

Fluxes of CO₂, CH₄ and N₂O from soil of burned grassland of central Africa

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1 Abstract

2 In the present study the impact of fire on post-burning soil fluxes of CO₂, CH₄ and N₂O was
3 investigated in a tropical grassland in Congo Brazzaville during two field campaigns. The first was
4 conducted in the middle of the dry season, one month after burning and the second eight months
5 after burning, at the end of the growing season. Gas fluxes and several soil parameters were
6 measured in each campaign from burned plots and from an area preserved from annual burning
7 (unburned). Rain events were simulated at each campaign to evaluate magnitude and length of the
8 generated GHG flux pulses. In laboratory experiments, soil samples from the two treatments were
9 analysed for microbial biomass, net N mineralization, net nitrification, N₂O, NO and CO₂ emissions
10 under different soil water and soil temperature regimes. Results showed that fire had only a
11 transient effect on CO₂ soil emissions and CH₄ fluxes. In fact, one month after burning CO₂ fluxes
12 were significantly lower in the burned plots and the average daily CH₄ flux shifted from net
13 emission in the unburned area to net consumption in the burned plots. After eight months from
14 burning, no significant difference of both average daily CO₂ and CH₄ fluxes was observed between
15 treatments. No significant effect of fire was observed in the field on N₂O fluxes. In fact, laboratory
16 data showed that such difference could be seen only above 70% of maximum soil water holding
17 capacity. This condition of soil water content would be hardly reached in the field even after the
18 rain simulation, due to the sandy texture of this soil. In the laboratory, a stimulating effect of fire on
19 NO soil emissions at low soil water content was observed. Increasing the incubation temperature
20 from 25°C to 37°C affected negatively microbial growth, mineralization and nitrification activities
21 but stimulated N₂O and CO₂ production. Overall, data indicate that fire would have a minor impact
22 on soil GHG emissions during the post-burning period in tropical grasslands having similar soil
23 characteristics (acidic, well drained, nutrient poor)..

24

25 1 Introduction

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

13 Savanna fires also influence nutrient cycling patterns by modifying plant cover and biodiversity
14 (Menault, 1977; Swaine et al., 1992; Sankaran et al., 2005) and by changing the chemical,
15 biological and physical characteristics of soil (Menault et al., 1993; Andersson et al., 2004a,b).
16 Enhanced rates of mineralization and nitrification have been reported in burned savannas at the
17 onset of rain season (Adedjii, 1983; Singh et al., 1991). Soil NH_4^+ concentration was found to
18 increase in savanna and shrubland soils after burning (Christensen, 1973; Singh et al., 1994;
19 Castaldi and Aragosa, 2002), as result of organic matter combustion and temperature induced
20 release from organo-mineral soil complexes (Raison, 1979; Kovacic et al., 1986; Prieto-Fernandez
21 et al., 2004). Andersson et al. (2004b) measured increased values of NH_4^+ , dissolved organic N and
22 C in savanna soil after burning, which supported higher rates of mineralization and nitrification
23 when soil water content allowed microbial activation. This generally coincides with rain events in
24 seasonally-dry ecosystems and is accompanied by pulses of NO_x , N_2O , CO_2 emissions (Davidson
25 et al., 1993; Breuer et al., 2000; Garcia-Montiel, 2003; Butterbach-Bahl et al., 2004; van Haren et
26 al., 2005), which have variable length and magnitude, depending on fire occurrence, plant cover,

1 soil nutrient status and soil matrix potential (Pinto et al., 2002; Rees et al., 2006; Williams et al.,
2 2009). They are generally enhanced by wetting-drying cycles (Davidson et al. 1993; Mills and Fey,
3 2004; van Haren et al., 2005; Jenerette et al., 2008).

4 Fire may influence the rate of soil CO₂ efflux by changing the contribution of autotrophic
5 respiration to total soil CO₂ emissions and by modifying the amount of soil organic matter in the
6 top soil. Burning of grasslands often results in earlier growth of grass in the growing season,
7 increasing the length of the biomass growth period (Ojima et al., 1994). Fire management, by
8 maintaining the dominance of grasses over shrubs and trees, increases detritus in the upper soil
9 centimetres, having grasses a shallower rooting system, compared with shrubs and trees (Ansley et
10 al., 2002). On the other hand, frequent fires (yearly to once every three year) have been reported to
11 lead to a decline in soil C as a result of fire combustion of aboveground biomass, leaf litter and soil
12 organic matter in the upper few centimeters of the soil (Fynn et al., 2003; Knicker, 2007). Fire
13 might also influence gas diffusivity, by changing soil porosity and water balance (Snyman, 2003;
14 Knicker, 2007), which influences soil potential for CH₄ oxidation. Most of the available studies on
15 tropical seasonally-dry ecosystems indicate that fire increases the net consumption of CH₄ (Castaldi
16 et al., 2006).

17 Savannas are generally regarded as modest carbon sinks (per surface unit area) (Bombelli et al.
18 2009), or, where fire frequency is high, they are considered to have an almost neutral carbon
19 balance (Saarnak, 2001, Bombelli et al. 2009). This value is quite uncertainty because it has not
20 been sufficiently investigated, particularly as regards the quantification of fluxes of N₂O and CH₄.
21 Data on post burning variations of soil greenhouse gas fluxes in savannas are relatively few and do
22 not give a clear and univocal answer. Few of these studies have been conducted in Africa (Levine et
23 al., 1996; Zepp et al., 1996; Andersson et al., 2004b; Michelsen et al., 2004), while most of them
24 refer to South American ecosystems (Castaldi et al., 2006).

25 The present work investigates the impact of fire on post-burning fluxes of CO₂, CH₄ and N₂O from
26 a grassland ecosystem of central-western Africa. Specific objectives were to verify (a) whether

1 burning increases the availability of extractable N substrates and stimulates microbial growth,
2 microbial activity, CO₂, N₂O and NO production, (b) whether rain events induce gas pulses of CO₂
3 and N₂O, the length and magnitude of which is higher in burned areas, and (c) whether fire
4 enhances the soil CH₄ sink.

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

10

11 2 Materials and methods

12 2.1 Study site

13 The research site is located in the littoral region of Congo, close to Tchizalamou (4° 17'20.61"13 S
14 and 11°39'22.78" E, Kouilou district, 82 m a.s.l.). The region is covered by a forest-savanna mosaic
15 situated between the coastline and the Mayombe forest (Favier et al., 2004). The present grass and
16 shrub savannas result from two interacting factors: seasonally-dry climate and expansion of
17 populations practising savanna burning. The climate of the Tchizalamou site is a two-season
18 transition equatorial type, characterized by a long dry and cloudy season from mid-May to mid-
19 October, followed by a rainy season from mid-October to mid-May. An optional short dry season
20 may occur around mid-February to mid-March. The mean annual rainfall is about 1200 mm and the
21 annual temperature is about 25° C, with seasonal variations of ca. 5° C (Pointe Noire airport
22 meteorological station 1982–2001). The herbaceous layer in these grasslands is dominated by taller
23 Poaceae such as *Loudetia simplex*, *Loudetia arundinacea*, or *Andropogon shirensis* with some
24 occurrence of short Poaceae, Joncaceae and Cyperaceae (e.g., *Ctenium newtonii*, *Bulbostylis*
25 *laniceps*). The Poaceae *Loudetia simplex* makes up more than 50% of the above ground biomass of
26 this grassland, which reached about 3.8 Mg ha⁻¹ of dry matter at the end of the rainy season in 2008

1 (de Grandcourt et al., submitted). Some shrubs of 1–2 m height are present, in particular *Annona*
2 *arenaria* (less than 5 ha⁻¹). The soils are Ferralic Arenosols (FAO classification), homogeneous in
3 the landscape in terms of colour (greyish in upper soil layers to ochre in deep layers), texture (the
4 sand content is > 85%), structure (always distinctive), chemical composition (CEC < 0.5 cmol_c kg⁻¹
5 whatever the soil layer). At site, soil presents a bulk density of 1.43 g.cm⁻³ and a water holding
6 capacity of 20%. The soils in this area have a low clay content, are poor in nutrients and therefore
7 unsuitable for agricultural purposes. Similar soil characteristics are found in most of the coastal
8 region of central Africa (Kouilou region in Republic of Congo, coastal Gabon). The studied area is
9 subjected to wide fire events every year, between mid-June and mid-July, but other fires may occur
10 during the short dry season in February. Fires are generally very rapid and of low intensity. No
11 agriculture or cattle grazing are conducted in the grassland. The only human activity is the
12 mushrooms harvest, occurring at the beginning of the wet season.

13

14 2.2 Experimental design

15 Two intensive experimental campaigns were organized, one starting two weeks after the day of
16 burning (11th June 2007), during the dry season, and the other eight months after burning, in
17 February 2008. This latter period corresponded to a particularly severe “short dry season”,
18 extending from mid-January to mid-April, within the period generally regarded as “wet season”. As
19 the area is subjected every year to large fires, to create “unburned treatment”, a grassland area of
20 about 1/2 hectare was isolated from fire by burning preventively a perimeter of vegetation around it.
21 To simulate dry-wet cycle events, a 20 m² area was rain-sheltered with a transparent plastic tent,
22 mounted at a height of two meters (to limit sun shading and temperature increase) in each treatment
23 (unburned and burned). The tent was supposed to limit partially the input of rain on the plots in the
24 15 days before the beginning of the gas sampling. Rain simulation was performed with 30 mm of
25 rain. This amount of water corresponds to typical large rain event, based on the analysis of rain
26 events occurring at the station in the two previous years. Each rain-sheltered area, was divided into

1 4 plots (2 m x 2.5 m), three of which were watered in different moments before the starting of gas
2 sampling (15, 7, 1 day prior gas sampling). One subplot in each treatment under the tent was left
3 unwatered. In order to verify the influence of the tent on gas fluxes and on some key soil
4 characteristics (water content and temperature), another subplot outside the tent was left unwatered.

5

6 2.3 Field GHG flux sampling and measurements

7 CO₂, N₂O and CH₄ fluxes were measured from each plot on 4 replicates. In order to measure N₂O
8 and CH₄ fluxes we used closed static chambers (Hutchinson and Mosier, 1981; Smith et al., 1995).
9 They were made of PVC collars (7 cm high with a diameter of 15 cm), inserted in the soil to about 5
10 cm depth in a slot previously dug by a metal cutting ring, and chamber lids (20 cm high with a
11 diameter of 15 cm), provided with a 2 way stopcock sampling port. To determine N₂O and CH₄
12 fluxes, gas (20 ml) was sampled using gas-tight syringes from the chamber headspace at 0, 20 and
13 60 minutes after closure, stored in pre-evacuated gas-tight vials, which were then sealed with
14 thermal glue and shipped at the end of the campaign to the DSA-SUN (ITALY) for gas
15 chromatographic analysis, which occurred within a month from gas sampling. Soil temperature
16 (HI93510 thermometer, Hanna Instruments Canada Inc., Laval, Quebec) and soil water content
17 (ThetaProbe ML2, Delta-T Device Ltd, Cambridge, UK) were measured in correspondence of each
18 chamber, 5 centimeters from the chamber edge, at each sampling date.

19 Soil respiration (Rs) was measured using the Li 8100 soil respiration infrared gas analyzer system
20 with a 8 cm-diameter chamber (LiCor Inc, Lincoln, NE, USA) which was placed on collars (7 cm
21 high, 4 collars per each plot) inserted in the soil to a depth of 5 cm inside the PVC collars used for
22 N₂O and CH₄ fluxes measurements. The increase of the CO₂ concentration was measured over an
23 interval of 2 minutes (including a dead band of 30 seconds) starting at the ambient CO₂
24 concentration. Soil temperature at 0-40 cm depth was monitored simultaneously with soil CO₂
25 efflux using a copper/constantan thermocouple penetration probe (Li6000-09 TC, LiCor Inc) in the
26 vicinity of the soil respiration chamber. This temperature was used to normalize soil respiration

1 values at 25°C, using the temperature function: $R_{(T)} = R_{(25^\circ)} \cdot Q_{10}^{\frac{(T-25)}{10}}$, with a Q_{10} value equal to 2.9,
2 as determined by Epron et al. (2004). This normalization was used to compare the effect of wetting
3 treatments independently from temperature effects on soil respiration. In fact, soil respiration was
4 measured in different hours of the day depending on the daily experimental load with a variation of
5 soil temperature in a range of 4.2°C in the first campaign and 2.7°C in the second campaign. The
6 volumetric soil water content in the topsoil layer (0–6 cm) was monitored just after respiration
7 measurements at 5 centimeters from chamber edge.
8 Gas fluxes were measured at least five times within two weeks in all plots (unburned and burned;
9 unwatered, and watered 1, 7 and 15 days before starting).

10

11 2.4 Soil sampling

12 Soil was sampled at 0-10 cm depth at the end of the two campaign periods from unburned and
13 burned control plots (unwatered) by taking 4 soil cores from each plot. Soil was immediately sieved
14 (2 mm mesh), air dried (2 day max required) and shipped to the department of environmental
15 sciences (DSA-SUN, Italy) and CEH UK for subsequent analyses.

16

17 2.5 Laboratory incubations

18 Two laboratory experiments were set up. In the first (at DSA-SUN, Italy), soil samples from the
19 two treatments were analysed for microbial biomass, net N mineralization, net nitrification, N_2O ,
20 NO and CO_2 emissions under different soil water and soil temperature regimes. For this purpose, 50
21 g of sieved soil samples were repacked into columns (10 cm height, 5.5 cm inner diameter, volume
22 occupied by the soil was about 50 cm³,) which were incubated in half litre flasks (on triplicate).
23 Soils were incubated for 15 days at 0, 25%, 50%, 100% of maximum water holding capacity,
24 WHC_{max} , (WHC_{max} 33% for burned and 37% for unburned soil measured on repacked sieved soil

1 columns) and at two temperatures (25°C and 37°C). In order to observe the pulse effect of water
2 addition on dry soil no pre-incubation was made. The flasks were kept open and losses of water
3 were corrected every day by gravimetric determination. Gas fluxes were measured on days 0, 1, 3,
4 10 and 15, by closing the flask with air tight lid and sampling gas at t_0 and after 24 hours. At the
5 beginning and at the end of the incubation (15 days) soil mineral N was determined to quantify
6 mineralization and nitrification net rates, soil microbial biomass was quantified at the end of the
7 experiment.

8 For the second incubation experiment (at Centre for Ecology and Hydrology, Edinburgh), sieved
9 soil samples (100 g on triplicate) repacked into clear Perspex columns (20 cm height, 5.5 cm inner
10 diameter, volume occupied by soil was around 100 cm³) were incubated at 25°C at 10% and 50% of
11 WHC_{max}. NO emissions were measured one hour, 5 days and 7 days after water addition, using a
12 gas flow-trough system described in details by Dick et al. (2001). NO was analysed by
13 chemiluminescence (42C model, Thermo-Environmental Instrument) and O₃ by UV absorption
14 (427 model, Thermo-Environmental Instrument). N₂O fluxes were determined in the same day (30
15 minutes after NO flux determination) closing the columns for 30 minutes and sampling 20 ml of gas
16 from the column headspace at t_0 and t_{30} min. N₂O concentration in the samples was determined by
17 gas chromatography. Soil cores were left uncapped in between gas sampling events and losses of
18 water were corrected everyday by gravimetric determination

19

20 2.6 Analytical technique

21 Soil mineral N was extracted with 0.5 M K₂SO₄ (1:4 soil extractant v/v) and filtered with Whatman
22 filter n°42. NH₄⁺ and NO₃⁻ were then determined on extracts by potentiometric analysis using
23 specific electrodes for NH₄⁺ (Model Orion 9512) and NO₃⁻ (Model Orion 9707). Soil total carbon
24 and nitrogen content were determined on homogenized soil sample by flash combustion-gas
25 chromatography on a NCS-soil Thermo FlashEA 1112. Net N mineralization and net nitrification
26 rates, over 15 days, were calculated as the difference between the mineral N (NH₄⁺ + NO₃⁻) or the

1 sole NO_3^- , respectively, measured after 15 days of incubation and that measured at time zero.
2 Spectrophotometric ninhydrin method, developed by Moore and Stein (1954), was used to quantify
3 the α -amino-N present in soil extracts (soil: extract 1:5) using 0.5 M K_2SO_4 . These techniques
4 allow to quantify soluble proteins, aminoacids and groups containing α -amino-group. To estimate
5 soil microbial biomass N a fumigation-extraction method was used. Soil (5g) was fumigated for 24
6 h with chloroform. The fraction of the cell constituents made available by the fumigation was
7 extracted by shaking the soil with 20 ml of 0.5 M K_2SO_4 for 1 hour and filtering the suspension
8 with paper filter (Whatman n°42). The ninhydrin-N deriving from the biomass was calculated as μg
9 ninhydrin-N g^{-1} dry soil of the fumigated samples minus the μg ninhydrin-N g^{-1} dry soil of the
10 unfumigated samples. From the value obtained it is possible to calculate the μg biomass N g^{-1} using
11 a conversion factor of 62 (Ocio and Brookes, 1990).

12 CH_4 , N_2O and CO_2 determinations on field and laboratory gas samples carried on at DSA-SUN
13 were made using a gas chromatograph (Fison series 800) equipped with an electron capture detector
14 (ECD) maintained at 280 °C and a flame ionization detector (FID) set up to analyse the three gases
15 on the same sample, using as well a frontflush and a backflush system (Castaldi et al 2004). Column
16 and precolumn were stainless steel packed with Porapak Q, both maintained at 60°C. N_2 was used
17 as carrier gas (flow rate 40 $\text{cm}^3 \text{min}^{-1}$). The injection port was provided with a 2 ml loop. N_2O
18 analysis at CEH were done using a gas chromatograph (Hewlett Packard, 5890) equipped with an
19 electron capture detector kept a 320°C, columns were stainless steel packed with Porapak Q (oven
20 60°C) and Ar/ CH_4 was used as carrier at a flow rate of 40 ml min^{-1} . Standards were injected every
21 20 samples to allow for GC drift.

22

23 2.7 Statistical analyses

24 As the same sites were sampled over consecutive days, a two-way “repeated measurements” analysis
25 of variance (ANOVA) was used to determine differences of gas fluxes, considering treatment and

1 season as sources of variation of gas fluxes. When the difference was significant ($P < 0.05$) an "all
2 pairwise" comparison was carried out using the "Student Newman-Keul test". When normality test
3 failed a Kruskal-Wallis ANOVA on ranks was performed. Simple linear regressions, multiple linear
4 regression and non- linear regression analysis were performed to find the relationship between
5 independent and dependent variables (Sigma Stat, Jandel Scientific). To test for significant difference
6 between slopes and intercepts of two different linear regressions an analysis of covariance
7 (ANCOVA) by the GLM procedure was performed using SAS/STAT 9.00 (SAS Institute Inc, Cary,
8 NC, USA). Significant differences were at the $P < 0.05$ level.

9

10 3 Results

11 3.1 Soil parameters

12 The analysed soil was characterized by a quite acidic pH, slightly higher in the burned plots, and
13 low content of total C and N (Table 1). Total C increased in both treatments after eight months but
14 no significant difference was observed between burned and unburned plots. Total N was
15 significantly higher after 8 months in burned plots, while one month after burning it was equally
16 low in both treatments (Table 1). NH_4^+ -N was the dominant form of soil mineral N in both sampling
17 campaigns (Table 1), and it was slightly higher in burned plots (Table 1). The soil content of
18 extractable organic N, measured as α -amino-N (aminoacids, peptides, proteins, etc), was much
19 higher than mineral N in both sampling periods, and it was significantly higher in burned plots
20 (Table 1). Soil water content (average of five sampling days over 2 weeks of campaign) measured
21 in unwatered plots was extremely low during both campaigns and, as also observed for soil
22 temperature, it did not change significantly between treatments (Table 1, Fig. 1). The rain-sheltering
23 tent did not seem to influence significantly soil water content and temperature in both campaigns, as
24 pointed out by the comparison of values in the unwatered plots outside and inside the tent ($P > 0.05$
25 two-way ANOVA).

1 When the rain event was simulated, the maximum soil water filled pore space (WFPS), measured in
2 the top 5 soil centimetres, was about 42.8 % during the first campaign and 51% during the second
3 campaign (Fig. 1). Soil WFPS decreased rapidly after water addition, going back to values
4 comparable to the control plots within 10 days in the first campaign and 7 days in the second
5 campaign (Fig. 1), with no significant difference between burned and unburned treatments (Fig 1).

6

7 3.2 Soil respiration

8 As no difference between CO₂ effluxes measured from unwatered controls outside and under the
9 tent was found, average daily fluxes of soil respiration were calculated using all the replicates from
10 unwatered plots. The average daily soil respiration was 7.7 ± 0.3 mg of CO₂ m⁻² day⁻¹ (unburned)
11 and 5.5 ± 0.1 (burned) mg of CO₂ m⁻² day⁻¹ (calculated from 12 days of measurements) one month
12 after burning. Eight months after burning it was 11.8 ± 0.9 mg of CO₂ m⁻² day⁻¹ (unburned) and
13 12.76 ± 1.7 (burned) mg of CO₂ m⁻² day⁻¹ (calculated from 6 days of measurements). The increase
14 of soil respiration observed in the second campaign for both treatments was statistically significant
15 ($P < 0.05$ using a two-way ANOVA). Only one month after burning (first campaign) the difference
16 between treatments was statistically significant ($P < 0.05$ using a two-way ANOVA). Watering
17 induced a pulse of CO₂ emission, which peaked in the first day after water addition and decreased
18 thereafter, getting back to control (unwatered) level within 10 days and 7 days in the 1st and 2nd
19 campaign, respectively, similarly to what observed for soil WFPS (Fig, 1 and 2). On a daily base
20 CO₂ emissions from unburned watered plots were usually significantly higher than emissions from
21 burned watered plots one month after burning (Fig. 2 A). The maximum soil respiration values
22 induced by watering were just slightly higher eight months after burning, for both treatments.

23 The normalized soil respiration increased linearly with the increase of soil water filled pores space
24 (WFPS) in both campaigns (Fig 3). The slope of the regression lines which fitted data from burned
25 and unburned plots (equations reported in the Fig. 3) was not significantly different ($P > 0.05$,
26 ANCOVA) in both campaigns, whereas the y-axis intercept was significantly higher in the

1 unburned plots compared with the burned plots ($P < 0.0001$, ANCOVA) in both campaign. No
2 significant difference was observed between the frequency distribution of soil respiration values for
3 unburned and burned treatments in both campaigns, and data were normally distributed
4 (Kolmogorov-Smirnov test)

5

6 3.3 Methane fluxes

7 CH_4 flux in unwatered plots (the average of data from outside and under the tent) resulted in a net
8 emission in the unburned plots ($0.70 \pm 0.62 \text{ mg CH}_4 \text{ m}^{-2} \text{ day}^{-1}$) and in a net, although weak, sink in
9 the burned plots ($-0.34 \pm 0.27 \text{ mg CH}_4 \text{ m}^{-2} \text{ day}^{-1}$) one month after burning (1st campaign). The
10 difference was statistically significant ($P < 0.05$). Eight months after burning (second campaign) the
11 site acted as a weak net CH_4 sink in both unburned ($-0.40 \pm 0.57 \text{ mg CH}_4 \text{ m}^{-2} \text{ day}^{-1}$) and burned ($-$
12 $0.75 \pm 0.75 \text{ mg CH}_4 \text{ m}^{-2} \text{ day}^{-1}$) plots, having here no significant difference between treatments. CH_4
13 fluxes were quite variable, as shown by the flux distribution of Fig. 4. A significant difference in the
14 distribution of CH_4 fluxes among flux size classes was observed for the two treatments one month
15 after burning (Fig. 4). In fact, a clear reduction in the frequency of fluxes above $0.80 \text{ mg CH}_4 \text{ m}^{-2}$
16 day^{-1} , and an increase of fluxes below $0 \text{ mg CH}_4 \text{ m}^{-2} \text{ day}^{-1}$ was observed in burned plots (Fig. 4).
17 Eight months after burning the frequency distributions in the two treatments were similar (Fig. 4).
18 Analysing the relationship between soil water filled pore space and CH_4 fluxes, obtained including
19 the watered plots (Fig. 5), we can observe that in the first month after burning most of the fluxes
20 were positive (net emission) in unburned plots and negative (net consumption) in burned plots for
21 values of WFPS below 15%. Above this threshold most of the measured fluxes were positive in
22 both treatments. Eight months after burning there was no clear difference between fluxes measured
23 in the two treatments. The increase of soil WFPS did not induce a clear shift from net CH_4 source
24 to net CH_4 sink, as observed in the previous campaign, although above 10% of WFPS the
25 frequency of net CH_4 emissions increased (Fig. 5).

26

1 3.4 Nitrous oxide fluxes

2 Nitrous oxide fluxes were quite low, and in the second campaign many of the analyzed fluxes were
3 below the detection limit of the used technique ($0.7 \mu\text{g}$ of $\text{N}_2\text{O m}^{-2} \text{h}^{-1}$ for single flux measurement),
4 which were hence assigned a value of zero. The daily average N_2O flux, calculated from 5 days of
5 measurements in both campaigns, was $0.02 \pm 0.13 \text{ mg}$ of $\text{N}_2\text{O m}^{-2} \text{day}^{-1}$ in unburned plots (range -
6 0.7 to 0.6) and $0.02 \pm 0.10 \text{ mg}$ of $\text{N}_2\text{O m}^{-2} \text{day}^{-1}$ in burned plots (range -0.4 to 0.5) one month after
7 burning and $-0.03 \pm 0.11 \text{ mg}$ of $\text{N}_2\text{O m}^{-2} \text{day}^{-1}$ in unburned plots (range -0.6 to 0.0) and 0.0 ± 0.4
8 mg of $\text{N}_2\text{O m}^{-2} \text{day}^{-1}$ in burned plots (range -0.1 to 0.2) eight months after burning. A slight shift of
9 flux frequency distribution toward more positive (emission) fluxes was observed in burned plots,
10 compared with unburned ones (Fig 6). The average flux in the two treatments, however, was not
11 significantly different (Table 2). Water addition did not produce any detectable increase of N_2O
12 emissions (data not shown) neither in unburned or in burned plots.

13

14 3.5 Laboratory incubations

15 In the first incubation experiment 92% of N_2O production occurred within the first day after water
16 addition, thereafter fluxes decreased exponentially within 2 days (data not shown). N_2O emission
17 was significantly stimulated in the soil sampled in burned plots but the difference with soil from
18 unburned plots could be evidenced only above 50% of WHC_{max} (Fig. 7). N_2O fluxes rose
19 exponentially with increasing water content, faster in the soil from burned plots (Fig. 7, insert). No
20 significant effect of rising temperature from 25°C to 37°C on N_2O emissions was instead observed
21 even at 100% of WHC_{max} . The pulse of CO_2 peaked the first day after water addition. This peak
22 accounted for about 50% of the total cumulative CO_2 emitted over about 15 days. At the end of the
23 incubation (day 15), CO_2 emissions from watered soil (25, 50 and 100% of WHC_{max}) were still
24 significantly higher than CO_2 emissions from unwatered soil (0% WHC_{max}) (data not shown). Fig.
25 7, reporting the cumulative CO_2 measured at day 1, 2, 3, 4, 5, 7 and 15 of incubation, shows that
26 respiration was significantly stimulated by water addition at 25% of WHC_{max} and further water

1 addition did not change significantly the rate of CO₂ production. The increase of incubation
2 temperature from 25°C to 37°C stimulated significantly CO₂ production and respiration was
3 significantly higher in the watered soil from burned plots (Fig. 7). Microbial biomass N, net N
4 mineralization and net nitrification were lower at 37°C than at 25°C (Fig. 8). Net nitrification was
5 close to zero at all tested soil water contents at 37°C. At 25°C both nitrification and microbial
6 biomass showed a maximum between 25 and 50% of WHC_{max}, whereas at 100% of WHC_{max}
7 biomass growth was significantly reduced and no net nitrification was observed. Net N
8 mineralization increased, with increasing soil water content up to 100% of WHC_{max} (Fig 7, Fig.8),
9 similarly to N₂O production. The effect of burning was in most cases not significant, although
10 values of microbial biomass N were slightly higher in burned plots. Soil from burned plots showed
11 higher NO emission than soil from unburned plots and NO fluxes were significantly higher at 10%
12 of WHC_{max} compared with 50% WHC_{max}, for both treatments (Fig 9). The NO pulse induced by
13 water addition (zero flux at time zero, 1 hour before watering, data not shown) reduced
14 significantly after 5 days of incubation. At 10% WHC_{max} no N₂O production was detected whereas
15 at 50% of WHC_{max} N₂O emissions never exceeded 0.10 ng N g⁻¹ h⁻¹.

16

17 4 Discussion

18 4.1 Soil respiration

19 Data indicate that fire had only transient effects on soil respiration. Burned plots emitted
20 significantly less CO₂ than unburned plots during the first campaign (one month after burning, dry
21 season) but this difference was no longer detectable eight months after burning (growing season).
22 A first possible explanation for the observed difference in CO₂ emissions one month after burning is
23 that fire destroyed the above-ground vegetation, which had still about 20% of green biomass at the
24 moment of fire occurrence, contributing to the maintenance activity of roots of these perennial grass
25 species. Hence, fire might have reduced the autotrophic component of the soil respiration in burned

1 plots during the first campaign. Fire also consumed most of the litter, which in these grasslands
2 typically dries out as standing litter before falling on the ground. This litter represents a source of C
3 for microbial respiration during the decomposition process. One season of fire exclusion was not
4 sufficient to vary significantly the soil content of total C in unburned plots. On the contrary, a
5 higher content of extractable α -amino-N was found in burned soil one month after fire. Andersson
6 et al. (2004) reported an increase of dissolved organic C in savanna soils immediately after burning,
7 which he suggested might in part include low molecular weight compounds released from the
8 microbial biomass killed by heating, generally including also peptides and proteins. Laboratory
9 analyses showed that in very dry soils (unwater soil treatment) microbial biomass still persisted
10 after more than a month from sampling, but its activity was limited. However, the addition of water
11 quickly stimulated microbial growth, activity and CO₂ production. A similar recovering capacity of
12 microbes is expected also in the field. The similar rate of increase of CO₂ emissions in function of
13 increasing soil water content observed in the burned and unburned plots (field data), despite the
14 very different condition of plant cover (20% of active plant standing tissue still present in unburned
15 plots), suggests that soil microbial activity might be the main contributor to the enhanced CO₂
16 efflux observed after water addition. The extra CO₂ flux occurring in the unburned plots at all water
17 contents (higher intercept with y-axis) might be due to root respiration. However, our experimental
18 design did not allow a conclusive partitioning of the CO₂ flux between autotrophic and
19 heterotrophic sources. Both laboratory and field data showed that no less than 10 days were
20 necessary to extinguish the CO₂ pulse generated by water addition in the dry season and that the
21 maximum emission occurred within a day after water addition. Several authors reported quick
22 response of the ecosystem respiration to rain pulses in dry conditions (Jenerette et al 2008, Xu and
23 Baldocchi 2004, Williams et al 2009), but pulses generally came to an end from one to three days.
24 Eight months after burning the pulse peak of CO₂ was comparable to the pulse obtained in the first
25 campaign, but its lifetime was shorter (7 days) and the background (unwatered) rate of soil
26 respiration was higher. In this period both treatments presented similar plant cover density and

1 grass height, and the soil showed a higher content total C and α -amino N, probably reflecting the
2 higher C and N inputs in the soil associated to roots growth, turnover and exudation. These
3 conditions might have stimulated microbial growth and activity resulting in higher rates of soil
4 respiration but also a faster consuming of substrates made available by water addition to the dry soil
5 (shorter pulse lifetime).

6 Previous studies in Nigeria, Venezuela and South Africa showed no difference of soil CO₂
7 emissions between burned and unburned savannas (Adedeji, 1983; Hao et al., 1988; Zepp et al.,
8 1996). Aslight stimulating effect of fire on soil respiration was found in Brazilian cerrado, but only
9 after wetting the soil (Poth et al., 1995). Michelsen et al. (2004) found higher soil respiration in
10 forest and woodland subject to sporadic burning compared with frequently burnt grasslands.
11 Similarly to the results found in the present study, lower soil respiration rates were found in burned
12 grassland savannah areas in Ethiopia compared with unburned areas (Andersson et al., 2004).

13

14 4.2 Methane fluxes

15 The very low water content of the soil at the time of measurements, and its loose structure, mainly
16 dominated by sand, is expected to create favourable conditions for significant methanotrophic
17 activity (Striegl et al., 1992; Potter et al., 1996; Castaldi and Fierro, 2005; Castaldi et al., 2006).
18 However, data showed that the unburned grassland plots were a CH₄ source rather than a good sink.
19 Similar results were previously reported for some tropical ecosystems (Hao et al., 1988; Poth et al.,
20 1995; Scharffe et al., 1990; Sanhueza et al., 1994; Zepp et al., 1996; Castaldi et al., 2004; Brümmer
21 et al., 2009). We observed net CH₄ emissions even at 7% of WFPS, hence at very dry conditions,
22 which makes quite unlikely for CH₄ source to derive from anaerobic hotspots of microbial activity,
23 as hypothesised in other studies (Castaldi et al., 2004; Verchot et al., 2000). A more probable source
24 of CH₄ might be termite activity, also considering that site presented a very high abundance of
25 termite nests. Care was taken at the moment of sampling to keep distant from termite nests,

1 however termite activity can occur several meters far from the nest, and the pattern of this source
2 cannot be easily predicted. Fire reduced significantly the frequency of net CH₄ emissions, and this
3 was particularly evident one month after burning. We could hypothesise that fire temporarily
4 reduced termite activity outside the nest. Indeed even CH₄ production inside the termite nests was
5 significantly reduced in the first months after burning (Castaldi and de Grandcourt, in preparation).
6 Other authors have evidenced that clearance of savanna soil surface (grasses and litter) by burning
7 produces a significant reduction of the methane emission from the soil-grass system (Poth et al.,
8 1995; Zepp et al., 1996). Indeed, with the destruction of most of the litter, burning reduces that
9 amount of palatable substrate that termites can use, either directly as litter or as soil organic matter.
10 Soil-feeding termites, as those belonging to the genus *Cubitermes* or those feeding on litter such as
11 *Nasutitermes*, both found at the site, may be affected by substrate reduction as a consequence of
12 fire. As observed for CO₂ fluxes, the effect of fire on CH₄ fluxes was transient. In fact eight months
13 after burning the frequency distribution of CH₄ fluxes in the two treatments was not different.

14 4.3 Nitrous oxide emissions

15 The analysed grassland ecosystem showed extremely low N₂O fluxes, as also found in previous
16 studies in savannas characterized by acidic and nutrient poor soils (Scholes et al., 1997; Andersson
17 et al. 2004a,b; Castaldi et al., 2006). In general, the range of fluxes measured in undisturbed
18 savanna ecosystems is quite narrow, going from small uptake values to few mg N₂O-N m⁻² day⁻¹
19 (Castaldi et al., 2006), except if soil receives significant amount of fertilizer (Brümmer et al., 2008).
20 Higher N₂O fluxes in undisturbed savannas characterized by nutrient poor soil were found only in
21 isolated patches of nutrient rich soil (Otter and Scholes, 2000) or in savannas located in valleys
22 characterized by higher soil water retention and accumulation of organic matter (Sanhueza et al.,
23 1990). Higher N₂O fluxes in seasonally dry tropical environments are reported only for forests
24 (Sanhueza et al., 1990; Verchot et al., 1999, Castaldi et al., 2006). A combination of environmental
25 factors contribute to keep N₂O fluxes low: good soil drainage, low pH and low nutrient status

1 (Castaldi et al., 2006), as reported in the present study. During the dry season the low soil water
2 content represents a strong controlling factor, which limits the possibility of development of
3 anaerobic microsites, where N₂O production could take place (Firestone and Davidson, 1989;
4 Smith, 1990). In the studied site, the water filled pore space (WFPS %) was always below 51%,
5 even immediately after a big rain event simulation. The very good drainage and sandy texture of the
6 soil would probably not allow much higher WFPS even in case of more frequent rain events (onset
7 of rain season), thus keeping the WFPS value below the level at which O₂ diffusion is sufficiently
8 reduced to allow a sharp increase of N₂O production (Davidson, 1991). In fact, N₂O production by
9 denitrification generally increases exponentially between 60% and 90% of WFPS, but also N₂O
10 production by nitrifiers improves as soil water content increases and aeration becomes restricted,
11 with optimum values around 60% of WFPS (Davidson, 1991). Coherently with these results, the
12 rain simulation at our site did not induce a significant increase of N₂O emissions. Laboratory
13 incubations showed that only above 75% of WHC_{max} the increase of N₂O production was sharper.
14 This water content was much higher than the content required to stimulate significantly CO₂
15 production. A second limiting factor for N₂O production in this ecosystem is represented by the low
16 content of soil C and N, in particular N in the form of mineral N. During the dry season,
17 mineralization and nitrification activity might produce low amounts of mineral N, as demonstrated
18 by laboratory data. At the onset of the rain season, the increase of soil water content might
19 significantly stimulate mineralization activity leading to an increase of mineral N availability. This
20 would favour the occurrence of pulses of NO and N₂O, although the resprouting of shrubs and
21 growth of herbaceous plants may lead to competition for mineral N between plants and microbes
22 (Bate, 1981). The manipulation rain experiments proposed in this and other studies are probably
23 not sufficient to simulate the conditions occurring at the onset of the rain season and hence lower
24 rates of N₂O emissions should be expected compared with those that could be measured when rain
25 season starts. Laboratory data showed one small rain event was sufficient to treble NO emission in
26 burned plots, although the pulse was quite short-lived. Similar results were shown in Brazilian

1 cerrado by Poth et al. (1995) who measured NO fluxes in watered burned sites (fire 1 day or 1
2 month before) up to three times higher than fluxes from unburned sites. As previously reported by
3 Levine et al. (1996) and Johansson et al. (1988) data suggest that N gaseous emissions in burned
4 savannas and tropical grassland might be dominated by NO and that N₂O might represent only a
5 minor fraction. Fast spreading fires, such as those occurring in grassland savannas, do not seem to
6 affect microbial biomass and activities involved in N transformations. Higher N₂O emissions
7 following burning might be expected only in soils with high clay content and poor drainage during
8 the rain season.

9

10 5 Conclusions

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

23

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1

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1 Table 1 – Some soil chemical characteristics of the unburned and burned plots soils measured
 2 during the first (one month after burning) and second field campaign (eight months after burning)
 3 for the top 10 cm of soil. Different superscript letters indicate significant differences (two-Way
 4 ANOVA, $P < 0,005$) among values in the same row.

	1 st campaign		2 nd campaign	
	Unburned	Burned	Unburned	Burned
pH	3.65 ^a ± 0.03	3.73 ^a ± 0.01		
Total soil C (%)	0.95 ^a ± 0.07	1.17 ^{ab} ± 0.99	1.53 ^b ± 0.22	2.03 ^b ± 0.30
Total soil N(%)	0.06 ^b ± 0.01	0.08 ^b ± 0.01	0.02 ^a ± 0.03	0.26 ^c ± 0.11
NH ₄ ⁺ -N µg N g ⁻¹ d.s.	2.72 ^b ± 0.01	3.44 ^b ± 0.70	0.14 ^a ± 0.19	5.41 ^c ± 0.23
NO ₃ -N µg N g ⁻¹ d.s.	0.34 ^{ab} ± 0.07	0.38 ^{bc} ± 0.04	0.18 ^a ± 0.10	0.58 ^c ± 0.16
α-amino-N µg N g ⁻¹ d.s.	13.50 ^a ± 5.92	26.42 ^c ± 1.64	21.71 ^b ± 0.28	27.68 ^c ± 0.92
Volumetric water content (%)*	3.6 ^{ab} ± 0.2	3.9 ^b ± 0.2	3.8 ^{ab} ± 0.1	2.8 ^a ± 0.2
Soil temperature °C*	26.7 ^a ± 1.2	26.5 ^a ± 1.2	26.7 ^a ± 1.2	26.9 ^a ± 0.6

5 * average of 5 days of field measurements taken during 2 weeks of campaign
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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 ($\text{g CO}_2 \text{ m}^{-2} \text{ d}^{-1}$) 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 ($\text{g CO}_2 \text{ m}^{-2} \text{ d}^{-1}$) 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 ($\text{mg CH}_4 \text{ m}^{-2} \text{ d}^{-1}$) 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 $0.2 \text{ mg CH}_4 \text{ m}^{-2} \text{ d}^{-1}$.

Figure 5 – Single chamber CH_4 fluxes ($\text{mg CH}_4 \text{ m}^{-2} \text{ d}^{-1}$) 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 ($y_0 = -0.53$; $a = 0.008$; $b = 1.54$; $R^2 = 0.76$).

1 Figure 6 - The frequency distribution of single-chambers estimates of N₂O fluxes (mg N₂O-N m⁻²
2 d⁻¹) measured in unburned and burned plots (no water treatment) during the 1st campaign (A) one
3 month after burning (n=32) and the 2nd campaign (B) eight months after burning (n=30). The range
4 of each size class interval is 0.2 mg N₂O-N m⁻² d⁻¹.

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6 Figure 7 – (A) Emissions of N₂O (ng N₂O g⁻¹ soil dry weight h⁻¹) measured during the day of
7 maximal N₂O production after water addition (day 1) and (B) cumulative CO₂ emissions (mg CO₂
8 g⁻¹ soil dry weight) over 15 days from soil of burned and unburned plots incubated at 0, 25, 50 and
9 100 % of water saturation (WS) and at two temperatures (25°C or 37°C). In the small insert is
10 plotted N₂O emissions (ng N₂O g⁻¹ soil dry weight h⁻¹) vs. % of WHCmax. Bars are one standard
11 deviation.

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

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18 Figure 9 - Emissions of NO (ng NO-N d⁻¹ soil dry weight h⁻¹) measured from soil of burned and
19 unburned plots incubated at 10% and 50% of WHCmax.

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