

Short-term effect of adding nitrogen in forest soil of an urban rainforest

Efeito a curto prazo da adição de nitrogênio no solo de uma floresta urbana

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ABSTRACT

The deposition of atmospheric nitrogen has been increased in urban forest ecosystems, yet it is not clear how this increase affects soil respiration in the short term. The soil respiration could contribute to CO₂ flux to the atmosphere; therefore, it is essential to understand how nitrogen addition affects soil respiration and its autotrophic and heterotrophic compartments. We established a randomized block experiment to investigate the effects of adding 2.5 kg ha⁻¹ (which corresponds to ~ 40% of the total annual deposition) in soil respiration during five days in an urban tropical forest. The CO₂ flux of the autotrophic and heterotrophic compartments was individualized and measured using an infrared gas analyzer (IRGA). Two measurements per day (9-11 and 21-23 hours) were assessed for five consecutive days. Days and nights show no difference in CO₂ flux among all compartments. The heterotrophic respiration was strong negatively affected by nitrogen addition, about 34%. Autotrophic respiration was positively impacted by nitrogen addition, but no significant differences were found. Heterotrophic respiration is the primary source of CO₂ from the forest soil.

Keywords: Autotrophic and Heterotrophic Respiration. Fertilized Experiment. Soil Respiration. CO₂ flux. Tijuca Forest.

RESUMO

A deposição de nitrogênio atmosférico aumentou nos ecossistemas florestais urbanos, mas não está claro como esse aumento afeta a respiração do solo a curto prazo. A respiração do solo pode contribuir para o fluxo de CO₂ na atmosfera, portanto, é essencial entender como a adição de nitrogênio afeta a respiração do solo e seus compartimentos autotróficos e heterotróficos. Estabelecemos um experimento em bloco randomizado para investigar os efeitos da adição de 2,5 kg ha⁻¹ (o que corresponde a ~ 40% da deposição anual total) na respiração do solo durante cinco dias em uma floresta tropical urbana. O fluxo de CO₂ dos compartimentos autotrófico e heterotrófico foi individualizado e medido usando um analisador de gás infravermelho (IRGA). Duas medidas por dia (9-11 e 21-23 horas) foram avaliadas por cinco dias consecutivos. Dias e noites não mostram diferença no fluxo de CO₂ entre todos os compartimentos. A respiração heterotrófica foi fortemente afetada negativamente pela adição de nitrogênio, cerca de 34%. A respiração autotrófica foi impactada positivamente pela adição de nitrogênio, mas não foram encontradas diferenças significativas. A respiração heterotrófica é a principal fonte de CO₂ do solo da floresta.

Palavras-chave: Respiração Autotrófica e Heterotrófica. Experiment de Fertilização. Respiração do Solo. Fluxo de CO₂. Floresta da Tijuca.

1 INTRODUCTION

Atmospheric deposition of nitrogen has grown in forest ecosystems (VITOUSEK et al., 1997) as a result of burning fossil fuels and fertilizer applications in agriculture (DAVIDSON, 2009). According to Magnani et al., (2007), nitrogen deposition is essential for carbon sequestration in forest ecosystems. However, the CO₂ emissions by autotrophic and heterotrophic respiration in tropical forest soils are still poorly studied. Soil respiration (Rs) represents the total flux of CO₂ emitted by the soil. It can be divided, in general, into two pools: i) Heterotrophic respiration (Rh) that represents the decomposition and mineralization process of organic materials in the soil (that is, organo-mineral fraction) and litter (that is, mostly organic fraction); ii) Autotrophic respiration (Ra) that mainly represents root growth and exudate production (BOND-LAMBERTY et al., 2004; SUBKE et al., 2006).

The Rs corresponds to the second-largest flux of carbon from the terrestrial system to the atmosphere (RAICH and SCHLESINGER, 1992; RAICH et al., 2002; SHAO et al., 2014), thus being an important indicator of climate change. Previous studies show that Rs is influenced by soil temperature and humidity (DAVIDSON et al., 2000; LIU et al., 2002; RUSTAD et al., 2001). Therefore, the contribution of each component of Rs must be known to understand the effects of global changes on net CO₂ exchanges between terrestrial ecosystems and the atmosphere (ZHOU et al., 2007).

In forest ecosystems, studies show that there is a reduction in heterotrophic respiration after the addition of N (JANSSENS et al., 2010). The addition of N can boost microbial activity and biomass; or decrease enzymatic activity and soil organic matter decomposition (JANSSENS et al., 2010; WANG et al., 2017). However, studies regarding the response of both heterotrophic and autotrophic respiration in a different forest and non-forest ecosystems after the addition of nitrogen have been the subject of considerable discussion (ALLISON et al., 2008; CHEN et al., 2017; GAO et al., 2014; JIA et al., 2010; LI et al., 2018; LIU and GREAVES, 2010; LU et al., 2011; MO et al., 2007; OLSSON et al., 2005; YAN et al., 2017; ZENG et al., 2018).

In a study conducted at the Tiantong Natural Forest Park GAO et al., (2014) points to a non-linear response between the amount of N added and the average flux of CO₂ from the soil. WANG et al., (2017) found a 27% drop in Ra after adding nitrogen.

To unravel the effect of nitrogen, we conducted an fertilized experiment with separation of Ra and Rh compartments in a tropical forest for five days. The objective is to understand how the addition of nitrogen separately affects respiration (i.e., Ra and Rh), as well as to verify possible differences between night and day fluxes from Ra and Rh pools.

2 MATERIALS AND METHODS

2.1 STUDY AREA

The city of Rio de Janeiro was established within two very uneven geomorphological compartments: the coastal massifs and the lowland areas. Among the massifs, Tijuca stands out (Figure 1), which among the massifs is what is found in the most densely populated area of the city of Rio de Janeiro. (FERNANDES; AVELAR; NETTO, 2006). The present study was carried out in the Tijuca Forest / Massif, one of the four (4) areas that make up the Tijuca National Park (PARNA-Tijuca) which is a conservation unit with 3,972 hectares located in the city of Rio de Janeiro between parallels 22 ° 55'S and 23 ° 00'S and the meridians 43 ° 11'W and 43 ° 19'W, its altitude is between 0 to 1,021m.

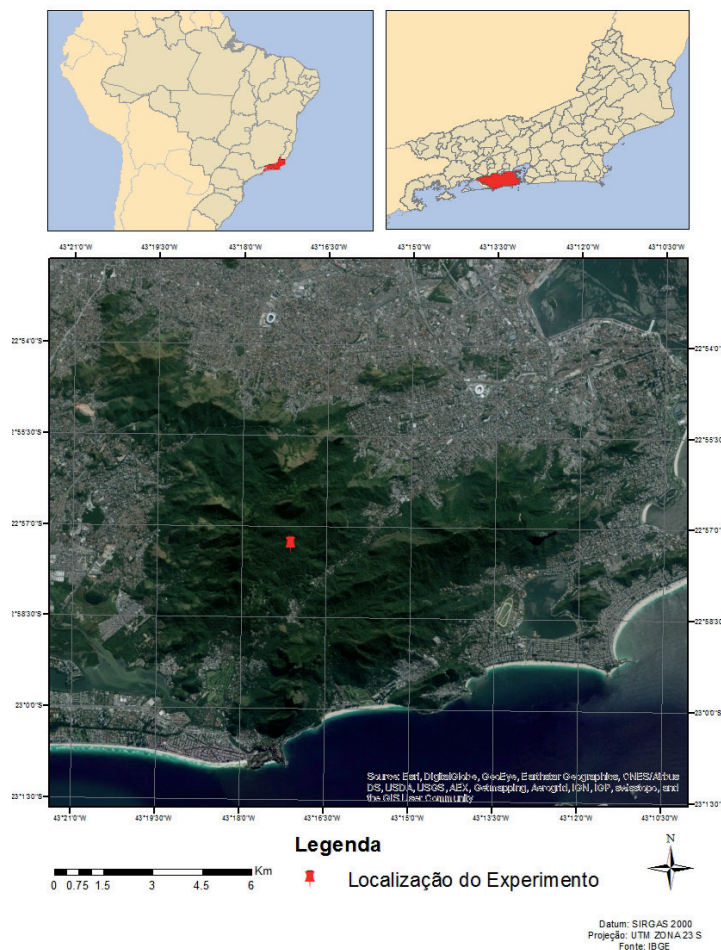


Figure 1 | Tijuca Forest massif and the location of the experiment (red pin).

Source: IBGE.

Currently, Tijuca landscape accommodates several types of land use and cover: grasses, rocky outcrops, areas occupied by buildings and forests in different successional stages (COELHO NETTO, 1992). According to COELHO NETTO (2005) the Tijuca massif is a formation from the Precambrian period, prevailing varied gneisses and granites. PARNA- Tijuca's climate is tropical in altitude with an average

annual temperature of 22°C. The average annual precipitation is between 2.000 and 2.500 mm, the rainiest period is concentrated in the first four months of the year while the months of May are characterized by the decrease in rainfall. PARNA-Tijuca is dominated by Oxisols with small pedological horizons differences and a deep profiles. In the lower lands there is a predominance of the red-yellow Oxisols, while in the lands with higher levels they may present small proportions of Leptosols and Cambisols (COELHO NETTO, 1992). Atmospheric nitrogen deposition is about 6.2 kg N ha⁻¹ year⁻¹ (A. DE SOUZA et al., 2017; PONETTE-GONZÁLEZ et al., 2017).

The vegetation is typical of the tropical evergreen-forest, characterized by large trees, palm trees, ferns, epiphytes and lianas (RIZZINI, 1979). The most frequent plant families are Leguminosae, Sapotaceae, Bombacaceae, Euphorbiaceae, Meliaceae, Lauraceae, Lecythidaceae, Moraceae and Melastomataceae (CCN, 1966). According to GÓES & QUINTELA, (2015) the families with the greatest number of individuals are: Asteraceae, Fabaceae, Myrtaceae; Bignoniaceae; Melastomataceae; Verbanaceae and Anacardiaceae. According to a survey by the Brazilian Forest Service (2018), the height of the individuals ranges from 1 to 12m, the average diameter is more than 10 cm, the average biomass is about 95.57 t ha⁻¹, the average basal area is 21.07 m² ha⁻¹ and the average density is about 360 individuals / ha⁻¹ (BRAZILIAN FOREST SERVICE, 2018).

2.2 EXPERIMENTAL DESIGN

The experiment is located on the southern slope of the Tijuca Forest, 530m high, the slope is oriented to the North. The experiment was established in half of a hectare (100 m x 50m) using a randomized complete block design that includes four replicate 5 x 5 m² (Figure 2) of each the following treatments:

- I. Heterotrophic Respiration (Rh);
- II. Autotrophic Respiration (Ra);
- III. Organic Soil Matter Respiration (Rsom);
- IV. Heterotrophic Respiration + Nitrogen addition (Rh+N);
- V. Autotrophic Respiration + Nitrogen addition (Ra+N);
- VI. Organic Soil Matter Respiration + Nitrogen addition (Rsom+N);

The blocks were allocated so that the canopy and the forest floor were homogeneous (Figure 2).

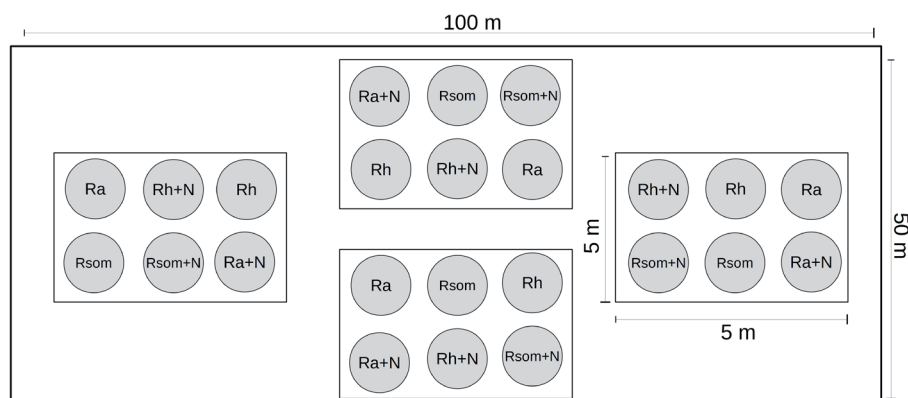


Figure 2 | Study site experimental plot layout. Plot treatment codes: Ra, Autotrophic respiration Rh, Heterotrophic respiration; Rsom, soil organic matter respiration; Ra+N, Autotrophic respiration + nitrogen addition; Rh+N, Heterotrophic respiration + nitrogen addition; Rsom+N, soil organic matter respiration + nitrogen addition.

Source: Personal archive.

2.3 TREATMENTS ESTABLISHMENT

Throughout this article study, we consider R_h to be the respiration with the presence of a litter and R_a to be the respiration due to root growth, both independent of R_{som} respiration. All treatments were established using PVC collars according to Marthewes et al., (2014). The treatments R_{som} , R_h , and their repetitions with the addition of nitrogen were established using collars with 100 x 400 mm, diameter, and height respectively. Before, the collar's insertion the litter was removed and reserved leaving the soil exposed (that is, an extra PVC collar of 100mm diameter was used to cut the litter precisely).

Then the collars were inserted into the soil to a depth of 350mm leaving 50mm above the exposed soil. Finally, the litter removed and previously reserved was replaced inside the collar (i.e., 100 x 400mm). In these treatments (i.e., R_{som} , R_h , R_{som+N} , and R_h+N) the collars have four side holes, that is, two holes on opposite sides. Each hole has a diameter of ~ 3.5cm. On each side, the first hole is located 5cm along the collar, and the second one 5cm away from the first. That is, these 400mm high collars have two holes on each side, 5cm apart and 5cm away from the beginning of the collar. A 35-41 μm mesh was used to cover all holes.

Regarding the R_a and $R_a + N$ treatments, these were established using PVC collars of 100 x 150 mm, diameter, and height respectively. The collars were inserted into the soil to a depth of 100mm, leaving 50mm above the ground. For these treatments, the litter was previously removed, but it was not (re) allocated, thus allowing the quantification of autotrophic respiration, originating by roots growing through the bottom of the collar.

For this experiment it is important to clarify that we are considering mycorrhizal respiration in all treatments, thus allowing the comparison between them, and all measurements were made according to Marthewes et al., (2014).

2.4 CO₂ FLUX MEASUREMENT

The measurement of the flux of carbon dioxide (CO₂) started on October 17, 2018, the flux of CO₂ in all treatments was measured for five days in two daily collections, between the periods from 9 to 11 and 21 to 23 hours.

The measurements of soil CO₂ flux were performed using a portable infrared gas analyzer - IRGA - (EGM-2, PP Systems, UK) connected to a CO₂ flux chamber that was attached to the PVC collars for reading the CO₂. The IRGA was calibrated to take CO₂ readings in 120 seconds or until the flux is stable. Calculations for CO₂ quantification were performed according to equation (1).

$$R = \frac{\frac{C_n - C_o * V}{T_n}}{A} \quad (1)$$

Where R is the assimilation rate (CO₂ flux / unit of area / unit of time), C_o is the concentration of CO₂ at time zero ($T = 0$) in hours and C_n is the concentration in the elapsed T_n , A is the area of the exposed soil (m²) and V the total volume of the system (ie, chamber, 0.0012287 m³).

The collars inserted into the ground do not have a uniform height inside because its height depends on the presence or absence of litter, and when the litter is present its thickness is not the same among the collars. Therefore, to correct the added volume (V_{add}) that may exist between the limit of the litter and the upper part of the collar, an iron disk with a diameter of ~ 9cm and a mass of 10g was used.

The disc was placed inside the collars, without pressure or compression of the litter, that is, only the weight of the disc exerted force on the litter. With the disc inside each collar in all treatments, the height between the disc and the upper limit of the collar was then measured. With the measured height (i.e., litter and collar limit) and the collar diameter, V_{add} was calculated. The V_{add} was used to correct the flux of each collar using equation 2, according to (MARTHEWS TR et al., 2014).

$$R_c = \frac{R_{uc} * V_d + V_{add}}{V_d} \quad (2)$$

Where, R_c is the corrected measurement, R_{uc} measurement without the correction of the additional volume of the collar (m^3), V_{add} additional volume of the collar measured in the field (m^3).

All measurements data were recorded in $g\ m^{-2}\ hr^{-1}$, and then converted to $umol\ m^{-2}\ s^{-1}$ by multiplying the values by 6,312 according to Marthews TR et al., (2014).

2.5 METEOROLOGICAL MEASUREMENT DATA

During the experiment period data of soil temperature, soil relative humidity, and precipitation were measured at 15-minute intervals, with an automatic weather station Vantage Pro2 Davis. The station was located 10m far from the blocks within the Tijuca forest canopy, located at 22 ° 57'S, longitude 43 ° 17'W, and an altitude of 520 m.

2.6 STATISTICAL ANALYSIS

Soil temperature and humidity data were calculated using the daily average. Precipitation was calculated by the daily sum. Regarding CO₂ fluxes, the normality of data distribution was assessed by Shapiro-Wilk, and the homogeneity of variance by the Fisher test (F) test. There is no difference among block, but the data did not show normality, so it was decided to use non-parametric tests. The Wilcoxon test was used to test differences in the CO₂ flux ($n = 4$).

3 RESULTS

3.1 SHORT-LIVED ABIOTIC CHARACTERISTICS

The total rainfall during the 5 days of exposure was 16.40 mm, with a daily average of 3.28 mm. The maximum fall occurred on the 19th and the minimum decrease on the 17th and 18th with 1.2 mm, respectively. On other days, the average was 1.4 mm of rain (Figure 3 b). Regarding the soil temperature, there were no major changes over the 5 days. The average soil temperature ($n = 5$) was 19.7°C, with a maximum and minimum temperature of 20.6 and 18.3°C on days 18 and 21, respectively (Figure 3 c). A soil density was measured with Meteorológica Vantage Pro2 Davis, during an experiment, on average 4.4%, with a maximum and minimum peak of 5 and 4% on days 18 and 20, respectively (Figure 3 d).

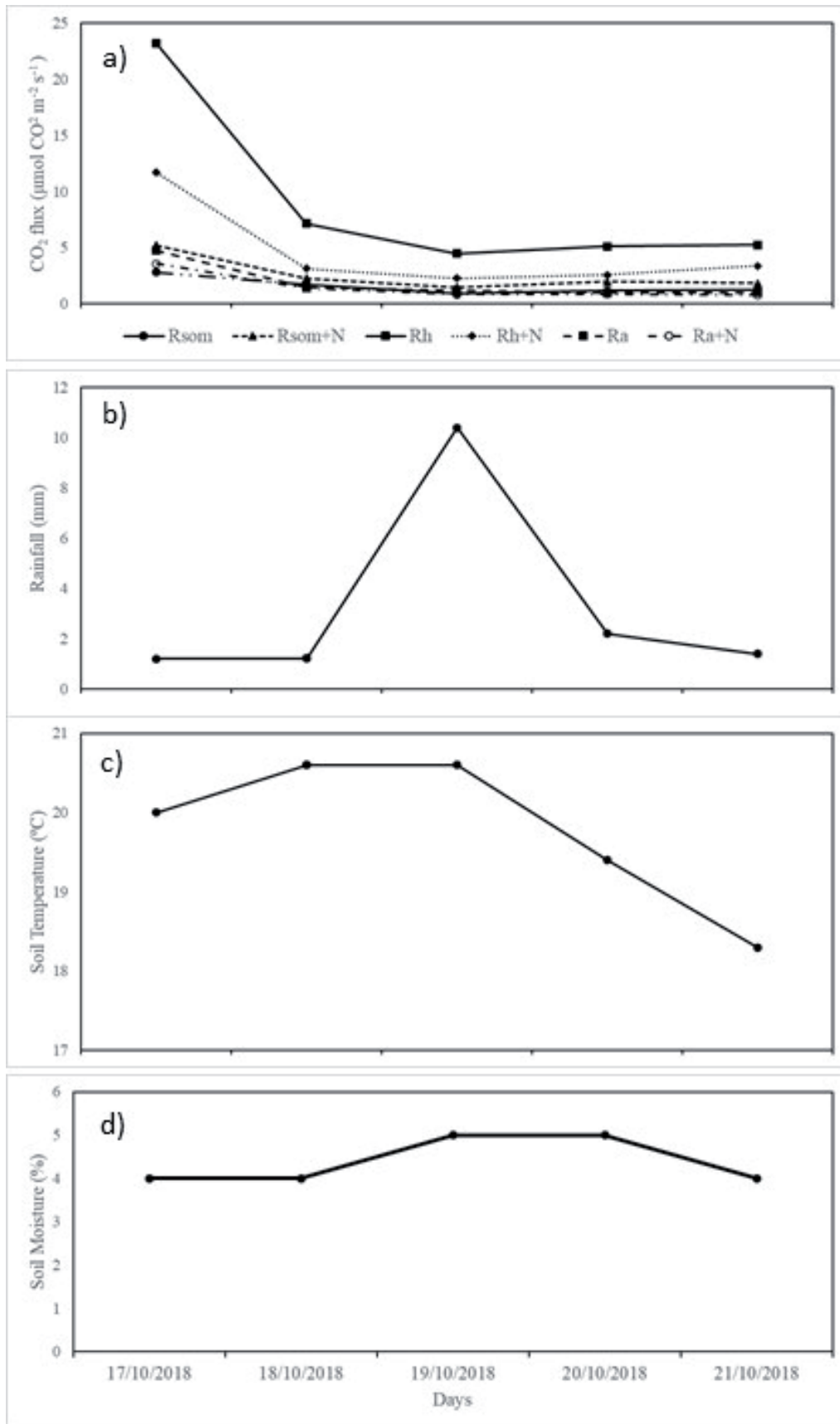


Figure 3 | (a) shows the CO₂ flux of each treatment, (b) shows the daily precipitation, (c) shows the average soil temperature and (d) shows the soil moisture. Whiskers represent standard deviation.

Source: Field experiment data.

3.2. NIGHT AND DAY FLUX OF CO₂

Figure 4 shows the results of the CO₂ flux for the day and night periods in the first five days of the experiment. The median of daytime and nighttime flux is 1.63 and 1.62 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, respectively. The daytime period showed a maximum flux of 13.76 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, while the maximum nighttime flux was only 10.89 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$. The minimum flux, on the other hand, show close values between the daytime and nighttime periods (Figure 4).

There was no significant difference between daytime and nighttime flux ($p = 0.4791$ - Wilcox test), and the difference in the median between daytime and nighttime flux is small as shown in figure 4.

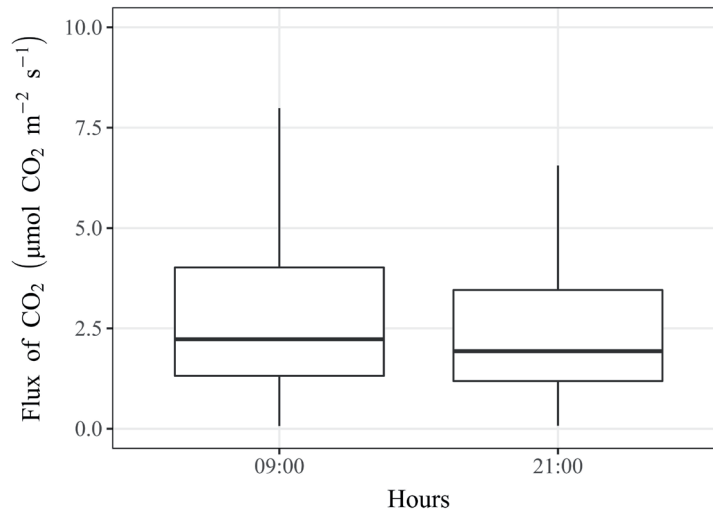


Figure 4 | Boxplot of comparison between daytime and nighttime CO₂ flux.

Source: Field experiment data.

3.3 EFFECT OF NITROGEN ADDITION ON ORGANIC SOIL MATTER RESPIRATION (RSOM)

Figure 5 shows the results of adding N to R_{som}. The results show that the addition of nitrogen increased the maximum CO₂ flux of R_{som} by 3 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, from 2.52 to 5.52 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, that is, when there was the addition of nitrogen the maximum CO₂ flux has increased considerably. However, the same trend was not followed by the minimum flux (Figure 5). The results show that there was no significant difference ($p = 0.2624$ - Wilcox test) between R_{som} and R_{som+N} treatments, indicating a small effect of nitrogen on R_{som} respiration.

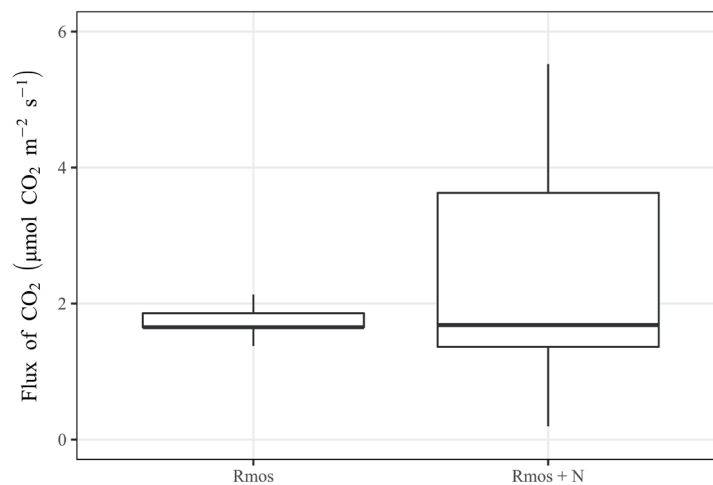


Figure 5 - Boxplot of comparison between R_{som} and R_{som + N} flux of CO₂.

Source: Field experiment data.

3.4 EFFECT OF ADDING NITROGEN ON HETEROTROPHIC RESPIRATION (RH)

Figure 6 shows the results of adding nitrogen to Rh. The results show that the median of treatments without and with nitrogen addition are 5.00 and 2.54 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, respectively, indicating that the addition of nitrogen decreased the performance of heterotrophic decomposers. In addition, a decrease in the minimum flux after the addition of nitrogen can also be observed, going from 2.34 to 0.93 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ (Figure 6). The results show a negative significant effect of nitrogen addition ($p < 0.0001$ Wilcox test) as show in Figure 6.

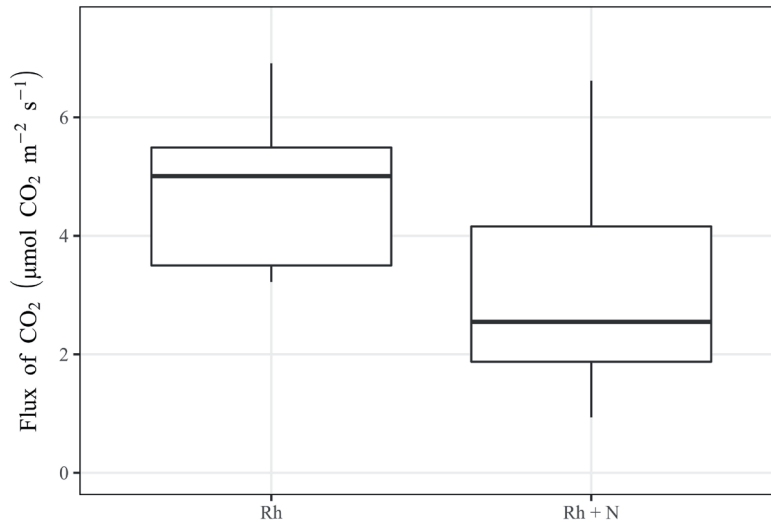


Figure 6 | Boxplot of comparison between Rh and Rh + N flux of CO₂.

Source: Field experiment data

3.5 EFFECT OF NITROGEN ON AUTOTROPHIC RESPIRATION

Figure 7 shows the effect of adding nitrogen on Ra. The results show that the median decreased after the addition of nitrogen, from 1.21 to 1.06 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ (Figure 7). However, there is a drop in the maximum flux achieved, going from 2.22 to 2.16 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ after the addition of nitrogen (Figure 7). The results indicate that there is no effect ($p = 0.2091$ - Wilcox test) of the addition of nitrogen in Ra respiration.

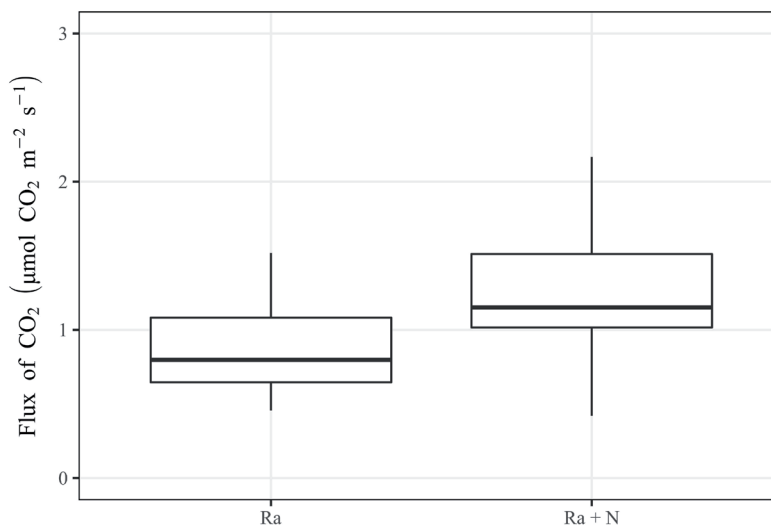


Figure 7 | Boxplot of comparison between Ra and Ra + N flux of CO₂.

Source: Field experiment data

3.6 COMPARISON BETWEEN AUTOTROPHIC (RA) AND HETEROTROPHIC (RH) RESPIRATION

Figure 8 shows the comparison between Ra and Rh without the addition of nitrogen. The results show that the difference between the medians of Ra and Rh is greater than 200%, going from 1.21 to 5.00 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, respectively. Additionally, the maximum flux of Rh is approximately 6 times greater than that of Ra (Figure 8). Figure 8 shows that the treatments differ statistically after the addition of nitrogen ($p < 0.0001$ - Wilcox test).

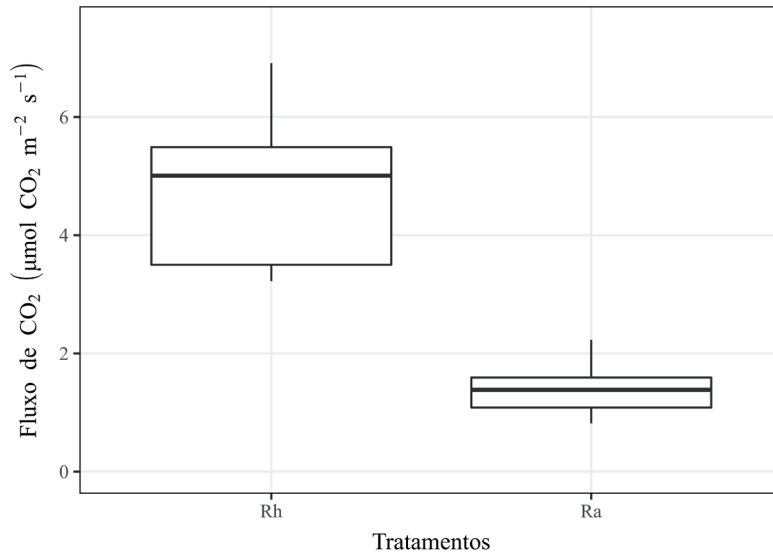


Figure 8 | Boxplot of comparison between autotrophic and heterotrophic respiration.

Source: Field experiment data

4. DISCUSSION

4.1 SOIL RESPIRATION AND ABIOTIC CLIMATIC ASSOCIATION.

Soil temperature and humidity might control respiration. Therefore, we correlate changes in soil temperature, humidity, and rainfall with CO2 flux measurements. In our results, no positive correlations were found between the variations and the treatments analyzed. However, we found a negative correlation between rainfall with R_{soil} and R_h (Figure 9). Some root traits are already known to be correlated to root recalcitrance (POIRIER; ROUMET; MUNSON, 2018), and these traits favor short term stabilization by slowing decomposition. Poirier et al., (2018) already show that root depth distribution is the most crucial trait to control root C storage, and therefore CO2 emissions.

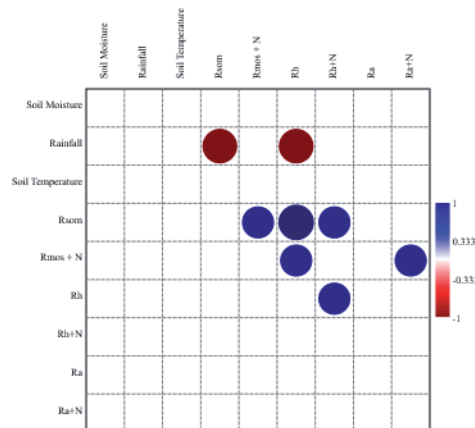


Figure 9 | Correlation between CO2 flux compartments and abiotic variables.

Source: Field experiment data.

4.2 DIFFERENCES BETWEEN DAY AND NIGHT CO₂ RESPIRATION

In our study, there was no significant difference ($p = 0.4791$ - Wilcox test) between day and night CO₂ fluxes (Figure 4), indicating that day and night abiotic factors might be acting with the same intensity on decomposition and autotrophic respiration. The average CO₂ flux occurred during the day, a similar result was found by Hu et al., (2016) who found fluxes ranging between 4.15 and 0.20 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in the daytime and 3.04 and 0, 13 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at night in a subalpine forest in Tibet.

On the other hand, Grahammer et al., (1991), in a prairie in Northeast Kansas, found a 20% greater CO₂ flux during the day. However, in a study carried out in a pasture area on Caatinga vegetation in Brazil, Renata et al., (2018) found no difference. Previous studies indicate that the daytime and nighttime variability of the CO₂ flux can be affected by the biological growth of plants, photosynthesis, microbial activity (KUZUYAKOV; CHENG, 2001; TANEVA, 2011; VARGAS et al., 2011), but in Tijuca forest we did not find differences between day and night fluxes, indicating a possible, stable vegetation growth along the entire day.

4.3 RESPONSES OF HETEROTROPHIC RESPIRATION BY NITROGEN ADDITION.

On average, the addition of nitrogen decreases Rh by 34% (Figure 6). However, in a meta-analysis study Janssens et al., (2010) points to a 15% increase in Rh in forest environments. There is a great variation range between the results suggesting that in highly productive sites the deposition of nitrogen causes stronger negative effects. According to Zhou et al., (2014) the nitrogen addition stimulates negative responses in most Biomes, except in forest biomes. The addition of nitrogen can lead to a decrease in the rates of "microbial mining", which has an effect on some microbes during the decomposition process of organic matter (CRAINE et al., 2007; FONTAINE et al., 2003; MICHEL & MATZNER, 2003)

4.4 RESPONSES OF AUTOTROPHIC RESPIRATION BY NITROGEN ADDITION

We did not find a significant difference in Ra after adding nitrogen (Figure 7). In a study carried out in a temperate steppe area Yan et al., (2010) shows that Ra increased due to the increase in plant productivity. Zeng et al., (2018) in a study carried out in a non-degraded pasture area showed an increase in Ra after the addition of nitrogen. In a meta-analysis study in different biomes Zhou et al., (2014) points out that the addition of nitrogen did not significantly affect Ra in forest environments. According to Xu and Shang., (2016) Ra is strongly influenced by the carbohydrates of photosynthesis and can thus increase as the productivity of plants increases.

5 CONCLUSION

The data show that in the analyzed period there was no significant difference between the flows of the day and the night. The addition of nitrogen did not affect R_{soil} and Ra, however, it reduced the average flux of Rh causing a significant difference. The data show that in our area of analysis, in a short period, the addition of nitrogen may have inhibited the action of microbial biomass, thus causing a reduction in Rh.

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