Fluxes of CO\textsubscript{2}, CH\textsubscript{4} and N\textsubscript{2}O from soil of burned grassland of central Africa

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Abstract

In the present study the impact of fire on post-burning soil fluxes of CO$_2$, CH$_4$ and N$_2$O was investigated in a tropical grassland in Congo Brazzaville during two field campaigns. The first was conducted in the middle of the dry season, one month after burning and the second eight months after burning, at the end of the growing season. Gas fluxes and several soil parameters were measured in each campaign from burned plots and from an area preserved from annual burning (unburned). Rain events were simulated at each campaign to evaluate magnitude and length of the generated GHG flux pulses. In laboratory experiments, soil samples from the two treatments were analysed for microbial biomass, net N mineralization, net nitrification, N$_2$O, NO and CO$_2$ emissions under different soil water and soil temperature regimes. Results showed that fire had only a transient effect on CO$_2$ soil emissions and CH$_4$ fluxes. In fact, one month after burning CO$_2$ fluxes were significantly lower in the burned plots and the average daily CH$_4$ flux shifted from net emission in the unburned area to net consumption in the burned plots. After eight months from burning, no significant difference of both average daily CO$_2$ and CH$_4$ fluxes was observed between treatments. No significant effect of fire was observed in the field on N$_2$O fluxes. In fact, laboratory data showed that such difference could be seen only above 70% of maximum soil water holding capacity. This condition of soil water content would be hardly reached in the field even after the rain simulation, due to the sandy texture of this soil. In the laboratory, a stimulating effect of fire on NO soil emissions at low soil water content was observed. Increasing the incubation temperature from 25°C to 37°C affected negatively microbial growth, mineralization and nitrification activities but stimulated N$_2$O and CO$_2$ production. Overall, data indicate that fire would have a minor impact on soil GHG emissions during the post-burning period in tropical grasslands having similar soil characteristics (acidic, well drained, nutrient poor).

1 Introduction
In the African continent fire is a widespread phenomenon. Its occurrence varies from "natural" events, based entirely on lightning as ignition source, to actively applied burning systems, based on rangeland management (Bothma and Du, 1996; Trollope, 1990). African savannas, which represent approximately half of the African land surface (Scholes and Walker, 2004), are mostly characterized by the co-dominance of trees and grasses (Sankaran et al., 2005), and are distributed in areas characterized by a clear dry season, followed by a rainy season (Huntley and Walker, 1982; Scholes and Hall, 1996). Mean annual precipitation, disturbance by fire and/or herbivory, duration of dry season and soil fertility are the key factors which determine the density of grasses, trees and shrubs (Sankaran et al., 2005; Bond, 2008). Above 650 mm of mean annual precipitation, the water input to the ecosystem would be sufficient for woody canopy closure, and the co-existence of trees and grasses is the result of burning or strong herbivory pressure (Sankaran et al., 2005). Pastoral activity is always combined with burning.

Savanna fires also influence nutrient cycling patterns by modifying plant cover and biodiversity (Menault, 1977; Swaine et al., 1992; Sankaran et al., 2005) and by changing the chemical, biological and physical characteristics of soil (Menaut et al., 1993; Andersson et al., 2004a,b). Enhanced rates of mineralization and nitrification have been reported in burned savannas at the onset of rain season (Adedejii, 1983; Singh et al, 1991). Soil NH$_4^+$ concentration was found to increase in savanna and shrubland soils after burning (Christensen, 1973; Singh et al., 1994; Castaldi and Aragosa, 2002), as result of organic matter combustion and temperature induced release from organo-mineral soil complexes (Raison, 1979; Kovacic et al., 1986; Prieto-Fernandez et al., 2004). Andersson et al. (2004b) measured increased values of NH$_4^+$, dissolved organic N and C in savanna soil after burning, which supported higher rates of mineralization and nitrification when soil water content allowed microbial activation. This generally coincides with rain events in seasonally-dry ecosystems and is accompanied by pulses of NO$_x$, N$_2$O, CO$_2$ emissions (Davidson et al., 1993; Breuer et al., 2000; Garcia-Montiel, 2003; Butterbach-Bahl et al., 2004; van Haren et al., 2005), which have variable length and magnitude, depending on fire occurrence, plant cover,
soil nutrient status and soil matrix potential (Pinto et al., 2002; Rees et al., 2006; Williams et al., 2009). They are generally enhanced by wetting-drying cycles (Davidson et al. 1993; Mills and Fey, 2004; van Haren et al., 2005; Jenerette et al., 2008).

Fire may influence the rate of soil CO$_2$ efflux by changing the contribution of autotrophic respiration to total soil CO$_2$ emissions and by modifying the amount of soil organic matter in the top soil. Burning of grasslands often results in earlier growth of grass in the growing season, increasing the length of the biomass growth period (Ojima et al., 1994). Fire management, by maintaining the dominance of grasses over shrubs and trees, increases detritus in the upper soil centimetres, having grasses a shallower rooting system, compared with shrubs and trees (Ansley et al., 2002). On the other hand, frequent fires (yearly to once every three year) have been reported to lead to a decline in soil C as a result of fire combustion of aboveground biomass, leaf litter and soil organic matter in the upper few centimeters of the soil (Fynn et al., 2003; Knicker, 2007). Fire might also influence gas diffusivity, by changing soil porosity and water balance (Snyman, 2003; Knicker, 2007), which influences soil potential for CH$_4$ oxidation. Most of the available studies on tropical seasonally-dry ecosystems indicate that fire increases the net consumption of CH$_4$ (Castaldi et al., 2006).

Savannas are generally regarded as modest carbon sinks (per surface unit area) (Bombelli et al. 2009), or, where fire frequency is high, they are considered to have an almost neutral carbon balance (Saarnak, 2001, Bombelli et al. 2009). This value is quite uncertainty because it has not been sufficiently investigated, particularly as regards the quantification of fluxes of N$_2$O and CH$_4$. Data on post burning variations of soil greenhouse gas fluxes in savannas are relatively few and do not give a clear and univocal answer. Few of these studies have been conducted in Africa (Levine et al., 1996; Zepp et al., 1996; Andersson et al., 2004b; Michelsen et al., 2004), while most of them refer to South American ecosystems (Castaldi et al., 2006).

The present work investigates the impact of fire on post-burning fluxes of CO$_2$, CH$_4$ and N$_2$O from a grassland ecosystem of central-western Africa. Specific objectives were to verify (a) whether
burning increases the availability of extractable N substrates and stimulates microbial growth,
microbial activity, CO\(_2\), N\(_2\)O and NO production, (b) whether rain events induce gas pulses of CO\(_2\) and N\(_2\)O, the length and magnitude of which is higher in burned areas, and (c) whether fire enhances the soil CH\(_4\) sink.

For this purpose gas fluxes were measured in the field one and eight months after the fire event in burned and unburned plots. A rain simulation experiment was also carried on. Lab experiments were performed with soil, from burned and unburned plots, incubated at different soil water contents and temperatures. The study site was chosen in Congo Brazzaville because of the presence of extended grassland areas where fire is yearly induced.

2 Materials and methods

2.1 Study site

The research site is located in the littoral region of Congo, close to Tchizalamou (4° 17'20.61"13 S and 11°39'22.78" E, Kouilou district, 82 m a.s.l.). The region is covered by a forest-savanna mosaic situated between the coastline and the Mayombe forest (Favier et al., 2004). The present grass and shrub savannas result from two interacting factors: seasonally-dry climate and expansion of populations practising savanna burning. The climate of the Tchizalamou site is a two-season transition equatorial type, characterized by a long dry and cloudy season from mid-May to mid-October, followed by a rainy season from mid-October to mid-May. An optional short dry season may occur around mid-February to mid-March. The mean annual rainfall is about 1200 mm and the annual temperature is about 25° C, with seasonal variations of ca. 5° C (Pointe Noire airport meteorological station 1982–2001). The herbaceous layer in these grasslands is dominated by taller Poaceae such as Loudetia simplex, Loudetia arundinacea, or Andropogon shirensis with some occurrence of short Poaceae, Joncaceae and Cyperaceae (e.g., Ctenium newtonii, Bulbostylis laniceps). The Poaceae Loudetia simplex makes up more than 50% of the above ground biomass of this grassland, which reached about 3.8 Mg ha\(^{-1}\) of dry matter at the end of the rainy season in 2008.
(de Grandcourt et al., submitted). Some shrubs of 1–2 m height are present, in particular *Annona arenaria* (less than 5 ha\(^{-1}\)). The soils are Ferralic Arenosols (FAO classification), homogeneous in the landscape in terms of colour (greyish in upper soil layers to ochre in deep layers), texture (the sand content is > 85%), structure (always distinctive), chemical composition (CEC < 0.5 cmol\(_c\) kg\(^{-1}\) whatever the soil layer). At site, soil presents a bulk density of 1.43 g.cm\(^{-3}\) and a water holding capacity of 20%. The soils in this area have a low clay content, are poor in nutrients and therefore unsuitable for agricultural purposes. Similar soil characteristics are found in most of the coastal region of central Africa (Kouilou region in Republic of Congo, coastal Gabon). The studied area is subjected to wide fire events every year, between mid-June and mid-July, but other fires may occur during the short dry season in February. Fires are generally very rapid and of low intensity. No agriculture or cattle grazing are conducted in the grassland. The only human activity is the mushrooms harvest, occurring at the beginning of the wet season.

2.2 Experimental design

Two intensive experimental campaigns were organized, one starting two weeks after the day of burning (11\(^{th}\) June 2007), during the dry season, and the other eight months after burning, in February 2008. This latter period corresponded to a particularly severe “short dry season”, extending from mid-January to mid-April, within the period generally regarded as “wet season”. As the area is subjected every year to large fires, to create “unburned treatment”, a grassland area of about 1/2 hectare was isolated from fire by burning preventively a perimeter of vegetation around it. To simulate dry-wet cycle events, a 20 m\(^2\) area was rain-sheltered with a transparent plastic tent, mounted at a height of two meters (to limit sun shading and temperature increase) in each treatment (unburned and burned). The tent was supposed to limit partially the input of rain on the plots in the 15 days before the beginning of the gas sampling. Rain simulation was performed with 30 mm of rain. This amount of water corresponds to typical large rain event, based on the analysis of rain events occurring at the station in the two previous years. Each rain-sheltered area, was divided into
4 plots (2 m x 2.5 m), three of which were watered in different moments before the starting of gas sampling (15, 7, 1 day prior gas sampling). One subplot in each treatment under the tent was left unwatered. In order to verify the influence of the tent on gas fluxes and on some key soil characteristics (water content and temperature), another subplot outside the tent was left unwatered.

2.3 Field GHG flux sampling and measurements

CO₂, N₂O and CH₄ fluxes were measured from each plot on 4 replicates. In order to measure N₂O and CH₄ fluxes we used closed static chambers (Hutchinson and Mosier, 1981; Smith et al., 1995). They were made of PVC collars (7 cm high with a diameter of 15 cm), inserted in the soil to about 5 cm depth in a slot previously dug by a metal cutting ring, and chamber lids (20 cm high with a diameter of 15 cm), provided with a 2 way stopcock sampling port. To determine N₂O and CH₄ fluxes, gas (20 ml) was sampled using gas-tight syringes from the chamber headspace at 0, 20 and 60 minutes after closure, stored in pre-evacuated gas-tight vials, which were then sealed with thermal glue and shipped at the end of the campaign to the DSA-SUN (ITALY) for gas chromatographic analysis, which occurred within a month from gas sampling. Soil temperature (HI93510 thermometer, Hanna Instruments Canada Inc., Laval, Quebec) and soil water content (ThetaProbe ML2, Delta-T Device Ltd, Cambridge, UK) were measured in correspondence of each chamber, 5 centimeters from the chamber edge, at each sampling date.

Soil respiration (Rs) was measured using the Li 8100 soil respiration infrared gas analyzer system with a 8 cm-diameter chamber (LiCor Inc, Lincoln, NE, USA) which was placed on collars (7 cm high, 4 collars per each plot) inserted in the soil to a depth of 5 cm inside the PVC collars used for N₂O and CH₄ fluxes measurements. The increase of the CO₂ concentration was measured over an interval of 2 minutes (including a dead band of 30 seconds) starting at the ambient CO₂ concentration. Soil temperature at 0-40 cm depth was monitored simultaneously with soil CO₂ efflux using a copper/constantan thermocouple penetration probe (Li6000-09 TC, LiCor Inc) in the vicinity of the soil respiration chamber. This temperature was used to normalize soil respiration.
values at 25°C, using the temperature function:  \( R_{(T)} = R_{(25)} \cdot Q_{10}^{(T-25)/10} \), with a \( Q_{10} \) value equal to 2.9, as determined by Epron et al. (2004). This normalization was used to compare the effect of wetting treatments independently from temperature effect on soil respiration. In fact, soil respiration was measured in different hours of the day depending on the daily experimental load with a variation of soil temperature in a range of 4.2°C in the first campaign and 2.7°C in the second campaign. The volumetric soil water content in the topsoil layer (0–6 cm) was monitored just after respiration measurements at 5 centimeters from chamber edge.

Gas fluxes were measured at least five times within two weeks in all plots (unburned and burned; unwatered, and watered 1, 7 and 15 days before starting).

2.4 Soil sampling

Soil was sampled at 0-10 cm depth at the end of the two campaign periods from unburned and burned control plots (unwatered) by taking 4 soil cores from each plot. Soil was immediately sieved (2 mm mesh), air dried (2 day max required) and shipped to the department of environmental sciences (DSA-SUN, Italy) and CEH UK for subsequent analyses.

2.5 Laboratory incubations

Two laboratory experiments were set up. In the first (at DSA-SUN, Italy), soil samples from the two treatments were analysed for microbial biomass, net N mineralization, net nitrification, \( N_2O \), NO and \( CO_2 \) emissions under different soil water and soil temperature regimes. For this purpose, 50 g of sieved soil samples were repacked into columns (10 cm height, 5.5 cm inner diameter, volume occupied by the soil was about 50 cm\(^3\)) which were incubated in half litre flasks (on triplicate). Soils were incubated for 15 days at 0, 25%, 50%, 100% of maximum water holding capacity, \( WHC_{\text{max}} \), (\( WHC_{\text{max}} \) 33% for burned and 37% for unburned soil measured on repacked sieved soil
columns) and at two temperatures (25°C and 37°C). In order to observe the pulse effect of water addition on dry soil no pre-incubation was made. The flasks were kept open and losses of water were corrected every day by gravimetical determination. Gas fluxes were measured on days 0, 1, 3, 10 and 15, by closing the flask with air tight lid and sampling gas at \( t_0 \) and after 24 hours. At the beginning and at the end of the incubation (15 days) soil mineral N was determined to quantify mineralization and nitrification net rates, soil microbial biomass was quantified at the end of the experiment.

For the second incubation experiment (at Centre for Ecology and Hydrology, Edinburgh), sieved soil samples (100 g on triplicate) repacked into clear Perspex columns (20 cm height, 5.5 cm inner diameter, volume occupied by soil was around 100 cm\(^3\)) were incubated at 25°C at 10% and 50% of WHC\(_{\text{max}}\). NO emissions were measured one hour, 5 days and 7 days after water addition, using a gas flow-trough system described in details by Dick et al. (2001). NO was analysed by chemiluminescence (42C model, Thermo-Environmental Instrument) and O\(_3\) by UV absorption (427 model, Thermo-Environmental Instrument). \( \text{N}_2\text{O} \) fluxes were determined in the same day (30 minutes after NO flux determination) closing the columns for 30 minutes and sampling 20 ml of gas from the column headspace at \( t_0 \) and \( t_{30} \) min. \( \text{N}_2\text{O} \) concentration in the samples was determined by gas chromatography. Soil cores were left uncapped in between gas sampling events and losses of water were corrected everyday by gravimetical determination

### 2.6 Analytical technique

Soil mineral N was extracted with 0.5 M K\(_2\)SO\(_4\) (1:4 soil extractant \( v/v \)) and filtered with Whatman filter n°42. NH\(_4^+\) and NO\(_3^-\) were then determined on extracts by potentiometric analysis using specific electrodes for NH\(_4^+\) (Model Orion 9512) and NO\(_3^-\) (Model Orion 9707). Soil total carbon and nitrogen content were determined on homogenized soil sample by flash combustion-gas chromatography on a NCS-soil Thermo FlashEA 1112. Net N mineralization and net nitrification rates, over 15 days, were calculated as the difference between the mineral N (NH\(_4^+\) + NO\(_3^-\)) or the
sole NO$_3^-$, respectively, measured after 15 days of incubation and that measured at time zero.

Spectrophotometric ninhydrin method, developed by Moore and Stein (1954), was used to quantify the $\alpha$-amino-N present in soil extracts (soil: extract 1:5) using 0.5 M K$_2$SO$_4$. These techniques allow to quantify soluble proteins, aminoacids and groups containing $\alpha$-amino-group. To estimate soil microbial biomass N a fumigation-extraction method was used. Soil (5g) was fumigated for 24 h with chloroform. The fraction of the cell constituents made available by the fumigation was extracted by shaking the soil with 20 ml of 0.5 M K$_2$SO$_4$ for 1 hour and filtering the suspension with paper filter (Whatman n°42). The ninhydrin-N deriving from the biomass was calculated as $\mu$g ninhydrin-N g$^{-1}$ dry soil of the fumigated samples minus the $\mu$g ninhydrin-N g$^{-1}$ dry soil of the unfumigated samples. From the value obtained it is possible to calculate the $\mu$g biomass N g$^{-1}$ using a conversion factor of 62 (Ocio and Brookes, 1990).

CH$_4$, N$_2$O and CO$_2$ determinations on field and laboratory gas samples carried on at DSA-SUN were made using a gas chromatograph (Fison series 800) equipped with an electron capture detector (ECD) maintained at 280 °C and a flame ionization detector (FID) set up to analyse the three gases on the same sample, using as well a frontflush and a backflush system (Castaldi et al 2004). Column and precolumn were stainless steel packed with Porapak Q, both maintained at 60°C. N$_2$ was used as carrier gas (flow rate 40 cm$^3$ min$^{-1}$). The injection port was provided with a 2 ml loop. N$_2$O analysis at CEH were done using a gas chromatograph (Hewlett Packard, 5890) equipped with an electron capture detector kept a 320°C, columns were stainless steel packed with Porapak Q (oven 60°C) and Ar/CH$_4$ was used as carrier at a flow rate of 40 ml min$^{-1}$. Standards were injected every 20 samples to allow for GC drift.

2.7 Statistical analyses

As the same sites were sampled over consecutive days, a two-way “repeated measurements” analysis of variance (ANOVA) was used to determine differences of gas fluxes, considering treatment and
season as sources of variation of gas fluxes. When the difference was significant (P<0.05) an "all pairwise" comparison was carried out using the “Student Newman-Keul test”. When normality test failed a Kruskal-Wallis ANOVA on ranks was performed. Simple linear regressions, multiple linear regression and non-linear regression analysis were performed to find the relationship between independent and dependent variables (Sigma Stat, Jandel Scientific). To test for significant difference between slopes and intercepts of two different linear regressions an analysis of covariance (ANCOVA) by the GLM procedure was performed using SAS/STAT 9.00 (SAS Institute Inc, Cary, NC, USA). Significant differences were at the P<0.05 level.

3 Results

3.1 Soil parameters

The analysed soil was characterized by a quite acidic pH, slightly higher in the burned plots, and low content of total C and N (Table 1). Total C increased in both treatments after eight months but no significant difference was observed between burned and unburned plots. Total N was significantly higher after 8 months in burned plots, while one month after burning it was equally low in both treatments (Table 1). NH$_4^+$-N was the dominant form of soil mineral N in both sampling campaigns (Table 1), and it was slightly higher in burned plots (Table 1). The soil content of extractable organic N, measured as α-amino-N (aminoacids, peptides, proteins, etc), was much higher than mineral N in both sampling periods, and it was significantly higher in burned plots (Table 1). Soil water content (average of five sampling days over 2 weeks of campaign) measured in unwatered plots was extremely low during both campaigns and, as also observed for soil temperature, it did not change significantly between treatments (Table 1, Fig. 1). The rain-sheltering tent did not seem to influence significantly soil water content and temperature in both campaigns, as pointed out by the comparison of values in the unwatered plots outside and inside the tent (P>0.05 two-way ANOVA).
When the rain event was simulated, the maximum soil water filled pore space (WFPS), measured in the top 5 soil centimetres, was about 42.8% during the first campaign and 51% during the second campaign (Fig. 1). Soil WFPS decreased rapidly after water addition, going back to values comparable to the control plots within 10 days in the first campaign and 7 days in the second campaign (Fig. 1), with no significant difference between burned and unburned treatments (Fig 1).

3.2 Soil respiration

As no difference between CO₂ effluxes measured from unwatered controls outside and under the tent was found, average daily fluxes of soil respiration were calculated using all the replicates from unwatered plots. The average daily soil respiration was 7.7 ± 0.3 mg of CO₂ m⁻² day⁻¹ (unburned) and 5.5 ± 0.1 (burned) mg of CO₂ m⁻² day⁻¹ (calculated from 12 days of measurements) one month after burning. Eight months after burning it was 11.8 ± 0.9 mg of CO₂ m⁻² day⁻¹ (unburned) and 12.76 ± 1.7 (burned) mg of CO₂ m⁻² day⁻¹ (calculated from 6 days of measurements). The increase of soil respiration observed in the second campaign for both treatments was statistically significant (P<0.05 using a two-way ANOVA). Only one month after burning (first campaign) the difference between treatments was statistically significant (P<0.05 using a two-way ANOVA). Watering induced a pulse of CO₂ emission, which peaked in the first day after water addition and decreased thereafter, getting back to control (unwatered) level within 10 days and 7 days in the 1ˢᵗ and 2ⁿᵈ campaign, respectively, similarly to what observed for soil WFPS (Fig. 1 and 2). On a daily base CO₂ emissions from unburned watered plots were usually significantly higher than emissions from burned watered plots one month after burning (Fig. 2 A). The maximum soil respiration values induced by watering were just slightly higher eight months after burning, for both treatments.

The normalized soil respiration increased linearly with the increase of soil water filled pores space (WFPS) in both campaigns (Fig 3). The slope of the regression lines which fitted data from burned and unburned plots (equations reported in the Fig. 3) was not significantly different (P>0.05, ANCOVA) in both campaigns, whereas the y-axis intercept was significantly higher in the
unburned plots compared with the burned plots (P<0.0001, ANCOVA) in both campaign. No significant difference was observed between the frequency distribution of soil respiration values for unburned and burned treatments in both campaigns, and data were normally distributed (Kolmogorov-Smirnov test)

3.3 Methane fluxes

CH$_4$ flux in unwatered plots (the average of data from outside and under the tent) resulted in a net emission in the unburned plots (0.70 ± 0.62 mg CH$_4$ m$^{-2}$ day$^{-1}$) and in a net, although weak, sink in the burned plots (-0.34 ± 0.27 mg CH$_4$ m$^{-2}$ day$^{-1}$) one month after burning (1$^{st}$ campaign). The difference was statistically significant (P<0.05). Eight months after burning (second campaign) the site acted as a weak net CH$_4$ sink in both unburned (-0.40 ± 0.57 mg CH$_4$ m$^{-2}$ day$^{-1}$) and burned (-0.75 ± 0.75 mg CH$_4$ m$^{-2}$ day$^{-1}$) plots, having here no significant difference between treatments. CH$_4$ fluxes were quite variable, as shown by the flux distribution of Fig. 4. A significant difference in the distribution of CH$_4$ fluxes among flux size classes was observed for the two treatments one month after burning (Fig. 4). In fact, a clear reduction in the frequency of fluxes above 0.80 mg CH$_4$ m$^{-2}$ day$^{-1}$, and an increase of fluxes below 0 mg CH$_4$ m$^{-2}$ day$^{-1}$ was observed in burned plots (Fig. 4). Eight months after burning the frequency distributions in the two treatments were similar (Fig. 4).

Analysing the relationship between soil water filled pore space and CH$_4$ fluxes, obtained including the watered plots (Fig. 5), we can observe that in the first month after burning most of the fluxes were positive (net emission) in unburned plots and negative (net consumption) in burned plots for values of WFPS below 15%. Above this threshold most of the measured fluxes were positive in both treatments. Eight months after burning there was no clear difference between fluxes measured in the two treatments. The increase of soil WFPS did not induce a clear shift from net CH$_4$ source to net CH$_4$ sink, as observed in the previous campaign, although above 10% of WFPS the frequency of net CH$_4$ emissions increased (Fig. 5).
3.4 Nitrous oxide fluxes

Nitrous oxide fluxes were quite low, and in the second campaign many of the analyzed fluxes were below the detection limit of the used technique (0.7 μg of N₂O m⁻² h⁻¹ for single flux measurement), which were hence assigned a value of zero. The daily average N₂O flux, calculated from 5 days of measurements in both campaigns, was 0.02 ± 0.13 mg of N₂O m⁻² day⁻¹ in unburned plots (range 0.7 to 0.6) and 0.02 ± 0.10 mg of N₂O m⁻² day⁻¹ in burned plots (range -0.4 to 0.5) one month after burning and -0.03 ± 0.11 mg of N₂O m⁻² day⁻¹ in unburned plots (range -0.6 to 0.0) and 0.0 ± 0.4 mg of N₂O m⁻² day⁻¹ in burned plots (range -0.1 to 0.2) eight months after burning. A slight shift of flux frequency distribution toward more positive (emission) fluxes was observed in burned plots, compared with unburned ones (Fig 6). The average flux in the two treatments, however, was not significantly different (Table 2). Water addition did not produce any detectable increase of N₂O emissions (data not shown) neither in unburned or in burned plots.

3.5 Laboratory incubations

In the first incubation experiment 92% of N₂O production occurred within the first day after water addition, thereafter fluxes decreased exponentially within 2 days (data not shown). N₂O emission was significantly stimulated in the soil sampled in burned plots but the difference with soil from unburned plots could be evidenced only above 50% of WHC_max (Fig. 7). N₂O fluxes rose exponentially with increasing water content, faster in the soil from burned plots (Fig. 7, insert). No significant effect of rising temperature from 25 °C to 37°C on N₂O emissions was instead observed even at 100% of WHC_max. The pulse of CO₂ peaked the first day after water addition. This peak accounted for about 50% of the total cumulative CO₂ emitted over about 15 days. At the end of the incubation (day 15), CO₂ emissions from watered soil (25, 50 and 100% of WHC_max) were still significantly higher than CO₂ emissions from unwatered soil (0% WHC_max) (data not shown). Fig. 7, reporting the cumulative CO₂ measured at day 1, 2, 3, 4, 5, 7 and 15 of incubation, shows that respiration was significantly stimulated by water addition at 25% of WHC_max and further water
addition did not change significantly the rate of CO₂ production. The increase of incubation
temperature from 25°C to 37°C stimulated significantly CO₂ production and respiration was
significantly higher in the watered soil from burned plots (Fig. 7). Microbial biomass N, net N
mineralization and net nitrification were lower at 37°C than at 25°C (Fig. 8). Net nitrification was
close to zero at all tested soil water contents at 37°C. At 25°C both nitrification and microbial
biomass showed a maximum between 25 and 50% of WHC_max, whereas at 100% of WHC_max
biomass growth was significantly reduced and no net nitrification was observed. Net N
mineralization increased, with increasing soil water content up to 100% of WHC_max (Fig 7, Fig.8),
similarly to N₂O production. The effect of burning was in most cases not significant, although
values of microbial biomass N were slightly higher in burned plots. Soil from burned plots showed
higher NO emission than soil from unburned plots and NO fluxes were significantly higher at 10%
of WHC_max compared with 50% WHC_max, for both treatments (Fig 9). The NO pulse induced by
water addition (zero flux at time zero, 1 hour before watering, data not shown) reduced
significantly after 5 days of incubation. At 10% WHC_max no N₂O production was detected whereas
at 50% of WHC_max N₂O emissions never exceeded 0.10 ng N g⁻¹ h⁻¹.

4 Discussion

4.1 Soil respiration

Data indicate that fire had only transient effects on soil respiration. Burned plots emitted
significantly less CO₂ than unburned plots during the first campaign (one month after burning, dry
season) but this difference was no longer detectable eight months after burning (growing season).
A first possible explanation for the observed difference in CO₂ emissions one month after burning is
that fire destroyed the above-ground vegetation, which had still about 20% of green biomass at the
moment of fire occurrence, contributing to the maintenance activity of roots of these perennial grass
species. Hence, fire might have reduced the autotrophic component of the soil respiration in burned
plots during the first campaign. Fire also consumed most of the litter, which in these grasslands typically dries out as standing litter before falling on the ground. This litter represents a source of C for microbial respiration during the decomposition process. One season of fire exclusion was not sufficient to vary significantly the soil content of total C in unburned plots. On the contrary, a higher content of extractable α-amino-N was found in burned soil one month after fire. Andersson et al. (2004) reported an increase of dissolved organic C in savanna soils immediately after burning, which he suggested might in part include low molecular weight compounds released from the microbial biomass killed by heating, generally including also peptides and proteins. Laboratory analyses showed that in very dry soils (unwater soil treatment) microbial biomass still persisted after more than a month from sampling, but its activity was limited. However, the addition of water quickly stimulated microbial growth, activity and CO₂ production. A similar recovering capacity of microbes is expected also in the field. The similar rate of increase of CO₂ emissions in function of increasing soil water content observed in the burned and unburned plots (field data), despite the very different condition of plant cover (20% of active plant standing tissue still present in unburned plots), suggests that soil microbial activity might be the main contributor to the enhanced CO₂ efflux observed after water addition. The extra CO₂ flux occurring in the unburned plots at all water contents (higher intercept with y-axis) might be due to root respiration. However, our experimental design did not allow a conclusive partitioning of the CO₂ flux between autotrophic and heterotrophic sources. Both laboratory and field data showed that no less than 10 days were necessary to extinguish the CO₂ pulse generated by water addition in the dry season and that the maximum emission occurred within a day after water addition. Several authors reported quick response of the ecosystem respiration to rain pulses in dry conditions (Jenerette et al 2008, Xu and Baldocchi 2004, Williams et al 2009), but pulses generally came to an end from one to three days. Eight months after burning the pulse peak of CO₂ was comparable to the pulse obtained in the first campaign, but its lifetime was shorter (7 days) and the background (unwatered) rate of soil respiration was higher. In this period both treatments presented similar plant cover density and
grass height, and the soil showed a higher content total C and α-amino N, probably reflecting the higher C and N inputs in the soil associated to roots growth, turnover and exudation. These conditions might have stimulated microbial growth and activity resulting in higher rates of soil respiration but also a faster consuming of substrates made available by water addition to the dry soil (shorter pulse lifetime).

Previous studies in Nigeria, Venezuela and South Africa showed no difference of soil CO₂ emissions between burned and unburned savannas (Adedeji, 1983; Hao et al., 1988; Zepp et al., 1996). A slight stimulating effect of fire on soil respiration was found in Brazilian cerrado, but only after wetting the soil (Poth et al., 1995). Michelsen et al. (2004) found higher soil respiration in forest and woodland subject to sporadic burning compared with frequently burnt grasslands.

Similarly to the results found in the present study, lower soil respiration rates were found in burned grassland savannah areas in Ethiopia compared with unburned areas (Andersson et al., 2004).

4.2 Methane fluxes

The very low water content of the soil at the time of measurements, and its loose structure, mainly dominated by sand, is expected to create favourable conditions for significant methanotrophic activity (Striegl et al., 1992; Potter et al., 1996; Castaldi and Fierro, 2005; Castaldi et al., 2006). However, data showed that the unburned grassland plots were a CH₄ source rather than a good sink. Similar results were previously reported for some tropical ecosystems (Hao et al., 1988; Poth et al., 1995; Scharffe et al., 1990; Sanhueza et al., 1994; Zepp et al., 1996: Castaldi et al., 2004; Brümmer et al., 2009). We observed net CH₄ emissions even at 7% of WFPS, hence at very dry conditions, which makes quite unlikely for CH₄ source to derive from anaerobic hotspots of microbial activity, as hypothesised in other studies (Castaldi et al., 2004; Verchot et al., 2000). A more probable source of CH₄ might be termite activity, also considering that site presented a very high abundance of termite nests. Care was taken at the moment of sampling to keep distant from termite nests,
however termite activity can occur several meters far from the nest, and the pattern of this source
cannot be easily predicted. Fire reduced significantly the frequency of net CH₄ emissions, and this
was particularly evident one month after burning. We could hypothesise that fire temporarily
reduced termite activity outside the nest. Indeed even CH₄ production inside the termite nests was
significantly reduced in the first months after burning (Castaldi and de Grandcourt, in preparation).
Other authors have evidenced that clearance of savanna soil surface (grasses and litter) by burning
produces a significant reduction of the methane emission from the soil-grass system (Poth et al.,
1995; Zepp et al., 1996). Indeed, with the destruction of most of the litter, burning reduces that
amount of palatable substrate that termites can use, either directly as litter or as soil organic matter.
Soil-feeding termites, as those belonging to the genus Cubitermes or those feeding on litter such as
Nasutitermes, both found at the site, may be affected by substrate reduction as a consequence of
fire. As observed for CO₂ fluxes, the effect of fire on CH₄ fluxes was transient. In fact eight months
after burning the frequency distribution of CH₄ fluxes in the two treatments was not different.

4.3 Nitrous oxide emissions
The analysed grassland ecosystem showed extremely low N₂O fluxes, as also found in previous
studies in savannas characterized by acidic and nutrient poor soils (Scholes et al., 1997; Andersson
et al. 2004a,b: Castaldi et al., 2006). In general, the range of fluxes measured in undisturbed
savanna ecosystems is quite narrow, going from small uptake values to few mg N₂O-N m⁻² day⁻¹
(Castaldi et al., 2006), except if soil receives significant amount of fertilizer (Brümmer et al., 2008).
Higher N₂O fluxes in undisturbed savannas characterized by nutrient poor soil were found only in
isolated patches of nutrient rich soil (Otter and Scholes, 2000) or in savannas located in valleys
characterized by higher soil water retention and accumulation of organic matter (Sanhueza et al.,
1990). Higher N₂O fluxes in seasonally dry tropical environments are reported only for forests
(Sanhueza et al., 1990; Verchot et al., 1999, Castaldi et al., 2006). A combination of environmental
factors contribute to keep N₂O fluxes low: good soil drainage, low pH and low nutrient status
(Castaldi et al., 2006), as reported in the present study. During the dry season the low soil water content represents a strong controlling factor, which limits the possibility of development of anaerobic microsites, where N₂O production could take place (Firestone and Davidson, 1989; Smith, 1990). In the studied site, the water filled pore space (WFPS %) was always below 51%, even immediately after a big rain event simulation. The very good drainage and sandy texture of the soil would probably not allow much higher WFPS even in case of more frequent rain events (onset of rain season), thus keeping the WFPS value below the level at which O₂ diffusion is sufficiently reduced to allow a sharp increase of N₂O production (Davidson, 1991). In fact, N₂O production by denitrification generally increases exponentially between 60% and 90% of WFPS, but also N₂O production by nitrifiers improves as soil water content increases and aeration becomes restricted, with optimum values around 60% of WFPS (Davidson, 1991). Coherently with these results, the rain simulation at our site did not induce a significant increase of N₂O emissions. Laboratory incubations showed that only above 75% of WHC\textsubscript{max} the increase of N₂O production was sharper. This water content was much higher than the content required to stimulate significantly CO₂ production. A second limiting factor for N₂O production in this ecosystem is represented by the low content of soil C and N, in particular N in the form of mineral N. During the dry season, mineralization and nitrification activity might produce low amounts of mineral N, as demonstrated by laboratory data. At the onset of the rain season, the increase of soil water content might significantly stimulate mineralization activity leading to an increase of mineral N availability. This would favour the occurrence of pulses of NO and N₂O, although the resprouting of shrubs and growth of herbaceous plants may lead to competition for mineral N between plants and microbes (Bate, 1981). The manipulation rain experiments proposed in this and other studies are probably not sufficient to simulate the conditions occurring at the onset of the rain season and hence lower rates of N₂O emissions should be expected compared with those that could be measured when rain season starts. Laboratory data showed one small rain event was sufficient to treble NO emission in burned plots, although the pulse was quite short-lived. Similar results were shown in Brasilian
cerrado by Poth et al. (1995) who measured NO fluxes in watered burned sites (fire 1 day or 1 month before) up to three times higher than fluxes from unburned sites. As previously reported by Levine et al. (1996) and Johansson et al. (1988) data suggest that N gaseous emissions in burned savannas and tropical grassland might be dominated by NO and that N₂O might represent only a minor fraction. Fast spreading fires, such as those occurring in grassland savannas, do not seem to affect microbial biomass and activities involved in N transformations. Higher N₂O emissions following burning might be expected only in soils with high clay content and poor drainage during the rain season.

5 Conclusions

Laboratory and field observations showed that fire increases soil availability of extractable N, both as mineral N and organic compounds, and significantly stimulate NO production even at low soil water content. On the contrary, only at very high soil water content (above 70% of WHCmax) we observed significant N₂O fluxes, which resulted higher in the burned area soil. Hence, isolated rain events in the field may not be sufficient to support significant N₂O losses from both unburned and burned areas. On the contrary, even small rain pulse seem to stimulate CO₂ emissions. However, no enhancement of soil respiration was observed in the burned plots in the two field campaigns. The studied soil was not a CH₄ sink as expected on the base of soil and climatic characteristics, and during at least part of the year, it was a slight CH₄ source, even in very dry conditions. However, fire shifted the CH₄ source/sink towards more negative values (consumption). Although data showed that fire did not increase post-burning soil GHG emissions these findings would need a more extended spatial replication and repeated temporal observations to be generalized.

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References


Table 1 – Some soil chemical characteristics of the unburned and burned plots soils measured during the first (one month after burning) and second field campaign (eight months after burning) for the top 10 cm of soil. Different superscript letters indicate significant differences (two-Way ANOVA, $P<0.005$) among values in the same row.

<table>
<thead>
<tr>
<th></th>
<th>1\textsuperscript{st} campaign</th>
<th>2\textsuperscript{nd} campaign</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unburned</td>
<td>Burned</td>
</tr>
<tr>
<td>pH</td>
<td>3.65\textsuperscript{a} ± 0.03</td>
<td>3.73\textsuperscript{a} ± 0.01</td>
</tr>
<tr>
<td>Total soil C (%)</td>
<td>0.95\textsuperscript{a} ± 0.07</td>
<td>1.17\textsuperscript{ab} ± 0.99</td>
</tr>
<tr>
<td>Total soil N(%)</td>
<td>0.06\textsuperscript{b} ± 0.01</td>
<td>0.08\textsuperscript{b} ± 0.01</td>
</tr>
<tr>
<td>$\text{NH}_4$-N $\mu$g N g$^{-1}$ d.s.</td>
<td>2.72\textsuperscript{b} ± 0.01</td>
<td>3.44\textsuperscript{b} ± 0.70</td>
</tr>
<tr>
<td>$\text{NO}_3$-N $\mu$g N g$^{-1}$ d.s.</td>
<td>0.34\textsuperscript{ab} ± 0.07</td>
<td>0.38\textsuperscript{bc} ± 0.04</td>
</tr>
<tr>
<td>$\alpha$-amino-N $\mu$g N g$^{-1}$ d.s.</td>
<td>13.50\textsuperscript{a} ± 5.92</td>
<td>26.42\textsuperscript{c} ± 1.64</td>
</tr>
<tr>
<td>Volumetric water content (%)*</td>
<td>3.6\textsuperscript{ab} ± 0.2</td>
<td>3.9\textsuperscript{b} ± 0.2</td>
</tr>
<tr>
<td>Soil temperature °C*</td>
<td>26.7\textsuperscript{a} ± 1.2</td>
<td>26.5\textsuperscript{a} ± 1.2</td>
</tr>
</tbody>
</table>

\* average of 5 days of field measurements taken during 2 weeks of campaign
Figure captions

Figure 1 - Soil water filled pore space (%) (0-10 cm soil depth) in function of time after watering (days) during the 1st campaign (A) one month after burning and the 2nd campaign (B) eight months after burning. The vertical bars correspond to one standard error (n=3 to 12).

Figure 2 - Soil respiration normalized at 25°C (g CO$_2$ m$^{-2}$ d$^{-1}$) in function of time after watering during the 1st campaign (A) one month after burning and the 2nd campaign (B) eight months after burning. The vertical bars correspond to one standard error (n=3 to 9).

Figure 3 - Soil respiration normalized at 25°C (g CO$_2$ m$^{-2}$ d$^{-1}$) in function of water filled pores space (%) during the 1st campaign (A) one month after burning and the 2nd campaign (B) eight months after burning. One point is one single-chamber measurement. Lines (continuous unburned, dotted burned) represent linear regressions indicated by the correspondent equations.

Figure 4 - The frequency distribution of single-chamber estimates of CH$_4$ fluxes (mg CH$_4$ m$^{-2}$ d$^{-1}$) measured in unburned and burned plots (no water treatment) during the 1st campaign (A) one month after burning (n=32) and the 2nd campaign (B) eight months after burning (n=30). The range of each size class interval is 0.2 mg CH$_4$ m$^{-2}$ d$^{-1}$.

Figure 5 – Single chamber CH$_4$ fluxes (mg CH$_4$ m$^{-2}$ d$^{-1}$) plotted versus soil water filled pore space (%) reported for the 1st campaign (A) one month after burning and the 2nd campaign (B) eight months after burning. In graph A line represents the fit of data from burned plots ($y_0 = -0.53; a = 0.008; b = 1.54; R^2 = 0.76$).
Figure 6 - The frequency distribution of single-chambers estimates of \( \text{N}_2\text{O} \) fluxes (mg \( \text{N}_2\text{O}-\text{N} \text{ m}^{-2} \text{ d}^{-1} \)) measured in unburned and burned plots (no water treatment) during the 1\(^{st}\) campaign (A) one month after burning (n=32) and the 2\(^{nd}\) campaign (B) eight months after burning (n=30). The range of each size class interval is 0.2 mg \( \text{N}_2\text{O}-\text{N} \text{ m}^{-2} \text{ d}^{-1} \).

Figure 7 – (A) Emissions of \( \text{N}_2\text{O} \) (ng \( \text{N}_2\text{O} \text{ g}^{-1} \text{ soil dry weight h}^{-1} \)) measured during the day of maximal \( \text{N}_2\text{O} \) production after water addition (day 1) and (B) cumulative \( \text{CO}_2 \) emissions (mg \( \text{CO}_2 \text{ g}^{-1} \text{ soil dry weight} \)) over 15 days from soil of burned and unburned plots incubated at 0, 25, 50 and 100 % of water saturation (WS) and at two temperatures (25°C or 37°C). In the small insert is plotted \( \text{N}_2\text{O} \) emissions (ng \( \text{N}_2\text{O} \text{ g}^{-1} \text{ soil dry weight h}^{-1} \)) vs. % of WHCmax. Bars are one standard deviation.

Figure 8 – Microbial biomass N (µg N g\(^{-1}\) soil dry weight) and rates of net N mineralization (µg N g\(^{-1}\) soil dry weight) and net nitrification (µg N g\(^{-1}\) soil dry weight) measured after 2 weeks of incubation in soil from burned and unburned plots incubated at 0, 25, 50 and 100 % of WHCmax and at two temperatures (25°C or 37°C).

Figure 9 – Emissions of NO (ng NO-N d\(^{-1}\) soil dry weight h\(^{-1}\)) measured from soil of burned and unburned plots incubated at 10% and 50% of WHCmax.