (Högberg, 1997; Boddey et al., 2000), the B values in this work were not estimated. However, according to the suggestion by Högberg (1997), the importance of using extreme B values in the %Ndfa calculations was tested. With the absence of data for the studied species, values of 0‰ and -2‰ were used which are commonly found in studies of tree legumes (Schulze et al., 1991; Raddad et al., 2005; Roggy et al.,1999).

The quantities of fixed N (kg ha^{-1} $year^{-1}$) were estimated using %Ndfa and %N content results, obtained in this work, and the annual production of leaf mass of the fixing species, estimated from information in other available works only for the Remígio and Serra Talhada fragments. Due to the low number of replications per species the mean %Ndfa and %N of all the fixing species for each site were used. For the Remígio fragment the primary liquid production of the fixing legumes was estimated based on the relative basal area of these species, determined by Pereira et al. (2002), and on the annual leaf production estimated by Dantas (2002). For the Serra Talhada fragment, the estimates were carried out using basal area determinations done by Ferraz et al. (2003), and the leaf mass measurements made by Kauffmann et al. (1993).

Results

Identification of diazotrophic species

In the four sites studied, species of legumes with isotopic signals ($\delta^{15}N$) statistically similar to the mean of the control species (*Bauhinia cheilanta*, *Caesalpinia pyramidalis*, *Aspidosperma pyrifolium* and *Commiphora leptophloeos*), as well as species with inferior signals were identified (Table 2). The species with statistically lower signals than the reference species were considered as species that were able to obtain part of their N supply through biological fixation of atmospheric N_2. All the plants of *Pithecellobium parviflorum*

Benth, *Piptadenia stipulacea* (Benth.) Ducke and *Mimosa* spp (*M. arenosa* (Willd.) Poir., *M. tenuiflora* (Willd.) Poir., *M. paraibana* Barneby and five other non identified species) presented ^{15}N impoverished leaves in relation to the reference species. The plants of *Anadenanthera columbrina* (Vell.) Brenan collected in Caruaru were considered as fixing, but those collected in Santa Teresinha and Serra Telhada presented isotopic signals equal to those of the reference species. *Piptadenia zehntneri* (Harms) M.P. de Lima & Lima and *Acacia paniculata* Willd. had an isotopic signal equal to those of the control plants.

BNF estimate

Species with great N fixation capacity (Ndfa>50%) were found in Serra Talhada, Remígio and Caruaru, even considering more conservative estimates (%Ndfa calculated considering B=-2). The most remarkable ones were *Mimosa tenuiflora*, in Serra Telhada (58% of Ndfa) and in Remígio (54% of Ndfa), and *Mimosa arenosa* (72% of Ndfa) and *Piptadenia stipulacea* (70% of Ndfa), in Caruaru. The mean biological fixation contributions for plant nitrogen were 27% or 36%, in Santa Teresinha, 41% or 49%, in Remígio, 52% or 64%, in Caruaru, and 52% or ¨68%, in Serra Telhada, according to calculations that were done considering B=-2 or B=0, respectively (Table 2).

The lowest N content of leaves (2.01%) was found in the non-legume reference species (*Aspidosperma pyrifolium* and *Commiphora leptophloeos*) (Table3). Among the legumes, the N leaf content differed significantly ($p \leq 0.01$) according to occurrence of N_2 fixation. The fixing legumes had a mean content of 3.10%, while the non fixing had 2.48% of N in the dry biomass of the leaves. The highest N content was found in the Remígio trees (3.23%) while the lowest was found in Santa Teresinha (1.93%).

Utilizing the available total biomass leaf data (Dantas, 2002) and proportion of fixing species (Pereira et al., 2002), for Remígio, the annual production of leaves of fixing species was estimated at 625 kg ha^{-1} (Table 4). A similar estimation, for Serra Talhada, using published data of leaves mass (Kauffmann et al., 1993) and fixing species proportion (Ferraz et al., 2003), resulted in 170 kg ha^{-1}. From these results and averages, per site, of N content and %Ndfa, the quantities of N added annually to the systems were estimated. In Serra Talhada, the total quantity of N fixed from the atmosphere by the tree legumes would be 3.3 kg ha^{-1} year^{-1}, considering B=0, or 2.5 kg ha^{-1} year^{-1}, considering B=-2 (Table 4). In Remígio, these values varied between 11.2 kg ha^{-1} year^{-1}, adopting B=0, and 9.3 kg ha^{-1} year^{-1} adopting B=-2.

Discussion

Identification of diazotrophic species

Among the 14 leguminous target species, 8 species of *Mimosa* spp (*M. arenosa*, *M. tenuiflora*, *M. paraibana* and another five non identified species), *Pithecellobium parviflorum*, *Anadenanthera columbrina* and *Piptadenia stipulacea* presented ($\delta^{15}N$) isotopic signals significantly inferior to the reference species, being identified as diazotrophic species. Although there are no previous reports on the nodulation capacity of *M. paraibana*, *P. parviflorum* and *P. stipulacea* and that the lack of identification of five species of *Mimosa* had impeded the comparison with literature, these results are consistent with the nodulation capacity information on other species of these genera (Allen and Allen, 1981; Faria et al., 1984; 1994; Faria and Lima, 1998). *Anadenanthera columbrina* (a nodulating species, according to Faria et al., 1984) varied in signal among the sites, in Caruaru being considered as a fixer and in Serra Talhada and Remígio as non fixer. *Piptadenia viridiflora*, a nodulating species (Faria and Lima, 1998), presented a non fixing signal. The behavior of these Mimosoideae point to the fact that the occurrence of N_2 atmospheric fixation in legumes with nodulating capacity is influenced by environmental and/or physiological conditions. In systems where the N stock is well conserved, fixing legumes may obtain insignificant quantities of BNF (Boddey et al., 2000). In mature forests, large size plants can present physiological limitations to the BNF (Gehring et al., 2005).

The fixation status of *Parapiptadenia zehntneri* and *Acacia paniculata* can not be defined with certainty because, although they presented non fixing signals, they were collected from only one site for each one (Serra Talhada and Caruaru, respectively) and there are no registers in literature on their nodulation capacities. Considerations can be taken by the taxonomic position of these species. Nodulation has already been observed in two species of *Parapiptadenia* (Allen and Allen, 1981; Faria et al., 1984), an indigenous genus of the Brazilian Northeast and very close to the nodulating genera *Piptadenia* and *Anadenanthera* (Allen e Allen, 1981). In this way, it is probable that the high ^{15}N signal of *Parapiptadenia zehntneri* plants is due to some physiological and/or environmental restriction for the N_2 fixation and not to lack of nodulating capacity. In the contrary manner, *Acacia paniculata* is probably not a diazotrophic species, since the majority of the *Acacia* species of the Americas, in contrast with the African ones, do not form symbiosis with rhizobia (Allen and Allen, 1981; Faria et al., 1984; 1994; Faria and Lima, 1998). Direct observation studies of radical nodules need to be done for definitive identification of the nodulation capacity or these species.

Estimations of BNF

Besides the identification of diazotrophic plants, the results of the isotopic signals give very promising conditions for calculating the N percentage that is derived from the air through biological fixation. The signals of the reference plants were high in all the sites studied (Table 2) and those of the fixing plants were more than two units of δ^{15}N‰ inferior of those of the reference plants. The differences varied between 2.28‰ (*Piptadenia stipulacea*, in Santa Teresinha) and 7.98‰ (*Mimosa arenosa*, in Caruaru). This condition, that permits safety in fixation studies (Högberg, 1997), is not often found, principally in humid forests (Roggy et al., 1999; Gehring and Vlek, 2004). Even in semi-arid environments the differences between the δ^{15}N signals of the potentially fixing species and non fixing species can be ambiguous, generating doubts as to whether the fixing species are fixing a little or if the method does not work (Handley et al., 1994).

High contributions of BNF were estimated for the tree legumes of the caatinga, which had means %Ndfa, in the minimum, of 27% in Santa Teresinha, and, in the maximum, 52% in Caruaru and Serra Talhada, considering B=-2 (Table 2). If the calculations had been done with B=0, these values would increase to 36 e 68%, respectively. Comparison of these results with those found in literature, as much for site averages as for the species, were done utilizing B=-2, that is a value commonly used in tree legume studies (Schearer et al., 1983; Sclulze et al., 1991; Sprent et al., 1996; Ndiaye e Ganry 1997; Roggy et al. 1999; Raddad et al., 2005).

Although higher values of %Ndfa are found in some highly efficient species in symbiotic nitrogen fixation, as much under natural conditions (Sprent et al., 1996) as in plantations (Gathumbi et al., 2002), the mean values for sites with natural vegetation are generally much lower than those found for the caatinga. Ndiaye and Ganry (1997) found very small contributions (Ndfa=10% and 20%) of BNF in two ecological zones siteized in semi-arid regions of Senegal, while Schulze et al.(1991) found a mean of 32% of BNF contribution in an arid gradient in Nambibia. Similar values (43 to 65%) to those found in Serra Telhada and the Agreste fragments were found in some desertic areas of California covered by *Prosopis* spp (Schearer et al., 1983). However, it would be relevant to say that such plants, in spite of being in a desertic environment, have a phreatrophic habit, growing under more favorable conditions for the BNF. In a humid forest in French Guyana, Roggy et al.(1999) found 44% of Ndfa for the Mimosoideae species, a mean that includes high fixing species (Ndfa=68%) and species with practically no fixation. Highly fixing species (Ndfa greater than 50%, even considering B=-2) were found in Serra Talhada, Remígio and Caruaru, the most remarkable ones being *Mimosa temuiflora*, *Mimosa arenosa* and *Piptadenia stipulacea*. They have a much superior performance than *Acacia senegal* that is cultivated in the semi-arid region of Sudan (Raddad et al., 2005), demonstrating the high potential of these species for insertion into agroforestry systems and for recuperation of degraded areas.

Although the lowest value of %Ndfa found in legumes in Santa Teresinha could be related to the worse conditions for plant growth, due to lower availability of water, this isolated fact does not explain the variations of N_2 contributions for the total N of the plants in the sites studied. The highest mean contribution per site was found in Serra Talhada, which is in the physiographical zone of the Sertão, which is drier than the Agreste sites (Caruaru and Remígio). Factors that were not evaluated in this work, such as nutrient and micorriza availability (Cardoso and Kuyper, 2006), also affect the BNF and they could be more favorable in Serra Talhada. The importance of BNF for nitrogenous nutrition of the plants also had a direct relationship with the N contents. Although the plants of Santa Teresinha are of lower %Ndfa and %N, the plants of Serra Talhada, with high levels of %Ndfa do not differ from them regarding the N contents (Table 3).

The N content of the legume leaves differed from the non legume leaves content. Among the legumes, the content differed according to the $\delta^{15}N$ signal (fixing or non fixing legumes). Fixing legumes presented 3.1% of N in their leaves and those from the Remígio fragments reached 3.9%. Even among non fixing species, the legumes were still richer in N (2.48%) than the non legumes (2.01%). This richness of N in leaves of legume species, even in non fixing ones, also observed in species of the Cerrado and Amazonia (Sprent et al ., 1996; Martinelli et al., 2000; Bustamante et al., 2004), is attributed to their growth habit (McKey, 1994), which demands large quantities of nitrogen and maximizes the rates of photosynthesis per leaf area. Our results demonstrate that, in the caatinga, the capacity of BNF guarantees even more elevated content of N in the leaves.

The quantities of N added annually by the BNF vary from 2.5 kg ha^{-1} $year^{-1}$, in Serra Talhada to 9.3 kg ha^{-1} $year^{-1}$, in Remígio, with a small increase (3.3 or 11.2 kg ha^{-1} $year^{-1}$) if B=0 were adopted. The impact of different B values has little significance in the final estimations of BNF in sites where the ^{15}N abundance of reference species is very different from zero (>+5‰ or <-5‰) (Boddey et al., 2000; Högberg, 1997). In humid tropical forests, the estimates made with different B values would signify considerable differences in the final fixed N contribution. In a humid forest of French Guyana, Roggy et al. (1999) observed a variation of 33% in the estimate of annual N fixation when using B=0 or B=-2, in a situation where the reference species had a signal of 5.01%.

These estimates indicate that the BNF contribution in well preserved caatinga is small, reaching a little more than 11 kg ha^{-1} $year^{-1}$. Although this quantity of N could be considered small, it is the same order of magnitude of estimates for one humid forest, carried out by Roggy et al. (1999), in French Guyana (7 kg ha^{-1} $year^{-1}$). Due to the scarcity of specific studies, Cleveland et al. (1999) estimated that the BNF for Brazilian xeromorphic forests should be of the same order of magnitude as those in deciduous tropical forests (between 7.5

and 17.6 kg ha^{-1} $year^{-1}$), considering that they have a similar floristic composition. The results for the Remígio fragment are on the lower limits of these estimates and those for the Serra Talhada fragment are even lower than the lower limit. Tropical and subtropical deciduous forests present more favorable moisture and soil fertility conditions for N fixation than the caatinga. In this work, the crucial factor in the low fixation estimates was the low number of fixing plants in the vegetation (2.4% in Serra Talhada and 11.8% in Remígio). An analysis of many works that quantify density proportions of basal areas or wood volume in the caatinga (Sampaio, 1996) confirm that in the majority of cases, the proportion of fixing legumes is low. Gehring et al. (2005) considered that the BNF in Amazonia was high in a secondary forest and low in a mature forest, based on the observation that there were a greater number of potentially fixing trees in the secondary than in the mature forest. In a similar way, after disturbances, the regeneration of the native caatinga vegetation is dominated by fixing species during a great part of the succession process (Sampaio et al., 1998; Pereira et al., 2003). In these situations, in which the biomass is predominantly composed of fixing legumes, the total input of N can reach 130 kg ha^{-1} $year^{-1}$, by adopting the same levels of %Ndfa as found in this work. These quantities are considerable and important in the reestablishment of stocks of N in the areas that are exploited for firewood and slash and burn agriculture. In semi-arid climate conditions, pure stands of *Acacia senegal*, with a capacity of 48% of Ndfa introduced 36 kg of N ha^{-1} $year^{-1}$at four years of age, in the Sudan (Raddad et al., 2005). Although there are no estimates for annual biomass production from Mimosoideae cultivation in the Brazilian semi-arid, the high nitrogen fixation levels found in this work indicate a great potential for considerable inputs of N in such systems.

Very few studies make simultaneous estimates of %Ndfa and primary production of the systems and this is one of the most important problems for estimating total fixed N quantities in communities (Boddey et al., 2000). Thus, quantifications of N added to natural ecosystems are scarce in literature (Cleveland et al., 1999). In spite of the limitations imposed by using estimates of biomass fixation taken from data of other works, the quantification of added N presented in this work has the importance of being the first attempt for caatinga vegetation and gives an idea of the order of magnitude of annually fixed N through the process of symbiotic fixation in tree legumes.

Conclusion

The results suggest that in the caatinga there is a good potential for use of the ^{15}N natural abundance methodology for evaluating the fixation ability of tree legumes under field conditions. Not only it is possible the identification of diazotrophic species but also reasonable estimates of %Ndfa can be made. The legumes of the caatinga have the potential of fixing great quantities of nitrogen, in particular *Mimosa tenuiflora*, *Mimosa arenosa* and *Piptadenia stipulacea*. The mean contributions of BNF for plant nitrogen were high, reaching up to 68%. However, the estimated quantities of N added annually to the systems were low, between 2.5 and 11.2 kg ha^{-1} year^{-1}, because of the low proportions of fixing plants in the vegetation (2.4 to 11.8%). In situations of native vegetation regeneration, where the succession is dominated by fixing species, N fixation could reach 130kg ha^{-1} year^{-1}.

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Table 1 – General characteristics of the four chosen municipalities, in the States of Paraíba (PB) and Pernambuco (PE), Brazil.

Characteristics	Municipality (state)			
	Remígio (PB)	Santa Teresinha (PB)	Caruaru (PE)	Serra Talhada (PE)
Coordinates	6°52’S 35°47’W	07º03'S 37º29'W	08º15'S 35º57'W	07º59'S 38º18'W
Altitude (m)	596	380	545	500
Soil type	Regolithic Neosol	Litholic Neosol	Yellow Argisol	Luvisol
Annual rainfall (mm)	700	824	696	768
Months with water deficit	4 - 5	9 - 10	4 - 5	6 - 7
Average temperature (°C)	22	26	24	24

Table 2: Leaf δ^{15}N, nodulation and nitrogen derived from the atmosphere (%Ndfa) in plants collected at four different sites in the semi-arid caatinga of Paraíba and Pernambuco States, Brazil.

Species	n(1)	δ^{15}N‰ (average±standard deviation)		Nodulation(2)	%Ndfa(3)	
					(I)	(II)
Santa Teresinha	**24**				**36**	**27**
Reference plants(4)	12	6,71±1,14				
Piptadenia stipulacea	5	4,43±1,68	**(5)	w.i.	34	26
Mimosa tenuiflora	5	4,22±0,91	**	nod	37	28
Anadenanthera colubrina	2	6,73±0,78	ns	nod	-	-
Serra Talhada	**45**				**68**	**52**
Reference plants(4)	20	6,58±1,41	ns			
Parapiptadenia zehntneri	4	4,00±1,11	ns	w.i.	-	-
Anadenanthera colubrina	4	5,27±1,62	ns	nod	-	-
Mimosa tenuiflora	4	1,59±1,06	**	nod	76	58
Mimosa sp1	4	2,05±0,64	**	w.i.	69	53
Mimosa sp2	5	2,39±0,32	**	w.i.	64	49
Mimosa sp3	4	2,75±1,05	**	w.i.	63	48
Remígio	**42**				**49**	**41**
Reference plants(4)	19	9,84±0,65				
Mimosa paraibana	1	4,50	**	w.i.	54	45
Mimosa arenosa	5	5,59±0,96	**	nod	43	36
Mimosa sp4	3	5,38±2,57	**	w.i.	45	38
Mimosa sp5	2	4,18±1,70	**	w.i.	58	48
Mimosa tenuiflora	3	3,47±1,02	**	nod	65	54
Piptadenia viridiflora	5	9,99±2,31	ns	nod	-	-
Piptadenia stipulacea	4	7,09±0,78	**	w.i.	28	23
Caruaru	**34**				**64**	**52**
Reference plants(4)	10	9,03±1,81				
Anadenanthera colubrina	5	5,34±2,01	**	nod	41	33
Pithecellobium parviflorum	4	5,28±0,93	**	w.i.	42	34
Acacia paniculata	4	10,39±1,07	ns	w.i.	-	-
Mimosa arenosa	6	1,04±0,74	**	nod	88	72
Piptadenia stipulacea	5	1,28±0,47	**	w.i.	86	70

(1) Number of plants

(2) Literature information on nodulation occurrence: w.i..= without information; nod = nodulating

(3) %Ndfa calculated with B value of 0 (I) or -2 ‰ (II)

(4) *Croton sonderianus*, *Aspidosperma pyrifolium* (non legume species), *Bauhinia cheilanta* and *Caesalpinia pyramidalis* (non nodulating legume species)

(5) Averages significantly different (**) or not (ns) of reference plants averages, at 0,01 probability level

Table 3: Average leaf nitrogen concentrations of plants collected at four caatinga sites of Paraíba and Pernambuco States, Brazil. Numbers in parentheses refer to replicates.

Sites	Fixing legumes	Non-fixing legumes	Non legumes	Overall averages
Remígio	3.90±0.51 (18)	2.87±0.30 (13)	2.21±0.36 (11)	3.13±0.86a
Caruaru	2.95±0.54 (20)	2.24±0.50 (9)	1.81±0.20 (5)	2.60±0.66b
Serra Talhada	2.88±0.74 (17)	2.26±0.44 (18)	1.78±0.27 (10)	2.38±0.69bc
Santa Teresinha	2.19±0.35 (10)	1.80±0.36 (10)	1.60±0.15 (4)	1.93±0.40c
Averages	3.10±0.83 A	2.48±0.69 B	2.01±0.37 C	

Averages followed by the same capital letter in the line and small letter in the column are not significantly different at the 0.05 level (Tukey test).

Table 4: Total biomass, total nitrogen and fixed nitrogen in leaves of fixing legumes at two caatinga sites (Serra Talhada and Remígio) of semi-arid Northeast Brazil.

Variable	Serra Talhada	Remígio
Total leaf biomass (ton ha^{-1})	6.6(1)	5.3(2)
Proportion of fixing legume plants (%)	2.4(3)	11.8(4)
Biomass of fixing plants (kg ha^{-1})	170	625
N content in fixing legume leaves (%)	2.88	3.90
Stock of N in fixing plants (kg ha^{-1})	4.9	24.4
%Ndfa, with B=0	68	49
%Ndfa, with B=-2	52	41
Estimate of fixed N, with B=0 (kg ha^{-1})	3.3	11.2
Estimate of fixed N, with B=-2 (kg ha^{-1})	2.5	9.3

(1) Kauffmann et al. (1993)
(2) Dantas (2002)
(3) Ferraz et al (2003)
(4) Pereira et al (2002)

CONSIDERAÇÕES FINAIS

As folhas de plantas arbóreas não fixadoras da caatinga apresentaram sinais de $\delta^{15}N$ elevados, indicando a ocorrência de um ciclo aberto do N, com importantes processos de perda do elemento. Os locais de Agreste, com médias de precipitação anual menores que os do Sertão, apresentaram os maiores valores de $\delta^{15}N$ foliar, entre os maiores reportados na literatura. Este resultado está de acordo com a tendência global de locais com menores precipitações serem mais enriquecidos isotopicamente. Entretanto, as médias anuais de precipitação têm sido usadas como indicadores da disponibilidade hídrica ao longo do ano. Neste sentido, os locais de Sertão, apesar das maiores médias anuais, são mais secos devido à maior concentração das chuvas. Esta menor disponibilidade hídrica é comprovada pelo menor porte da vegetação e pelo menor número de espécies nestes locais que nos do Agreste. Assim, se fosse considerada a disponibilidade hídrica, os resultados seriam o contrário do esperado. Na verdade, os sinais são a resultante do balanço de entradas e perdas de N no sistema solo – planta. Teoricamente, sinais maiores têm sido explicados como a conseqüência de maiores perdas de formas depletadas de ^{15}N ou maior fixação de N_2 atmosférico. Embora menos considerado, também poderiam vir de perdas maiores de formas enriquecidas em ^{15}N. Assim, é provável que os maiores valores do Agreste possam ter resultado de maiores perdas de gases empobrecidos em ^{15}N ou menores perdas de material enriquecido em ^{15}N.

Além de isotopicamente enriquecidas, as plantas não fixadoras apresentaram um sinal isotópico bastante uniforme, tanto espacialmente quanto entre espécies. Esta ocorrência facilita a escolha de plantas referência, o esquema de amostragem e a detecção de diferença significativa entre os $\delta^{15}N$ de indivíduos fixadores e não-fixadores, em estudos da fixação biológica do N_2 utilizando a metodologia da abundância natural do ^{15}N. Em todos os locais estudados foram identificadas espécies fixadoras, com sinais isotópicos inferiores em mais de duas unidades de $\delta^{15}N$‰ em relação ao das espécies controle não fixadoras. Esta condição permitiu segurança nas estimativas do %Ndda.

As leguminosas da caatinga têm o potencial de fixar quantidades grandes de nitrogênio, com destaque para *Mimosa tenuiflora*, *Mimosa arenosa* e *Piptadenia stipulacea*. As contribuições médias da fixação biológica do N_2 para o nitrogênio das plantas foram altas, podendo chegar a 68%. No entanto, estimativas das quantidades de N adicionadas anualmente aos sistemas, feitas a partir de dados publicados de biomassas produzidas e proporções de plantas fixadoras na vegetação, resultaram em valores baixos. As quantidades fixadas estariam entre 2.5 e 11.2 kg ha^{-1} ano^{-1} e seriam fortemente influenciadas pelas baixas proporções de plantas fixadoras no conjunto geral da vegetação (2.4 a 11.8 %). Em situações de regeneração da vegetação nativa, nas quais a literatura aponta o domínio da sucessão por espécies fixadoras, as estimativas indicam que as quantidades fixadas poderiam ser muito maiores, chegando a 130 kg^{-1} ha^{-1} ano^{-1}.