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Soil microbial activities in tree-based cropping systems and natural forests of the Central Amazon, Brazil

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Abstract Little information is available about the factors controlling soil C and N transformations in natural tropical forests and tree-based cropping systems. The aim of this work was to study the effects of single trees on soil microbiological activities from plantations of timber and non-timber species as well as species of primary and secondary forests in the Central Amazon. Soil samples were taken in the primary forest under *Oenocarpus bacaba* and *Eschweilera* spp., in secondary regrowth with *Vismia* spp., under two non-timber tree species (*Bixa orellana* L. and *Theobroma grandiflorum* Willd.), and two species planted for wood production (*Carapa guianensis* Aubl. and *Ceiba pentandra*). In these soils, net N mineralization, net nitrification, denitrification potential, basal and substrate-induced respiration rates were studied under standardized soil moisture and temperature conditions. Individual tree species more strongly affected N transformations, particularly net nitrification, than C respiration. Our results suggest that soil C respiration can be affected by tree species if inorganic N becomes a limiting factor. We found a strong correlation among almost all microbiological processes suggesting close inter-relationship between C and N transformations in the studied soils. Correlation analysis between soil chemical properties and microbiological activities suggest that such strong inter-relationships are likely due to competition between the denitrifying and C-mineralizing communities for NO_3^-, which might be an important N source for the microbial population in the studied soils.

Keywords Greenhouse gases · Denitrification · Microbial activities · N fertilizers · Tropical soils

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Introduction

Tropical forest soils are known to be one of the main natural sources of NO and N_2O (Matson et al. 1990). Recent estimates suggest that emissions of N_2O from tropical forest soils account for 20–50% of all global sources of atmospheric N_2O (Potter et al. 1996). In recent years large areas of tropical rain forests have been converted to continuous farming, grasslands or tree-based cropping systems. Changes in land-use systems affect the activities of microorganisms responsible for C and N transformations and fluxes of greenhouse gases. The effect of the conversion of primary forests to timber and non-timber tree plantations, as in the Brazilian Amazon, has received considerably less attention, especially the factors controlling microbial activities related to gaseous N losses.

We have reported before that different tree species of temperate forests with varying litter C-to-N ratios can have different effects on concentrations and dynamics of denitrifying enzymes, which influence N_2O emissions (Menyailo and Huwe 1999a); they also altered soil chemical properties (Menyailo et al. 2002a) and C and N mineralization rates (Menyailo et al. 2002b). This suggests that in the Amazon region also, tree species with different litter quality used in plantations and in natural forest may have distinct effects on soil microbiological activities. This is not only important from a global environmental point of view (emission of greenhouse gases), but also for farming practices, as the activities of microorganisms affect soil nutritional status and the efficiency of applied fertilizers. In central Amazonian upland soils, the availability of inorganic N fertilizers to annual crops was reduced by 30% due to denitrification

losses (Alfaia 1997); similar values were estimated from the N balance using ^{15}N applications to fruit trees (Lehmann et al. 2000). The mineralization of organic matter may also be linked to denitrification and/or nitrification providing inorganic N and easily available C for anaerobic nitrate reduction.

The aim of the present study was to examine the effects of timber and non-timber tree species in plantation systems and primary and secondary forests on Brazilian Central Amazonian upland soils on C and N mineralization and N_2O formation (denitrification). We intended (a) to assess relationships between potential microbial activities and (b) to explain variations of microbial activities by variations of soil chemical properties.

Materials and methods

Research site

The experimental plots of the Empresa Brasileira de Pesquisa Agropecuaria (Embrapa–Amazonia Ocidental) are located 40 km north of Manaus (Central Amazon). The soil type is a Xanthic Ferralsol (FAO 1990), which is typical for the "terra firme" ecosystems of the Central Amazon, Brazil. The climate is humid tropical with an average annual precipitation of 2,503 mm. This soil is characterized by a high clay content (80%), low nutrient content and acidic reaction (pH <4.5).

The experiment was set up in a completely randomized blocked design with three blocks, each of them consisting of four plots. Plots with dimensions of 35×45 m were established for the tree cropping systems (one plot with timber and one with non-timber species) and the secondary forest (one plot) on an abandoned rubber plantation after slash and burn. Adjacent to the three plots was another plot within the natural forest; together these four plots constituted one block. We analyzed the data using a completely randomized design, where species was the only treatment and blocks were replicates.

The studied trees were two non-timber tree species—*Bixa orellana* L. (annatto; used as natural dye) and *Theobroma grandiflorum* Willd. (ex Spreng.) K. Schum. (cupuaçu; for ice-cream and juice production)—growing together on the one plot of the tree cropping system, and two species planted for wood production—*Carapa guianensis* Aubl (andiroba) and *Ceiba pentandra*. Plots with secondary forests consisted of one species, *Vismia sp*. In the plots of primary forest two species were chosen, *Oenocarpus bacaba* and *Eschweilera* spp. Both trees are typical of the terra firme forest.

The planting density of the timber trees was 3×3 m. *Bixa* was planted in rows 4 m apart and *Theobroma* 7 m apart alternating with Brazil nut (*Bertholletia excelsa* Humb. Bonpl.). Total planting density was 93.3 and 156.3 trees ha^{-1} for *Theobroma* and *Bixa*, respectively. At the natural forest sites no other tree species were closer than 4 m from the tested species stems and neighboring trees could not affect the microbial activities or soil chemistry as much as *Oenocarpus* and *Eschweilera*. The tree plantations and the secondary forest were 7 years old at the time of the experiment.

In February 1999, soil cores of the 0- to 5-cm depth were taken at a 50-cm distance from tree stems using a soil auger. We took 4 soil samples under 2 trees in each plot and these 8 samples were mixed. All the 21 soil samples (3 aggregated samples for each of the 7 species) were air-dried, sieved (2 mm) and transported to the laboratory.

Study of soil chemical properties

All samples were analyzed for pH in water solution (1:2.5), with an extraction time of 2 h. The major ions in water solution (1:5, extraction time of 1 day at 4°C; Na^+, K^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{3+} and Al^{3+} were measured by atomic absorption spectrometry (AAS 4100, Perkin Elmer), NH_4^+, NO_3^-, Cl^- and SO_4^{2-} by flow injection analyzer (Lachat) and dissolved organic carbon (DOC) as CO_2 by infrared detection after persulfate oxidation. In the water extract, total dissolved nitrogen was also determined using a Total Nitrogen Analyzer (TN-05, Mitsubishi Kasei Corp.). Dissolved organic nitrogen (DON) was determined as the difference between total dissolved N and inorganic N. Total C and N were determined by gas chromatography with an elemental analyzer (Heraeus). Chemical analyses were done in duplicate for each soil sample. All chemical characteristics (except pH, C and N) were determined in the central analytical laboratory of BITÖK (Bayreuth Institute of Terrestrial Ecosystem Research). Total C, N and pH were analyzed at the Department of Soil Science of the University of Bayreuth.

Net N mineralization and net nitrification

From each of the 21 soil samples 15 g were taken to study the initial concentration of NH_4^+-N and NO_3^--N in 1 M KCL solution (1:5) with a flow injection analyzer (Lachat). Additionally, 15 g from each soil sample were placed in plastic flasks, moistened to 60% of water holding capacity (WHC), closed with stoppers and incubated at 28°C for 14 days. To avoid anaerobic conditions (oxygen deficiency), the flasks were opened every 3 days for 5 min. On day 14, soil samples were analyzed for NH_4^+-N and NO_3^--N as described above.

The net mineralization rate was calculated as the difference in NO_3^- + NH_4^+ after and before the incubation and was expressed as mg NO_3^- + NH_4^+-N $kg^{-1}day^{-1}$. Net nitrification was calculated as the difference of NO_3^- after and before the incubation and was expressed as mg NO_3^--N $kg^{-1}day^{-1}$.

Potential denitrification rates

Ten grams of soil were placed in glass flasks (25 ml) and pre-incubated at 28°C for 3 days to initiate microbial activity. Thereafter, 5 ml distilled water with KNO_3 and glucose were added to each sample. The resulting concentration of nitrate was 100 mg NO_3-N kg^{-1}, that of glucose 100 mg C kg^{-1} soil. The flasks were closed with air-tight rubber stoppers, fixed with clamps. Anaerobic conditions were induced by changing the gas phase with He for 15 min. After removing O_2 from the flasks, 2.5 ml C_2H_2 (10% v/v) were added as an inhibitor of N_2O-reductase. All samples were than incubated at 28°C and after 24 h the headspace of each flask was sampled and analyzed for N_2O using a gas chromatograph (Shimadzu 14A) equipped with an electron capture detector (ECD ^{63}Ni; Menyailo and Huwe 1999b). The sample volume was 5 ml. The results were expressed as mg N_2O-N kg^{-1} day^{-1}.

Substrate-induced respiration

Substrate-induced respiration (SIR) was measured to obtain an indicator of microbial biomass, as potential CO_2 production in the absence of substrate limitation corresponds to biomass of heterotrophic microorganisms. Five grams of soil were placed in 25-ml flasks and moistened to 40% of WHC with distilled water. The flasks were closed with rubber stoppers, fixed with clamps and pre-incubated at 28°C for 3 days. Thereafter, water solution with glucose as a C-source was added. The resulting soil moisture was 60% of WHC and the resulting concentration of glucose was 100 mg C kg^{-1} soil. Soil samples were then incubated for 24 h at 28°C and 1 ml headspace air of each flask was sampled and

analyzed for CO_2 as described above. The results are expressed as g CO_2-C $kg^{-1}day^{-1}$.

Basal respiration

Five grams of soil were placed in a 25-ml flask and water was added to achieve 60% of WHC. The flasks were closed with rubber stoppers, fixed with clamps and pre-incubated at 28°C for 3 days. After that they were opened and closed again for a 24-h incubation under the same temperature. After that, a gas sample of the headspace (1 ml) from each flask was taken for analysis of the CO_2 concentrations using gas chromatography as described above. The results are expressed as g CO_2-C $kg^{-1}day^{-1}$.

Statistical data analysis

All measurements of microbiological activities were done in duplicate for each soil sample. Mean values for one soil sample were taken. All measured microbial activities and chemical parameters had normal distributions. The effects of tree species were determined by one-way analysis of variance (ANOVA) with three replicates (three blocks). First, the main effect was calculated. We considered the effect significant at $P < 0.05$. Where the main effect was significant, post hoc comparisons with the LSD test were done. Spearman rank order correlation coefficients were used to study relationships between microbial activities and chemical parameters, and within microbiological activities themselves. When computing correlations all soil samples were considered together (n =21) and after that mean values representing plots with different species (n =7). Principal component analysis was then used to cluster all variables studied and to discern common factors affecting groups of variables. Scores of Varimax rotated principal components were compared using one-way ANOVA to test for species effects. All statistics were carried out with the statistical package STATISTICA (5.0 for Windows, StatSoft 1997).

Results

Soil chemistry

Chemical properties of the studied soils are presented in Table 1. Tree species strongly affected total N contents (P =0.001) and C-to-N ratio (P <0.001). Soil under *Carapa* had the highest N content (2.7%), *Carapa* differed from all other species (P <0.05) except *Ceiba*, which had significantly (P <0.05) higher N contents than under all other species except *Oenocarpus*. Tree species had no effect on total soil C content (P =0.219). The main effect of tree species was also significant for NH_4^+ (P =0.038), Ca (P =0.020), Mg (P =0.029), Na (P <0.001), Cl (P =0.029) and SO_4^{2-} (P =0.017). Tree species had no effect on soil pH, NO_3^-, DON, DOC, Al, Fe, K and Mn concentrations (for all, the main effect P >0.05).

Net N mineralization and net nitrification

Tree species significantly affected net N mineralization (P <0.001) and net nitrification (P =0.040). The highest rate of net N mineralization was found beneath *Ceiba* and *Oenocarpus* (Fig. 1a). *Ceiba* significantly differed from all other species, except *Oenocarpus* (P =0.559). *Oeno-*

Table 1 Chemical properties of studied soils beneath the different tree species (n =3; *DON* dissolved organic nitrogen, *DOC* dissolved organic carbon). Values are means of three plots; standard errors are given in *parentheses*; values in one row *followed by the same letter* are not significantly different from each other (P <0.05)

Soil chemical properties	Tree species						
	Bixa	*Theobroma*	*Carapa*	*Ceiba*	*Eschweilera*	*Oenocarpus*	*Vismia*
N (mg g^{-1})	2.1 (0.1) c	2.0 (0.1) c	2.7 (0.1) a	2.5 (0.1) ab	2.1 (0.1) c	2.3 (0.1) bc	2.1 (0.1) c
C (mg g^{-1})	28.1 (2.1)	27.4 (2.2)	32.8 (1.2)	30.8 (1.4)	30.7 (1.3)	35.6 (3.7)	29.3 (2.7)
C-to-N ratio	13.23 (0.40) ce	13.70 (0.48) bc	12.36 (0.17) e	12.20 (0.14) e	14.58 (0.24) ab	15.74 (0.71) a	14.16 (0.58) bc
pH (H_2O)	4.48 (0.01)	4.39 (0.06)	4.36 (0.27)	4.37 (0.08)	4.25 (0.08)	3.89 (0.15)	4.26 (0.06)
NH_4^+-N (mg kg^{-1})	20.69 (2.75) b	19.93 (1.01) b	13.35 (0.70) b	17.07 (1.84) b	45.48 (4.76) a	31.65 (14.82) ab	31.30 (5.04) ab
NO_3^--N (mg kg^{-1})	13.22 (2.55)	15.45 (1.73)	22.11 (12.35)	9.32 (0.77)	21.86 (3.30)	17.93 (1.21)	15.38 (1.44)
DON (mg kg^{-1})	42.38 (9.47)	61.50 (2.93)	59.98 (16.03)	49.80 (15.06)	62.00 (4.92)	58.48 (9.55)	47.42 (5.58)
DOC (mg kg^{-1})	222.2 (90.7)	802.3 (230.3)	350.2 (235.8)	520.5 (225.2)	455.8 (262.5)	591.5 (211.9)	250.3 (73.4)
Al (mg kg^{-1})	2,615.0 (1,024.2)	2,803.3 (755.2)	4,557.1 (4,471.7)	971.8 (604.3)	1,891.2 (928.0)	752.5 (701.5)	848.5 (411.2)
Ca (mg kg^{-1})	38.82 (13.84) a	21.62 (4.32) ab	15.78 (5.90) bc	11.05 (5.42) bc	8.78 (7.26) bc	1.87 (0.33) bc	0.66 (0.66) c
Fe (mg kg^{-1})	557.2 (221.7)	589.7 (170.9)	921.1 (907.0)	189.5 (118.9)	340.9 (165.0)	144.8 (136.4)	240.4 (135.1)
K (mg kg^{-1})	14.27 (1.04)	17.68 (2.37)	15.73 (0.33)	12.78 (0.91)	11.58 (0.23)	36.35 (13.77)	10.02 (1.27)
Mg (mg kg^{-1})	2.61 (0.16) b	2.96 (0.59) b	9.33 (3.10) a	3.51 (0.36) b	3.09 (0.71) b	3.54 (1.55) b	1.74 (0.16) b
Mn (mg kg^{-1})	0.38 (0.09)	0.60 (0.10)	1.01 (0.51)	0.26 (0.05)	0.29 (0.02)	0.28 (0.10)	0.11 (0.06)
Na (mg kg^{-1})	1.57 (0.12) b	2.65 (0.14) b	2.07 (0.06) b	1.73 (0.27) b	9.83 (2.41) a	8.20 (1.31) a	1.33 (0.86) b
Cl (mg kg^{-1})	6.80 (0.19) a	9.60 (1.10) a	5.13 (0.58) a	5.14 (0.39) a	9.33 (0.84) a	21.80 (8.23) b	6.21 (1.32) a
SO_4^{2-} (mg kg^{-1})	13.77 (0.75) c	14.70 (1.79) c	19.17 (4.64) abc	17.63 (1.45) bc	25.68 (3.75) ab	27.18 (4.27) a	12.77 (0.43) c

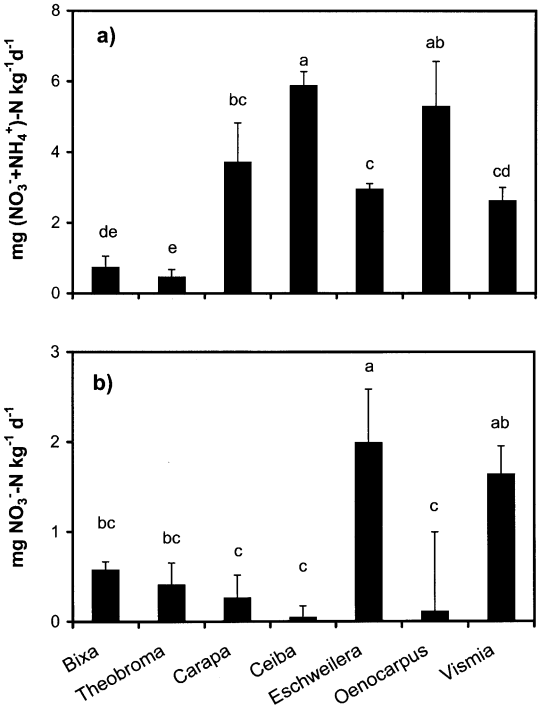

Fig. 1 **a** Net N mineralization rates, and **b** net nitrification rates in soil samples beneath the different tree species. *Bars with the same letter* are not significantly different at $P < 0.05$ (means and standard errors, $n = 3$)

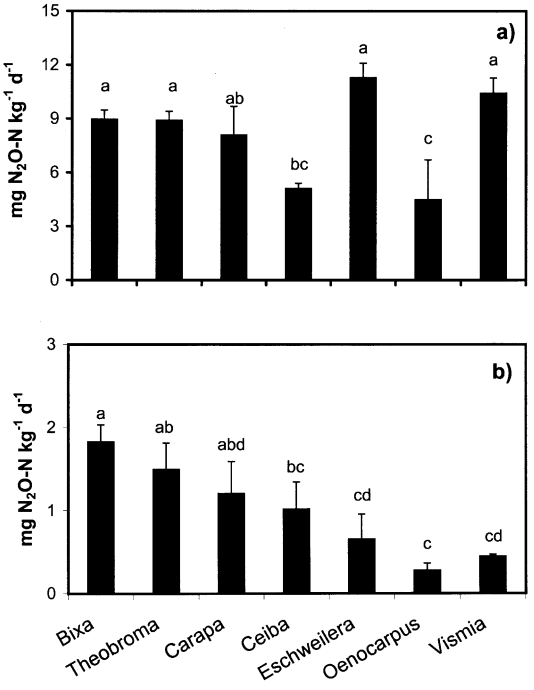

Fig. 2 **a** Potential denitrification rates, and **b** N_2O evolved in incubation experiments for basal respiration (aerobic conditions, no glucose, nitrate, or C_2H_2 addition) in soil samples beneath the different tree species. *Bars with the same letter* are not significantly different at $P < 0.05$ (means and standard errors, $n = 3$)

carpus did not differ from *Carapa* and *Ceiba*; other species had significantly lower values. The lowest values were for *Bixa* and *Theobroma*; they differed from all other species ($P < 0.050$), except that *Bixa* and *Vismia* had equal values ($P > 0.050$).

Soils under *Eschweilera* had significantly ($P < 0.001$) higher net nitrification rates compared to soils from all other species, except *Vismia* (Fig. 1b). The lowest mean values of net nitrification were measured in soils from *Carapa*, *Ceiba* and *Oenocarpus*, where the highest rate of net N mineralization was found. The last three species significantly differed from *Vismia* (for all, $P < 0.050$).

It is important to note that species with high net N mineralization did not have the highest net nitrification rates. Thus, the proportion of net nitrification to net N mineralization depended on the individual tree species. Beneath the non-timber species (*Bixa* and *Theobroma*), net nitrification accounted for almost all net N mineralization (90–100%), under *Eschweilera* and *Vismia* for 70%, and beneath the timber species (*Carapa* and *Ceiba*) and *Oenocarpus* for only 10%.

Denitrification potential and N_2O evolved from basal respiration

Denitrification was also strongly affected by tree species (main effect, $P = 0.007$; Fig. 2a). *Oenocarpus* and *Ceiba* had lower denitrification rates than *Bixa* and *Theobroma* ($P < 0.050$), and *Eschweilera* and *Vismia* ($P < 0.010$). *Oenocarpus* also significantly differed from *Carapa* ($P = 0.044$). Denitrification potential did not significantly differ between *Carapa* and *Ceiba*, and between *Ceiba* and *Oenocarpus* ($P > 0.050$). During the basal respiration incubation, a 4–10 times smaller amount of N_2O was formed (Fig. 2b) because of aerobic conditions and no addition of C_2H_2, NO_3^- or glucose. Species also differed in the N_2O produced ($P < 0.001$) and the pattern of N_2O formation was not the same as the denitrification potential.

Basal and substrate-induced respiration

Rates of substrate-induced respiration (Fig. 3a) were 3 times higher than those of basal respiration (Fig. 3b). Tree species significantly affected SIR ($P = 0.046$) but not basal respiration ($P = 0.092$). SIR under *Ceiba* was significantly higher than under *Bixa* and *Theobroma* (for both, $P < 0.05$), and *Eschweilera* and *Vismia* (for both, $P < 0.01$).

Interrelationships of microbiological activities and chemical parameters

The relationships among microbial activities in all soil samples are presented in Table 2. The potential denitrification was closely related to SIR (negative relation) and

Table 2 Spearman rank order coefficients of correlations between microbiological activities of soils beneath the different tree species (*SIR* substrate-induced respiration)

	Net N mineralization	Net nitrification	Denitrification	N_2O-Basal respiration	SIR	Basal respiration
All soil samples (n =21)						
Net N mineralization	1.00					
Net nitrification	–0.33	1.00				
Denitrification	–0.54*	0.73***	1.00			
N_2O-Basal respiration	–0.55**	–0.11	0.18	1.00		
SIR	0.70***	–0.62**	–0.78***	–0.10	1.00	
Basal respiration	0.74***	–0.59**	–0.62**	–0.27	0.80***	1.00
Mean values for different species (n =7)						
Net N mineralization	1.00					
Net nitrification	–0.64	1.00				
Denitrification	–0.61	0.96***	1.00			
N_2O-Basal respiration	–0.46	–0.03	0.10	1.00		
SIR	0.71	–0.93**	–0.89**	0.10	1.00	
Basal respiration	0.93***	–0.64	–0.67	–0.42	0.78*	1.00

* P <0.05, ** P <0.01, *** P <0.001

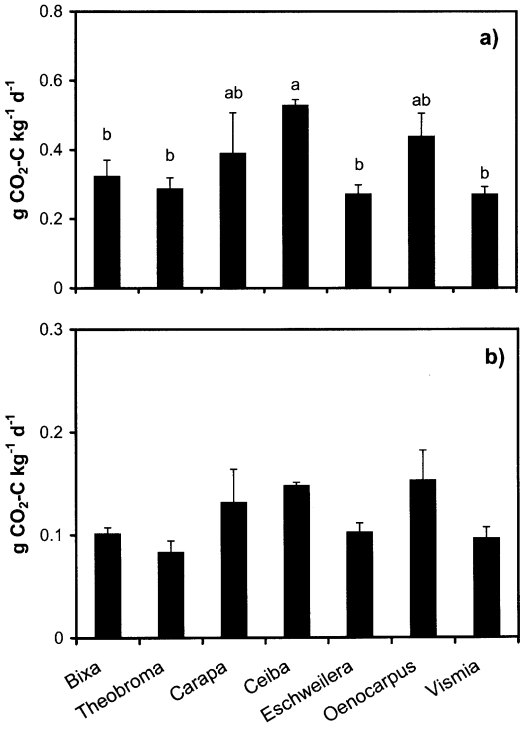

Fig. 3 **a** Substrate-induced respiration rates and **b** basal respiration rates in soil samples beneath the different tree species. *Bars with the same letter* are not significantly different at P <0.05 (means and standard errors, n =3)

to net nitrification rate (positive relation). Net nitrification showed significant (P <0.01) negative correlation with basal respiration and SIR. In contrast to net nitrification, net N mineralization was related to both respiration rates positively (P <0.001). N_2O production from basal respiration incubation (N_2O-basal respiration) was negatively correlated to net N mineralization (–0.55, P <0.010). When analyzing mean values only (Table 2), fewer significant relationships were obtained. Overall, the relationships were the same, except that net N mineralization was not significantly related to denitrification and SIR, and basal respiration was not correlated to net nitrification and denitrification.

In an attempt to understand the causes of variations in the different microbial activities, we analyzed the linear relationships between soil microbial processes and soil chemical properties (Table 3). Net N mineralization was positively related to total N, C and SO_4^{2-} concentrations and negatively to Ca. Net nitrification rate was significantly (P <0.001) and positively related to soil NH_4^+ concentrations and negatively to K concentrations. Denitrification potential was positively correlated (P <0.05) with only two soil properties—initial NO_3^- and NH_4^+ concentrations. N_2O-basal respiration was correlated to pH, Ca and SO_4^{2-}. Both SIR and basal respiration were negatively and significantly related to soil NO_3^- concentrations.

Correlation of mean values for each species (n =7) resulted in fewer significant relationships (data not shown). Total carbon was correlated with net N mineralization and basal respiration (0.89 and 0.93, respectively, for both P <0.01). Total nitrogen content was correlated to net N mineralization and SIR (for both, 0.79, P <0.05) and basal respiration (0.82, P <0.01).

We used principal components analysis (PCA) to describe overall patterns of interrelationships among individual soil properties and to extract common factors responsible for total variation. PCA explained 72% of the total variation of all soil chemical and biological properties examined with four principal components (Table 4). The first principal component (PC1) explained 25.6% of the total variance, and had strong loadings for C, C/N, Na, Cl, SO_4^{2-}, pH, Ca and N_2O-basal respiration. The second principal component (PC2) explained 22% of the total variation, and was primarily associated with variance in microbial activities and NH_4^+ contents. This is due to strong interrelationship between microbial activities and

Table 3 Spearman rank order coefficients of correlations between microbial activities and chemical properties of soils under the different tree species (n =21)

Chemical properties	Microbiological activities					
	Net N mineralization	Net nitrification	Denitrification	N_2O-Basal respiration	SIR	Basal respiration
N	0.58**	–0.28	–0.23	–0.14	0.36	0.39
C	0.50*	0.02	0.03	–0.29	0.07	0.09
C/N	0.00	0.42	0.33	–0.37	–0.33	–0.26
pH	–0.31	–0.12	–0.01	0.64**	0.18	0.14
NH_4^+-N	–0.22	0.70***	0.51*	–0.15	–0.36	–0.28
NO_3^--N	–0.25	0.39	0.52*	–0.28	–0.59**	–0.49*
DON	0.10	0.19	0.02	0.01	0.11	0.03
DOC	0.02	–0.04	–0.13	0.19	0.09	–0.09
Al	–0.36	–0.01	0.16	0.41	0.13	0.03
Ca	–0.44*	–0.30	–0.09	0.50*	0.01	–0.05
Fe	–0.38	0.00	0.18	0.42	0.08	0.03
K	–0.05	–0.57**	–0.37	0.20	0.21	0.18
Mg	0.29	–0.42	–0.24	0.04	0.33	0.40
Mn	–0.30	–0.22	0.06	0.37	0.04	–0.08
Na	0.11	0.08	0.08	–0.24	–0.08	0.10
Cl	–0.18	0.15	0.13	–0.07	–0.26	–0.13
SO_4^{2-}	0.60**	–0.05	–0.40	–0.47*	0.32	0.40

* P <0.05, ** P <0.01, *** P <0.001

Table 4 Principal components loadings after Varimax rotation. Chemical parameters are grouped according to the maximum fittings to principal components (correlation coefficients >0.60; n =21)

	PC 1	PC 2	PC 3	PC 4
Total variability explained	25.60%	22%	14%	10.20%
C	0.71	0.29	–0.43	–0.10
C/N	0.78	–0.23	0.08	–0.29
Na	0.73	–0.33	0.17	0.15
Cl	0.74	0.11	0.11	–0.17
SO_4^{2-}	0.79	0.25	0.10	0.02
N_2O-Basal respiration	–0.67	–0.11	–0.08	–0.07
pH	–0.61	–0.05	0.44	0.53
Ca	–0.60	–0.03	–0.08	0.11
Net N mineralization	0.49	0.75	0.05	0.02
Net nitrification	0.15	–0.79	0.22	–0.02
Denitrification	–0.15	–0.92	–0.08	0.09
SIR	0.08	0.90	0.24	0.18
Basal respiration	0.28	0.83	0.00	0.31
NH_4^+-N	0.48	–0.64	0.40	0.06
NO_3^--N	0.23	–0.48	–0.79	–0.12
Mn	–0.16	–0.15	–0.87	0.12
Al	–0.17	0.06	0.06	0.92
Fe	–0.19	0.05	0.07	0.91
Mg	–0.02	0.33	–0.46	0.78

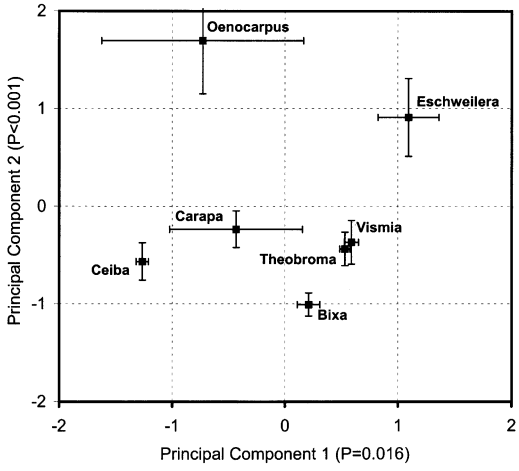

Fig. 4 Principal components 1 and 2, summarizing the data of relative contribution of each species included in factor analysis (means and standard errors, n =21)

because of strong correlation between net nitrification and NH_4^+ (Table 3). Only the first two principal components were affected by species (Fig. 4). The other two principal components (PC3 and PC4) explained less variability and were not affected by species (P >0.050).

Discussion

The primary aim of the research was to determine the effect of tree species of natural, secondary forests and artificially planted species on soil microbial activities, soil chemical properties and their interrelationships. Based on our previous studies in the Siberian artificial afforestation experiment (Menyailo et al. 2002a, 2002b), we submit that the interrelationships between C and N cycling processes and soil chemistry dramatically change with the region (temperate versus tropical). Such region-specific interactions are important to understand to predict changes in element cycles and greenhouse gases fluxes under a changing land use system.

Species effects

Tree species strongly affected net N mineralization and net nitrification rates. The proportion of NO_3^- in total inorganic N (NH_4^+ + NO_3^-) accumulated varied consid-

erably (from 10% to 100%) depending on species. Vitousek and Matson (1988) found NH_4^+-N pools to be 47 times larger than NO_3^--N pools in the root mat of similar terra firme forests near Manaus. They determined that only 38% of the net N mineralization was due to net nitrification in the root mat, while net nitrification contributed 100% of net N mineralization in surface soils. They demonstrated that the role of nitrification in N mineralization also varies within one region. In the present study, however, only surface mineral soil (0–5 cm) was sampled and the differences in the nitrification-to-mineralization ratio cannot be attributed to different soil horizons. Smith et al. (1998) reported large differences in the surface soil inorganic N pool under *Pinus caribaea*, *Carapa guianensis* and *Euxylophora paraensis* in lowland Amazonia throughout the year. They found the smallest NH_4^+-N and largest NO_3^--N pools under *P. caribaea*, and relatively small NO_3^-- N pools under *C. guianensis* and *E. paraensis*. These findings support our observations and allow us to conclude that tree species significantly affect net nitrification and N mineralization in the Brazilian Amazon, and the proportion of net nitrification to net N mineralization is species dependent.

For an explanation of such pronounced single-species effects, it is important to come closer to the mechanisms by which the plant species affected net N mineralization and net nitrification rates. One of the possible mechanisms is that plant species directly affect groups of microorganisms in the rhizosphere, promoting nitrification, ammonification, or N immobilization. An early hypothesis was that plants inhibited nitrification by exudation of chemicals. This explanation was, however, refuted in most cases (Aber and Melillo 1991). The current theory holds that nitrification is mainly controlled by a combination of soil pH and ammonium availability. This is in agreement with the high rate of net nitrification under *Eschweilera* and *Vismia* in our experiments, where relatively high NH_4^+ concentrations were also found.

The denitrification potential was also significantly affected by tree species. The denitrification potential was significantly higher beneath non-timber (*Bixa* and *Theobroma*) than beneath timber-tree species (*Ceiba*). Moreover, *Bixa* and *Theobroma* have shown large net N_2O production even without NO_3^- addition (Fig. 2b). As non-timber tree species in this region usually receive inorganic N fertilizer, whereas timber species are usually not fertilized, much larger gaseous N losses under fruit trees could be expected, especially when inorganic N fertilizers are used.

C respiration activities responded differently to tree species: if SIR was affected by species, basal respiration was not. One of the possible explanations of the different response is that by adding easily available organic C for SIR measurements, inorganic N could be a limiting factor for C respiration. In the incubation experiments, the amount of inorganic N was determined by net N mineralization and net nitrification, which were strongly affected by species and thereafter SIR was affected by species. Soil net N mineralization and net nitrification appear thus to be more sensitive to species than C mineralization if no glucose is added. Surprisingly, this is in agreement with our findings in Siberia where tree species have a much stronger affect on N cycling processes than on C respiration (Menyailo et al. 2002b).

Interrelationships of microbial activities

Using correlation analysis, we examined the relationships between soil microbial activities themselves and soil chemistry. We used two ways to assess these relationships: first, all soil samples were considered together independent of tree species and, second, mean values for different species were taken for correlation. In the first case, the relationships were determined not only by species but also by other factors, for example, spatial variability. In the second case, relationships were more affected by individual species effects. As expected, more significant relationships among microbial activities and activities versus chemical parameters were received when all samples were considered due to the larger sample number (21 versus 7). Overall, all microbial activities studied were closely interrelated in our soils. Only net nitrification was not correlated with net N mineralization. One of the possible explanations for the lack of correlation would be that net N mineralization and net nitrification were influenced by different rates of immobilization of NH_4^+ and NO_3^- (Chen and Stark 2000). We found a negative correlation between net nitrification and respiratory activities, suggesting that heterotrophic microorganisms affected the net accumulation of NO_3^- by immobilizing either NO_3^- or NH_4^+. In contrast, net N mineralization was positively correlated to SIR and basal respiration.

A positive correlation between net N mineralization and respiratory activities is commonly observed, as most heterotrophic C-mineralizing microorganisms participate in the first stage of N mineralization (ammonification). Also here, the positive relationship indicates that soil heterotrophic microorganisms contribute to net NH_4^+ accumulation more than to NH_4^+ immobilization. This might indicate that the microbial C-mineralizing community in our soils consumed relatively more NO_3^- than NH_4^+. It has been suggested that soil microorganisms, in contrast to higher plants, prefer NH_4^+ to NO_3^- as their N source (Walley et al. 1996). In contrast, Stark and Hart (1997) reported that soil microorganisms also immobilize NO_3^- in large amounts in undisturbed coniferous forests of the USA. We found correlative evidence that NO_3^- may also be an important form of inorganic N for soil microorganisms in weathered soils of the humid tropics.

Analyzing correlations of the means did not change the overall pattern of relationships among microbial activities, it only reduced the amount of significant correlations, making least significant correlations not significant. The strong correlations between the potential activities studied demonstrate that C and N transformations in these tropical soils are closely linked, and with the knowledge

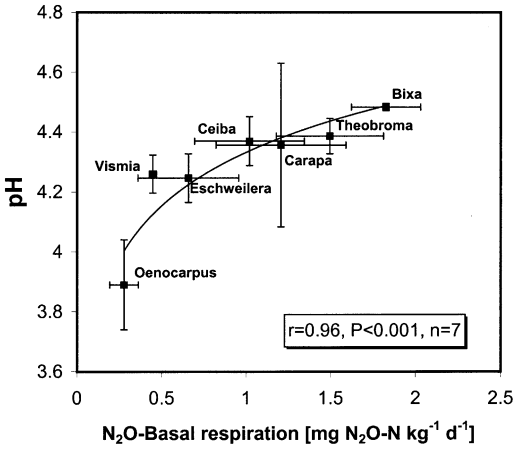

Fig. 5 Relationship between N_2O produced during basal respiration measurement and soil pH beneath the different tree species (means and standard errors, n =3)

of the activities of some processes, it can be possible to predict the rates of the others.

Correlations between microbial activities and soil chemical properties

Net N mineralization was correlated with total soil N, C, Ca and SO_4^{2-} contents. The relationship between net N mineralization and organic matter quality (C-to-N or lignin-to-N ratios) and quantity is well known (Vitousek et al. 1982; Aber et al. 1990; Aber and Melillo 1991; Attiwill and Adams 1993), but the relations to Ca and SO_4^{2-} are rarely reported. One of the possible explanations of the correlation with Ca would be that Ca concentrations are often correlated with soil pH. In our soils, however, the relationship of net N mineralization with pH was weak. The co-variation of net N mineralization and Ca in our study may be indicative of stress through a lack of nutrients rather than through acidic soil reaction. As mentioned above, our soils are both acidic and poor in nutrients, and it is difficult to distinguish whether low pH or low nutrient contents were more important for determining net N mineralization rates. We were unable to find any explanation for a positive relationship between the net N mineralization and SO_4^{2-}.

Strong correlation between net nitrification and NH_4^+ concentrations is in agreement with the current theory of the regulation of net nitrification by NH_4^+ (Aber and Melillo 1991). The negative correlation between nitrification and soil K concentrations may have been caused by antagonisms between NH_4^+ and K at the soil exchange surfaces and uptake by plants. In this case, low K concentrations were not directly responsible for high nitrification, but simply co-varied with net nitrification. Another possible explanation is the response of net-nitrification-related microorganisms (nitrifying bacteria or N-immobilizing organisms) to K deficiency (Walters and Joergensen 1991).

Denitrification potential was correlated with initial NO_3^- and NH_4^+ concentrations in soil. The same result was obtained by Robertson and Klemedtsson (1996) for an organic forest soil in Sweden. N_2O-basal respiration negatively correlated to net N mineralization, suggesting that net N_2O production was not C limited in these soils, and also that N_2O losses contributed to the amount of inorganic N accumulated during N mineralization incubations. Among chemical parameters, N_2O-basal respiration correlated most to soil pH (Table 3), and this relationship becomes even stronger when mean values are analysed (Fig. 5).

Both respiratory activities were negatively correlated with initial NO_3^- concentrations. The strong correlation indicates that soil heterotrophic microorganisms likely preferred NO_3^- as an N source and that nitrification most likely controlled C mineralization rates in this experiment. It is still not clear whether growth and activities of soil microorganisms are N- or C-limited in tropical forest soils. Most models developed during the last 10–20 years consider availability of organic C as the main factor governing N mineralization. The control of C mineralization by the availability of inorganic N is less recognized (Paustian et al. 1992; Blagodatsky and Richter 1998). Our results suggest that inorganic N may be a limiting factor for C mineralization even in a tropical forest with generally high N mineralization rates.

To conclude, individual tree species of natural and secondary forests and species used in tree-based crop plantations of Brazilian Amazon more strongly affect N transformations in soil, particularly net nitrification, than C respiration. The soil C respiration can be affected by tree species if inorganic N becomes a limiting factor. Overall, there were close negative relationships between two groups of processes: on the one hand, basal respiration, SIR and net N mineralization and, on the other, net nitrification and denitrification. Correlation analysis between soil chemical properties and microbiological activities suggest that the negative relationships between the two groups of processes are likely due to competition between the denitrifying and C-mineralizing communities for NO_3^-, which appears to the important N source for microbial growth in the studied soils. However, the preference of heterotrophic microorganisms for NH_4^+ or NO_3^- might be species-dependent and should be tested in future research with ^{15}N.

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References

Aber JD, Melillo JM (1991) Terrestrial ecosystems. Saunders College Publishing, Philadelphia, Pa.

Aber JD, Melillo JM, McClaugherty CA (1990) Predicting long-term patterns of mass loss, nitrogen dynamics, and soil organic matter formation from initial fine litter chemistry in temperate forest ecosystems. Can J Bot 68:2201–2208

Alfaia SS (1997) Destino de adubos nitrogenados marcados com ^{15}N em amostras de dois solos da Amazônia central. Rev Bras Cien Solo 21:379–385

Attiwill PM, Adams MA (1993) Nutrient cycling in forests. New Phytol 124:561–582

Blagodatsky SA, Richter O (1998) Microbial growth in soil and nitrogen turnover: a theoretical model considering the activity state of microorganisms. Soil Biol Biochem 30:1743–1755

Chen J, Stark JM (2000) Plant species effects and carbon and nitrogen cycling in a sagebrush-crested wheatgrass soil. Soil Biol Biochem 32:47–57

FAO (1990) Soil map of the world, revised legend. FAO, Rome

Lehmann J, da Silva JP Jr, Schroth G, Gebauer G, da Silva LF (2000) Nitrogen use in mixed tree crop plantations with legume cover crop. Plant Soil 225:63–72

Matson PA, Vitousek PM, Livingstone GP, Swanberg NA (1990) Sources of variation in nitrous oxide fluxes in Amazonian ecosystems. J Geophys Res 95:16789–16798

Menyailo OV, Huwe B (1999a) Activity of denitrification and dynamics of N_2O-release in soils under six tree species and grassland in central Siberia. J Plant Nutr Soil Sci 162:533–538

Menyailo OV, Huwe B (1999b) C, N-mineralization and denitrification as function of temperature and water potential in organic and mineral horizons of forest soil. J Plant Nutr Soil Sci 162:527–531

Menyailo OV, Hungate BA, Zech W (2002a) Tree species mediated soil chemical changes in a Siberian artificial afforestation experiment. Plant Soil 242:171–182

Menyailo OV, Hungate BA, Zech W (2002b) The effect of single tree species on soil microbial activities related to C and N cycling in the Siberian artificial afforestation experiment. Plant Soil 242:183–196

Paustian K, Parton WG, Persson J (1992) Modelling soil organic matter in organic amended and nitrogen fertilized long-term plots. Soil Sci Soc Am J 56:476–488

Potter CS, Matson PA, Vitousek PM, Davidson EA (1996) Process modeling of controls on nitrogen gas emissions from soils world-wide. J Geophys Res 101:1361–1377

Robertson K, Klemedtsson L (1996) Assessment of denitrification in organogenic forest soil by regulating factors. Plant Soil 178:49–57

Smith CK, Gholz HL, Oliveira F de Assis (1998) Soil nitrogen dynamics and plant induced soil changes under plantations and primary forest in lowland Amazonia, Brazil. Plant Soil 200:193–204

Stark JM, Hart SC (1997) High rates of nitrification and nitrate turnover in undisturbed coniferous forests. Nature 385:61–64

StatSoft (1997) STATISTICA for Windows (computer program manual). Tulsa, Okla.

Vitousek PM, Matson P (1988) Nitrogen transformations in tropical forest soils. Soil Biol Biochem 20:361–367

Vitousek PM, Gosz JR, Grier CC, Melillo JM, Reiners WA (1982) A comparative analysis of potential nitrification and nitrate mobility in forest ecosystems. Ecol Monogr 52:155–177

Walley FL, Kessel C, Pennock DJ (1996) Landscape-scale variability of N mineralization in forest soils. Soil Biol Biochem 28:383–391

Walters V, Joergensen RG (1991) Microbial carbon turnover in beech forest soils at different stages of acidification. Soil Biol Biochem 23:897–902