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# Effects of Different Treatments of Pasture Restoration on Soil Trace Gas Emissions in the Cerrados of Central Brazil

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**ABSTRACT:** Planted pastures (mainly *Brachiaria* spp) are the most extensive land use in the cerrado (savannas of central Brazil) with an area of approximately  $50 \times 10^6$  ha. The objective of the study was to assess the effects of pasture restoration on the N dynamics (net N mineralization/nitrification, available inorganic N and soil N oxide gas fluxes—NO and N<sub>2</sub>O), C dynamics (CO<sub>2</sub> fluxes and microbial biomass carbon), and diversity of the soil bacterial community using denaturing gradient gel electrophoresis (DGGE) profiles. Sampling was done monthly on a farm in Planaltina, Goiás, Brazil (15°13'S, 47°42'W) from November 2001 to April 2002. Three areas of cerradão (dense cerrado) were converted to pasture (*Brachiaria brizantha*) in 1991, and after 8 years degradation was evident with the decreasing plant biomass production. Methods to restore these pastures were investigated for their sustainability, principally their effects on trace gas emissions. The pastures have been managed since 1999 as follows: 1) fertilized plot (N = 60 kg ha<sup>-1</sup> yr<sup>-1</sup>, P = 12 kg ha<sup>-1</sup> yr<sup>-1</sup>); 2) grass–legume plot, *Brachiaria* associated with a legume (*Stylosanthes guianensis*) with addition of P (12 kg ha<sup>-1</sup> yr<sup>-1</sup>); and 3) a traditional plot without management. A fourth area of cerradão was converted to pasture in 1999 and was not managed (young pasture). Ammonium was the predominant inorganic N form in the soils (~76 mg N kg<sup>-1</sup>) for all treatments throughout the study. In December 2001 a reduction in average soil N-NH<sub>4</sub><sup>+</sup> was observed (~30 mg N kg<sup>-1</sup>) compared to November 2001, probably related to plant demand. All plots had high variability of soil N gases emissions, but during the wet season, the NO and N<sub>2</sub>O soil fluxes were near zero. The results of the water addition experiment made during the dry season (September 2002) indicated that the transition of dry to wet season is an important period for the production of N gases in the fertilized pasture and in the young pasture. Soil CO<sub>2</sub> fluxes also increased after the water addition and the grass–legume plot had the highest increase in soil respiration (from ~2 to 8.3 μmol m<sup>-2</sup> s<sup>-1</sup>). The lowest values of soil respiration and microbial biomass carbon (~320 mg C kg<sup>-1</sup> soil) tended to be observed in the young pasture, because the superficial layer of the soil (0–10 cm) was removed during the conversion to pasture. Trace gas emissions measured after the water addition experiment corresponded to rapid changes in the soil bacterial community. The young pasture sample showed the lowest level of similarity in relation to the others, indicating that the bacterial community is also influenced by the time since conversion. This study indicates that the restoration technique of including *Stylosanthes guianensis* with *B. brizantha* increases plant productivity without the peaks of N oxide gas emissions that are often associated with the use of N fertilizers. Additionally, the soil bacterial community structure may be restored to one similar to that of native cerrado grasslands, suggesting that this restoration method may beneficially affect bacterially mediated processes.

**KEYWORDS:** Brazilian savanna; Pasture reformation; Trace gases

## 1. Introduction

Pastures planted with African grasses cover approximately  $50 \times 10^6$  ha in the cerrado region (savannas of central Brazil) (Sano et al. 2000). Generally, pasture productivity declines after a few years. According to Oliveira et al. (Oliveira et al. 2004) degraded pastures in the tropical region of Brazil occupy  $25 \times 10^6$  ha,

representing a loss of approximately \$10 billion (U.S. dollars) in terms of beef production.

The conversion of native areas to pasture causes substantial changes in soil properties and biogeochemical processes (Reiners et al. 1994). In fertilized pastures on clayey soils in the cerrado region, long-term soil C stocks can surpass levels under native vegetation (da Silva et al. 2004). However, poor management practices, especially overgrazing, lead to pasture degradation. Advanced degradation of the natural resources stems from efforts to sustain the forage quantity and quality necessary for the animals (Barcellos 1996). Degradation is attributed to the reduction of the fertility of the soil after conversion, associated with an increase in the C:N of the litter, which leads to the immobilization of nutrients and slower nutrient cycling (Macedo 1995; Boddey et al. 1996). Müller et al. (Müller et al. 2004), studying the relationship between pasture degradation and soil properties in the Brazilian Amazon, concluded that soil bulk density increased with pasture decline in the topsoil layer, reflecting the decrease in soil cover by pasture biomass and litter.

Little information exists on the trace gas emissions from pasture soils in the cerrado. In a degraded, 20-yr-old pasture (*Brachiaria brizantha*), Varella et al. (Varella et al. 2004) estimated an annual soil emission of 14 Mg C-CO<sub>2</sub> ha<sup>-1</sup> yr<sup>-1</sup>. The few existing data for N oxides suggest a decrease in the emissions with pasture age as observed in the Amazon (Garcia-Montiel et al. 2001), but the annual emissions of nitrogen oxides are much lower in the cerrado. Saminêz (Saminêz 1999) estimated an annual N<sub>2</sub>O soil emission of 0.5 kg N ha<sup>-1</sup> yr<sup>-1</sup> in a native cerrado and in a 5-yr-old pasture (*Andropogon gayannus*). Low N<sub>2</sub>O fluxes (0.2 ng N-N<sub>2</sub>O cm<sup>-2</sup> h<sup>-1</sup>) were also measured by Nobre (Nobre 1994) in a 10-yr-old pasture planted with *Paspalum* sp. Fluxes of NO in a 20-yr-old pasture (*Brachiaria brizantha*) of central Brazil were measured by Varella et al. (Varella et al. 2004). They estimated annual emissions of 0.1 kg N ha<sup>-1</sup> yr<sup>-1</sup>, and the emissions of soil N<sub>2</sub>O were below the detection limit (0.6 ng N-N<sub>2</sub>O cm<sup>-2</sup> h<sup>-1</sup>). Although small, these nitrogen losses can lead to decreased soil fertility and are a source of greenhouse gases.

Studies of the restoration of degraded pastures demonstrated that the productivity and the persistence of the African grasses are associated with an increase in soil nutrient availability (Embrapa 1981; Embrapa 1983; Arruda et al. 1987; Soares Filho et al. 1992; Oliveira et al. 1997). The combination of grasses and legumes and the rotation of crop to pasture are alternative management techniques considered to be economically viable for the improvement of the soil fertility (Toledo 1985). Nitrogen fertilizers are very expensive and potentially harmful to the environment due to their loss to the atmosphere and surface waters via leaching.

There are no data, at least to our knowledge, about the impacts of restoration treatments on soil trace gas fluxes. Trace gases contribute to greenhouse gases in the atmosphere and represent a loss of N and C from the pasture, both of which are needed to maintain a sustainable system. In this sense, the objectives of this study were to evaluate the effect of different pasture restoration treatments on 1) soil N dynamics (fluxes of N<sub>2</sub>O and NO and N-mineralization rates), 2) soil C dynamics (fluxes of CO<sub>2</sub> and microbial biomass carbon), and 3) diversity of soil bacterial communities. Pasture function (C and N dynamics) and bacterial structure were

compared with native cerrado areas to evaluate the sustainability of pasture restoration.

## 2. Material and methods

### 2.1. Study area

The study was carried out at the Fazenda Rio de Janeiro, located at Planaltina (federal state of Goiás, Brazil) (15°14'S, 47°42'W), at 826-m altitude. The soil is classified as Latossolo Vermelho in the Brazilian soil taxonomy (Embrapa 1999). *Brachiaria brizantha* cv. Marandu was introduced on this farm in 1990 after removal of the native vegetation. Information about the native former vegetation was not available, but the remaining vegetation in an adjacent area to the experimental plots until 1999 was defined as cerradão (dense woodland savanna).

After 9 yr of use, the pastures exhibited degradation characteristics. The carrying capacity of *B. brizantha* during the rainy season was less than 1 animal unit (AU = 450 kg live weight per hectare) (Corazza 2002). This is much lower than that of a new pasture during the rainy season, which produces forage sufficient for 2.6 AU per hectare (Ayarza et al. 1998). The low weight gain in the rainy season and weight loss in dry season were indications of low cattle production capacity.

In 1998–99, a study coordinated by Embrapa Cerrados (Brazil) and Institut de Recherche pour le Développement (IRD; France) was initiated to test restoration treatments that included fertility improvement and control of grazing rates by rotation. In the first year, 3000 kg ha<sup>-1</sup> of lime were applied. In 1999, plots of 5 ha were replanted with *Brachiaria brizantha* and six treatments were established. In the present study, measurements were made in three treatments: (i) fertilized plot (fertilized with NPK and S); (ii) grass–legume consortium (fertilized with P, K, and S and the legume *Stylosanthes guianensis* cv. Mineirão added to the *B. brizantha* pasture); and (iii) traditional plot (no management of a 9-yr-old *B. brizantha* degraded pasture). Fertilized and grass–legume plots received 205 kg ha<sup>-1</sup> triple superphosphate and 75 kg ha<sup>-1</sup> sulfur. Fertilization was made in February 1999, followed by incorporation with a heavy plow to ~10 cm depth. In May 2001, a second fertilization was performed (NPK in fertilized and only PK in the grass–legume plot) (Table 1).

In the rainy season of 1999/2000, the native vegetation (cerradão) of an adjacent area was removed using the traditional method in the region (two tractors were connected side by side with a 50-m chain between them for removing the woody species). Thereafter the organic material was organized in piles 50 m apart from each other. The wood of economic interest was separated and the rest was burned. This process generally removes the superficial soil layer (0–10 cm). Soil acidity was corrected through the application of 2 tons ha<sup>-1</sup> of lime and fertility improved with 12 kg P ha<sup>-1</sup> of simple superphosphate. We refer to this area as the young pasture due to its short time since conversion (2 yr) in relation to the other three study plots. Resprouts of native woody species were observed in the young pasture, giving the area a similar aspect of native shrubland. The main soil characteristics of the studied plots are presented in Table 2.

**Table 1. Amounts of nutrients added to soil in the different treatments of pasture (*Brachiaria brizantha*) restoration at the Fazenda Rio de Janeiro, Planaltina, Goiás, Brazil (Corazza 2002).**

Treatment	February 1999		March 2001		
	Element (kg ha <sup>-1</sup> yr <sup>-1</sup> )		Element (kg ha <sup>-1</sup> yr <sup>-1</sup> )		
	P*	S*	P*	N*	K*
Fertilized	40	74	12	60	33
Grass–legume	40	74	12	—	29
Traditional	—	—	—	—	—

\* Type of fertilizer used: P, triple superphosphate (44% P<sub>2</sub>O<sub>5</sub>); N, urea (45% N); K, KCl (60% K<sub>2</sub>O); and S, elemental sulfur (98% S).

## 2.2. Measurements of soil fluxes

Soil surface fluxes of N<sub>2</sub>O, NO, and CO<sub>2</sub> were measured using chamber techniques (Pinto et al. 2002). In October 2001, eight collars (366 cm<sup>2</sup> area) per plot were installed in two transects 3 m apart from each other. The distance between collars within each transect was 3 m. These collars were inserted to 5-cm depth in the soil and the top edges of the collar formed a U-shape groove into which a vented polyvinyl chloride (PVC) chamber (23.5 cm diameter × 20 cm height) could be set. Water in the groove provided a seal for the chamber. The combined volume of the chamber plus collar was about 8.3 L.

Trace gas fluxes were measured monthly during the rainy season in the older pastures, between November 2001 and April 2002. Measurements started approximately 8 months after the last fertilization. In the young pastures, measurements started 1 month earlier, in October 2001 (transition dry to wet season) and continued until April 2002. Ambient air, chamber air, and soil (2.5-, 5-, and 10-cm

**Table 2. Soil properties (0–10 cm) and plant biomass in the different treatments of pasture restoration (*Brachiaria brizantha*) at the Fazenda Rio de Janeiro, Planaltina, Goiás, Brazil. All pastures were 9 yr old at the beginning of the experiment (1999), except the young pasture that was cleared earlier in 2002. The fertilized pasture received N, P, K, and S; the grass–legume pasture (*B. brizantha* + *Stylosanthes guianensis*) received P, K, and S. The traditional and young pastures were not fertilized. Mean values between October 2001 and May 2002 (1-m<sup>2</sup> plots). Sampling was done monthly. Plants were cut 30 cm above soil surface, simulating cattle grazing. Different letters mean significant differences (Tukey, *P* < 0.05).**

	Treatment			
	Fertilized	Grass–legume	Young	Traditional
Clay (%)	63.7	58.1	63.1	62.2
Silt (%)	12.8	18.5	11.2	21.3
Sand (%)	23.5	23.4	25.7	16.5
Organic-C (g kg <sup>-1</sup> )	21	22	18	29
pH (H <sub>2</sub> O 1:2.5)	5.9	5.9	5.3	5.9
Bulk density (g cm <sup>-3</sup> )	1.21	1.29	1.08	1.17
Plant biomass (g m <sup>-2</sup> )*	40.2 <sup>b</sup>	49.5 <sup>a</sup>	ND	32.4 <sup>b</sup>

\* D. Brunet, unpublished data (brunet@mpl.ird.fr).

depth) temperatures were measured during the gas flux measurements. Soil water content was gravimetrically determined. Three soil samples (0–5 cm) per treatment were collected monthly near the installed collars. Gravimetric water content was converted to water-filled pore space (WFPS) according to Linn and Doran (Linn and Doran 1984). The particle density value was  $2.7 \text{ g cm}^{-3}$  as used by Embrapa Cerrados researchers.

### 2.3. NO and N<sub>2</sub>O fluxes

Nitric oxide was measured using a dynamic chamber technique. Air was circulated in a closed loop between the chamber and the analyzer. Nitric oxide was analyzed using a Scintrex LMA-3, after first converting NO to NO<sub>2</sub> by passing the gas sample through CrO<sub>3</sub>. Nitrogen dioxide reacts with a Luminol solution to produce a luminescent reaction that is functionally related to the mixing ratio of NO<sub>2</sub>. The NO concentration was recorded over a 5-min period. Fluxes were calculated from the rate of increase of NO concentration using the linear portion of the accumulation curve. The instrument was calibrated twice daily using mixtures of a NO standard (0.4 ppm) with NO- and NO<sub>2</sub>-free air.

The N<sub>2</sub>O fluxes were measured with a static chamber technique (Matson et al. 1990). Four gas samples were collected from the chamber headspace at intervals of 10 min using 60-mL polypropylene syringes with siliconized polypropylene plungers and Teflon gas-tight valves. In the laboratory, the samples were analyzed with a gas chromatograph (Shimadzu GC-14A) fitted with a <sup>63</sup>Ni electron capture detector. Gases were separated on a 1-m precolumn (Hayesep N) and a 2-m analytical column (Hayesep Q) operated at 70°C with a 5% CH<sub>4</sub> – 95% Ar carrier. The samples were analyzed the same day of collection, to avoid loss of N<sub>2</sub>O from the syringes. The N<sub>2</sub>O fluxes were calculated from linear regression of the rate of concentration change.

### 2.4. CO<sub>2</sub> fluxes

Carbon dioxide fluxes were measured using a dynamic chamber technique. Over a 3-min period, CO<sub>2</sub> was analyzed using a LiCor 6200 photosynthesis system with integrated infrared gas analyzer and data system.

### 2.5. Carbon microbial biomass

Soil carbon microbial biomass was determined from samples collected in November 2001 and January, February, and April 2002. Ten soil samples (0–5 cm) were collected per plot to build three composite samples. The soil microbial biomass C (SMB) was determined by the chloroform fumigation–incubation method proposed by Jenkinson and Powlson (Jenkinson and Powlson 1976).

### 2.6. Water addition experiment

To verify the effects of the first rain events after the dry season on soil trace gas fluxes and structure of the microbial community, an artificial water addition experiment in all restoration treatments was conducted from 17 to 19 September 2002. Although the transition between dry to wet season has been found to be



important for microbe-mediated processes, such as trace gas production (Varella et al. 2004; Pinto et al. 2002; Davidson et al. 1993), it is difficult to capture this event naturally in the field. Also, to sample the potential changes in the microbial community throughout the seasonal transition and wet season would have been labor-intensive. The experiment allowed for more control over the temporal aspect of the water addition and for comparison among the treatments. Trace gas measurements were made in six of the eight collars used for the monthly determinations. The collars were located in the middle of six subplots (50 cm × 50 cm) used for soil measurements. This allowed sampling for determination of soil water content and bacterial community structure during the experiment without disturbing the soil inside the collars. On the first day of the experiment (17 September) 1.25 L of water (equivalent to a 5-mm precipitation) were added to three subplots and the other three were used as control. Trace gas measurements were made 15 and 60 min, 1 and 2 days after the water addition.

### **2.7. Diversity of soil bacterial community**

Ten soil samples at a 0–5-cm depth were collected before and 2 days after the water addition to make composite samples for analyses of the bacterial community using denaturing gradient gel electrophoresis (DGGE) technique (Muyzer 1999).

Soil DNA was obtained by direct extraction with a protocol that included mechanical lyses of cells, phenol and chloroform extractions; a potassium acetate precipitation; and a final purification using the Wizard DNA clean-up kit (van Elsas et al. 1997, modified by Silva 2004). The 16S rDNA primers 968F and 1401R were used for DNA amplification with polymerase chain reaction (PCR). These primers are commonly used to analyze bacterial communities. DGGE was carried out using a BioRad Dcode Universal Mutation Detection System at 70 V and 60°C for 18 h in 0.5× TAE buffer. The 6% (w/v) polyacrilamide gels were made with a denaturing gradient ranging from 40% to 70% and used with PCR products. After electrophoresis, gels were stained for 40 min with SYBR green I and photographed on a UV transillumination table with a Kodak digital camera.

Gel pictures were manually converted to 1/10 matrices, which were used for clustering by the unweighted pair group method with mathematical averages (UPGMA; Dice coefficient of similarity), followed by tree inference. A soil sample collected in native campo sujo (grassland with sparse trees and shrubs) was used as reference.

### **2.8. Inorganic-N availability and net rates of mineralization and nitrification**

The nitrogen mineralization rates were determined by the in situ incubation method described by Adams and Attiwill (Adams and Attiwill 1986) and Adams et al. (Adams et al. 1989). Soil samples were collected adjacent to the collars used for collecting trace gas emissions. Sampling occurred at the beginning of each incubation period and after 7 days of incubation. The mineralization rates were measured monthly, and calculated as the difference between the  $\text{NO}_3^-$ -N +  $\text{NH}_4^+$ -N concentrations at the end and the beginning of each incubation period.

Field-moist soil samples were extracted with 1N KCl for 1 h and the inor-

ganic-N concentrations were determined by colorimetry.  $\text{NH}_4^+$ -N was determined through reaction with Nessler reagent and  $\text{NO}_3^-$ -N was determined by UV absorption (Meier 1991). The results are expressed on a 105°C oven-dry basis [dry weight (DW)].

## 2.9. Statistical analyses

Data normality was tested using the Kolmogorov–Smirnov test and homogeneity of variances was evaluated by the Levene test. Data were log transformed when not normally distributed. Net nitrification,  $\text{N}_2\text{O}$ , and NO fluxes were not statistically analyzed because no transformation could normalize the data. Repeated measures analysis was used to compare soil  $\text{CO}_2$  fluxes, WFPS, inorganic-N, net mineralization rates, and soil microbial biomass means along time in the same treatment or between treatments in the same month. A *t* test was performed to compare the dry and wet treatments in the first day of the water addition experiment. Relationships between NO fluxes and soil temperature, moisture, and N availability were tested by Spearman correlation.

## 3. Results

### 3.1. Precipitation and soil moisture

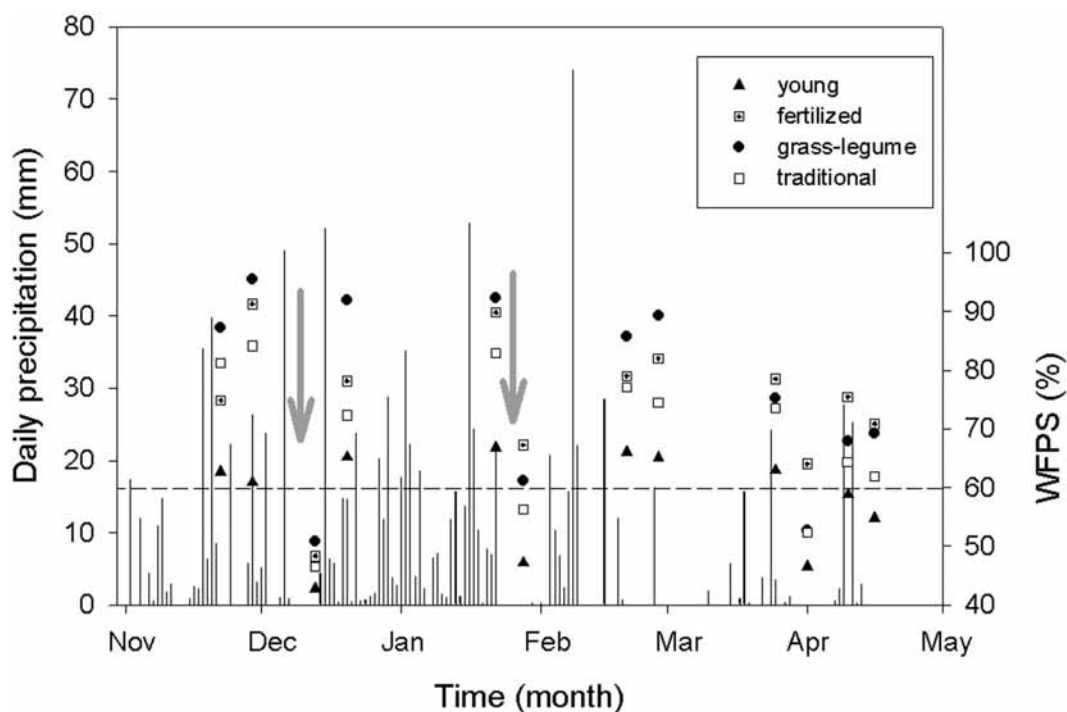
The total precipitation between May 2001 and April 2002 was 1315 mm (Figure 1). Precipitation peaks were observed in December (273.5 mm) and January (282.1 mm). In March and April precipitation decreased to 57.4 and 58.9 mm, respectively.

The pattern of rainfall distribution along the month affected soil moisture. In November 2001, WFPS values were about 80%, decreasing in the next month to about 47% in spite of the high precipitation amount observed in December (Figure 1). A dry spell of 7 days occurred in December 2001. A week after this measurement, the WFPS increased to about 77%. In February, another dry spell occurred. In the following months the soil moisture decreased with the transition to the dry season. Over the 6-month study, the average WFPS was highest in grass–legume and fertilized plots (76.5% and 74.9%, respectively) ( $F = 453.882$ ,  $DF = 3$ ,  $P < 0.001$ ), with lower averages for traditional and young pastures (68.9% and 58.3%, respectively).

### 3.2. Inorganic-N availability and rates of net mineralization and nitrification

In all treatments,  $\text{NH}_4^+$  was the predominant inorganic-N form available in the soil. In November, the average concentration was about 81 mg N- $\text{NH}_4^+$   $\text{kg}^{-1}$ , but all plots tended to decrease in December. Concentrations varied from 31.2 mg N  $\text{kg}^{-1}$  and 18.2 mg N- $\text{NH}_4^+$   $\text{kg}^{-1}$  in the grass–legume and young pastures to 16.1 and 11.9 mg N  $\text{kg}^{-1}$  in the traditional and fertilized pastures, respectively (Figure 2). In the following months (January and February), the concentrations increased with the highest averages of N- $\text{NH}_4^+$  observed in February in the young pasture (85.4 mg N  $\text{kg}^{-1}$ ), grass–legume (87.3 mg N  $\text{kg}^{-1}$ ), and fertilized (98.2 mg N  $\text{kg}^{-1}$ ) while in the





**Figure 1.** Daily precipitation (bars in mm) and WFPS (%) (symbols) between November 2001 and April 2002 of soil samples from different restoration treatments of degraded pastures in Fazenda Rio de Janeiro, Planaltina, Goiás, Brazil. The arrows represent dry spells (drought periods in the rainy season).

traditional pasture the highest mean value ( $109.7 \text{ mg N kg}^{-1}$ ) was measured in March.

Between November and January, all treatments had net N-mineralization (Figure 3). During this period mineralization in the traditional, fertilized, grass-legume, and young plots peaked in November [ $127.9$ ,  $126.8$ ,  $80.3$ , and  $67.3 \text{ mg N kg}^{-1} \text{ DW (7 days)}^{-1}$ , respectively]. In February, strong N-immobilization was observed in all treatments, ranging from  $-67.5 \text{ mg N kg}^{-1} \text{ DW (7 days)}^{-1}$  in the fertilized plot to  $-57.7 \text{ mg N kg}^{-1} \text{ DW (7 days)}^{-1}$  in the grass-legume plot.

The highest  $\text{N-NO}_3^-$  averages were observed in November (fertilized and grass-legume =  $11.6 \text{ mg N kg}^{-1}$ ; traditional and young =  $13.7 \text{ mg N kg}^{-1}$ ) (Figure 2). Peaks of  $\text{NO}_3^-$  immobilization were observed in November in all treatments (Figure 3). Nitrate immobilization was also found in February and March, except in the young plot, which had a peak of nitrification in March [ $3.9 \text{ mg N kg}^{-1} \text{ DW (7 days)}^{-1}$ ]. Nitrification in fertilized, grass-legume, and traditional plots was observed in December and April.

Comparing the overall mean of N indices, there was no difference in  $\text{N-NH}_4^+$  concentration between treatments (Table 3) but the young plot had higher  $\text{N-NO}_3^-$  concentrations ( $10.3 \text{ mg N kg}^{-1}$ ) ( $F = 15.675$ ,  $DF = 3$ ,  $P < 0.001$ ). Net N-mineralization was higher in the fertilized pasture [ $37.3 \text{ mg N kg}^{-1} \text{ (7 days)}^{-1}$ ] and

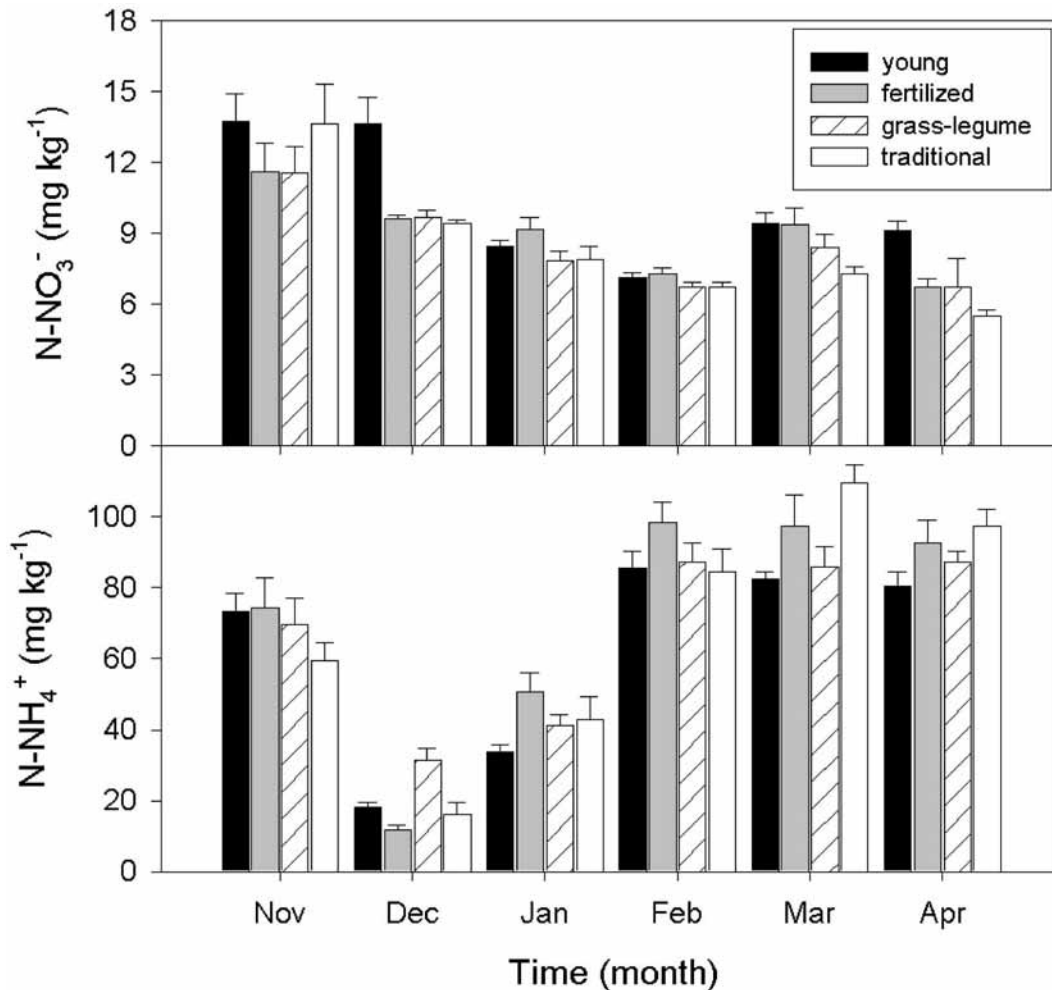


Figure 2. Concentrations of soil inorganic-N (N-NO<sub>3</sub><sup>-</sup> and N-NH<sub>4</sub><sup>+</sup>) between November 2001 and April 2002 in different restoration treatments of degraded pastures at Fazenda Rio de Janeiro, Planaltina, Goiás, Brazil.

the grass-legume pasture [36.2 mg N kg<sup>-1</sup> (7 days)<sup>-1</sup>], but lower in the young pasture [8.4 mg N kg<sup>-1</sup> (7 days)<sup>-1</sup>] and the traditional pasture [13.6 mg N kg<sup>-1</sup> (7 days)<sup>-1</sup>] ( $F = 10.796$ ,  $DF = 3$ ,  $P < 0.001$ ). All treatments had net NO<sub>3</sub><sup>-</sup> immobilization.

### 3.3. Soil NO and N<sub>2</sub>O fluxes

In general, soil NO fluxes in all treatments were low, but there was a very high variability of fluxes between the collars. The highest average was 3.6 ng N-NO cm<sup>-2</sup> h<sup>-1</sup>, in the traditional plot in November 2001 (Figure 4). However, this value was due to a single collar with a flux of 29.0 ng N-NO cm<sup>-2</sup> h<sup>-1</sup> while fluxes were near zero in the others.

The study period (November 2001–April 2002) included 48 flux measurements

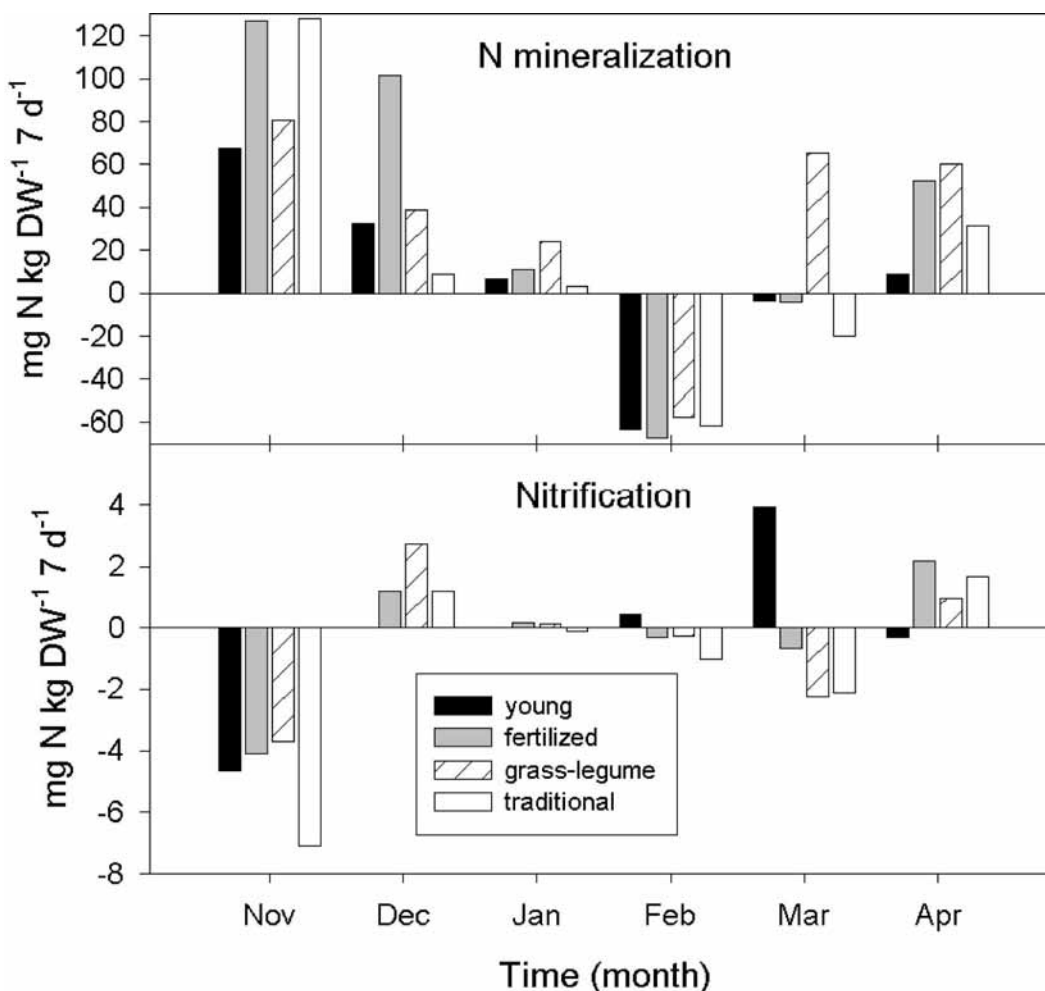


Figure 3. Net mineralization and nitrification rates between November 2001 and April 2002 in different restoration treatments of degraded pastures at Fazenda Rio de Janeiro, Planaltina, Goiás, Brazil.

per treatment. Considering only fluxes equal to or higher than  $0.1 \text{ ng N-NO cm}^{-2} \text{ h}^{-1}$ , the traditional and fertilized treatments had only one measurement within this range, and the grass-legume plot had only two. Many more measurements (20) were in this range in the young plot, with a maximum value of  $0.7 \text{ ng N-NO cm}^{-2} \text{ h}^{-1}$ .

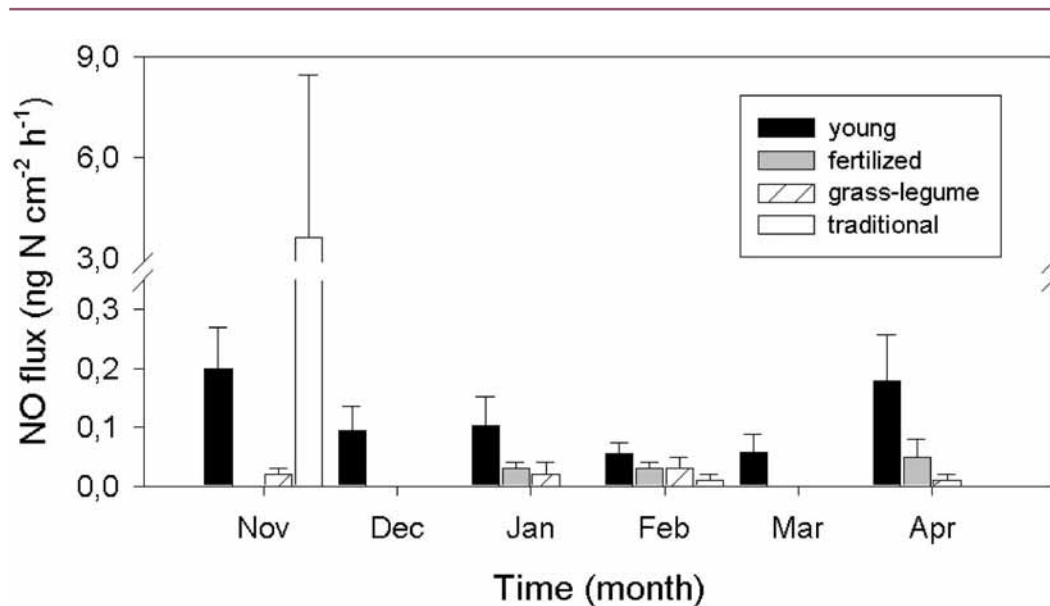
Most  $\text{N}_2\text{O}$  flux measurements were below the detection limit of our analytical system ( $0.6 \text{ ng N-N}_2\text{O cm}^{-2} \text{ h}^{-1}$ ) (Figure 5). As in the case of the observed fluxes for NO, higher mean fluxes of  $\text{N}_2\text{O}$  were found due to values measured in a single collar. For example, the highest average value ( $6.7 \text{ ng N-N}_2\text{O cm}^{-2} \text{ h}^{-1}$ ) observed in the traditional plot (March 2002), was dominated by one collar that had a flux of  $52.0 \text{ ng N-N}_2\text{O cm}^{-2} \text{ h}^{-1}$ . In the grass-legume plot the highest mean fluxes were measured in November and January (both  $2.0 \text{ ng N-N}_2\text{O cm}^{-2} \text{ h}^{-1}$ ) with one collar producing fluxes of  $14.0 \text{ ng N-N}_2\text{O cm}^{-2} \text{ h}^{-1}$  on each date.

**Table 3.** Average concentration (with standard error in parentheses) of available soil inorganic-N and net N-mineralization and nitrification rates between November 2001 and April 2002 in different restoration treatments of degraded pastures at Fazenda Rio de Janeiro, Planaltina, Goiás, Brazil. All pastures were 9 yr old at the beginning of the experiment (1999), except the young pasture that was cleared that same year. The fertilized pasture received N, P, K, and S; the grass-legume pasture (*B. brizantha* + *Stylosanthes guianensis*) received P, K, and S. Traditional and young pastures were not fertilized. In the same column, different letters represent significant differences.

Treatment	N-NH <sub>4</sub> <sup>+</sup>	N-NO <sub>3</sub> <sup>-</sup>	Mineralization	Nitrification
	mg N kg <sup>-1</sup>		Mg N kg <sup>-1</sup> (7 days) <sup>-1</sup>	
Young	63.1 <sup>a</sup> (3.7)	10.3 <sup>a</sup> (0.4)	8.4 <sup>a</sup> (5.7)	-0.1 (0.5)
Fertilized	70.8 <sup>a</sup> (4.8)	8.9 <sup>ab</sup> (0.3)	37.3 <sup>b</sup> (9.5)	-0.3 (0.4)
Grass-legume	67.0 <sup>a</sup> (3.5)	8.5 <sup>b</sup> (0.4)	36.2 <sup>b</sup> (7.7)	-0.4 (0.5)
Traditional	69.4 <sup>a</sup> (4.7)	8.4 <sup>b</sup> (0.5)	13.6 <sup>a</sup> (8.2)	-1.3 (0.5)

### 3.4. Carbon in soil microbial biomass

The average mean of carbon in soil microbial biomass, independently of pasture treatment, ranged from 160 to 760 mg C kg of soil. No differences were found between pasture treatments, and no interactions were observed between pasture treatment and time (months). In general, lower values of carbon in soil microbial biomass tended to be observed in the young plot for all four-sample dates (ranging from 160 to 420 mg C kg of soil). The lower value of this range was observed in



**Figure 4.** Soil NO fluxes (mean + 1 standard error (SE)) between November 2001 and April 2002 in different restoration treatments of degraded pastures at Fazenda Rio de Janeiro, Planaltina, Goiás, Brazil.

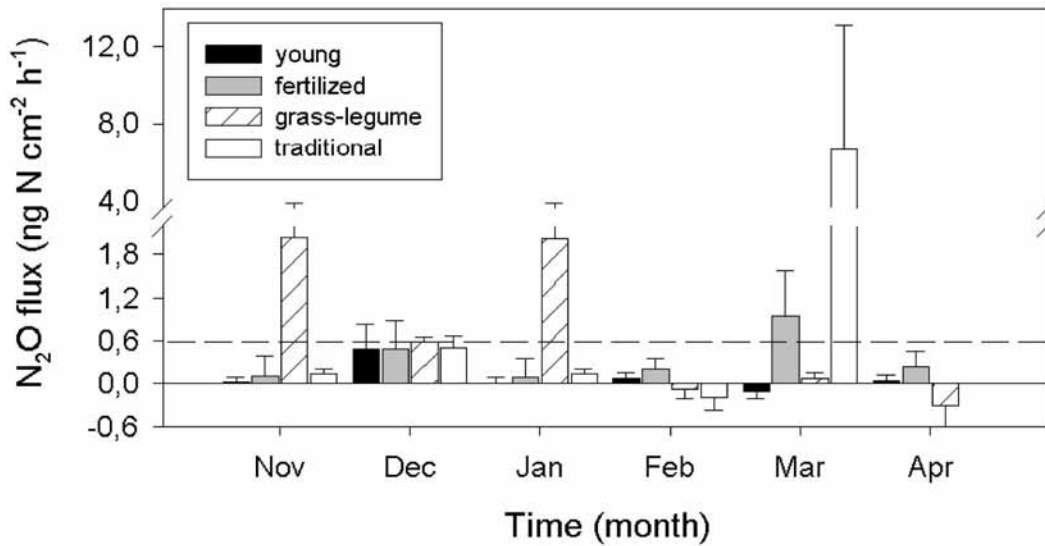


Figure 5. Soil N<sub>2</sub>O fluxes (mean + 1 SE) between November 2001 and April 2002 in different restoration treatments of degraded pastures at Fazenda Rio de Janeiro, Planaltina, Goiás, Brazil. Dotted line represents the detection limit of the gas chromatography system.

February, mean values of microbial biomass tended to be lower in February in the traditional plot (410 mg C kg of soil), young plot (160 mg C kg of soil), grass–legume plot (270 mg C kg of soil), and fertilized plot (320 mg C kg of soil).

### 3.5. Soil CO<sub>2</sub> fluxes

Soil CO<sub>2</sub> fluxes measured during the study period were similar between treatments (Figure 6). In January, the trend of CO<sub>2</sub> fluxes was higher in the grass–legume plot (9.2 μmol m<sup>-2</sup> s<sup>-1</sup>), intermediate in the fertilized and traditional plots (8.5 and 7.1 μmol m<sup>-2</sup> s<sup>-1</sup>, respectively), and lower in the young plot (6.7 μmol m<sup>-2</sup> s<sup>-1</sup>). In April 2002, all treatments had similar values of soil respiration (about 6.9 μmol m<sup>-2</sup> s<sup>-1</sup>).

A significant correlation was observed between CO<sub>2</sub> fluxes and soil temperature only in the fertilized plot ( $r = 0.50$ ,  $P = 0.005$ ).

### 3.6. Water addition experiment

In dry collars of the same treatment, there were no differences in soil moisture during the experiment. Water addition caused an increase in soil moisture ( $F = 72.235$ ,  $DF = 1$ ,  $P < 0.001$ ) (Figure 7); however, there was no interaction between moisture and pasture treatment. Comparing dry and wet collars in the same treatment during the first day of the experiment, the grass–legume plot WFPS increased from 28.5% to 47.2% ( $t = 7.05$ ,  $P < 0.002$ ), the young plot from 25.2% to 47.0% ( $t = 5.65$ ,  $P = 0.005$ ), the fertilized plot from 16.7% to 37.5% ( $t = 3.38$ ,  $P =$



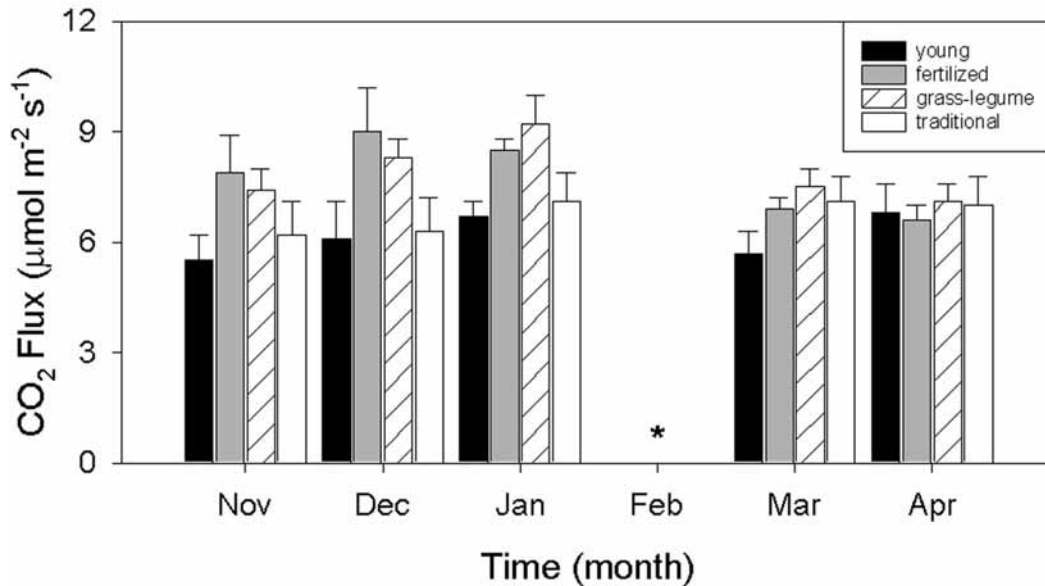


Figure 6. Soil CO<sub>2</sub> (mean + 1 SE) fluxes between November 2001 and April 2002 in different restoration treatments of degraded pastures at Fazenda Rio de Janeiro, Planaltina, Goiás, Brazil. An asterisk means no data.

0.028), and the traditional plot from 15.7% to 39.7% ( $t = 7.95$ ,  $P < 0.001$ ). One day after the water addition, WFPS values tended to decrease.

### 3.7. Soil N availability and fluxes of N oxides

There was no difference between pasture plots regarding inorganic-N concentration in dry collars. No effect of artificial water addition was observed on inorganic-N concentration once dry and wet collars had similar values (Figure 8).

The young pasture's mean NO fluxes were about 1 ng N cm<sup>-2</sup> h<sup>-1</sup> in dry collars during the moisture addition experiment while the other plots had somewhat lower fluxes (Figure 9). The young and fertilized plots had greatly increased soil NO flux in wet collars on the first day of the experiment, reaching mean values of 6.8 and 4.2 ng N cm<sup>-2</sup> h<sup>-1</sup>, respectively, while in the traditional and grass-legume plots fluxes reached values of 0.3 and 0.6 ng N cm<sup>-2</sup> h<sup>-1</sup>, respectively. In the second day, there was a reduction of NO flux in the fertilized plot to 0.4 ng N cm<sup>-2</sup> h<sup>-1</sup>, but substantially elevated NO fluxes persisted in wet collars of the young plot during the 3-day experiment (5.7 ng N cm<sup>-2</sup> h<sup>-1</sup> in the second day and 3.0 ng N cm<sup>-2</sup> h<sup>-1</sup> in the third day).

Soil N<sub>2</sub>O fluxes during the water addition experiment were below the detection limit (0.6 ng N cm<sup>-2</sup> h<sup>-1</sup>), independent of treatment.

### 3.8. Soil CO<sub>2</sub> fluxes

Soil CO<sub>2</sub> fluxes increased with the artificial water addition ( $F = 14.095$ ,  $DF = 1$ ,  $P < 0.013$ ). The dry collars had CO<sub>2</sub> fluxes of about 2 µmol m<sup>-2</sup> s<sup>-1</sup> (Figure 10).

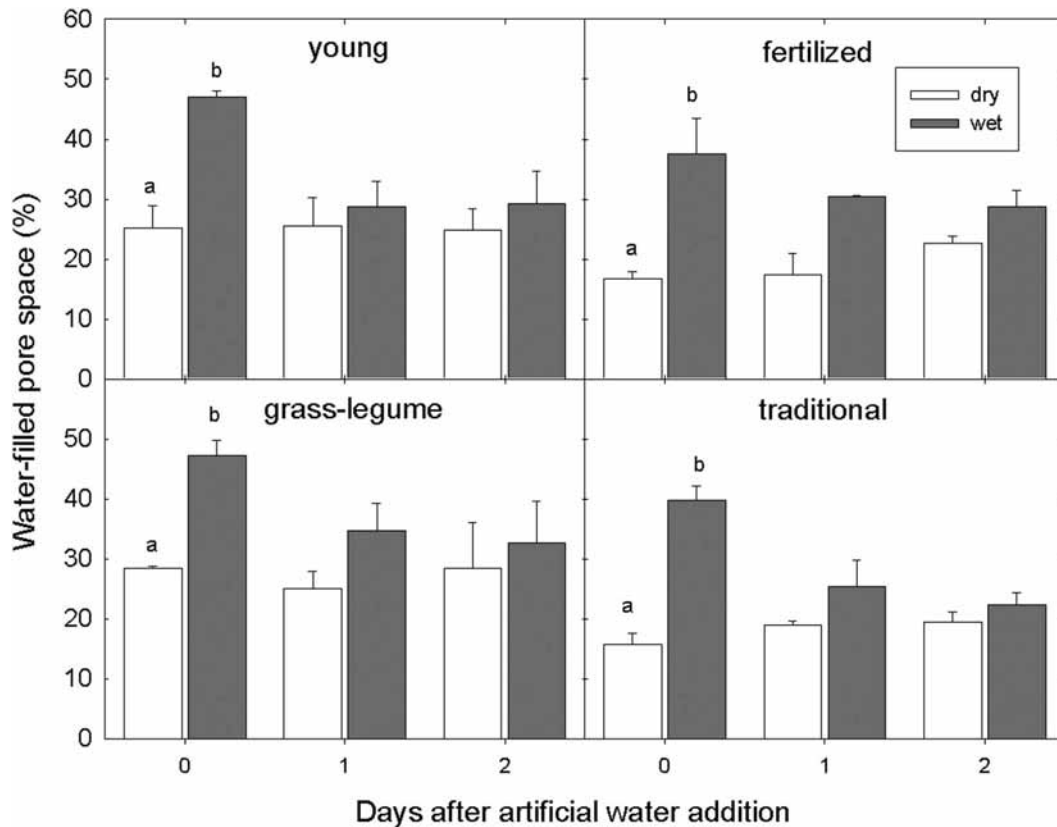
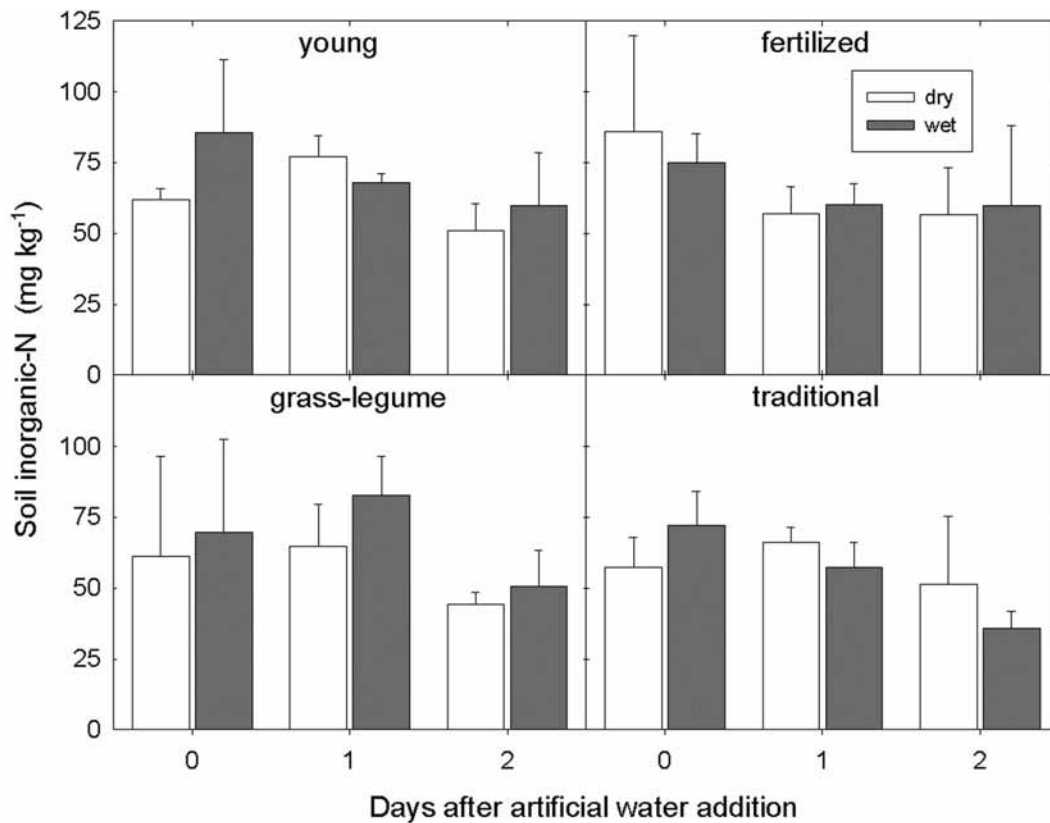


Figure 7. Effects of artificial water addition (simulating 5-mm rain) on WFPS (%) in different restoration treatments of degraded pastures at Fazenda Rio de Janeiro, Planaltina, Goiás, Brazil. Different letters mean a significant difference (*t* test,  $P < 0.05$ ). The experiment was conducted in September 2002.

The grass-legume plot had the highest increase of soil respiration after water addition, reaching  $8.3 \mu\text{mol m}^{-2} \text{s}^{-1}$  ( $t = 8.30$ ,  $P = 0.001$ ). The other treatments had values of  $5.4 \mu\text{mol m}^{-2} \text{s}^{-1}$  (young plot,  $t = 3.97$ ,  $P = 0.007$ ),  $5.2 \mu\text{mol m}^{-2} \text{s}^{-1}$  (fertilized plot,  $t = 4.49$ ,  $P = 0.021$ ), and  $5.0 \mu\text{mol m}^{-2} \text{s}^{-1}$  (traditional plot,  $t = 3.538$ ,  $P = 0.024$ ). One day after the water addition, soil  $\text{CO}_2$  fluxes tended to decrease.

### 3.9. Diversity of bacterial soil community

The soil bacterial community changed rapidly after an experimental water addition, showing clear differences between communities before and 2 days after water addition (Figure 11). Bacterial communities of soil samples collected before water addition showed a high level of similarity (>80%). Communities from samples collected after the addition separated from the preaddition samples at similarity levels lower than 58%. In the case of the young pasture sample the separation



**Figure 8.** Effects of artificial water addition (simulating 5-mm rain) on inorganic-N availability in different restoration treatments of degraded pastures in Fazenda Rio de Janeiro, Planaltina, Goiás, Brazil. The experiment was conducted in September 2002.

occurred at a similarity level of 47%, indicating that besides the response to the water addition, the bacterial community is influenced by the time since conversion. Another interesting aspect is that the grass–legume bacterial community had the highest level of similarity with the community extracted from a native area soil.

## 4. Discussion

### 4.1. Soil N dynamics

Pastures with higher productivity, such as the grass–legume treatment, may prevent surface soil evaporation. Plant demand for water and inorganic-N also could contribute to the reductions observed in December 2001, since the peak of live biomass production of grasses occurred between January and February (D. Brunet 2003, personal communication).

Conversion of tropical forests to pasture results in lower nitrification rates and a change in the dominant form of soil inorganic-N from  $\text{NO}_3^-$  to  $\text{NH}_4^+$  (Verchot et

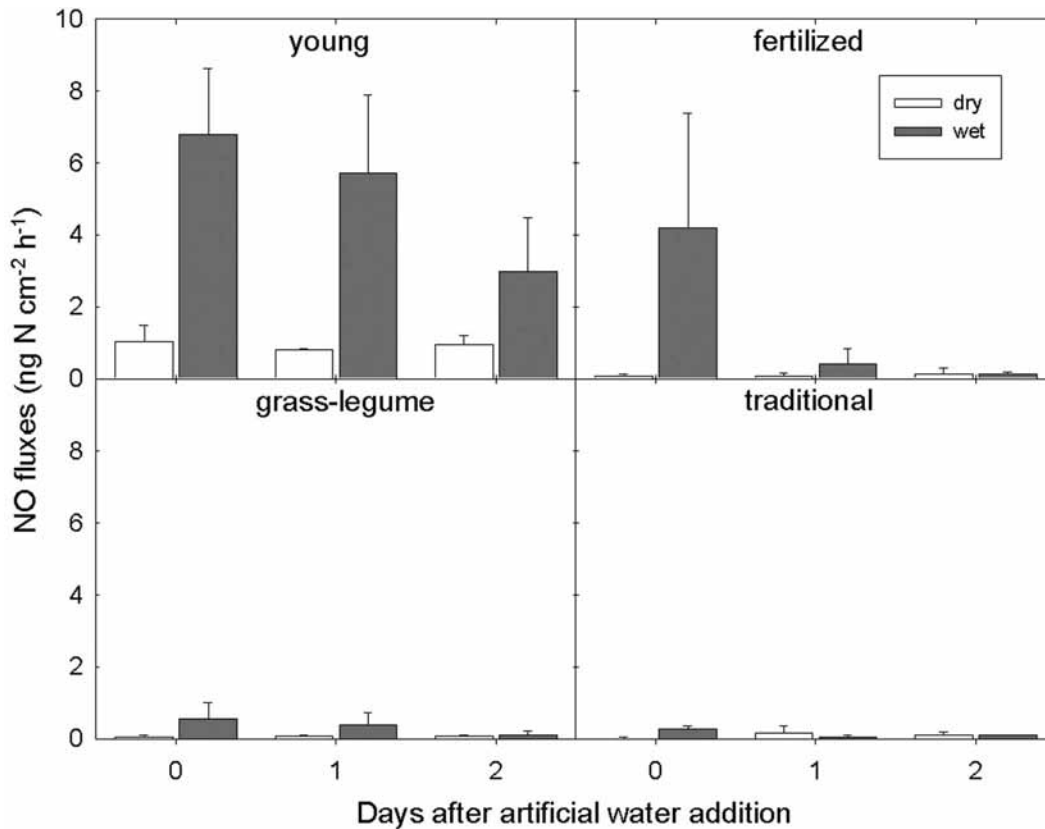


Figure 9. Effects of artificial water addition (simulating 5-mm rain) on soil NO fluxes in different restoration treatments of degraded pastures in Fazenda Rio de Janeiro, Planaltina, Goiás, Brazil. The experiment was conducted in September 2002.

al. 1999; Neill et al. 1995). However, as observed in recent studies, the dominant form of inorganic-N in cerrado soils either under native vegetation or pasture is  $\text{NH}_4^+$  (Pinto et al. 2002; Nardoto and Bustamante 2003; Varella et al. 2004). The high concentrations of  $\text{NH}_4^+$  found in the pasture soils of the present study, even when nitrogen fertilizer was not used, could be a consequence of a higher cation exchange capacity after liming that was applied in all of the plots including the traditional pasture.

Soil fluxes of NO and  $\text{N}_2\text{O}$  are thought to be regulated by a combination of soil moisture and nitrogen availability. According to Davidson (Davidson 1991; Davidson 1993), soil NO production is higher when values of WFPS are between 30% and 60% and nitrification becomes the dominant process. When values of WFPS are higher than 60%, denitrification becomes more important and more  $\text{N}_2\text{O}$  is produced in relation to NO.

During the wet season (November 2001–April 2002), the WFPS values in the studied sites were frequently higher than 60%, that is, good moisture conditions for denitrification. Two possibilities could account for the absence of  $\text{N}_2\text{O}$  fluxes: (i)

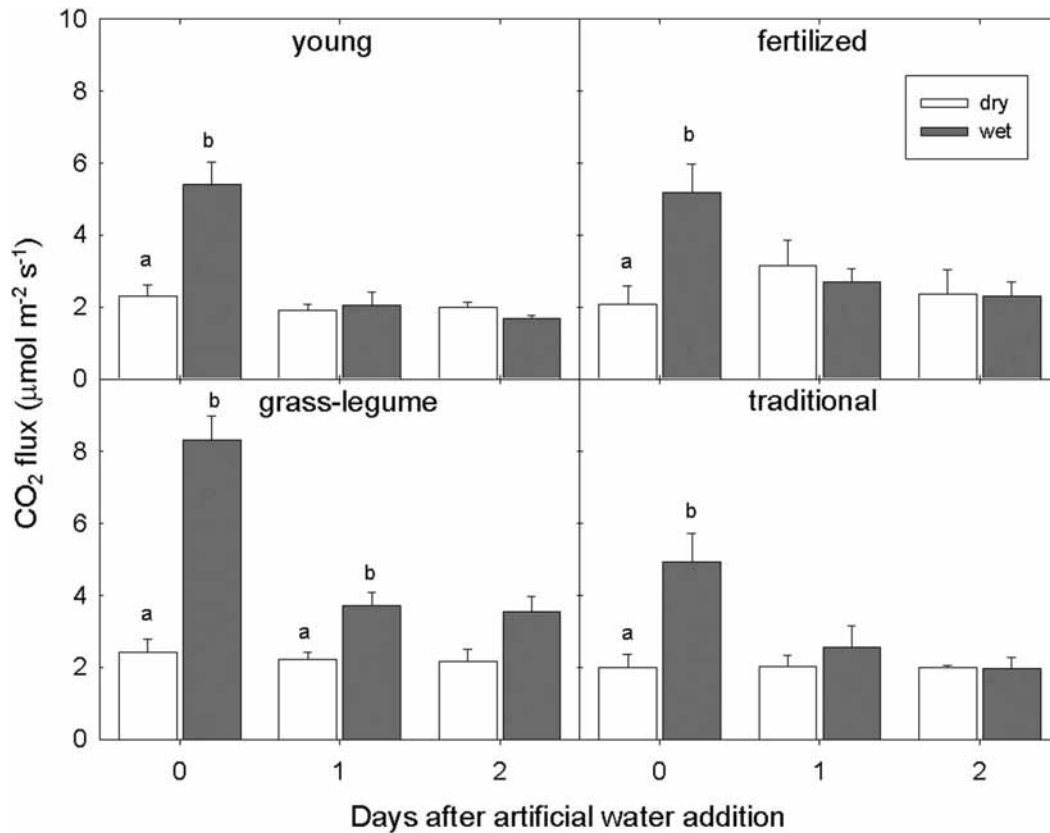


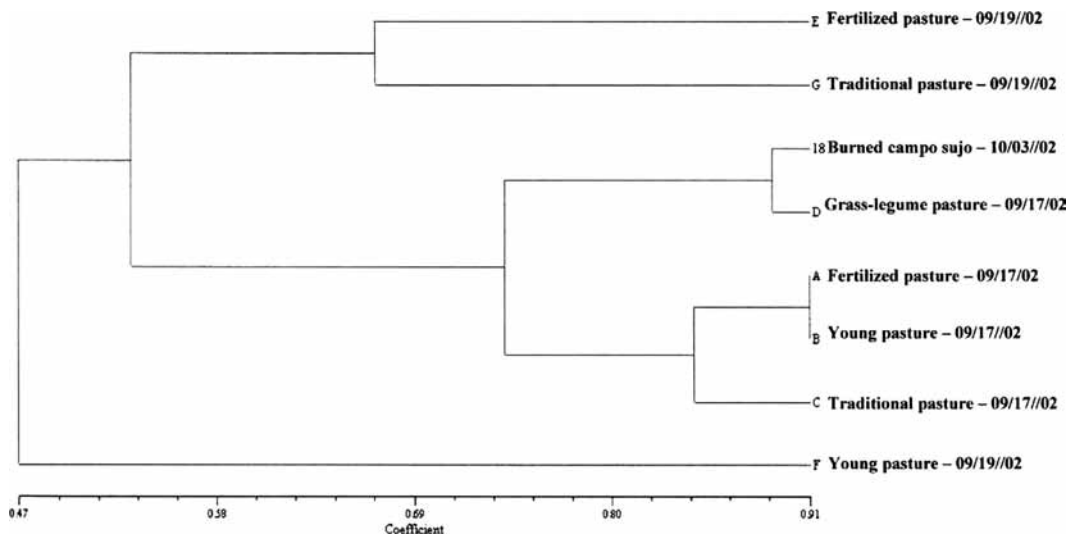
Figure 10. Effects of artificial water addition (simulating 5-mm rain) on soil CO<sub>2</sub> fluxes in different restoration treatments of degraded pastures in Fazenda Rio de Janeiro, Planaltina, Goiás, Brazil. Different letters mean a significant difference (*t* test, *P* < 0.05). The experiment was conducted in September 2002.

denitrification could be limited by the low concentration of the substrate (NO<sub>3</sub><sup>-</sup>), or (ii) denitrification occurred and N<sub>2</sub>O was completely reduced, forming N<sub>2</sub>. The higher values measured in some of the collars could be a consequence of cattle activity. Dias Filho et al. (Dias Filho et al. 2001) suggested that cattle activity could result in great spatial variability in P and N availability, because the excreta are not uniformly distributed on the soil.

The low concentrations of NO<sub>3</sub><sup>-</sup>, in comparison of NH<sub>4</sub><sup>+</sup>, observed could be due to lower densities of nitrifiers, by inhibition of nitrification, in spite of NH<sub>4</sub><sup>+</sup> availability, or by low O<sub>2</sub> availability in the soil when values of WFPS are high. Low rates of nitrification would also be expected to result in low soil NO fluxes.

Differences in N-oxide emissions between treatments were not detected during the wet season but might occur in the transition from the dry to the wet season. During the artificial water addition experiment, it was observed that the values of WFPS did not exceed 60%. Consequently, NO fluxes were higher in relation to N<sub>2</sub>O fluxes. Differences were observed in the treatments with peaks of NO emis-





**Figure 11.** Inferred similarity of bacterial communities using 16S rDNA-DGGE profiles from soil samples (0–5 cm) under *Brachiaria brizantha*. The pasture restoration treatments were as follows: traditional pasture (control plot without treatment), fertilized pasture (with NPK addition), grass-legume pasture (combination of *B. brizantha* and *Stylosanthes guianensis*), and a young pasture (2-yr-old pasture). The sampling dates correspond to before (17 Sep 2002) and after (19 Sep 2002) an artificial water addition. A soil sample collected in burned campo sujo (shrubland) was used as marker. Dendrograms were constructed from the DGGE gel photo using 1/0 clustering and the UPGMA (Dice coefficient of similarity) algorithm.

sion only in the young pasture during the 3 days of observations and in the fertilized pasture only during the first day. Actually, NO fluxes in the young pasture ( $1.0 \text{ ng N-NO cm}^{-2} \text{ h}^{-1}$ ) were higher in the dry collars during the moisture addition experiment than were the fluxes measured in the wet season. The water addition experiment was performed at the end of the dry season (September), but sparse rain events had occurred before the experiment dates. Better conditions for gas diffusivity, due to lower values of WFPS, in the dry-wet transition than in the middle of the wet season and time since conversion could explain the differences in NO flux dynamics between the young pasture and the others. Veldkamp et al. (Veldkamp et al. 1999) observed a reduction in N-cycling index (net N-mineralization, potential nitrification, and soil  $\text{NO}_3^-$  availability) and in emissions of  $\text{N}_2\text{O}$  and NO with pasture age in a pasture chronosequence in Costa Rica. There are no studies of pasture chronosequence in the cerrado region, but Viana (Viana 2002) found a 20-yr-old pasture to have lower  $\text{N-NH}_4^+$ ,  $\text{N-NO}_3^-$ , and net N-mineralization rates than soils of native cerrado. In the same pasture, Varella et al. (Varella et al. 2004) observed average fluxes of  $0.2 \text{ ng N-NO cm}^{-2} \text{ h}^{-1}$  over a year and a peak of  $1.27 \text{ ng N-NO cm}^{-2} \text{ h}^{-1}$  after an artificial water addition.

Measurements made in October 2001 only in the young pasture showed that values of WFPS reached 60% (Table 4). The mean  $\text{N}_2\text{O}$  flux measured at that time was the highest we are aware of in cerrado soils ( $9.5 \text{ ng N-N}_2\text{O cm}^{-2} \text{ h}^{-1}$ ) with

**Table 4. Soil N<sub>2</sub>O and NO fluxes, inorganic-N availability, and WFPS (%) from the young pasture (*Brachiaria brizantha*) in October 2001 in the Fazenda Rio de Janeiro, Planaltina, Goiás, Brazil. This pasture was converted in 1999, after a removal of the native vegetation (dense savanna woodland). Values represent mean (SE).**

Soil flux <sup>a</sup> (ng N cm <sup>-2</sup> h <sup>-1</sup> )		Inorganic-N availability <sup>b</sup> (mg N kg <sup>-1</sup> )		
N <sub>2</sub> O	NO	NH <sub>4</sub>	NO <sub>3</sub>	WFPS (%) <sup>c</sup>
9.5 (2.7)	2.1 (0.7)	114.5 (9.0)	15.9 (4.8)	60.6 (0.8)

<sup>a</sup>  $n = 6$ .

<sup>b</sup>  $n = 3$ .

<sup>c</sup>  $n = 4$ .

individual values ranging from 3.8 to 19.2 ng N-N<sub>2</sub>O cm<sup>-2</sup> h<sup>-1</sup>. Higher soil NO fluxes were also observed, but lower than the fluxes measured in the water addition experiment. Concentrations of N-NH<sub>4</sub><sup>+</sup> were higher than in the other studied months, probably due to the accumulation of dry matter during the dry season.

Previous laboratory studies showed that nitrification is important to the emission of N gas when the NO:N<sub>2</sub>O ratio is higher than 1 (Lipschultz et al. 1981). On the other hand, when denitrification is important, this ratio is lower than 1, and frequently about 0.01 (Anderson and Levine 1986). During the water addition experiment, nitrification seemed to be the dominant process. However, chemodenitrification can also be an important source of NO, as demonstrated by Anderson and Poth (Anderson and Poth 1998). These authors showed that additions of nitrite to sterilized cerrado soils promoted large pulses of NO. In measurements taken in October, the NO:N<sub>2</sub>O ratio was equal to 0.2; that is, denitrification could be the predominant process, due to higher WFPS value and anoxic conditions promoted by intense biological activity at the beginning of the growth period.

Slemr and Seiler (Slemr and Seiler 1991) suggested that NO emissions are higher when ammonium and urea fertilizers are applied. However, in a review by Veldkamp and Keller (Veldkamp and Keller 1997) it was observed that in temperate systems the emission of NO was linearly related to the amount of N applied, but it was not possible to separate the effects of different N fertilizers. In tropical soils in Costa Rica, Veldkamp et al. (Veldkamp et al. 1998) observed that the use of fertilizers promoted higher fluxes of nitrogen oxide gases and that the soil moisture at the moment of fertilization was more critical for the emissions than the fertilizer type used. In the present study, the fertilized pasture where urea was used did not produce higher NO fluxes than the other treatments. However, measurements were taken approximately 8 months after the fertilization. Peaks of NO emissions probably occurred in a short period (days to weeks) after the fertilization depending on precipitation and soil moisture as observed by Skiba et al. (Skiba et al. 1997).

Comparisons shown in Table 5 indicate that N-NO emissions were higher in native areas [between 0.10 and 0.26 kg N-NO ha<sup>-1</sup> (4 months)<sup>-1</sup>] (Pinto et al. 2002) than in pasture areas investigated in the present study [0.01 kg N-NO ha<sup>-1</sup> (4 months)<sup>-1</sup>] although the studies were performed in different years. The only exception was the burned campo sujo that produced NO flux values similar to those of the pastures.

**Table 5.** Mean NO and CO<sub>2</sub> fluxes, N and C emissions of the cerrado soils under native vegetation (cerrado ss. and campo sujo) at the Reserva Ecológica do IBGE (RECOR/IBGE), Brasília, and pastures (*Brachiaria brizantha*) at the Fazenda Rio de Janeiro, Planaltina, Goiás, Brazil. At the RECOR, the unburned areas were protected from fire since 1973, while the burned areas were subjected to prescribed biennial burning since 1992. At the Fazenda Rio de Janeiro, the pastures were part of an experiment of restoration of degraded pastures and were 9 yr old at the beginning of the experiment (1999) with the exception of the young pasture that was converted in that year.

Local		NO flux <sup>a</sup>	N emission <sup>a</sup>	CO <sub>2</sub> <sup>b</sup> flux	C <sup>b</sup> emission
		ng N cm <sup>-2</sup> h <sup>-1</sup>	kg N ha <sup>-1</sup>	μmol m <sup>-2</sup> s <sup>-1</sup>	Mg C ha <sup>-1</sup>
Cerrado	Unburned	0.37	0.11	4.1	6.4
	Burned	0.33	0.10	5.2	8.1
Campo sujo	Unburned	0.05	0.01	4.2	6.5
	Burned	0.90	0.26	5.5	8.6
Pasture	Fertilized	0.03	0.01	7.7	12.0
	Grass–legume	0.02	0.01	7.9	12.3
	Young	0.10	0.03	6.5	10.1
	Traditional	0.00	0.00	6.1	9.5

<sup>a</sup> To average NO flux and N emissions, data between January and April 2001 were used for the cerrado areas and between January and April 2002 for the pastures.

<sup>b</sup> To average CO<sub>2</sub> flux and the C emissions, data between November 2000 and April 2001 were used for the cerrado areas (except for February 2001) and between November 2001 and April 2002 for the pastures.

## 4.2. Soil C dynamics

The removal of the superficial soil layer (0–10 cm) with pasture clearing could have a great influence on soil carbon dynamics. Microbial biomass was lower in the young plot (158 mg C kg of soil) but did not significantly differ from the other treatments (mean values of about 526 mg C kg of soil). Varella et al. (Varella et al. 2004) found that microbial biomass in a 20-yr-old pasture in the cerrado ranged from ~240 to 700 mg C kg of soil along a year of study. The highest values were found at the beginning of the wet season.

Comparing the restoration treatments, the fertilized and grass–legume plots had higher values of soil CO<sub>2</sub> fluxes (9.0 and 9.2 μmol m<sup>-2</sup> s<sup>-1</sup>, respectively). The highest CO<sub>2</sub> fluxes measured in the young and traditional plots (6.8 and 7.1 μmol m<sup>-2</sup> s<sup>-1</sup>, respectively) were similar to those observed in a native cerrado (highest average = 6.8 μmol m<sup>-2</sup> s<sup>-1</sup>) by Pinto et al. (Pinto et al. 2002). During the dry season (September 2002), the soil respiration measurements in all treatments were similar to native cerrado (2 μmol m<sup>-2</sup> s<sup>-1</sup>). The seasonal CO<sub>2</sub> fluxes in this study slightly larger than those measured in an Amazon pasture (Fernandes et al. 2002) and Venezuelan savannas (Sanhueza et al. 1994).

However, the higher CO<sub>2</sub> fluxes measured in the grass–legume pasture after the water addition can also be due to the root respiration of *Stylosanthes* that remains green during the dry season. In terms of pasture management the maintenance of a green cover during the dry season is important to provide quality forage for the cattle. In the wet season, the grass–legume and fertilized pasture had higher soil

CO<sub>2</sub> fluxes, probably associated with the higher plant productivity in these areas. Santos et al. (2004) measured net ecosystem productivity using eddy covariance in a 5-yr-old pasture close to our study plots. They reported that both canopy photosynthetic rates and nighttime ecosystem CO<sub>2</sub> efflux rates were much greater than has been observed for cerrado native vegetation in both wet and dry seasons. Actually, the levels of ecosystem gas exchange measured by them were equivalent to fertilized C<sub>4</sub> crops growing in the temperate zone.

Chen et al. (Chen et al. 2002) measured emissions of 14.3 Mg C ha<sup>-1</sup> yr<sup>-1</sup> in savanna soils in northern Australia, and they observed that 70% of these emissions happened in the rainy season, while 30% happened in the dry season, showing the importance of the rainy season for soil C emission. Considering similar periods of CO<sub>2</sub> flux measurements during the wet season (5 months) made in this study and in that made by Pinto et al. (Pinto et al. 2002) (although in a different year), higher soil emissions of C were observed in the pastures of greater productivity (grass–legume and fertilized plots with ~12 Mg C ha<sup>-1</sup> in 5 months). The other pasture plots (young and traditional with ~10 Mg C ha<sup>-1</sup>) had slightly higher emissions than burned native areas. The lowest C emissions were observed in the unburned areas (cerrado = 6.4 Mg C ha<sup>-1</sup> and campo sujo = 6.5 Mg C ha<sup>-1</sup>).

Lardy et al. (Lardy et al. 2002) observed that the C soil stocks (0–100 cm) tend to be larger where the cerrado vegetation is dominated by grasses (~20 kg C m<sup>-2</sup> versus ~15 kg C m<sup>-2</sup> in a dense woodland savanna). Corazza et al. (Corazza et al. 1999) showed that the C stocks (0–100 cm) in an 18-yr-old pasture were larger in comparison with native cerrado soils (15 and 13.4 kg C m<sup>-2</sup>, respectively) showing that a high productivity pasture, in the long term, can accumulate more C than native cerrado areas.

### 4.3. Bacterial community structure

The comparison of DGGE profiles of soil samples before and after the water addition indicated a rapid response of bacterial community structure. The CO<sub>2</sub> flux data indicate that microbial and/or plant root activity also increased with water addition. This rapid response of the bacteria suggests that the primary mechanism for increased soil nitrogen oxide fluxes with water addition is biological. Although time since conversion to pasture affects the bacterial community, the pasture restoration methods resulted in bacterial communities more or less similar to that of native cerrado. The bacterial community structure of the grass–legume treatment had the highest similarity with that of a native cerrado soil. Assuming that similar bacterial community structure corresponds with similar bacteria-mediated processes, this indicates the grass–legume restoration treatment may be more sustainable.

## 5. Conclusions

Comparing the net N-mineralization and nitrification rates; N-availability; and fluxes of NO and N<sub>2</sub>O in traditional, grass–legume, and fertilized plots 8 months after the last fertilization, it can be concluded that the restoration treatments did not significantly change N cycling. Addition of *Stylosanthes guianensis* to *B. brizantha* pastures would probably be a more sustainable management technique for

degraded pastures in the region, by providing higher plant biomass productivity and a soil bacterial profile similar to native cerrado, without the peaks of nitrogen oxide gas emissions that are often associated with the use of N fertilizers. The results from the young plot indicated that the conversion of native areas to pasture could result in higher N emissions. However, the recently converted pasture did not show higher CO<sub>2</sub> fluxes and microbial biomass probably due to the technique of removing soil surface layer and burning the coarse wood debris. Based on the results of this and other studies, it appears that, in terms of net productivity, reduction of nitrogen oxide gas emissions, and resources demand, pasture restoration, especially with the addition of legumes, represents a viable alternative instead of new conversion of native areas to pasture.

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