Biogeochemistry **65:** 275–294, 2003.

Land use and nitrogen export in the Piracicaba River basin, Southeast Brazil

SOLANGE FILOSO[1,2,*], LUIZ A. MARTINELLI[2], MICHAEL R. WILLIAMS[1], LUCIENE B. LARA[2], ALEX KRUSCHE[2], MARIA VICTORIA BALLESTER[2], REYNALDO VICTORIA[2] and PLINIO B. DE CAMARGO[2]

*[1]Marine Biological Laboratory, The Ecosystems Center, Woods Hole, MA 02543, USA; [2]Centro de Energia Nuclear na Agricultura (CENA), Universidade de São Paulo, Av. Centenário 303, Piracicaba, 13416-000, SP, Brazil; *Author for correspondence*

Received 26 April 2002; accepted in revised form 4 September 2002

Key words: Brazil, Land use, Nitrogen, Piracicaba River, South America

Abstract. Anthropogenic N inputs and riverine export were determined for a meso-scale river basin in one of the most developed and economically important regions of South America. The Piracicaba River basin is located in southeastern Brazil and drains into a tributary of the Paraná River. The basin supports over 3 million people (about 2% of the population of Brazil) with intensive agricultural and industrial activities. During two years from 1995 to 1997, biweekly samples were collected at 10 stations along the Piracicaba River and its tributaries for analyses of dissolved and particulate N. The average annual flux of dissolved inorganic N and total N increased by a factor of 15 and 20 times, respectively, from the headwaters to the lower reaches of the main channel, whereas discharge increased by only 7 times. On a per area basis, the export of TN varied according to land use and was significantly correlated to the net input of anthropogenic N. Among 10 sub-catchments composing the basin, areas mostly covered by pasture and forest had the lowest export, whereas more agricultural and urban areas had higher export. The amount of N exported from each sub-catchment varied widely, but inputs were consistently higher than fluvial outputs. Losses and retention of N occurred throughout the basin but were especially high in the sub-catchment with a main-stem reservoir, suggesting that aquatic processing plays an important role in controlling riverine N export. Total net anthropogenic input to the Piracicaba River basin was 4,500 (± 900) kg N km^{-2} yr^{-1} of which about 40% was exported via fluvial outputs.

Introduction

In the past century, rates of synthetically-fixed N have increased dramatically because of human activities such as the production of fertilizer, energy, and cultivation of leguminous crops (Galloway 1998). Although 78% of the atmosphere is composed of N, biological systems are N limited because most biota are unable to use N_2, which is the largest form of N in the atmosphere (Vitousek and Howarth 1991). High amounts of fixed or reactive N in the environment can lead to increased primary productivity, decreased species diversity, and changes in foodweb structure (Vitousek et al. 1997). In the atmosphere, anthropogenic emissions of nitrous oxide contribute to global warming and stratospheric ozone depletion (Kroeze 1994; Galloway et al. 1995; Kroeze and Seitzinger 1998).

While the fate and accumulation rate of this excess N in the environment is poorly understood for many regions of the world (Galloway 1998), studies conducted in North America and Europe indicate that export of N in rivers is highly correlated to net anthropogenic inputs of N in watersheds (Howarth et al. 1996). Moreover, only a small percent of the net anthropogenic inputs is generally exported in rivers (Boyer et al. 2002), while the remaining is retained or lost in the watershed before reaching the aquatic system.

In tropical ecosystems, terrestrial environments are typically less nitrogen-limited than those of the temperate zone (Martinelli et al. 1999a). Consequently, the export of N in tropical rivers tends to be relatively high in comparison to terrestrial inputs (Lewis et al. 1999). In contrast, aquatic environments of the tropics are commonly more nitrogen-limited and therefore have a greater potential response to nutrient loading than aquatic environments of the temperate zone (Talling and Lemoalle 1998). Small catchments studies in pristine regions such as the Amazon have shown dramatic increases in stream N exports with deforestation (Williams and Melack 1997; Williams et al. 1997a). However, different land use characteristics, the relative importance of different N sources, and climatological factors ultimately determine the extent to which N is exported in rivers (Turner and Rabalais 1991; Howarth et al. 1996; Jordan et al. 1997; Vizcarra and Lavkulich 1997; Fowler et al. 1998; Boyer et al. 2002). Therefore, site-specific studies that assess the effects of anthropogenic activities on N inputs and outputs in impacted tropical regions are needed, especially at meso and regional scales.

In this study, we determined the export of N in the Piracicaba River, São Paulo, Brazil, which drains a densely populated and highly agricultural basin. We compared the export of N from 10 sub-catchments of the basin with different land uses and population sizes to assess the relationship between anthropogenic N inputs and riverine export and the effects of N inputs in this tropical mesoscale watershed.

Study area

The Piracicaba River basin is a mesoscale watershed (12,400 km^2) located mostly in the state of São Paulo, southeastern Brazil (Figure 1). The main stem of the Piracicaba River is formed by the confluence of three major tributaries and discharges about 156 $m^3 s^{-1}$ into the Barra Bonita reservoir. The river system drains from east to west and, ultimately, into the Tietê and Paraná rivers. Average annual precipitation in the basin is 1400 mm, most of which occurs between October and March (Williams et al. 2001).

The basin can be classified into three topographically distinct regions that are representative of different land use practices. The eastern portion of the basin is hilly with elevations up to 2000 m and large areas of forest, silviculture, and pasture. The central region has moderate elevation, extensive areas of sugarcane plantations, and high population and industrial densities in large urban centers. The western portion is mostly lowland with sugarcane plantations and some pasture in

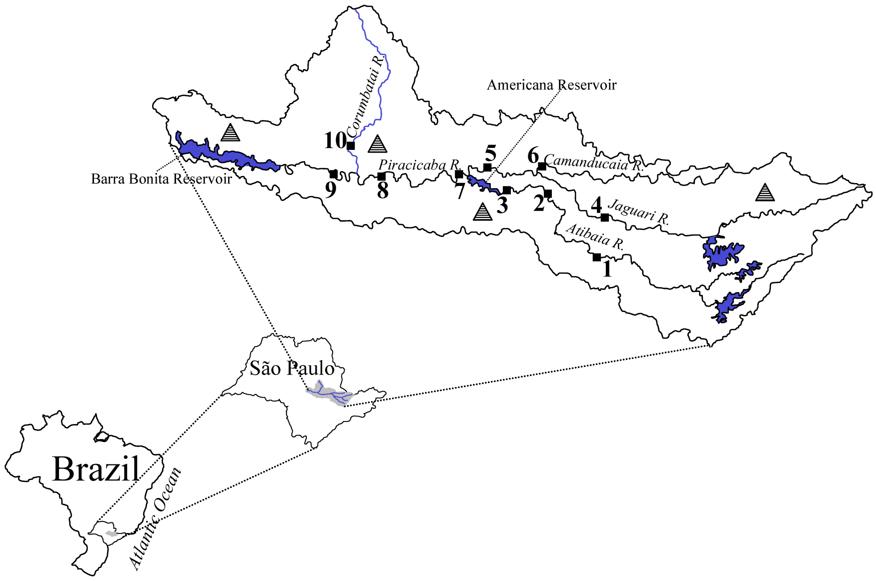

Figure 1. The Piracicaba River basin is located in the state of São Paulo, Brazil. River sampling stations (1–10), the location of rain collectors (solid triangles), and the names of major rivers and reservoirs of the basin are indicated.

the higher elevation areas. Overall, the dominant land cover types in the basin are pasture (45%), agriculture (31%), natural forest and silviculture (16%), and urban (6%). In the last 20 years, urban and agricultural (e.g., sugarcane plantations) areas have steadily increased throughout the basin largely at the expense of pasture and natural forests.

The basin sustains a population of approximately 2.9 million people located mainly in urban centers (92%). Although almost 95% of the urban households were sewered as of 1994, only 4% of the total domestic sewage was treated before being discharged into rivers. Presently, 16% of the domestic sewage is treated and point sources are an important factor contributing to N inputs in this river system.

Methods

Sample collection and water analyses

Water samples were collected from June 1995 to June 1997 at 10 stations of the Piracicaba River and its tributaries. Daily discharges were measured by the Water and Electric Energy Department for the state of São Paulo (DAEE) at each station (Figure 1). Samples were collected from the river surface (30 cm) with a Niskin bottle and stored on ice in the dark while transported to the laboratory. For dissolved N, subsamples were filtered through pre-combusted cellulose acetate filters (nominal pore size 0.45 μm) in the laboratory and stored at 4 °C prior to analysis. Nitrate concentrations were determined within 24 hours with a Shimatzu ion chromatograph (model LC-10AD). Ammonium was analyzed within 5 to 10 days from collection by ion chromatography using aliquots preserved with $HgCl_2$. Total dissolved nitrogen (TDN) was determined by persulfate digestion (Valderrama 1981) followed by ion chromatography, and dissolved organic nitrogen (DON) estimated as the difference between TDN and dissolved inorganic nitrogen (DIN = NO_3 + NO_2 + NH_4).

Samples for analyses of particulate nitrogen (PN) were collected with a depth-integrating sampler in two to three vertical profiles, depending on channel width. After collection, the water from each profile was homogenized through a US Geological Survey splitter. In the laboratory, 1-L sub-samples were filtered through pre-weighed filters (0.1 μm pore size) for determination of PN by an elemental CHNS analyzer (EA 1110). Total N (TN) is defined as the sum of PN, DON and DIN.

Daily discharges at each sampling station were obtained from DAEE for calculation of discharge-weighted means and riverine fluxes. Annual discharge-weighted means (DWM) were calculated for each sampling station as:

$$DWM = (\Sigma C_i Q_i)/\Sigma Qi \quad (1)$$

where C_i is the observed concentration of instantaneous river flow i, Q_i is the discharge volume (liters) for the two-week period with sample date as the midpoint of the period i, and the denominator is the annual Σ of discharge volume.

The annual flux of N (F_j) at each sampling station was calculated as:

$$F_j = [DWM][Q_j] \quad (2)$$

where DWM is the concentration at each station and Q is the annual discharge.

The annual N export per unit of area of each sampling station (E_j) was calculated by dividing the annual N flux at the sampling station (F_j) by the area of its respective sub-basin (A_j). Nitrogen fluxes at each station were not cumulative (i.e., they excluded the flux from any upstream sampling station(s)). Nitrogen fluxes were calculated as:

$$E_j = F_j - F_i/A_j \quad (3)$$

Delineation and characterization of the basin

Using Arc/Info Geographic Information System (GIS), the Piracicaba basin was divided into 10 sub-catchments corresponding to the drainage area of each sampling station along the main river and tributaries (Figure 2). Total area, land use types and cover, and population size were determined for each of the sub-catchments.

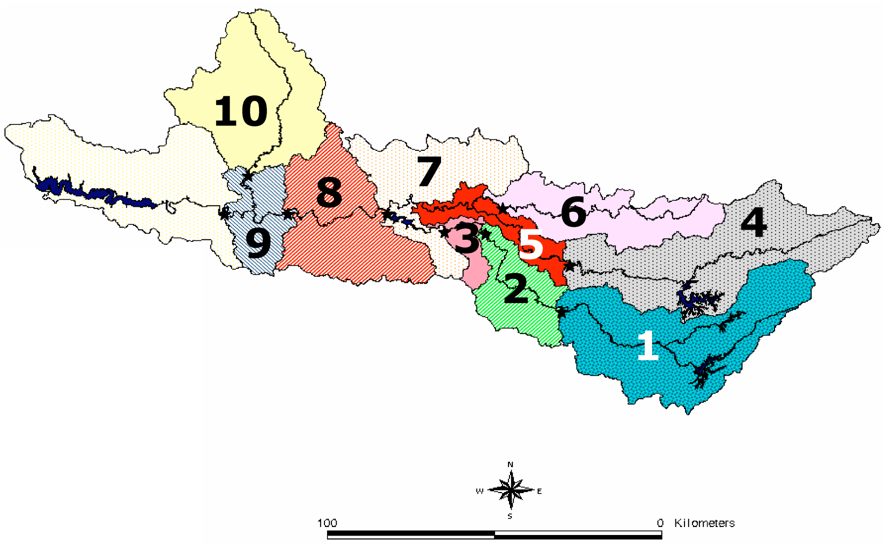

Figure 2. The Piracicaba River basin was divided into 10 sub-catchments corresponding to the drainage areas of each sampling station along the main channel and tributaries.

The delineation of the sub-catchments was based on the Arc/Info Digital Elevation Model (DEM) derived from an elevation map with 20-m vertical resolution obtained from the Brazilian Institute of Geography and Statistics (IBGE). Land cover classification was performed on a Landsat thematic mapper (TM) image from 1997.

Nitrogen inputs

Based on the area, land types and cover, and population size of each sub-catchment, we calculated the inputs of human-derived N from different sources such as atmospheric deposition, fertilizer application, and food for human consumption (sewage) and industrial waste (Table 1). The sub-catchment of the Barra Bonita reservoir was not used in our export calculations (total basin area without this sub-catchment = 10,797 km^2).

Inputs of N through atmospheric deposition was estimated using N concentrations in precipitation measured in rain samples collected from August 1997 to July 1998 at four sites in the Piracicaba basin (Figure 1). Rain samples were collected on an event basis during 24 hours using a wet-only collector (Aerochem Metrics). A total of 272 samples were collected and analyzed for nitrate, nitrite and ammonium by ion chromatography (Dionex 2010i). Dry deposition inputs were estimated based on values of N deposition in rain, using the method used by Caraco and Cole (1999), where N inputs from dry deposition equal those of wet deposition. Lovett and Rueth (1999) and Boyer et al. (2002) have confirmed that this assumption is valid for the northeastern United States. Although there are no available data of dry deposition for the Piracicaba River basin, we used this assumption to estimate dry

Table 1. Physical and geographical characteristics of sub-catchments in the Piracicaba Basin.

Sampling Station/sub-catchment	Drainage Area	Cumulative water discharge (10^6 m^3 yr^{-1})		Population			Land Use (km^2)		
	(km^2)	Year 1	Year 2	Total (10^3 ind)	Rural (%)	Agriculture	Pasture	Forest	Urban
1	1930	897	762	232	20	37	1257	540	50
2	642	1025	998	426	6	39	413	101	81
3	198	1236	1080	199	1	117	25	4.8	46
4	1951	873	670	237	21	38	1310	521	33
5	442	1481	1283	242	9	194	177	40	23
6	992	518	456	57	20	68	741	166	13
7	1256	3148	2689	456	5	909	88	21	91
8	1366	4377	3723	843	3	1088	40	11	222
9	554	4664	4008	327	4	406	51	20	73
10	1596	861	674	210	9	518	774	247	55
TOTAL	10767	4664	4008	3177	10	3414	4878	1672	687

deposition inputs in our budget because dry deposition is likely high from intensive agricultural and industrial activities. For instance, the common land-use practice of burning sugarcane fields occurs over a period of seven months or more per year, and can be an important regional source of nitrogen (Lewis 1981).

Background deposition, or the deposition that occurred in the basin prior to anthropogenic activities, was estimated for nitrate and ammonium using concentrations from the Amazon basin (Williams et al. 1997b; Filoso et al. 1999). The estimated background deposition was subtracted from the atmospheric deposition measured in the Piracicaba basin in order to account only for anthropogenic N. Volatilized N from fertilizer application (15%) in agricultural fields of the basin was also subtracted from atmospheric deposition based on ^{15}N marked fertilizer experiments (Cabezas 1987; Oliveira et al. 1999).

Inputs of N from fertilizers were estimated for each sub-catchment as the product of the amount of fertilizer used per hectare for the main agricultural land use types in the area (Gonçalves 1995; Van Raij et al. 1996; Yamada and Lopes 1998) and the total area of each land use type (Table 1). The amount of N in fertilizer recommended for sugarcane cultivation in the Piracicaba basin is between 60 to 100 kg ha^{-1} (Yamada and Lopes 1998); therefore, we used an average of 80 kg ha^{-1}. This amount of N in fertilizer is applied in sugarcane fields of the Piracicaba basin every two years, when cuttings from mature cane stalks are planted in the soil. According to the local practice in the basin, four successive crops of mature sugarcane are harvested with each planting. During the period of successive crop harvests, fertilization of up to 60 kg N ha^{-1} yr^{-1} is recommended (Korndörfer et al. 1997). In the Piracicaba basin, N fertilization is uncommon during this period because of the high cost:benefit ratio. Instead, application of fertilizer with phosphorus is preferred since this is the most limiting nutrient in sugarcane fields of Brazil (Orlando Filho et al. 2001). Accordingly, we calculated inputs of fertilizer in the basin using an average between a minimum rate of 40 kg N ha^{-1} yr^{-1} and a maximum of 100 kg N ha^{-1} yr^{-1}. The fertilization of pasture was considered to be negligible in the Piracicaba basin as it is an uncommon practice in the region and Brazil in general (A. Abdalla, University of São Paulo, pers. comm.; Boddey et al. (1997)). Fertilization of eucalyptus plantation is also minimal in the region, as only 60 kg ha^{-1} is applied every seven years when plants are in the seedling stage (Pereira et al. 1984; Reis et al. 1987).

The input of N in the basin from food consumed by the human population was estimated based on the load of N in human waste. We calculated the N load in human waste as the product of the annual per capita load of 3.3 kg N (Meybeck 1982) and the population number in the whole basin and in each of the sub-catchments. Although human waste does not represent newly fixed or imported N to many basins of the world (Howarth et al. 1996), we estimated that part of the N in the human waste of the Piracicaba basin originates from imported foods and, therefore, represents newly fixed N.

Because the main agricultural products in the Piracicaba basin are either inedible by humans (wood) or do not contain protein (sugar and alcohol), we assumed that all the vegetarian protein consumed by the human population in the basin was imported. This type of protein accounted for about 60% of the total consumed by the population, based on Brazilian averages (Smil 2000), while the remaining 40% came from animal protein.

The main sources of animal protein for the human population in the Piracicaba basin, as for Brazilians in general, are bovine meat (55%), poultry (34%), and eggs (9%) (FAO 2002). According to our calculations based on the total area of pasture in the basin (Table 1), which is the only source of feed to cattle in the region, and on the average number of cattle per area of pasture (e.g. ~ 2 heads ha^{-1}) and the weight at the time of slaughter (150 kg of meat for human consumption), the production of bovine meat is about 28,000 tons yr^{-1}. Such production sustains only about half of the average per capita bovine meat consumption of 19 kg yr^{-1} in the basin, hence, the other 50% of the meat consumed by the population must be imported.

There are no accurate data for the percentage of imported versus locally produced poultry and eggs in the basin. However, a map from the São Paulo State Poultry Association (www.apa.com.br) shows that out of the six major counties located in the Piracicaba basin, four of them are major centers of poultry production. All the egg production centers are located out of the basin. On the basis of this information, we assumed that about two-thirds or 67% of the poultry consumed in the basin is locally produced while the remaining poultry is imported. The origin of fish consumed in the basin is unknown, but it constitutes only 0.6% of the protein consumption of Brazilians (FAO 2002). In summary, 42% of the total amount of protein consumed by the population in the Piracicaba basin is imported whereas 56% is produced locally.

Inputs from industrial waste produced within the basin were obtained from the environmental sanitation technology company for the state of São Paulo (CETESB – Companía Estadual de Tratamento de Esgoto e Saneamento Básico). Considering that the main industries in the basin produce sugar, ethanol, paper and cellulose, it is likely that some of the N in industrial waste originates from fertilizer application in sugarcane fields.

Estimation of nitrogen losses

Losses of N through the export of agricultural products from the Piracicaba basin were estimated for citrus and silviculture products. Since there are no available data on what percentage of wood harvested in the basin is exported as agricultural products, we assumed that all of the wood produced in the basin is exported. All the by-products of paper and cellulose production in the basin were accounted for as industrial waste.

The N exported in wood originating from silviculture in the basin was calculated based on the N contents of several species of Eucalyptus cultivated in the region. According to several studies (Bellote 1979; Pereira et al. 1984; Reis et al. 1987), the average N content in the wood of Eucalyptus stands 7 years of age is 373 kg N ha^{-1}. We assumed that the remaining parts of the trees are not removed from the forest and, therefore, are decomposed and remineralized in the forest floor.

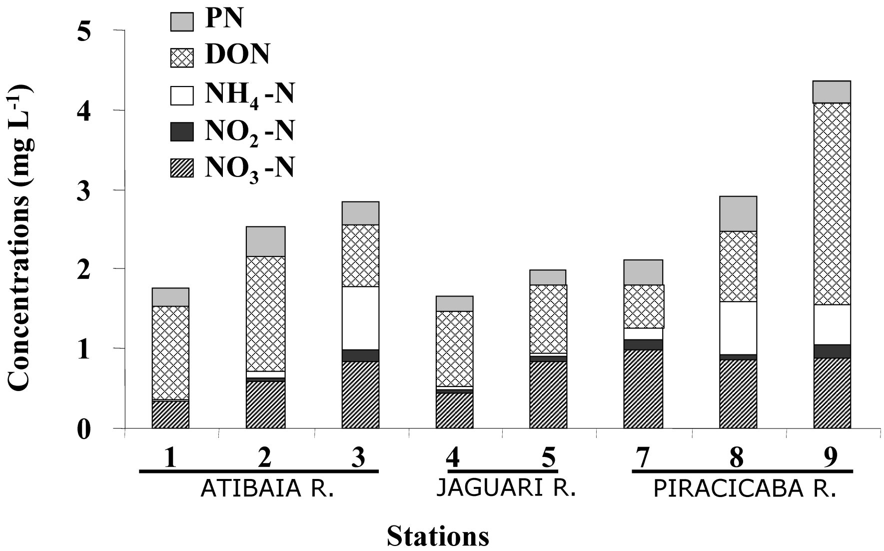

Figure 3. Volume-weighted mean concentrations of N partitioned into particulate, dissolved organic (DON) and inorganic forms (NO_3-N, NO_2-N, and NH_4-N). Sampling stations 1–3, 4–5, and 7–9 are located along the Atibaia, Jaguari, and Piracicaba rivers, respectively.

The export of N in citrus (2 kg ha^{-1} yr^{-1}) was estimated by using literature values of N exported per ton of product (kg N ton^{-1}) (Malavolta and Viloante Netto 1989; Gonçalves 1995). Export in sugarcane products was considered negligible because of the low N content in the end products of sugarcane that are exported.

Results

N concentrations

Discharge-weighted mean concentrations of TN increased from upstream to downstream reaches of the tributaries and main channel of the Piracicaba River (Figure 3). In the tributaries, TN concentrations increased with DIN, whereas the proportion of DON:TN decreased. In the last downstream sampling station of the Atibaia River, ammonium concentrations contributed to about half of the DIN. In the main channel, DON concentrations contributed to most of the TN increase downriver, followed by ammonium and nitrate. Except for Station 9, the highest ratios of DON:TN were associated with stations 1 and 4 that drain the largest percent area of forest and pasture combined with low population densities in the basin (Table 1). Conversely, sampling stations draining areas with the highest percent cover of agricultural lands combined with urban areas had the highest ratios of DIN:TN, except for Station 9 (Figure 3).

Land use and N export

The average annual flux of nitrogen for the two-year study was about 18,900 tons N yr^{-1} (Table 2). On a per area basis, the watershed exported approximately 1,750 kg N km^{-2} yr^{-1}. Export values were generally higher in sub-catchments with relatively more agricultural and urban land cover and lower in sub-catchments where the landscape was mostly pasture and forest (Figure 4). Hence, there was a significant positive correlation between N export versus agricultural ($r = 0.75$; $n = 10$) and urban land use ($r = 0.69$; $n = 10$), and a negative correlation between N export versus pasture ($r = -0.60$; $n = 10$) and forest land use ($r = -0.56$; $n = 10$) (Figure 5). These correlations between land use types and N export were statistically significant ($p < 0.05$) only after we excluded outlier values of sub-basin 7 from the analyses. We justified the removal of such values by considering that samples from Station 7 did not belong to the population of samples representing the lotic system of the Piracicaba River basin. Water samples from Station 7 were collected immediately downriver from the Americana Reservoir (Figure 1) and, therefore, were more representative of a lacustrine system.

Nitrogen budget

The total net anthropogenic N inputs in the sub-catchments of the basin varied from about 1,500 to over 9,000 kg km^{-2} yr^{-1} (Table 2), with the highest amounts in sub-catchments 3 and 8. The relative importance of each source varied, with atmospheric deposition accounting for most of the inputs in sub-catchments located in areas with relatively more forest and pasture, while fertilizer and/or point sources were largest in predominantly agricultural and urban sub-catchments (Figure 6).

The export of N in the river sectors draining each of the sub-catchments was significantly related to the total amount of net anthropogenic inputs ($r^2 = 0.40$; $n = 9$) (Figure 7), although the N exported from sub-catchment 10 was considerably higher than expected. The lowest riverine export in relation to inputs was observed for sub-catchment 7, where the Americana Reservoir is located. As indicated previously, because N processing in the Americana Reservoir masked the effect of anthropogenic inputs in the sub-catchment 7, we excluded this station from our analysis in Figure 7. For all the sub-catchments, the N exported in the river sectors ranged between 3% and 86% of the inputs (Table 2).

Although fertilizer application was the largest source of N inputs to the Piracicaba basin as a whole (Table 2), contribution from food and industrial waste (as point sources) were substantial. In total, over 42,000 (± 9,000) tons of N were added to the entire basin while the N export in the Piracicaba River was only about 45 ± 8% of the net inputs (Table 2). Hence, about half of the anthropogenic N inputs added to the landscape was retained or lost from the basin before reaching the last downriver sampling station of the Piracicaba River.

Table 2. Main anthropogenic N inputs and export in the entire Piracicaba basin and by sub-catchment. All values are in kg N km^{-2} yr^{-1}, except for those in Basin Totals.

Sampling Stations/ Sub-catchments	Fertilizer	Net Atmo-spheric deposition	Imported food	Industrial waste	**TOTAL INPUTS**	Wood export	Citrus Export	**TOTAL PROD-UCT EXPORT**	**NET INPUT**	**RIVER EXPORT**	**% RIVER EXPORT**
1	311	1346	232	0	1889	379	0	379	1510	725	48 ± 1
2	565	1421	1284	698	3968	298	0	298	3670	1834	49 ± 1
3	4321	1109	1940	2225	9595	10	0	10	9585	2391	26 ± 5
4	329	1346	235	0	1910	489	0	489	1421	644	45 ± 1
5	3318	1100	1056	1119	6593	267	0	267	6326	1502	27 ± 6
6	736	1322	336	0	2394	533	119	652	1742	798	46 ± 2
7	5763	1040	782	1683	9268	0	2897	2897	6371	199	3 ± 2
8	5306	843	1193	530	7872	5	0	5	7867	4667	63 ± 16
9	5130	880	1141	406	7557	11	0	11	7546	2688	39 ± 10
10	2286	1125	254	351	4016	143	0	143	3873	3159	85 ± 21
Area-weighted average	2,381	1,198	610	465	4,653	257	349	606	3,929	1,757	45 ± 7
BASIN TOTAL *(Tons)*	25,634	12,894	6,564	5,006	50,098	2,767	3,757	6,523	42,308	18,917	45 ± 5

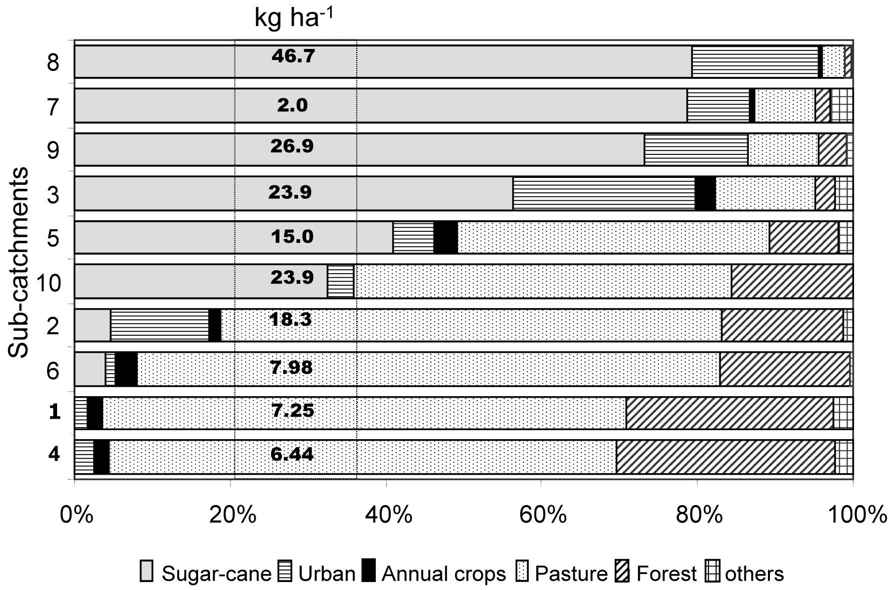

Figure 4. Percent cover of the main land use types in each sub-catchment of the Piracicaba River basin and their respective export of TN (kg ha^{-1} yr^{-1}).

Discussion

Similar to several other regions of the world (Howarth et al. 1996; Boyer et al. 2002), anthropogenic inputs to the Piracicaba River basin has increased riverine N export. The export of N for each sub-catchment of the basin was significantly related to the magnitude and relative importance of the N sources, which varied according to land use. For instance, the sub-catchments mostly covered by pasture and forest (1, 4, and 6) had relatively low N inputs from anthropogenic sources and, consequently, lower N export in their respective river sectors.

The riverine export of N in relation to inputs to the predominantly forested catchments was low, similar to watersheds of the temperate zone (Howarth et al. 1996; Boyer et al. 2002). Low N export from predominately forested sub-catchments is probably because of extensive cultivation of eucalyptus and pine trees, and a high N retention capacity of the ecosystem as they are subjected to repeated biomass removal (Vitousek et al. 1997). Silveira et al. (2001) determined that an average of 373 kg N ha^{-1} accumulated in the wood of several species of eucalyptus cultivated in the region is removed every seven years after cutting. Considering that these eucalyptus forests receive about 13 kg N ha^{-1} yr^{-1} in atmospheric deposition in addition to an average of 9 kg N ha^{-1} yr^{-1} in fertilizer, we assume that the forested sub-catchments of the Piracicaba basin are retaining significant amounts of N in contrast to natural forests of tropical regions (Martinelli et al. 1999a). Because the net input of N in the predominantly forested sub-catchments of the Piracicaba

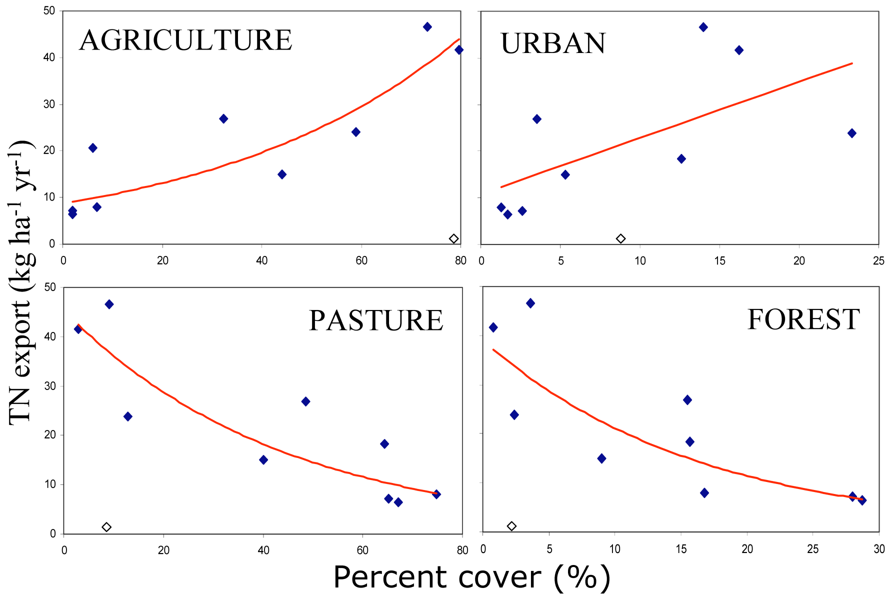

Figure 5. Plots of percent area of agricultural, urban, pasture and forested lands in the sub-catchments versus TN export at each sampling station. Agricultural lands represent sugar cane, citrus, and annual crops. Forested land includes natural stands and silviculture (eucalyptus and pine trees). All correlations were statistically significant ($p < 0.05$). Correlation analyses did not include samples from sub-catchment 7, marked with an open diamond (◇).

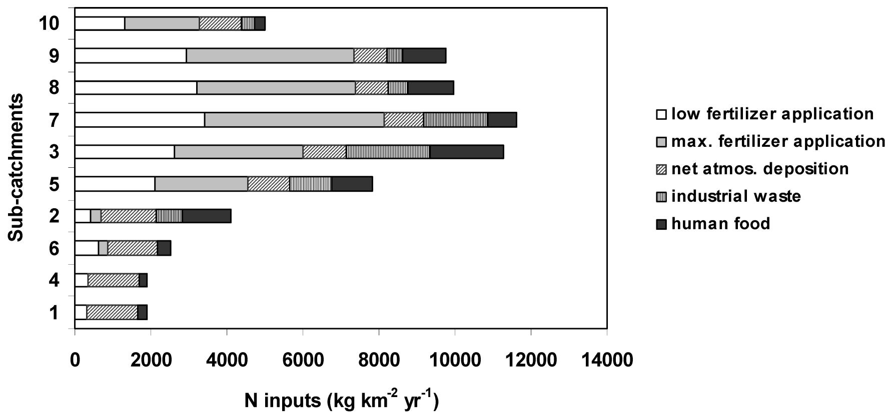

Figure 6. Sum of anthropogenic N inputs on a per area basis in each sub-catchment of the Piracicaba basin. Sub-catchments 1, 2 and 3 drain into the Atibaia River; 4, 5 and 6 drain into the Jaguari River; 7, 8 and 9 into the Piracicaba River, and 10 into the Corumbatai River.

basin was far above the baseline determined by Lewis et al. (1999) for minimally disturbed tropical watersheds with equivalent runoff, it is likely that cultivation of eucalyptus and pine trees buffer the impact of increasing anthropogenic N inputs in

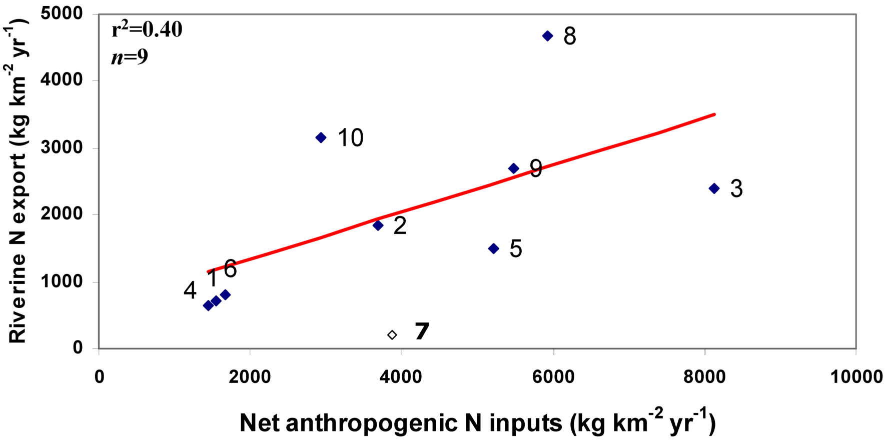

Figure 7. Export of TN at each sampling station versus net anthropogenic inputs in each sub-catchment. Net input is defined as the sum of anthropogenic inputs minus the N exported from the basin as agricultural products. The value from sub-catchment 7, marked with an open diamond (◇), was not included in the regression analysis.

the region. In areas of natural forest cover, the effect of high N inputs from atmospheric deposition, for instance, would probably be more conspicuous, especially in the aquatic system.

In the more agricultural and urban sub-catchments of the basin, net anthropogenic N inputs were higher than those in areas covered mostly by forest and pasture, although ratios of export in relation to net inputs varied considerably among these sub-catchments (Figure 7). Some of the variation could be explained by errors associated with the magnitude of the inputs and the origin of the data, such as for industrial waste, which are often collected by the industries themselves and underestimated. Alternatively, some of the variability may be associated with in-stream processes such as denitrification and/or burial. For instance, in sub-catchment 7 there is strong evidence that denitrification and/or burial of N in the Americana Reservoir regulate riverine N export. Williams et al. (2001) observed substantial DIN losses in and downriver of the Americana Reservoir of the Piracicaba River system, and anoxic conditions have been detected frequently in the reservoir, indicating that the environment is often suitable for denitrification (Martinelli et al. 1999b). In some preliminary measurements of N_2O emissions in the Americana Reservoir, emission rates from the water surface were on the order of 180 kg km^{-2} yr^{-1} (L. Martinelli – unpub. data), which could amount to significant losses of N from the aquatic system. Other evidence of the importance of aquatic denitrification controlling the riverine export of N was observed in sub-catchment 3, where more than 50% of the net anthropogenic inputs entered the river as point sources (i.e., industrial and human wastes), but the riverine export was equivalent to only 26% of the total inputs.

Because of the direct contribution of point sources, they commonly account for the majority of the N inputs in rivers of densely populated basins (Cole et al. 1993).

In agricultural river basins of North America and Europe, fertilizer application and agricultural runoff are frequently cited as the major causes of increased N loadings in rivers (Howarth et al. 1996; Boyer et al. 2002). In the Piracicaba basin, many of the predominantly agricultural sub-catchments have high population densities, making it difficult to determine which source contributes most of the N exported in the different river sectors. Although we estimated that fertilizer inputs of N were greater than those from point sources in dominantly agricultural sub-catchments, and that they also contributed between about 50 ± 10% of the net anthropogenic N inputs into the basin as a whole, ^{15}N marked fertilizer experiments with sugarcane conducted in the region indicated that leaching of N in sugarcane fields is low, on the order of about 5% for clayey arable soils (Salcedo et al. 1988; Oliveira et al. 1999) and 15% for sandy soils (Camargo 1989). Leaching is negligible in silviculture (Pereira et al. 1984; Lima et al. 1999). Moreover, soils in the basin can be deep (> 10 m), so much of the fertilizer leached out of the rooted zone may not enter the hydrologic system (Matson and Vitousek 1987), which is in contrast to what is often observed in temperate agricultural watersheds (Staver and Brinsfield (1990, 1996)).

Nevertheless, the highest export value of TN in the Piracicaba River was observed for sub-catchments 8 and 10, where sugarcane cultivation dominates most of the landscape, and fertilizer application contributed more N than the sum of inputs from all the other sources. We presume that several factors, besides high rates of fertilizer application, contribute to the high export of TN in these sectors of the river. For instance, the discharge of point sources directly into the main channel in sub-catchment 8, as opposed to small streams, probably hindered any immobilization and removal of the excess N in the river (Alexander et al. 2000). Also, increased water velocity in this sector of the river, caused by abrupt elevation changes and larger water volume, could have reduced important processes such as denitrification and burial of N.

Biological N fixation associated with the plant root system in areas highly cultivated with sugarcane may be another important factor contributing the high N export in this sector of the river. This source of N has not been accounted for in our budget because of the lack of available data and ongoing debate among Brazilian scientists about its magnitude. However, some estimates show that up to 100 kg N ha^{-1} yr^{-1} can be fixed in ten varieties of sugarcane commercially used in Brazil given a sufficient supply of phosphorus and good irrigation (Urquiaga et al. 1992). Biological fixation could be particularly significant in areas where burning practices are less common such as in the region surrounding the city of Piracicaba, located in sub-catchment 8.

Another potentially unaccounted-for source of N input to agricultural fields is the irrigation of fields with vinasse, a N-rich byproduct of sugar and alcohol. Vinasse from sugar cane and alcohol industries cannot be disposed directly into rivers because of national regulations in Brazil, so it accumulates in storage tanks and eventually is applied to sugarcane fields as organic fertilizer or leached under the tanks to the hydrologic system (Vianna 2001). Many of the large sugar and alcohol industries in the Piracicaba River basin are located in the sub-catchments with the highest riverine N exports, which receive N from sugarcane harvested elsewhere in the basin.

Using an average value of nitrogenous fertilizer application in sugarcane, we estimate that fertilizer contributes about 51% of the N inputs followed by atmospheric deposition (21%), imported food (15%), and industrial waste (13%). Fertilizer inputs in the Piracicaba basin could account for more than 60% of the anthropogenic inputs, although we believe that this is unlikely because the response of sugar cane plants during the second re-growth phase of the plant to the application of nitrogenous fertilizer is small (Oliveira 1999) and, therefore, the cost-benefit ratio of fertilizer application is too high. The causes for such a poor response of sugar cane plants is still unknown but has been linked to the mineralization of organic matter (Demattê 1997) and nitrogen fixation (Urquiaga et al. 1992).

Biological fixation in sugarcane fields could potentially account for twice as much input of reactive N in the Piracicaba River basin as fertilizer application. If this were the case, agricultural practices would overwhelmingly be the dominant source of N to the basin, similar to the Mid-Atlantic region of the US where agriculture is the dominant land use type (Boyer et al. 2002). The contribution of atmospheric deposition to the inputs of N in the Piracicaba basin (Table 2) was comparable to that reported for many catchments in the Northeastern US (Boyer et al. 2002) and was the single most important source of N to forested areas. The relatively high deposition rates in the Piracicaba River basin are probably related to intensive industrial activity, fuel combustion in densely populated areas and the burning of sugarcane fields. Volatilization from sugarcane fields can also contribute significant amounts of N to the atmosphere (Cabezas 1987; Kwong and Deville 1994; Trivelin et al. 1996; Oliveira et al. 1999), even after the percentage originating from fertilizer is subtracted from the total. However, it is possible that the amount of newly fixed N in atmospheric deposition has been overestimated because some of the fertilizer applied in sugarcane fields and assimilated in plant tissues is released to the atmosphere during biomass burning. Alternatively, the long-range transport of NH_x from the basin may reduce the contribution of dry deposition and the overall importance of atmospheric deposition.

The average export of TN in the Piracicaba River for the two-year study was over 1,750 kg km^{-2} yr^{-1}, with about two-thirds of the N in the form of DON. Still, the river exported over 520 kg km^{-2} yr^{-1} of N in inorganic dissolved forms, ranking the basin among those with the highest yields of DIN in the world, along with China, India, northeastern and central Europe and northeastern US (Howarth et al. 1996; Seitzinger and Kroeze 1998). Problems associated with high DIN concentrations include phytoplankton blooms, anoxic conditions, and massive mortality of fish and benthic invertebrates (Gabric and Bell 1993; Jordan et al. 1997). In the Piracicaba River, these problems are accompanied by the export of large amounts of readily available N and P to two important reservoirs that supply water and energy for several cities of the basin. Euthrophication of large reservoirs in Brazil has been a major problem in the country in the past several years, and continues to threaten water supplies and energy production in the state of São Paulo (Tundisi and Matsumura-Tundisi 1990).

Although the Piracicaba River exports large amounts of N from the basin, about half of the net inputs were retained or lost. High retention rates of net inputs from fertilizer application, atmospheric deposition, and fixation by leguminous crops are common in temperate regions (Howarth et al. 1996; Boyer et al. 2002). This input-output deficit can be attributed to storages such as accumulation in soil and plant tissues, and to losses such as leaching below the root zone, volatilization and denitrification in soils (Boyer et al. 2002). Also, Seitzinger et al. (2002) suggest that up to 40% of N losses in temperate zone watersheds occurs via aquatic denitrification.

Because pristine tropical, terrestrial systems tend to be less retentive (Howarth et al. 1996; Martinelli et al. 1999a), relatively large anthropogenic inputs are likely to increase the flux of diffuse N inputs to receiving waters (Downing et al. 1999). Moreover, there are large N inputs from point sources in the Piracicaba basin. Accordingly, we expected the riverine N export:N input ratio to be higher than observed. Although the lower-than-expected ratios may have been the result of overestimated inputs, this is unlikely since our budgets did not include inputs from N fixation and vinasse application to sugarcane fields. Alternatively, our N budget analysis and the findings of other studies done on the Piracicaba River (Martinelli et al. 1999b; Williams et al. 2001) suggest that rates of denitrification and burial in the aquatic system are high, particularly in a main-stem reservoir immediately downriver of concentrated urban centers. Eutrophic conditions observed in many tropical river systems that include main-stem reservoirs (Tundisi and Matsumura-Tundisi 1990) suggest that anoxia is common and, therefore, aquatic denitrification in these rivers is potentially high. Although recent estimates of N losses range from about 10% to 67% of total N loading to temperate aquatic systems (Howarth et al. 1996; Seitzinger et al. 2002; van Breemen et al. 2002), rigorous studies that specifically address the relative importance of aquatic N processing in tropical rivers and reservoirs are still needed in order to make robust comparisons between tropical and temperate systems.

Acknowledgements

We thank A. Abdalla, A. Boaretto, R. Boddey, E. Malavolta, M. deOliveira, W. Paula Lima, J. Salvador and P. Trivelin, T. Yamada for providing data and suggestions, and E. Fischer from CETESB for point-source data. We thank J. Galloway for insightful comments, and C. Hopkinson for his review of a previous version of this manuscript. Support was provided by a FAPESP post-doctoral fellowship to S. Filoso and the following grants: 94.0529-9 and 98.10269-5 (FAPESP), 62.0363/92.4 (CNPq/PADCT), and INT-9901158, DEB-9726862 and OCE 9726921 (NSF).

References

Alexander R.B., Smith R.A. and Schwarz G.E. 2000. Effect of stream channel size on the delivery of nitrogen to the Gulf of Mexico. Nature 403: 758–761.

Bellote A.F. 1979. Concentração, acumulação e exportação de nutrientes pelo *Eucalyptus grandis* (Hill ex Maiden) em função da idade. Master Thesis, University of São Paulo, Piracicaba, Brazil.

Boddey R.M., Sá J.C.D., Alves B.J.R. and Urquiaga S. 1997. The contribution of biological nitrogen fixation for sustainable agricultural systems in the tropics. Soil Biol. Biochem. 29: 787–799.

Boyer E.W., Goodale C.L., Jaworski N.A. and Howarth R.W. 2002. Anthropogenic nitrogen sources and relationships to riverine export in the northeastern USA. Biogeochemistry 57/58: 137–169.

Cabezas W.A.R. 1987. Calibração de um método para estimar perdas por volatilização de N-NH_3 de fertilizantes nitrogenados aplicados no solo. PhD Dissertation, University of São Paulo, Piracicaba, Brazil.

Camargo P.B. 1989. Dinâmica do nitrogênio dos fertilizantes: uréia (^{15}N) e aquamônia (^{15}N) incorporados ao solo na cultura de cana-de-açúcar. Masters Thesis, University of São Paulo, Piracicaba, Brazil.

Caraco N.F. and Cole J.J. 1999. Human impact on nitrate export: an analysis using major world rivers. Ambio 28: 167–170.

Cole J.J., Peierls B.L., Caraco N.F. and Pace M.L. 1993. Nitrogen loading of rivers as a human-driven process. In: McDonell M.J. and Pickett S.T.A. (eds), Humans as components of ecosystems: The ecology of subtle human effects and populated areas. Springer-Verlag, New York, pp. 141–157.

Demattê J.L.I. 1997. Considerações a respeito da adubação nitrogenada e seu parcelamento em cana-planta. Stab 15: 14–15.

Downing J.A., McClain M., Twilley R., Elser J., Rabalis N.N., Lewis W.M. et al. 1999. The impact of accelerating land-use change on the N-Cycle of tropical aquatic ecosystems: Current conditions and projected changes. Biogeochemistry 46: 109–148.

Filoso S., Williams M.R. and Melack J.M. 1999. Composition and deposition of throughfall in a flooded forest archipelago (Negro River, Brazil). Biogeochemistry 45: 169–195.

Fowler D., Sutton M.A., Smith R.I., Pitcairn C.E.R., Coyle M., Campbell G. et al. 1998. Regional mass budgets of oxidized and reduced nitrogen and their relative contribution to the nitrogen inputs of sensitive ecosystems. Environ. Poll. 102: 337–342.

Gabric A.J. and Bell P.R.F. 1993. Review of the effects of non-point nutrient loading on coastal ecosystems. Aust. J. Mar. Freshwater Res. 44: 421–483.

Galloway J.N. 1998. The global nitrogen cycle: changes and consequences. Environ. Poll. 102: 15–24.

Galloway J.N., Schlesinger W.H., Levy H. II, Michaels A. and Schnool J.L. 1995. Nitrogen fixation: Anthropogenic enhancement-environmental response. Glob. Biogeochem. Cycl. 9: 235–252.

Gonçalves J.L.M. 1995. Recomendações de adubação para *Eucalyptus*, *Pinus* e espécies típicas da Mata Atlântica. Docum. Florest 15: 1–23.

Howarth R.W., Billen G., Swaney D., Townsend A., Jaworski N., Lajtha K. et al. 1996. Regional nitrogen budgets and riverine N and P fluxes for the drainages in the North Atlantic Ocean: Natural and human influences. Biogeochemistry 22: 1–65.

Jordan T.E., Correll D.L. and Weller D.E. 1997. Effects of agriculture on discharges of nutrients from Coastal Plain watersheds of Chesapeake Bay. J. Environ. Qual. 26: 836–848.

Korndörfer G.H., Valle M.R., Martins M. and Trivelin P.C.O. 1997. Aproveitamento do nitrogênio da uréia pela cana-planta. Rev. Brasil. Cienc. Sol. 21: 23–26.

Kroeze C. 1994. Nitrous oxide and global warming. Sci. Tot. Environ. 143: 193–208.

Kroeze C. and Seitzinger S.P. 1998. The impact of land use on N_2O emissions from watersheds draining into the Northeastern Atlantic Ocean and European Seas. Environ. Poll. 102: 149–158.

Kwong K.F.K. and Deville J. 1994. Application of ^{15}N-labelled urea to sugarcane through a drip-irrigation system in Mauritius. Fert. Res. 39: 223–228.

Lima W.P., Zakia M.J., Camara C.D., Ferraz F.F.B. and Cezare C.H.G. 1999. Indicators of soil and water conservation for sustainable forest management in the neotropics. Conferencia y Taller Interna-

cional sobre Indicadores para el Manejo Forestal Sostenible en el Neotropico, 9–12 November. Catie. Turrialba, Costa Rica.
Lewis W.M. Jr 1981. Precipitation chemistry and nutrient loading by precipitation in a tropical watershed. Water Resources Research 17: 169–181.
Lewis W.M. Jr, Melack J.M., McDowell W.H., McClain M. and Richey J. 1999. Nitrogen yields from undisturbed watersheds in the Americas. In: Townsend A.R. (ed.), New perspectives on nitrogen cycling in the temperate and tropical Americas. Kluwer Academic Publishers.
Lovett G.M. and Rueth H. 1999. Soil nitrogen transformations in Beech and Maple stands along a nitrogen deposition gradient. Ecol. Appl. 9: 1330–1344.
Malavolta E. and Viloante Netto A. 1989. Nutrição mineral, calagem, gessagem e adubação de citros. Potafos., Piracicaba, Brazil.
Martinelli L.A., Piccolo M.C., Townsend A.R., Vitousek P.M., Cuevas E., McDowell W.H. et al. 1999a. Nitrogen stable isotopic composition of leaves and soil: tropical versus temperate forests. Biogeochemistry 46: 45–65.
Martinelli L.A., Krusche A.V., Victoria R.L., de Camargo P.B., Bernardes M., Ferraz E.S. et al. 1999b. Effects of sewage on the chemical composition of Piracicaba River, Brazil. Wat. Air Soil Poll. 110: 67–79.
Matson P.A. and Vitousek P.M. 1987. Cross-system comparisons of soil nitrogen trasformations and nitrous oxide flux in tropical forest ecosystems. Global Biogeochemical Cycles 1: 163–170.
Meybeck M. 1982. Carbon, nitrogen and phosphorus transport by world rivers. Am. J. Sci. 282: 401–450.
Oliveira M.W. 1999. Dinâmica do nitrogênio da uréia (^{15}N) no sistema solo-cana-de-açúcar colhida com ou sem queima da palhada. PhD Dissertation, University of São Paulo, Piracicaba, Brazil.
Oliveira M.W., Trivelin P.C.O., Gava G.J.C. and Vitti A.C. 1999. Lixiviação de nitrogênio em solo cultivado com cana-de-açúcar: experimento em lisímetro. Stab. Álcool, Açúcar e Sub-produtos 18: 28–31.
Orlando Filho J., Macedo N. and Tokeshi H. 2001. Balanço de nutrientes na agricultura brasileira. Arquivo do Agronomo 6: 1–6.
Pereira A.R., Andrade D.C., Leal P.G.L. and Teixeira N.C.S. 1984. Produção de biomassa e remoção de nutrientes em povoamentos de *Eucalyptus citriodora* e *E. saligna* cultivados na região do cerrado de Minas Gerais. Rev. Flores 5: 8–16.
Reis M.G.F., Barros N.F. and Kimmins J.P. 1987. Acúmulo de nutrients em uma seqüência de idade de *Eucalyptus grandis* W. Hill (ex-Maiden) plantado no cerrado em duas áreas com diferentes produtividades em Minas Gerais. Rev. Arvor. 11: 1–15.
Salcedo I.H., Sampaio E.V.S.B. and Carneiro C.J.G. 1988. Nutrient cycling in sugarcane. 4: Leaching losses of N in plant-cane fertilized with urea-N-15. Pesquisa Agropecuaria Brasileira 23: 725–732.
Seitzinger S.P. and Kroeze C. 1998. Global distribution of nitrous oxide production and N inputs in freshwater and coastal marine ecosystems. Glob. Biogeochem. Cycl. 12: 93–113.
Seitzinger S.P., Styles R.V., Boyer E., Alexander R.B., Billen G., Howarth R. et al. 2002. Nitrogen retention in rivers: model development and application to watersheds in the northeastern USA. Biogeochemistry 57/58: 199–237.
Silveira R.L.V.A., Higashi E.N., Sgarbi F. and Muniz M.R.A. 2001. Seja doutor de seu eucalipto. Arquivo Agronômico Potafos 93: 1–23.
Smil V. 2000. Feeding the world: a challenge for the twenty-first century. MIT Press, Cambridge, Massachusetts, USA.
Staver K.W. and Brinsfield R.D. 1990. Patterns of soil nitrate availability in corn production systems – implications for reducing groundwater contamination. J. Soil Wat. Conserv. 45: 318–323.
Staver K.W. and Brinsfield R.D. 1996. Seepage of groundwater nitrate from a riparian agroecosystem into the Wye River estuary. Estuaries 19: 359–370.
Talling J.F. and Lemoalle J. 1998. Ecological Dynamics of Tropical Inland Waters. Cambridge University Press, Cambridge.

Trivelin P.C.O., Rodrigues J.C.S. and Victoria R.L. 1996. Utilização por soqueira de cana-de-açúcar de início de safra do N da aquamônia uréia ^{15}N aplicado ao solo em complemento à vinhaça. Pesq. Agropec. Bras. 31: 89–99.
Tundisi J.G. and Matsumura-Tundisi T. 1990. Limnology and eutrophication of Barra Bonita Reservoir, Sao Paulo state, southern Brazil. Arch. Fur. Hydrobiol. 33: 661–676.
Turner R.E. and Rabalais N.N. 1991. Changes in Mississippi River water quality this century. BioSci. 41: 140–147.
Urquiaga S., Cruz K.H.S. and Boddey R.M. 1992. Contribution of nitrogen fixation to sugarcane: nitrogen-15 and nitrogen balance estimates. Soil Sci. Soc. Am. 22: 104–114.
Valderrama J.C. 1981. The simultaneous analysis of total nitrogen and total phosphorus in natural waters. Mar. Chem. 10: 109–122.
van Breemen N., Boyer E.W., Goodale C.L., Jaworski N.A., Paustian K., Seitzinger S.P. et al. 2002. Where did all the nitrogen go? Fate of nitrogen inputs to large watersheds in the northeastern USA. Biogeochemistry 57/58: 267–293.
Van Raij B., Cantarella H., Quaggio J.A. and Furlani A.M.C. 1996. Recomendações de adubação e calagem para o Estado de São Paulo. Instituto Agronômico de Campinas, São Paulo, Brasil.
Vianna M.R.V. 2001. Estudo em escala de laboratório, da adsorção de componentes orgânicos do vinhoto em argila organofílica. Master Thesis, University of São Paulo, Piracicaba, Brazil.
Vitousek P.M. and Howarth R.W. 1991. Nitrogen limitation on land and in the sea: How can it occur? Biogeochemistry 13: 87–115.
Vitousek P.M., Aber J.D., Howarth R.W., Likens G.E., Matson P.A., Schindler D.W. et al. 1997. Human alteration of the global nitrogen cycle: sources and consequences. Ecol. Applic. 7: 737–750.
Vizcarra A.T. and Lavkulich L.M. 1997. Nitrogen balance in the lower Fraser River basin of British Columbia. Environ. Manag. 21: 269–282.
Williams M.R. and Melack J.M. 1997. Solute export from forested and partially deforested catchments in the central Amazon. Biogeochemistry 38: 67–102.
Williams M.R., Fisher T.R. and Melack J.M. 1997a. Solute dynamics in soil water and groundwater in a central Amazon catchment undergoing deforestation. Biogeochemistry 38: 303–335.
Williams M.R., Fisher T.R. and Melack J.M. 1997b. Chemical composition and deposition of rain in the central Amazon, Brazil. Atmos. Env. 31: 207–217.
Williams M.R., Filoso S., Martinelli L.A., Luciene L.B. and Carmago P.B. 2001. Precipitation and river chemistry of the Piracicaba River basin in southern Brazil. J. Environ. Qual. 30: 967–981.
Yamada T. and Lopes A.S. 1998. Balanço de nutrientes na agricultura brasileira. Informações agronômicas 84: 1–8.