



# Impact of nitrogen additions on soil microbial respiration and temperature sensitivity in native and agricultural ecosystems in the Brazilian Cerrado



Suellen Pereira Espíndola<sup>a,\*</sup>, Lenka Bobuřská<sup>b</sup>, Adão de Siqueira Ferreira<sup>a</sup>

<sup>a</sup> Institute of Agrarian Science, Federal University of Uberlândia, Uberlândia 38400902, Brazil

<sup>b</sup> Department of Ecology, University of Prešov, 17. Novembra 1, 080 16 Prešov, Slovakia

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## ABSTRACT

Soil microbial respiration (SMR<sup>1</sup>) is a major flux of CO<sub>2</sub> from terrestrial ecosystems into the atmosphere, which depends on several abiotic factors, including soil temperature and nutrient availability. A nutrient of great interest in soil ecology is nitrogen (N) because of its use in agriculture and an expected increase of depositions in soils. Models on the relationship between SMR and temperature may be able to describe the effects of N addition on the temperature sensitivity of soil respiration (Q<sub>10</sub><sup>2</sup>). Hence, this study aimed to investigate the effects of N addition on SMR and Q<sub>10</sub> values in soils of native Brazilian Cerrado (NC<sup>3</sup>), and of an agricultural ecosystem (AE<sup>4</sup>) cultivated over the past 17 years. SMR was stimulated by N additions (100 and 200 mg N kg dwt soil<sup>-1</sup>) in samples of Cerrado sites depending on the land use type and temperature assessed. The addition of 200 N kg dwt soil<sup>-1</sup> to NC showed higher SMR values at 25 °C compared to soil without N input, whereas the same happened in AE at 30 °C. Overall, NC presented higher Q<sub>10</sub> values than AE. N inputs increased Q<sub>10</sub> in the Cerrado sites. In NC, the highest Q<sub>10</sub> value occurred when 200 mg N kg dwt soil<sup>-1</sup> was added to soil, whereas in AE the greatest value was observed for a lower dose (100 mg N kg dwt soil<sup>-1</sup>). This study shows that N additions to tropical soils alter Q<sub>10</sub> values depending on dose and land use. These are important findings to compare the fragility of pristine and cultivated Brazilian Cerrado ecosystems in the perspective of climate change.

## 1. Introduction

Terrestrial ecosystems play a key role in the carbon dioxide (CO<sub>2</sub>) efflux between soil and atmosphere (Crowther et al., 2015; Kirschbaum, 1995; Litton et al., 2011; Stoffel et al., 2010). On a global scale, the soil CO<sub>2</sub> efflux resulting from roots and soil microorganism's metabolism, commonly referred to as soil respiration, is considered to be the second largest carbon (C) flux (Raich and Potter, 1995; Schlesinger, 1977). With the phenomenon of global warming, research on the positive soil CO<sub>2</sub> feedback to the atmosphere caused by a warming-induced increase in soil respiration has received a great concern (Crowther et al., 2016; Davidson and Janssens, 2006; Jiang et al., 2015). However, little has been reported over soil microbial respiration (SMR) in tropical soils, including savanna ecosystems. SMR is a substantial carbon efflux affecting atmospheric CO<sub>2</sub> concentrations, which is accounted for approximately 91 Pg C yr<sup>-1</sup> (Hashimoto et al., 2015). Therefore, small changes in the factors controlling SMR may result in high net CO<sub>2</sub>

effluxes to the atmosphere, thus, contributing to soil C losses and intensifying climate change effects (Crowther et al., 2015; Erhagen et al., 2015; Raich and Schlesinger, 1992).

SMR depends on many factors, such as soil moisture, temperature and organic substrate availability (Raich and Schlesinger, 1992; Yonemura et al., 2009). However, the temperature is described as the primary factor affecting microbial activity and soil respiration rates (Fang and Moncrieff, 2001; Janssens and Pilegard, 2003; Koch et al., 2007). Many reports have shown an exponential correlation between soil respiration and temperature, but there is still no consensus on the exact form of this relationship in tropical soils (Fierer et al., 2006; Karhu et al., 2014; Lloyd and Taylor, 1994) due to the heterogeneity and complexity of soils and ecosystems. The temperature sensitivity (Q<sub>10</sub>), measured by the response of soil respiration to an increase of 10 °C in soil temperature, is an important indicator for assessing the fragility of ecosystems to abiotic factors (Hursh et al., 2017; Liang et al., 2015; Qi et al., 2002). Few experiments have explicitly determined the

\* Correspondence to: Izaú Rangel de Mendonça, 112, Uberlândia 38408136, Brazil.

E-mail address: [suellenesp@gmail.com](mailto:suellenesp@gmail.com) (S.P. Espíndola).

<sup>1</sup> SMR: Soil microbial respiration.

<sup>2</sup> Q<sub>10</sub>: Temperature sensitivity of soil microbial respiration.

<sup>3</sup> NC: native Brazilian Cerrado.

<sup>4</sup> AE: Agricultural ecosystem.

$Q_{10}$  of SMR and particularly between 10 and 30 °C (Hamdi et al., 2011). Many studies have been carried out measuring the  $Q_{10}$  factor in different ecosystems (Crowther et al., 2016; Davidson and Janssens, 2006; Jenkins and Adams, 2011; Peng et al., 2009). Nevertheless, there is a shortage of studies on tropical Brazilian soils (Vinhai-Freitas et al., 2013).

Soil microbial activity depends on quantity and quality of the soils organic matter (Liu et al., 2016; Sjögersten and Wookey, 2002; Yuste et al., 2003). Likewise, the microbial use of C sources is also dependent on nutrient availability, such as nitrogen (N) (Cao et al., 2011; Fang et al., 2017; Gnankamary et al., 2008). Soil microorganisms actively participate in organic matter decomposition and regulate nutrient cycling (Ilstedt and Singh, 2005; Mary et al., 1996). Nitrogen availability is particularly important due to its role in controlling soil microbial activity, as it is required in the microbial metabolism (Galloway et al., 2004). Nitrogen is an essential compound in the formation of amino acids, proteins and nucleic acids (Harper, 1987; Mifflin and Lea, 1976). This nutrient cycle is very dynamic; therefore, soil N losses are expected due to the processes of denitrification, erosion, and volatilization (Galloway et al., 2004; López-López et al., 2012). Hence, N availability to microorganisms controls the process of soil organic matter transformation in many ecosystems (Allison et al., 2008; Bayer et al., 2000).

Terrestrial ecosystems have received more than 50 kg accumulated N ha<sup>-1</sup> between 2000 and 2010 (Peñuelas et al., 2013). Nitrogen deposition is an increasing global environmental problem that occurs mostly due to anthropogenic causes and agricultural practices, such as fertilization (Gruber and Galloway, 2008; Peng et al., 2009; Tian and Niu, 2015). Chronic N deposition often results in nitrate loss and base cation depletion occasioning in soil acidification and biomass reduction, which pose a threat to biological diversity and terrestrial ecosystem functioning (Corre et al., 2003; Gaudio et al., 2015; Tian and Niu, 2015).

In the context of global warming, one of the main challenges is to understand how this increasing atmospheric N inputs to terrestrial environments influences soil C dynamics and soil respiration (Fang et al., 2017; Tu et al., 2013). Anthropogenic N deposition can lead to an N saturated state, what may also alter the efficiency of soil C use and decomposition processes (Corre et al., 2003; Saiya-Cork et al., 2002); directly affecting SMR. Therefore, the effects of N deposition on SMR can greatly change the direction and extent of the C balance response (Ramirez et al., 2010). In the Brazilian Cerrado biome, the effects of N deposition on the temperature sensitivity of SMR,  $Q_{10}$ , have never been reported, as well as whether it would have a higher impact on native forest or agricultural soils.

The Brazilian Cerrado covers over 200 million hectares and is equivalent to 22% of the country's territory (Batlle-Bayer et al., 2010; Klink and Machado, 2005). Moreover, the region is a global biodiversity hotspot (Carranza et al., 2014). The ongoing conversion of this ecosystem into agricultural lands is of high concern (Maia et al., 2013; Rada, 2013). Land use changes are reported to greatly affect SMR and the C cycle, altering the CO<sub>2</sub> efflux to the atmosphere and soil C stocks (Gong et al., 2014; Jiang et al., 2015; Zhang et al., 2015). Furthermore, Brazilian Cerrado soils are acidic, nutrient-poor and characterized by low cation and P availability (Copeland et al., 2012; Costa, 2011; Rada, 2013). Under these conditions, an increase on N deposition can alter soil pH and, therefore, increase aluminum toxicity and decrease cation and P availability (Copeland et al., 2012; Saiya-Cork et al., 2002; Tian and Niu, 2015). There are limited studies on the effects of nutrients on  $Q_{10}$  of SMR (Erhagen et al., 2015; Liu et al., 2016; Tu et al., 2013). In this sense, research on these processes and particularly in Brazilian Cerrado soils are relevant. Therefore, this research aims to: (1) determine the SMR under different temperature conditions and N additions; and (2) evaluate the effects of N addition on soil  $Q_{10}$  indexes in pristine and cultivated Brazilian Cerrado sites.

## 2. Material and methods

### 2.1. Sampling sites

The study was performed in the Cerrado region in Minas Gerais state, southwest in Brazil. The climate of this region is classified as “Cwa” according to Köppen (Alvares et al., 2013), characterized by a well-defined dry season during fall-winter (April to September) and a hot and rainy season during spring-summer (October to March). The mean annual precipitation is in between 1500 and 1600 mm, with 50% falling during the rainy season from December to February. The mean annual air temperature during the winter season is of 18 °C whereas in summer it is approximately of 23 °C with maximum temperatures ranging from 28 to 29 °C (Silva et al., 2003). Soil sampling was performed in sites of native Cerrado (NC, 19°20′42″S and 48°0′59″W, 975 m) and agricultural ecosystem (AE, 19°20′54″S and 48°01′06″W, 948 m). Representative plant species from NC can be found in Vinhai-Freitas et al. (2013). The studied AE was an area recently gained by land use conversion from a forest ecosystem, cultivated with soya in the past 17 years and alternating with corn each 5-year.

### 2.2. Soil sampling

The soil sampling in AE was performed just before soya plantation in November 2012. The soil in the study area was classified as clayey Oxisols (Typic Acrustox) according to the USA Soil Taxonomy (USDA, 1992). Soil samples from each study area were collected during the rainy season from the top 0–5 cm of soil. In each area, three points were sampled within roughly a 1 ha area (as can be seen by the coordinates). Three subsamples of 600 cm<sup>2</sup> (20 cm × 30 cm), spaced 5 m apart, were randomly collected from each point to form a composite sample, summing three field samples per area studied. The samples were conditioned in sealed plastic bags and transported to the laboratory where they were immediately sieved (< 3 mm), stored and kept in a refrigerator at 4 °C until analyses were performed. SMR incubation tests were initiated within two days.

### 2.3. Soil characterization

Soil physical and chemical characterization analysis was performed for each study area. Particle size distribution was determined in air-dried samples (< 2 mm) using the pipette method (Gee and Or, 2002) and the gravimetric water content by oven drying soil samples (20 g) for 24 h at 105 °C. The following chemical analyses were carried out: pH in water (1:2.5 fresh soil: water slurry); soil organic carbon (SOC) using the potassium dichromate heating method (Yeomans and Bremner, 1988); total nitrogen (TN) using the Kjeldahl method (Jones, 1991); available phosphorous (P) and potassium (K<sup>+</sup>) were extracted in acid solution (0.05 M HCl and 0.0125 M H<sub>2</sub>SO<sub>4</sub>) and quantified using a colorimetry assay and flame spectrophotometry respectively (Tedesco et al., 1995), employing wet-sieved soil samples. Microbial biomass carbon (MBC) was extracted by an irradiation-extraction method using a potassium sulfate solution according to Ferreira et al. (1999). Differences in C concentration between irradiated and non-irradiated extracts were used to determine MBC (Vance et al., 1987). The carbon content of soil samples was obtained by potassium dichromate reaction with s-diphenylcarbazine (Cai et al., 2011) using a spectrophotometer (Biomate 3, Thermo Fisher Scientific, Waltham, MA) at 540 nm.

### 2.4. Nitrogen addition and temperature assays

Treatments with N addition were performed in portions of 100 g of fresh, moist soil (40% water holding capacity (WHC)) placed in 500 mL hermetically sealed flasks. Subsequently, the samples were adjusted to a

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