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Fluxes of CO_2, CH_4 and N_2O from soil of burned grassland savannah of central Africa

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Abstract

Grassland savannah ecosystems subject to frequent fires are considered to have an almost neutral carbon balance, as the C released during burning mostly balance the C fixed by the photosynthetic process. However, burning might modify the net soil-atmosphere exchange of GHGs in the post burning phase so that the radiative balance of the site might shift from neutrality. In the present study the impact of fire on soil fluxes of CO_2, CH_4 and N_2O was investigated in a grassland savannah (Congo Brazzaville) where high frequency burning is the typical management form of the region. An area was preserved for one season from annual burning and was used as "unburned" treatment. Two field campaigns were carried on at different time length from the fire event, 1 month, in the middle of the dry season, and 8 months after, at the end of the growing season. CO_2, CH_4 and N_2O fluxes, as well as several soil parameters, were measured in each campaign from burned and unburned plots. Rain events were simulated at each campaign to evaluate magnitude and length of the generated GHG flux pulses. In laboratory experiments, on soil samples from the two treatments, microbial biomass, net N mineralization, net nitrification, N_2O, NO and CO_2 emissions were analyzed in function of soil water and/or temperature variations. Results showed that fire had a significant effect on GHG fluxes but the effect was transient, as after 8 months differences between treatments were no longer significant. One month after burning CO_2 soil emissions were significantly lower in the burned plots, CH_4 fluxes were dominated by net emissions rather than net consumption in the unburned area and fire shifted the CH_4 flux distribution towards more negative values. No significant effect of fire was observed in the field on N_2O fluxes. It was assumed that the low water content was the main limiting factor as in fact laboratory data showed that only above 75% of water saturation, N_2O emissions increased sharply and more strongly in the soil from burned plots. This soil water content was hardly reached in the field even in the watered plots. Burned also stimulated NO production in the laboratory, which was more evident at low water content. Differently from N_2O, 25% of water saturation was

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sufficient to significantly stimulate CO_2 production in the laboratory and rain simulation in the field stimulated soil respiration. However in the laboratory the highest fluxes were measured in burned soil whereas in the field the opposite was observed. Increasing the incubation temperature from 25 °C to 37 °C affected negatively microbial growth and activities (mineralization and nitrification) but stimulated gas production (N_2O and CO_2). Overall, data indicate that fire would have a reductive or null impact on soil GHG emissions in savannah sites presenting similar soil characteristics (acidic, well drained, nutrient poor) and land management (high fire frequency).

1 Introduction

In the African continent fire is a widespread phenomenon, which 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 coexistence of trees and grassed is the result of burning or strong herbivory pressure (Sankaran et al., 2005). Pastoral activity is always combined with burning.

Savannah fires do also influences 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

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have been reported in burned savannas at the onset of rainy season (Adedejii, 1983; Singh et al., 1991). Soil NH_4^+ concentration was found to increase in savannah 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 savannahs soil after burning, which supported higher rates of mineralized and nitrification as soon as soil water content allowed for microbial activation. This generally coincides with rain events in seasonally-dry ecosystems and is accompanied by pulses of NO_x, N_2O, CO_2 emissions (Davidson et al., 1993; Breuer et al., 2000; Garcia-Montiel, 2003; Butterbach-Bahl et al., 2004; van Haren et al., 2005). These have a variable length and magnitude which depends on fire occurrence, plant cover, soil nutrient status and soil matrix potential (Pinto et al., 2002; Rees et al., 2006; Williams et al., 2009), being generally enhanced by wetting-drying cycles (Davidson et al., 1993; Mills and Fey, 2004; van Haren et al., 2005; Jenerette et al., 2008). On an annual base fire might influence the rate of soil CO_2 efflux by changing the contribution of live roots to 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, which increases dry-matter production (Ojima et al., 1994). Fire management, by maintaining the dominance of grasses over shrubs and trees, increases detritus to the upper soil centimetres, having grasses a shallower rooting system, compared with shrubs and trees (Ansley et al., 2002). On the other hand, high frequency burning can also lead to a decline in soil C as a result of fire combustion of the SOM in the upper few cm of the soil, aboveground biomass and leaf litter (Fynn et al., 2003; Knicker, 2007), hence reducing the source of C for heterotrophic respiration. The balance among these processes depends on site characteristics and management. Fire might also influence gas diffusivity by changing soil porosity and water balance (Snyman, 2003; Knicker, 2007), which, together with increased NH_4^+ availability, which acts as a competing substrate for CH_4, might influence soil potential

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for CH_4 oxidation. However, 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 C sinks (per surface unit area) (Bombelli et al., 2009), and, where fire frequency is high, they are considered to have a almost neutral carbon balance (Saarnak, 2001; Bombelli et al., 2009). High uncertainty is associated to this value due to the lack of sufficient studies which also include the overall balance of GHG in unburned and burned conditions. Data on post burning variations of soil greenhouse gas (GHG) fluxes from savannahs 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 the 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_2O from a grassland savannah ecosystem of central-western Africa, with the specific objectives of verifying that: a) burning increases the availability of extractable N substrates and stimulates microbial growth, microbial activity, CO_2, N_2O and NO production; b) rain events induce gas pulses of CO_2 and N_2O, the length and magnitude of which is higher in burned areas; c) fire enhances the soil CH_4 sink.

For this purpose GHG fluxes were measured in the field at different time length from the fire event (1 and 8 months after burning) in burned and control plots manipulated with simulated rain events. Laboratory manipulation experiments of soil water content and temperature were also performed on burned and control soils. The study site was chosen in Congo Brazzaville which is highly representative for this type of ecosystem management. From 60 to 80% of the total land surface of the "Guinea Zone" savannah (humid savannah) is burned annually (Menault et al., 1991).

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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-savannah mosaic lying between the coastline and the Mayombe forest (Favier et al., 2004). The present grass and shrub savannahs result from two interacting factors: seasonally-dry climate and expansion of populations practising savannah burning. Before Upper Holocene, the open vegetation during dry climate periods was made by open forest or at least tree savannahs (Schwartz et al., 1995). The climate of the Tchizalamou site is a two-season transition equatorial type, characterized by a long cool 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 precipitation 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 savannahs 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 aerial biomass of this savannah, which reached about 3.8 Mg ha^{-1} of dry matter at the end of the rainy season (de Grandcourt et al., 2010). 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) chemically poor (CEC<0.5 $cmol_c$ kg^{-1} whatever the soil layer) with a bulk density of 1.43 g cm^{-3} and a water holding capacity of 20%. The soils in these savannahs have a low clay content, are poor in nutrients and therefore unsuitable for agricultural purposes. The studied area is interested by wide fires between mid-June and mid-July but other fires may

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occur during the short dry season in February. Fires are generally very rapid and superficial. No agriculture or cattle breeding 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 campaigns were organized at the experimental site, the first starting two weeks after the day of burning (11 June 2007), during the dry season, and the second 8 months after burning, in February 2008. This latter period corresponded to a particularly severe "short dry season", within a period generally regarded as "wet season", that extended from mid-January to mid-April. Being the area interested every year by large fires induced by villagers, the plots we used as control cannot be taken as representative of unburned savannah ecosystems, rather they can be used to compare plots which retain their plant cover and litter layer over the year (control) with plots where the plant cover disappears and an input of elements in form of partially of totally uncombusted material gets to the soil (burned). To create this "unburned treatment" a grassland area of about 1/2 hectare was isolated by preventively burning a perimeter of vegetation around it, which avoided flame propagation during the seasonal massive fires.

To simulate dry-wet cycles event, a 20 m^2 area was rain-shaded with a transparent plastic tent kept a two meters height (to limit sun shading and temperature increase) in each treatment (control and burned) so to ensure a quite low water content before simulating a rain event. This was performed as 30 mm of rain which, based on the analysis of rain events at the station from the two previous years, corresponded to typical large rain events. Within each rain-shaded area 3 subplots were watered in different moments before the starting of gas sampling (15, 7, 1 day prior gas sampling) so to have a gradient of soil water contents to follow at the beginning of the gas flux measurement campaigns. One subplot in each treatment under the tent was left unwatered. An other subplot outside the tent was left unwatered, in order to verify the influence of the tent on gas fluxes and some key soil characteristics (water content and temperature).

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2.3 Field GHG flux sampling and measurements

CO_2, N_2O and CH_4 fluxes were measured from each plot on 4 replicates. To measure N_2O and CH_4 fluxes we used closed static chambers (Hutchinson and Mosier, 1981; Smith et al., 1995) made of PVC collars (7 cm high for 31.5 cm diameter) inserted in the soil to about 5 cm depth, in a slot previously formed by a metal cutting ring and chamber lids (20 cm high for 31.5 cm diameter), which were provided with a 2 way stopcock sampling port. To determine N_2O and CH_4 fluxes, gas samples (20 ml) were withdrawn using Hamilton syringes from the chamber headspace at 0, 20 and 60 min after closure, stored in pre-evacuated glass tight vials and then shipped at the end of the campaign to the DSA-SUN (ITALY) for gas chromatographic analysis. 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 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 eight) inserted in the soil to a depth of 5 cm. The increase of the CO_2 concentration was measured over an interval of 2 min (including a dead band of 30 s) starting at the ambient CO_2 concentration. Soil temperature at 0–40 cm depth was monitored simultaneously with soil CO_2 efflux using a copper/constantan thermocouple penetration probe (Li6000-09 TC, LiCor Inc) in the vicinity of the soil respiration chamber and was used to normalize soil respiration values at 25 °C, using the Arrhenius relation: $R_{(T)} = R_{(25°)} Q_{10}^{\frac{(T-25)}{10}}$, with a Q10 value equal to 2.9, as determined by Epron et al. (2004). This normalization was used to compared the effect of wetting treatments on soil respiration getting independent of the temperature factor. 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 measurement at the chamber

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location.

All gases were measured at least 5 times within 2 weeks from all the treatments (unburned, burned x control, and 3 different timing of water addition).

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 plots. 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. The first (at DSA-SUN, Italy) aimed at investigating the effect of burning on temperature and water content responses of N_2O and CO_2 fluxes, as well as some related microbial parameters (net N mineralization, net nitrification, microbial biomass). For this purpose 50 g of sieved soil samples were repacked into columns (10 cm height, 5.5 cm inner diameter) which were incubated in half litre conic flasks (on triplicate). Soils were incubated for 15 days at 0, 25%, 50%, 100% of water saturation (33% for burned and 37% for unburned soil measured on repacked sieved soil columns) and at two temperatures (25 °C and 37 °C) in thermostated cells. In order to observe the pulse effect of water addition on dry soil no pre-incubation was made. Flask were kept open and losses of water were corrected everyday by gravimetrical 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 t0 and after 24 h. 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.

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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) were incubated at 25 °C at 10% and 50% of water saturation. NO emissions were measured at T_0 and 1 h, 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). N_2O fluxes were determined in the same day (30 min after NO flux determination) closing the columns for 30 min and sampling 20 ml of gas from the column headspace at T_0 and T_{30} min. N_2O 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 gravimetrical determination

2.6 Analytical technique

Soil mineral N was extracted with 0.5 M K_2SO_4 (1:4 soil extractant v/v) and filtered with Wathmann filter no. 42. NH_4^+ and NO_3^- were then determined on extracts by potenziometric analysis using specific electrodes for NH_4^+ (Model Orion 9512) and NO_3^- (Model Orion 9707). Soil water saturation was determined gravimetrically on repacked soil column using sieved soil. 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 α-amino-N present in soil extracts (soil: extract 1:5) using 0.5 M K_2SO_4. This techniques allow to quantify soluble proteins, aminoacids and groups containing α-amino-group. To estimate soil microbial biomass N a fumigation-extraction method was used. Soil (5 g) was fumigated for 24 h with chloroform. The

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fraction of the cell constituents made available by the fumigation was extracted shaking the soil with 20 ml of 0.5 M K_2SO_4 for 1 h and filtering the suspension with paper filter (Whatman no. 42). The ninhydrin-N deriving from the biomass was calculated as the ninhydrin-N of the fumigated samples minus the ninhydrin-N of the unfumigated samples. From the value obtained it is possible to calculate biomass N using a conversion factor of 62 (Ocio and Brookes, 1990).

CH_4, N_2O and CO_2 determination 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 2 ml sample, using as well a fronthflush 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_2O analysis at CEH were done using a gas chromatograph (Hewlett Packard, 5890) equipped with an electron capture detector kept a 350 °C, columns were stainless steel packed with Porapak Q (oven 50 °C) and Ar/CH_4 was used as carrier at a flow rate of 40 ml min^{-1}.

2.7 Statistical analyses

As the same sites were sampled over consequent 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). Significant differences were at the $P < 0.05$ level.

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3 Results

3.1 Soil parameters

The analysed soil was characterized by a quite acid pH, slightly higher in the burned plots, and low content of total C and N (Table 1). Total C increased in both treatments after 8 months but no significant difference was observed between burned and unburned plots. Total N was significantly higher after 8 months in the burned plots, on the contrary 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 the 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). The average soil water content (mean of five sampling days over 2 weeks of campaign) measured in the un-watered 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 presence of the rain-shading tent did not seem to influence soil conditions as in fact both soil water content and temperature measured in the unwatered plots outside the tent were not significantly different from those measured in the unwatered control under the tent, in both seasons (data not shown).

When the rain event was simulated, the maximum volumetric water content obtained within 5 h after water addition was about 19.7±0.79% during the first campaign and 23,5±0.92% during the second campaign in the upper 5 soil centimetres (Fig. 1), which corresponded to a water filled pore spaces of 42.8% for the first campaign and 51.0% for the second campaign. Soil water content decreased rapidly after water addition, going back to values comparable to control plots within 10 days during the first campaign and 7 days for the second campaign (Fig. 1) with no significant differences between burned and unburned treatments (Fig. 1). A significantly higher potential evapotranspiration was measured during the second campaign (ETo=101.5 mm, data from metereological station present at site) compared with first campaign (ETo=57.9 mm).

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3.2 Soil respiration

As no difference between CO_2 effluxes measured from unwatered controls outside and under the rain-shading tent was found, average soil respiration daily fluxes were calculated using all the replicates from unwatered plots. The average daily soil respiration, calculated from 12 days of measurements in the 1st campaign and 6 days during the 2nd campaign, was 7732.1±316.1 mg of CO_2 m^{-2} day^{-1} (unburned) and 5478.3±101.3 (burned) mg of CO_2 m^{-2} day^{-1}, in the 1st campaign and 11797.6±905.7 mg of CO_2 m^{-2} day^{-1} (unburned) and 12663.5±1711.1 (burned) mg of CO_2 m^{-2} day^{-1}, during the second campaign. The increase of soil respiration observed in the 2nd campaign for both treatments was statistically significant ($P < 0{,}05$ two-way ANOVA). Only in the 1st campaign the difference between treatments was statistically significant ($P < 0.05$ Two-way ANOVA). To compare the effect of watering on soil respiration in burned and unburned plots, being independent from the effect of soil temperature on CO_2 emissions, these were normalized at 25 °C, as described in the method section. Watering induced a pulse of CO_2 emissions which had its maximum in the first day after water addition and decreased with a trend comparable to that observed for soil water content (Figs. 1 and 2), getting back to control (unwatered) levels within 10 days and 7 days in the 1st and 2nd campaign, respectively. On a daily base CO_2 emissions from unburned watered plots were usually significantly higher than emissions from burned watered plots during the first campaign (Fig. 2a). The maximum soil respiration values induced by watering were just slightly higher in the second campaign, 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 rate of increase (see equations reported in the Fig. 3) was similar in the two treatments one month after burning, although the intercept was significantly higher in the unburned plots compared with burned plots. During the second campaign, the positive relation between soil respiration and water filled pores space was weak but still significant for the unburned

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treatment (Fig. 3). The rate of increase per unit of soil water content was slower compared with the first campaign, although the intercept was higher. 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

The average CH_4 flux calculated from control (unwatered) plots, also in this case using average daily fluxes from both control outside and inside the tent (no statistical difference, Two-way ANOVA, $P > 0.05$), 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 (1st campaign). The difference was statistically significant ($P < 0.05$). In the 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 this time no significant difference between treatments. Fluxes showed an elevated variability (Fig. 4). A significant difference in the distribution of CH_4 fluxes among size classes was observed for the two treatments one month after burning (Fig. 4). In fact, a significant 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 the 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 content and CH_4 fluxes obtained including the watered plots (Fig. 5) it can be observed that in the 1st month after burning most of the fluxes were positive (net emission) in the unburned plots and negative (net consumption) in the burned plots for values of WFPS below 15% (6.9% vol. water content). Above this threshold most of the measured fluxes were positive in both treatments. In the second campaign there was no clear difference between fluxes measured in the two treatments and the increase of soil water content did not induce a clear shift from source to sink

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as observed in the previous campaign, although above 10% of WFPS the frequency of positive fluxes 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 N_2O m^{-2} h^{-1} for single flux measurement), which were hence assigned a value of zero. The daily average N_2O flux, calculated from 5 days of measurements in both campaigns, was 0.02±0.13 mg of N_2O m^{-2} day^{-1} in unburned (range −0.7 to 0.6) and 0.02±0.10 mg of N_2O m^{-2} day^{-1} in burned plots (range −0.4 to 0.5) in the 1st campaign and −0.03±0.11 mg of N_2O m^{-2} day^{-1} in unburned (range −0.6 to 0.0) and 0.0±0.4 mg of N_2O m^{-2} day^{-1} in burned plots (range −0.1 to 0.2) during the 2nd campaign. A slight shift of flux frequency distribution toward more positive (emission) fluxes was observed in burned plots, compared with unburned ones (Fig. 6), however the average flux in the two treatments was not significantly different (Table 2). Water addition did not produced any detectable increase of N_2O emissions (data not shown) neither in unburned or in burned plots.

3.5 Laboratory incubations

The 92% of N_2O production in the first incubation experiment occurred within the first day after water addition, thereafter fluxes decreased exponentially within 2 days (data not shown). N_2O emission was significantly stimulated by burning but the difference with unburned plots could be appreciated only above 50% of water saturation (WS) (corresponding to about 75% of water holding capacity) (Fig. 7). In fact, fluxes of N_2O raised exponentially with increasing water content, faster in the burned plots (Fig. 7, insert). No significant effect of rising temperature from 25 °C to 37 °C was instead observed even at saturation. The pulse of CO_2 peaked the first day after water addition. This peak accounted for about 50% of the total cumulative CO_2 emitted over about 15

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days. At the end of the incubation (day 15), CO_2 emissions from watered plots (25, 50 and 100% of WS) were still significantly higher than CO_2 emissions from unwatered (0% WS) soil (data not shown). Figure 7, which reports the cumulative CO_2 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 WS (40% of water holding capacity) and further water addition did not change significantly the rate of CO_2 production. The increase of incubation temperature from 25 °C to 37 °C stimulated significantly CO_2 production. At 25 °C the effect of burning on soil CO_2 emission was not significant, whereas at 37 °C soil respiration was significantly higher in the burned watered soil (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 almost completely blocked 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 WS, whereas 100% of WS reduced biomass growth and blocked net nitrification. Net N mineralization increased similarly to N_2O production for increasing soil water content up to 100% of WS (Figs. 7, 8). The effect of burning was in most cases not significant although values of microbial biomass N were slightly higher in the burned plots. Burning significantly increased the potential of soil NO emission, which was significantly higher at 10% of WS compared with 50% WS (Fig. 9). The NO pulse induced by water addition (zero flux at time zero, 1 h before watering, data not shown) was significantly reduced already after 5 days of incubation and at all sampling times NO emissions were the dominant form of N gas measured. In fact at 10% WS no N_2O production was detected whereas at 50% of WS N_2O emissions never exceeded 0.10 ng N g^{-1} h^{-1}.

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4 Discussion

4.1 Soil respiration

Data indicate that fire had some transient effect on one or both the components of soil respiration, microbial activity (heterotrophic component) and root respiration (autotrophic component) (Singh and Gupta, 1977). Burned plots emitted significantly less CO_2 than unburned plots during the 1st campaign (one month after burning, dry season) but this difference was not detectable any longer in the 2nd campaign (growing season). The clearest effect of fire was the immediate disappearance of the aboveground vegetation, which despite being partially dry due to the strong water limitation, had still about 20% of green biomass. This was most probably supporting the maintenance activity of roots of these perennial grass species, rather than growth, which typically stops during the driest period of the year, as demonstrated by ingrowth core methodology (de Grandcourt et al., 2010). Hence, fire might have partially reduced this source of soil respiration by destroying the remaining active photosynthetic tissues. Fire also consumed most of the litter, which in these grasslands typically dries out as aerial litter before falling on the ground. This litter would represent a source of C for microbial respiration during the decomposition process. One sole season of fire exclusion was not sufficient to vary significantly the soil content of total C in the unburned plots, compared with the burned plots, on the contrary a higher content of extractable α-amino-N products was found in the burned soil 1 month after fire. Andersson et al. (2004) reported an increase of dissolved organic C in savannah 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 soil analyses showed that in very dry soil (no water addition) microbial biomass still persisted after more than a month from sampling, but its activity was limited. However the addition of water, even at moderate rates (25% WS) quickly stimulated microbial growth, activity and CO_2 production. A similar capacity of microbes to recover as soon as rain arrives could be expected also

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in the field. The similar rate of increase of CO_2 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 CO_2 efflux induced by water addition, whereas the extra CO_2 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 for a conclusive partitioning of the CO_2 flux between autotrophic and heterotrophic sources. Both laboratory and field data showed that around 10 days were necessary to extinguish the CO_2 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 a rain pulse in dry conditions (Jenerette et al., 2008; Xu and Baldocchi, 2004; Williams et al., 2009), but the pulse was generally ending within one to three days. In the second campaign the pulse peak was comparable to that obtained in the first campaign, although the pulse lifetime was shorter (7 days) and the background (unwatered) rate of soil respiration was higher. No significant difference was evidenced in this second campaign between burned and unburned plots. At this time of the year both treatments presented similar plant cover density and grass height. The higher biomass corresponded also to higher soil total C and α-amino N content, probably reflecting the increase of C in the soil associated to root growth, turnover and exudation. These 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 in the second campaign (shorted pulse lifetime). Clearly also the autotrophic component contributed significantly to the CO_2 efflux during the growing period.

Overall the impact of burning on CO_2 emissions in the present experiment seemed not relevant in terms of stimulation of CO_2 losses from the soil. On the contrary fire seemed to reduce soil respiration, taking into account both plants and microbial contribution. Previous studies comparing soil CO_2 emissions from burned and unburned

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savannahs showed no difference in Nigeria, Venezuela and South Africa (Adedeji, 1983; Hao et al., 1988; Zepp et al., 1996) or a slight stimulating effect 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 grassland, 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 measurement, and its loose structure, mainly dominated by sand, should 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 CH_4 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). Net CH_4 emissions were observed even at 7% of WFPS, hence at very dry conditions, which makes quite unlikely that this CH_4 source might 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 CH_4 might be represented by termite activity, also considering that site presented a very high frequency 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 CH_4 emissions, and this was particularly evident in the first campaign, immediately after burning. We could hypothesise that fire temporarily reduced termite activity outside the nest. Indeed even CH_4 production inside the termite nests was significantly reduced in the first months after burning (Castaldi and de Grandcourt, in preparation). Eight months after burning the frequency distribution of CH_4 fluxes in the two treatments did not present

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differences in the burned and unburned plots, although fluxes were on average lower (more negative), compared with the previous dry season, probably as a consequence of the drier conditions which might have slowed termite activity overall and also facilitated CH_4 uptake in the deeper soil layers (Striegl et al., 1992; Castaldi and Fierro, 2005). Other authors have evidenced that clearance of savannah soil surface (grasses and litter) by burning produces a significant reduction of the methane production from the soil-grass system (Poth et al., 1995; Zepp et al., 1996). Indeed, destroying most of the litter, burning reduces that amount of palatable substrate that termites can use, either directly as litter or as SOM. Soil-feeding termites as those belonging to the genus Cubitermes or those feeding on litter such as Nasutitermes, both found at the site, would be affected by substrate reduction consequent to fire.

4.3 Nitrous oxide emissions

The analysed savannah ecosystem showed extremely low N_2O 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 N_2O-N m^{-2} day^{-1} (Castaldi et al., 2006) except if soil receives significant amount of fertilizer (Brümmer et al., 2008). Higher fluxes from undisturbed savannahs have been only measured in isolated patches of nutrient rich savannas in nutrient poor soils (Otter and Scholes, 2000) or savannas located in valleys characterized by higher soil water retention and accumulation of organic matter (Sanhueza et al., 1990). Higher N_2O fluxes in seasonally dry tropical environment are reported only for forests (Sanhueza et al., 1990; Verchot et al., 1999; Castaldi et al., 2006). A combination of environmental factors concur to keep N_2O 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 N_2O production could take place (Firestone and Davidson, 1989; Smith, 1990). In the studied site, the

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water filled pore space (WFPS %) was always below 51%, even immediately after a big rain event simulation. This WFPS value is below the value at which O_2 diffusion is sufficiently reduced to allow for a sharp increase of N_2O production (Davidson, 1991). In fact, N_2O production by denitrification generally increases exponentially between 60% and 90% of WFPS, but also N_2O 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 allow to observe a significant increase of N_2O emissions. Equally, laboratory incubations demonstrated that only above 75% of WS the increase of N_2O production was sharper. This value cannot be compared directly with field WFPS but gives an indication that the water content required to induce significant N_2O production is certainly higher than that required to stimulate significantly CO_2 production. However, a second limiting factor for N_2O production in these environments is represented by the very low contents of soil C and N, in particular N in the form of mineral N. During the dry season mineralization and nitrification activity might concur quite little to produced significant amounts of available mineral N, as demonstrated by laboratory data. When soil water content increases, during the wet season, the most intense mineralization activity would probably coincides with resprouting of shrubs and growth of herbaceous plants making the competition for N quite high (Bate, 1981). Burning grasslands often results in grass growth earlier in the growing season, (Ojima et al., 1994), which might enhance the competing effect of plant with microbes at the onset of the rainy season, when pulses of N_2O might occur with higher frequency.

Laboratory data showed that significant losses of NO could occur after smaller rain events (low soil water content) and that burning triplicated the emission measured in the control. The flush was much higher at water contents below 10% of WS, but most of the observed pulse could be assumed to occur within a couple of days, slowly decreasing thereafter. 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. The pulse of NO was quite long; however,

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the amount of mineral N was quite high compared with our study, where N could have been an important limiting factor for NO and N_2O emissions. Hence data suggest that the occurrence of burning in savannahs ecosystems such the one investigated in this study might lead mostly to have an increase of NO production but no or little N_2O production, as previously reported by Levine et al. (1996) and Johansson et al. (1988). Fast spreading fires, such as those occurring in grassland savannahs, do not seem to affect, on the other hand, microbial biomass and activity involved in N transformations. Higher N_2O emissions following burning might be expected only in clay reach and poorly drained soil following a rain event which might allow for transient saturation of soil pores.

5 Conclusions

Burning increased the soil availability of extractable N, both as mineral N and organic compounds containing amino groups. This might have lead to increased N_2O production in burned soils if enough water might have been retained in the soil during rain events, as shown by lab data. However field observation indicated that this condition never occurred in the field, so that no appreciable effect of fire on N_2O fluxes could be observed. Rain simulation stimulated a significant CO_2 pulse, which lasted up to 10 days in the dry season. The slightly stimulating effect of burning on microbial growth and activity, as observed in the lab, was probably balanced by the negative effect of fire on the autotrophic component of soil respiration, so that overall the burned soils tended to respire less even after water addition. The studied soil was not a CH_4 sink as expected on the base of soil and climatic characteristics, and during at least part of the year, it acted as a slight CH_4 source, even at very dry conditions, however, fire shifted the CH_4 source/sink towards more negative values (consumption).

Overall data indicate that fire might reduce the GHG emissions into the atmosphere when applied to grassland savannas characterized by similar soil characteristics (acidic, well drained, nutrient poor) and land management (high fire frequency).

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The only significant contribution to enhanced release of GHG gases at burned sites might hence derive by N_2O and CH_4 emissions produced during the flaming and smouldering phase of burning.

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

	1st campaign		2nd campaign	
	Unburned	Burned	Unburned	Burned
pH	$3.65^{a} \pm 0.03$	$3.73^{a} \pm 0.01$		
Total soil C (%)	$0.95^{a} \pm 0.07$	$1.17^{ab} \pm 0.99$	$1.53^{b} \pm 0.22$	$2.03^{b} \pm 0.30$
Total soil N(%)	$0.06^{b} \pm 0.01$	$0.08^{b} \pm 0.01$	$0.02^{a} \pm 0.03$	$0.26^{c} \pm 0.11$
NH_4^+-N µg N g^{-1} d.s.	$2.72^{b} \pm 0.01$	$3.44^{b} \pm 0.70$	$0.14^{a} \pm 0.19$	$5.41^{c} \pm 0.23$
NO_3-N µg N g^{-1} d.s.	$0.34^{ab} \pm 0.07$	$0.38^{bc} \pm 0.04$	$0.18^{a} \pm 0.10$	$0.58^{c} \pm 0.16$
a-amino-N µg N g^{-1} d.s.	$13.50^{a} \pm 5.92$	$26.42^{c} \pm 1.64$	$21.71^{b} \pm 0.28$	$27.68^{c} \pm 0.92$
Volumetric water content (%)*	$3.6^{ab} \pm 0.2$	$3.9^{b} \pm 0.2$	$3.8^{ab} \pm 0.1$	$2.8^{a} \pm 0.2$
Soil temperature °C*	$26.7^{a} \pm 1.2$	$26.5^{a} \pm 1.2$	$26.7^{a} \pm 1.2$	$26.9^{a} \pm 0.6$

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

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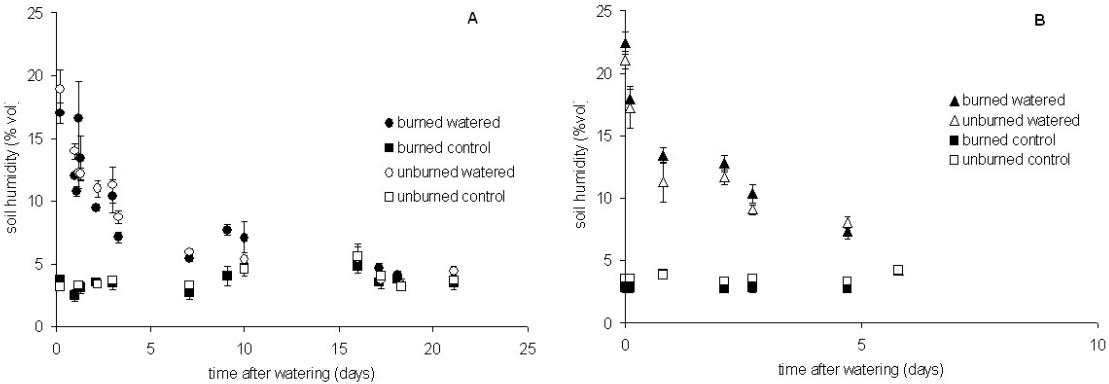

Fig. 1. Soil volumetric water content in function of time after watering (days) during the 1st campaign **(A)** and the 2nd campaign **(B)**. The vertical bars correspond to standard-errors (n=3).

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Fluxes of CO_2, CH_4 and N_2O from savannah

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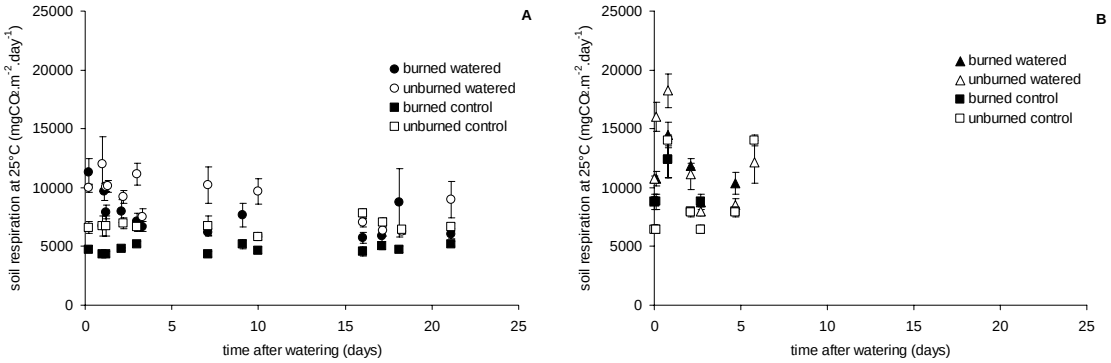

Fig. 2. Soil respiration normalized at 25 °C in function of time after watering during the 1st campaign **(A)** and the 2nd campaign **(B)**. The vertical bars correspond to standard-errors (n=3).

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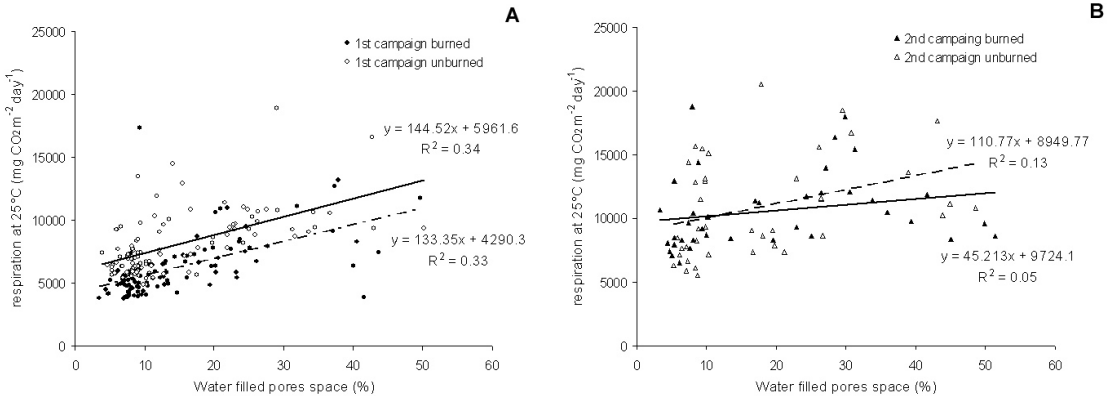

Fig. 3. Soil respiration normalized at 25 °C in function of water filled pores space (during the 1st campaign **(A)** and the 2nd campaign **(B)**. One point is one single-chamber measurement. Lines (continuous unburned, dotted burned) represent linear regressions indicated by the correspondent equations.

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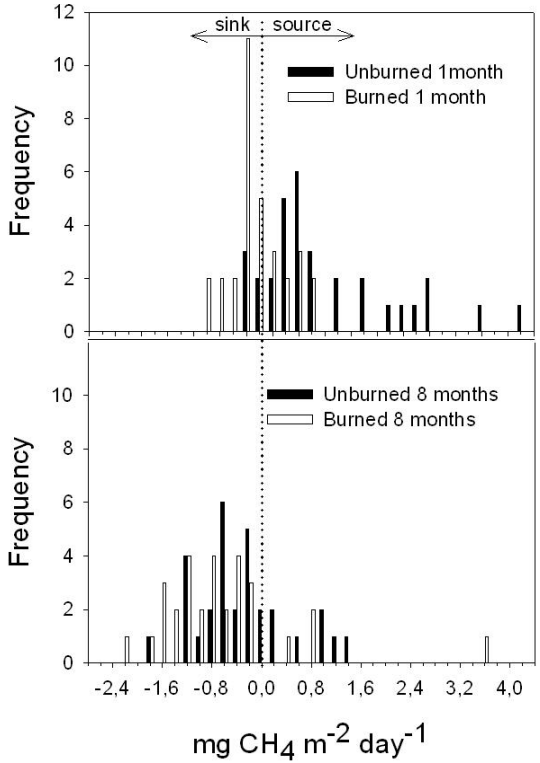

Fig. 4. The frequency distribution of single-chamber estimates of CH_4 fluxes (n=32 and 30 in the 1st and 2nd campaign, respectively) presented for the unburned and burned plots (not treated with water). The range of each size class interval is 0.2 mg CH_4 m^{-2} day^{-1}.

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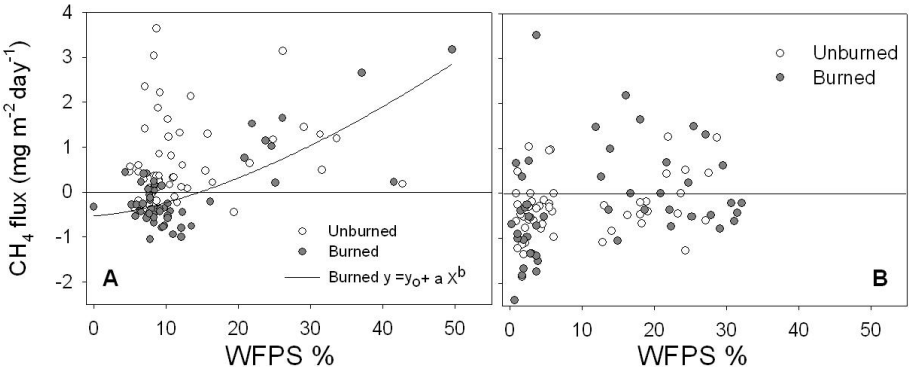

Fig. 5. Single chamber CH_4 fluxes plotted versus soil water filled pore space (%). In graph A line represents the fit of data from burned plots ($y_0 = -0.53$; a=0.008; b=1.54; R^2=0.76).

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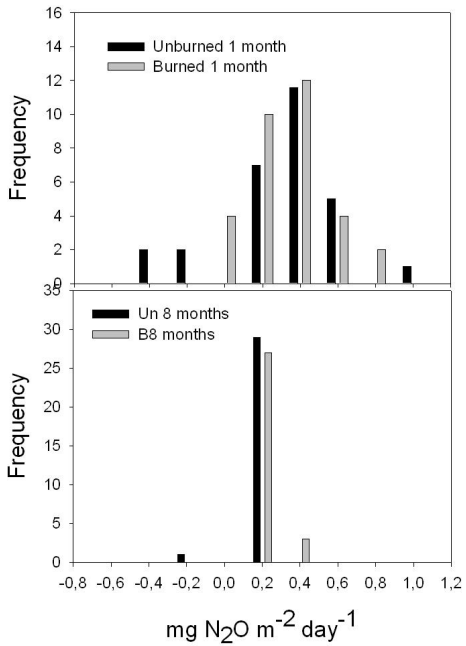

Fig. 6. The frequency distribution of single-chamber estimates of N_2O fluxes (n=32 and 30 in the 1st and 2nd campaign, respectively) presented for the unburned and burned plots (not treated with water). The range of each size class interval is 0.2 mg N_2O-N m^{-2} day^{-1}.

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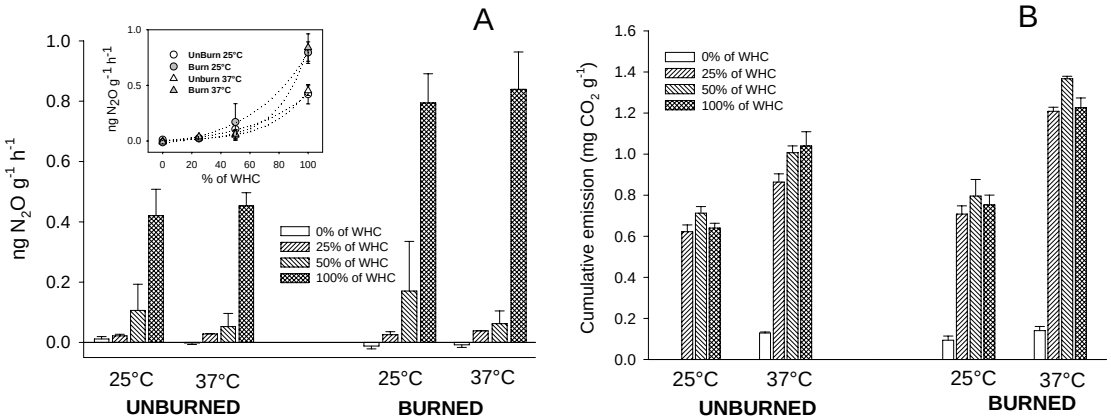

Fig. 7. (A) Emissions of N_2O measured during the day of maximal N_2O production after water addition (day 1) and **(B)** cumulative CO_2 emissions over 15 days from soil of burned and unburned plots incubated at 0, 25, 50 and 100% of water saturation (WS) and at two temperatures (25 °C or 37 °C). In the small insert is plotted N_2O emissions vs. soil water content expressed as % of maximal WS. Bars are one st.dev.

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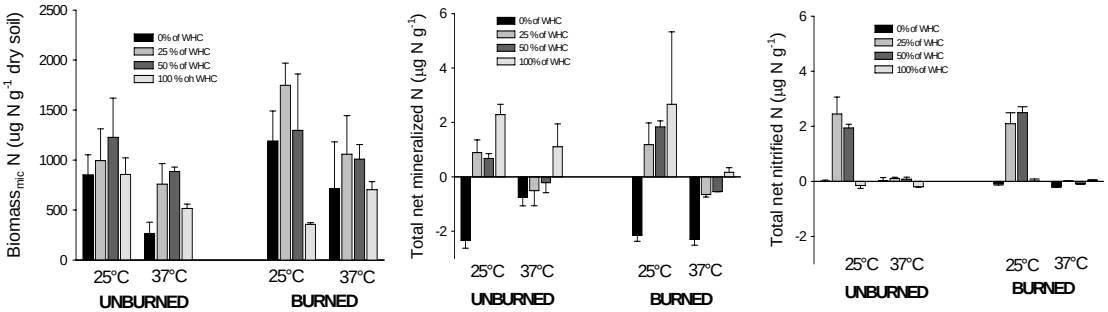

Fig. 8. Microbial biomass N and rates of net N mineralization and net nitrification measured after 2 weeks of incubation in soil from burned and unburned plots incubated at 0, 25, 50 and 100% of water saturation (WS) and at two temperatures (25 °C or 37 °C).

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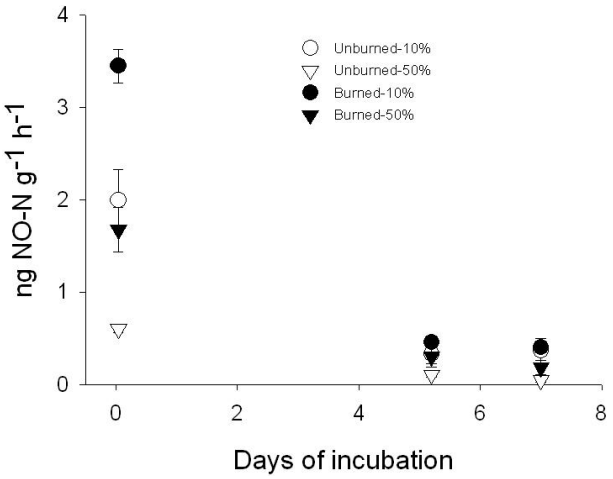

Fig. 9. Emissions of NO measured from burned and unburned Congo soils incubated at 10% and 50% of water saturation (WS).

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