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TESI DI DOTTORATO

in

BIOLOGIA APPLICATA

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GREENHOUSE GASES FROM AFRICAN SEASONALLY DRY ECOSYSTEMS

COORDINATORE

Ch.ma Prof. ssa AMALIA VIRZO DE SANTO

CANDIDATO

Dott. AMERICO RASILE

TUTORE

Ch.ma Dott. ssa SIMONA CASTALDI

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1 INTRODUCTION

1.1 The greenhouse effects and climate change

Atmospheric gas composition has considerably and quickly changed in the last decades due to the industrial emissions, fuel combustion, deforestation and land use change. All these activities have increased the natural background atmospheric level of greenhouse gases (GHGs), CO2, N2O, CH4, plus have added into the atmosphere some new greenhouse gases, previously absent, as the chlorofluorocarbons (CFCs). Carbon dioxide is the most abundant greenhouse gas (alone contributes to 60% of greenhouse effect), methane the second (it contributes to 15%), nitrous oxide, ozone and chlorofluorocarbons (CFC cause also ozone depletion phenomenon) contribute to the remaining enhanced atmospheric radiative increase. (Fig 1.1).

The greenhouse effect is a phenomenon which occurs as a result of absorption of infrared radiations emitted by the earth, by several gases present in the atmosphere, which induces a consequent increase of its average temperature. This effect have a fundamental role for life on earth, because it limits the energy dispersion and keeps the temperature constant on the earth in a range suitable for life. Arrehenius (1896) calculated that water vapour and carbon dioxide increased the earth's average temperature from – 18 °C (the temperature that should be present if there wasn't atmosphere) to + 15 °C.

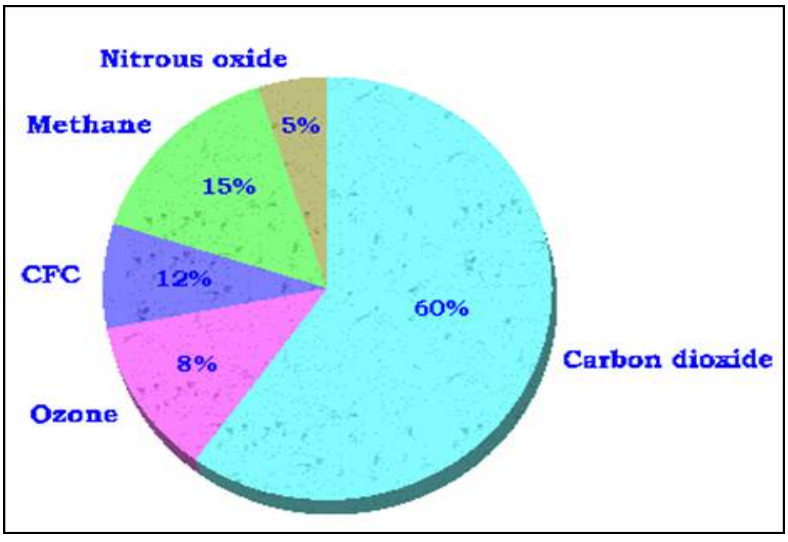


Figure 1.1 – Contribution of gases on greenhouse effect .

Earth can be compared to an heated wire that emitts energy. The amount of energy emitted and absorbed from earth must be the same to keep atmospheric temperature constant. Earth's surface and the atmosphere mainly warm, thanks to the sun energy, the upper layer of atmosphere in

particular, the stratosphere which spreads from 15 to 50 Km, absorbs short and middle-wavelenght ultraviolet radiation (< 400 nm). The ultraviolet radiation not absorbed from ozone in the stratosphere together with radiations visible to the human eye from 400 to 700 nm (useful to photosynthesis), and the infrared radiation from 800 to 4000 nm reach the Earth's surface.

Of the total amount of solar energy reaching the earth's atmosphere an average 20 % is absorbed by atmospheric gases in particular the ultraviolet component by stratospheric, ozone while the infrared component from CO_2, H_2O and others trace gases. About 30% of the energy is reflected back to the space by clouds, water, vegetation, desert, and iced surfaces, but 50% is absorbed by the surfaces (Fig 1.2).

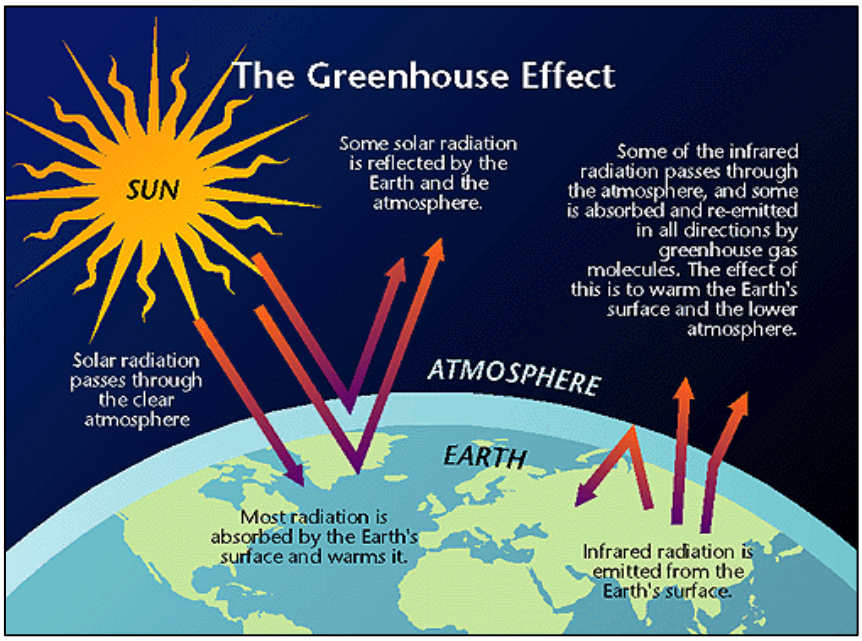


Figure 1.2 – The effect of solar irradiation on the Earth.

The energy absorbed from Earth returns to the atmosphere as radiations with a bigger wavelength in fact:

$$E = h\nu, \quad \text{being} \quad \nu = \frac{c}{\lambda} \Rightarrow \quad E = h\frac{c}{\lambda}$$

Where h is Planck constant ($6{,}626218 \times 10^{-34}$ Js), c is the speed of light in the vacuum ($2{,}997925 \times 10^{8}$ ms^{-1}) and λ is the wavelenght, so that if E decreases λ must increase because c and h are constant. The radiations emitted from Earth have a wavelength between 4 and 50 μm, this component is known as thermal infrared radiation.

The main constituents of the atmosphere N_2 O_2 don't absorb the infrared radiations. Gases, normally presented in the atmosphere, at environmental temperature, generally are at fundamental

state of low energy but if they are excited its energy increases. The excited state is unstable and shortlasting because atoms and molecules relax to the initial state and they back-radiate the absorbed energy to the atmosphere. During this phase the infrared radiation is emitted in all directions, so that some energy goes towards the space and other back to the surface, which an overall warming effect of the atmosphere.

The far-infrared (heat) energy re-irradiated is completely absorbed by molecules if its frequency is the same of that one produced by molecular vibration. The main vibrations in a polyatomic molecule depend on the oscillatory movement of two atoms A and B, this movement is called stretching movement. During this oscillation, the chemical bond increases over its average value R to come back to the initial condition (Fig 1.3). The stretching movement can be compared to the oscillatory movement of two bodies joint by a spring

Figure 1.3 – The stretching movement of a polyatomic molecule.

The other molecular vibration is due to the oscillation of the angle bond, known as bending movement. This last one generally occurs in the molecules with three or more atoms (Fig. 1.4).

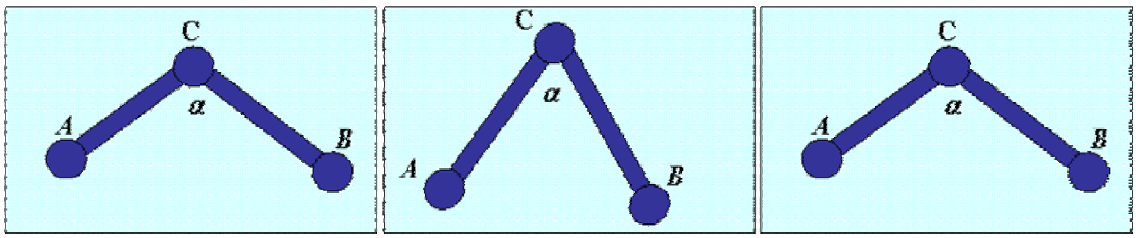


Figure 1.4 – The bending movement of a polyatomic molecule.

Carbon dioxide (CO_2), a natural component of the atmosphere, is a biologically reactive gas and represents the principal greenhouse gases with a residence time of about 120 years, its concentration before the industrialization was 280 ppm (Shallcross et al., 2003), now it's about 360 ppm (Prentice, 2001). Major natural sources to the atmosphere are heterotrophic respiration, microbial breakdown of dead organic matter and soil carbon, and ocean equilibrium of CO_2 and carbonates. CO_2 actual increase depends on increased deforestation and land use change rates but above all on the increasing use of combustible fuel (carbon, petrol and natural gas) formed from vegetal and animal organic matter in geological sediments. Part of these emissions are direct, for example those ones originated from domestic warming system that use fossil fuel and from the cars; the other part of the emissions are indirect and they depends on the petrol extraction and refinement. Globally,

more than 80% of human CO_2 emissions come from transportation and industrial sources. The remaining 20% comes primarily from deforestation and biomass burning.

The atmospheric concentrations of trace gases such as methane, nitrous oxide CFC-11 and CFC-12 are increasing more rapidly than in the case for CO_2 so that their relative importance in modifying climate is changing (Bolin *et al.*, 1986). The heat absorption potential of these gases depends on factors such as their relative concentration in the atmosphere, infrared absorption profile and atmospheric life-time, and as a consequence it varies widely with the gas; furthermore their contribution to the global warming is very important because they absorb radiations with a wavelength included between 8-13 μm that represent the ‘’window’’ through which the radiation can escape.

Though the concentration of CH_4 (Table 1.1) is smaller than CO_2 the global warming for each molecule is about 21 times bigger than each molecule of CO_2. Its atmospheric concentration increased by 150% since 1750 and is increasing rapidly by about 1 % per year. About half the current CH_4 emissions are from anthropogenic (human produced) sources. These sources include livestock production (incomplete digestion food), wetland rice cultivation, solid waste, landfills, and coal and have still a big uncertainty (Houghton et al., 2001).

Nitrous oxide (N_2O), despite of its low concentration, makes a significant contribution to the overall global warming. It originates from agricultural fertilizers, fossil-fuel combustion, and biomass burning, its atmospheric concentration has increased since the pre-industrial era by 16% and it continues to increase by about 0.25% (Table 1.1).

Table 1.1 - Tropospheric concentration, residence time, annual increase of the most important greenhouse gases. Source: IPCC [54, 55]

	CO_2	CH_4	$CFC-11$	$CFC-12$	N_2O
Atmospheric concentration	(ppmv)	(ppmv)	(pptv)	(pptv)	(ppbv)
Pre-industrial	280	0.8	0	0	288
Present (1990)	355	1.72	280	484	310
Current average yearly increase	0.5	0.9	4	4	0.25
Atmospheric lifetime	50-200	10	65	130	150
Greenhouse effect					
For each molecula	1	21	12400	15800	206
For mass unit	1	58	3970	5750	206

The post-industrial increase in greenhouse gases has resulted in an increase in global radiative forcing (warming) of 2.45 watts per square meter (Wm^{-2}). This is only about 1% of the net incoming solar radiation, but it corresponds to the energy content of about 1.8 billion of tonnes of oil $minute^{-1}$, or more than 100 times world's current rate of commercial energy consumption UNFCC, 2002).

1.2 Methane

Methane (CH_4) is one of the most important greenhouse gases that influences the photochemistry of atmosphere and it contributes for about 15% to global warming (JEA/EPA, 1990). Its increase in the air cause, for each molecule, a greenhouse effect 23 times higher than carbon dioxide because the molecule of CH_4 absorbs a bigger fraction of photons of thermal infrared radiation respect to CO_2.

Thanks to ice cores, extracted in Antarctica, it was possible to estimated that the concentration of CH_4 in atmosphere during the last glaciation (2000 years B.P.) was about 350 ppbv. It increased to 650 ppbv during pre-industrial period and reached today an average concentration of 1.7 ppm (Fig 1.4) (Stauffer *et al*., 1988).

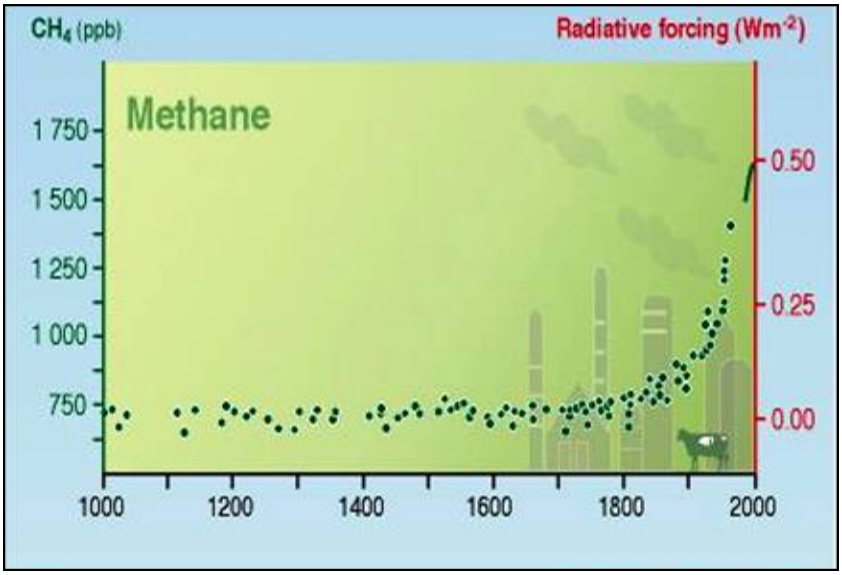


Figure 1.4 – Methane increase in atmosphere and its radiative forcing (Wm^{-2}).

CH_4 concentration in atmosphere is lower in Southern hemisphere then the Northern one due to the lower anthropogenic inputs and its spatial and temporal variability, which can be influenced by geographic location, seasonality, and diurnal cycles (Khail and Rasmussen, 1983; Blake and Rowland, 1988).

CH_4 increase in the atmosphere is due to the large intensive and extensive use of paddy fields, to the increase of ruminants, to the bigger escapes of CH_4 from coal mines, to the biomass burning and to the leakage of natural gas wells and distribution systems.

There are different sources of methane (Table 1.2), oceans produce 15±12 Tg CH_4 yr^{-1}, that represents about the 3% of the total budget, the biggest part of CH_4 emission, about half of it, is produced in the anaerobic soils, principally through microbial degradation of organic compounds, in

particular in rice paddies (100±50 $Tgyr^{-1}$), natural wetlands (100±50 $Tgyr^{-1}$) and landfills (50±20 $Tgyr^{-1}$). CH_4 sources in atmosphere not due to the soils include ruminants (85±15 $Tgyr^{-1}$), biomass burning (80±20 $Tgyr^{-1}$) and coal mining (233±60 $Tgyr^{-1}$) (Schütz *et al*.,1990) (Table 1.2). Methane can even form in hyper-saline environments as the Great Salt Lake and in thermophilic environments (Oremland, 1988).

Table 1.2 - Estimated sources and sinks of methane. (IPCC, 1992)

SOURCES	**$Tgyr^{-1}$**
NATURAL	
Wetlands	115 (100-200)
Termites	20 (10-50)
Oceans	10 (5-20)
Freshwater	5 (1-25)
CH_4 hydrate	0 (0-5)
ANTHROPOGENIC	
Coal mining, natural gas and petroleum industry	100 (70-120)
Rice paddies	60 (20-100)
Enteric fermentation	80 (65-100)
Animal wastes	25 (20-30)
Domestic sewage treatment	25 (??)
Landfills	30 (20-70)
Biomass burning	40 (20-80)
SINKS	
Reaction with OH	420 (340-500)
Removal by soil	30 (15-45)
Removal in stratosphere	10 (??)
ATMOSPHERE INCREASE	37 (34-40)

CH_4 is produced naturally through the methanogenesis, an important process that occurs in all anaerobic environment (lakes, wetlands, rice paddies) where the organic compounds can be decomposed but it can occur in the digestive tract of ruminants. CH_4 can be produced when carbohydrates are broken down in the digestive tract of animals. The volume of methane produced from this process, called enteric fermentation, is largest in those animals that possess a rumen, or

forestomach, such as cattle, sheep, and goats. The forestomach allows these animals to digest large quantities of cellulose found in plant material. Microorganisms in the rumen, some of which are methanogenic bacteria, accomplish this digestion.

In the rumen the production of methane depends on a biochemical process due to specific archeobacteria rather than eubacteria. The methanogens are very different respect to the other microorganisms presented in the rumen (principally eubacteria able to ferment fibers, sugars, peptides, amino acids and other little molecules) for their specialized metabolism; they include methanogens, sulphate reducers, thermophile, allophile bacteria. Some eubacteria can produce little amount of CH_4, but only the archeobacteria methanogens obtain energy from methanogenesis (Jones *et al*., 1987). In the rumen fermentative bacteria produce fermentative compounds as volatile fatty acids (VFAs), formate and H_2, then he methanogens use formate and $H_2 + CO_2$ to produce CH_4. Methane emitted from manure, produced mainly from reduction of acetate during the primary fermentation, is a process less important for its high time of turnover. Animals waste are a source of CH_4 and the amount of produced gas depends on the diet, on the and on waste management. If the animal waste are stored in lagoons or in closed tanks, methane production is strictly connected to the reduction of organic compounds.

1.2.1 Termites

Methane can be produced by termites too. They inhabit many different ecological regions, but they are concentrated primarily in tropical grasslands and forests. Symbiotic micro-organisms in the digestive tracts of termites (flagellate protozoa in lower termites and bacteria in higher termites) produce methane (CH_4). Estimates of the contribution to the global budget of CH_4 from termites vary widely, from negligible up to 15 percent. Termite CH_4 emissions estimates vary for several reasons.

Researchers have taken different approaches to approximate the number of termites per area for different ecological regions (e.g., cultivated land, temperate grassland, tropical forest) and different species. In Table 1.3 are reported the numbers of termites expected per Acre in different undisturbed ecosystem types. In Table 1.4 the strength of CH_4 emission associated to termite source is reported for each ecosystem type. The biggest difficulty comes from predicting the variation in termite biomass associated to the various types of land use.

Table 1.3 - Typical termite densities per ecological region (from zimmerman et al. 1982).

Ecological Region	10^6 Termites per Acre
Tropical wet forest	4.05
Tropical moist forest	18.01
Tropical dry forest	12.80
Temperate	2.43
Wood/shrub land	1.74
Wet savanna	17.81
Dry savanna	3.48
Temperate grassland	8.66
Cultivated land	11.38
Desert scrub	0.93
Clearing and burning	27.62

Table 1.4 – Emission factors associated to termite activity in different ecosystem types.

Ecological Region	Methane Emissions (lb CH_4/1000 lb termite/hr)
Tropical forest	4.2 E-03
Temperate forest	1.8 E-03
Savanna	8.0 E-03
Temperate grassland	1.8 E-03
Cultivated land	1.8 E-03
Desert scrub	1.0 E-03

From: Saegar, SAIC, to Lee Beck, Project Officer, U. S. Environmental Protection Agency, S*ummary Of Data Gaps Associated With County-Specific Estimates Of CH4 Emissions*, July 6, 1992. P. J. Frasser, *et al*., "Termites And Global Methane — Another Assessment", *Journal Of Atmospheric Chemistry, 4*:295-310, 1986. T. M. Lynch, *Compilation Of Global Methane Emissions Data, Draft Report*, Alliance Tech. Corp. for U. S. Environmental Protection Agency, Nov. 1991.

1.2.3 Methanogenesis

Methane of biogenic origin derive from metabolic activity of specific bacterial group that represent the terminal ring of the food chain in these environments; they transform fermentation products formed by other microorganisms in CH_4 when the conditions are strictly anaerobic.

The methanogens principally use acetate as carbon substrate, in this way about the 80% of methane is produced, but they can also use substrates as H_2, CO_2 and formate responsible of 10-30% of methane production.

The main bacterial reactions that produce CH_4 are essentially three, in particular:

1. H_2 reduction of CO_2 by obligate chemoautotrophic methanogens

   $$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$$

2. Reduction of HCOOH or CO in addiction to CO_2 and H_2 obtained through other methanogens:

   $$4HCOOH \rightarrow CH_4 + 3CO_2 + 2H_2O$$

   $$4CO + 2H_2O \rightarrow CH_4 + 3CO_2$$

3. Methanogenesis methylotrophic, in this reaction are used methyl groups of some compounds as methanol, acetate and trimethylamine:

   $$4CH_3OH \rightarrow 3CH_4 + CO_2 + 2H_2O$$

   $$CH_3COOH \rightarrow CH_4 + CO_2$$

   $$4(CH_3)_3N + 6H_2O \rightarrow 9CH_4 + 3CO_2 + 4NH_3$$

1.3 Processes regulating the transfer of CH_4 to the atmosphere

Methane produced in flooded soils and in the sediments can escape from surface and get into the atmosphere through three different ways (Fig 1.5):

1. Ebullition
2. Molecular diffusion
3. Transport through aerenchyma in some vascular plants

The ebullition consists in the formation of bubbles containing methane, in the sediments and in the peat bog, and it represents about 49-64% of methane fluxes from soil to atmosphere (Bartlett *et al.*, 1988).

In completely flooded environments, the gas exchange between soil and atmosphere can be reduced because the pressure applied by the water can entrap the methane in the soil reducing the ebullition process; but if the pressure of this gas increases, favoured by anaerobic condition, the pressure

enhances leading to the formation in the soil of ‘’vescicular structure’’ through which gases can escape as bubbles to the atmosphere (Moormann and van Breemen, 1978).

Methane emissions from ebullition generally are bigger in the soils where the vegetation is absent moreover, according to some authors, in rice paddies the ebullition prevails during the early stage of flooding, when rice plants are small, while vascular transport becomes more important as the rice plants grow (Takai and Wada, 1990).

In wet tropical ecosystems methane is generally emitted from soil to atmosphere, through bubbles formation, in particular during the onset of vegetative season and when there are high organic input (Denier van Der Gon and Neue, 1995). The ebullition is a principal transport mechanism of CH_4 in wetland (Wassman and Martius, 1997) and cultivated wet soils (Sass *et al.*, 2000). In soils with poor vegetation or where plants with aerenchyma are less developed this mechanism represents the principal way of methane transport but it happens only in the upper layers and it is regulated by CH_4 concentration, temperature and soil porosity (Li, 2002).

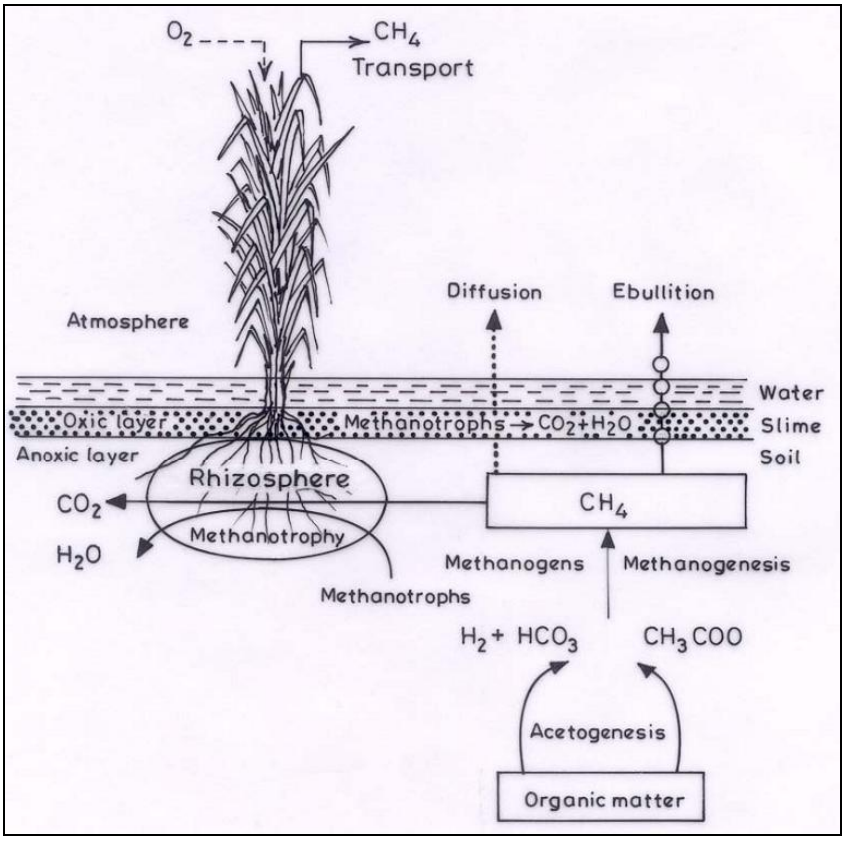


Figure 1.5 - Conceptual schematic diagram of methane production, oxidation and emission from paddy field. (Dubey, 2005)

Methane can be introduced in atmosphere also thanks to gas diffusion but this process is much slower in water than in the air, and its diffusion rate depends on the concentration gradient between

sediments and water and on other factors as the solar radiation, the temperature, wind speed and the atmospheric pressure.

In different aquatic plants, as the rice, CH_4 can be transported through the aerenchyma (Figure 1.6). These structures have evolved to transport oxygen needed for root respiration and cell division, but serve equally well as channels for the transport of methane from the root environment to the atmosphere.

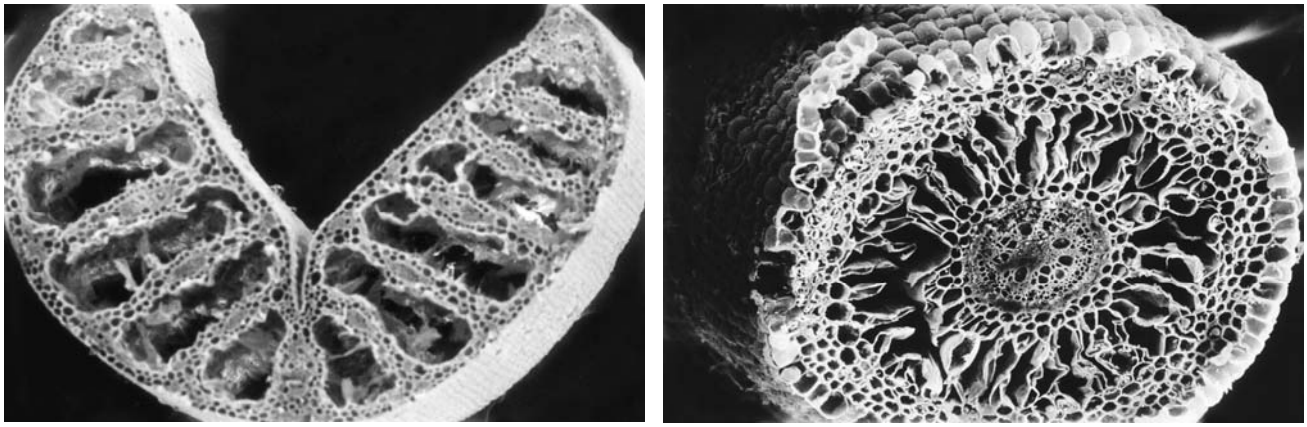


Figure 1.6 Electron micrographs showing aerenchymain (a) leaf of Eriophorum angustifolium and (b) root of Molinia caerulea at c.150mm depth. (Reprinted from Thomas et al. (1996) with permission from Elsevier).

Methane emissions considerable change with the type of vegetation present, but the greatest differences in flux seem to depend on the depth to the water table (MacDonald *et al.*, 1998). The methanogenesis in the rice paddies can be more intensive in the deeper layer thanks to higher anaerobic conditions than in the upper aerobic layer, where methane oxidation predominates.

Methane diffusion through aerenchyma in the rice paddies can fluctuate daily probably for the change of environment conditions and the ratio photosynthesis/respiration.

1.4 Methane sinks

The atmosphere represents the most important methane sink, which occurs in the troposphere thanks to the reactions with free hydroxyl radicals. This sink corresponds to about 460±100 Tg CH_4 yr^{-1}, the 90% of total budget removed yearly.

$$CH_4 + OH^{\cdot} \rightarrow CH_3 + H_2O$$

$$CH_4 + 5O_2 + 5NO^{\cdot} \xrightarrow{UV-A} CO_2 + H_2O + 5NO_2^{\cdot} + 2OH^{\cdot}$$

These oxidation reactions produce CO_2 e H_2O with an annual methane loss of about 420±80 Tg yr^{-1} (Crutzen, 1991).

The stratosphere represents a less important methane sink, being here a smaller percentage of CH_4 respect to the total atmospheric amount. Methane in the stratosphere reacts with $OH^{\cdot}$ or with excited oxygen atoms $O(^1D)$ or with chlorine and bromine atoms; the reaction with the latter produce hydroxyl radicals and at the end H_2O:

$$O(^1D) + CH_4 \rightarrow OH^{\cdot} + CH_3^{\cdot}$$

$$OH^{\cdot} + CH_4 \rightarrow H_2O + CH_3$$

Through this mechanism about 10±5 Tg CH_4 yr^{-1} are lost.

1.4.1 Methane consumption in soil

Some aerobic terrestrial ecosystems can be sinks for methane. About 30 Tg of atmospheric CH_4 are oxidized in CO_2 thanks to aerobic bacteria present in the soil and adapted to use small concentration of this substrate. Other microorganism are methylo- or methano-trophic Eubacteria that can use other compounds as methanol.

The principal reactions of oxidation, and the enzymes involved, are:

1. $CH_4 + NADH + H^+ + O_2 \rightarrow CH_3OH + NADH + H^+ + H_2O$ *methane-monooxygenase*
2. $CH_3OH + PQQ \rightarrow CH_2O + PQQH_2$ *methanoo-dehidrogenase*
3. $CH_2O + NAD^+ + H_2O \rightarrow HCOOH + NADH + H^+$ *formaldehyde-dehidrogenase*
4. $HCOOH + NAD^+ \rightarrow CO_2 + NADH + H^+$ *formate- dehidrogenase*

Where PQQ = methotaxin

Oxidation is most rapid in coarse-textured forest soils with well-developed soil structure and a surface organic layer through which gases can readily diffuse (Smith et al., 2000). However, Brumme & Borken (1999) have shown that the inhibition of litter decomposition in acid conditions in some forest soils can reduce substantially the entry of atmospheric CH_4 into the soil. The resulting accumulation of multiple layers of leaves from deciduous trees acts in wet conditions as an effective barrier against gas exchange by diffusion, and retards CH_4 oxidation considerably.

1.5 Nitrous oxide

Nitrous oxide (N_2O) is a gas that contributes to the anthropogenic greenhouse effect for about 5%. Despite its low concentration of about 0.320 ppm (World Metereological Organization, 2006), it has a greenhouse effect 296 times higher than CO_2 (Intergovernmental Panel on Climate Change, 2001), its residence time is about 130 years (IPCC 1995). At present nitrous oxide is increasing with a rate of 0.2%-0.3% per year in atmosphere (Fig. 1.7).

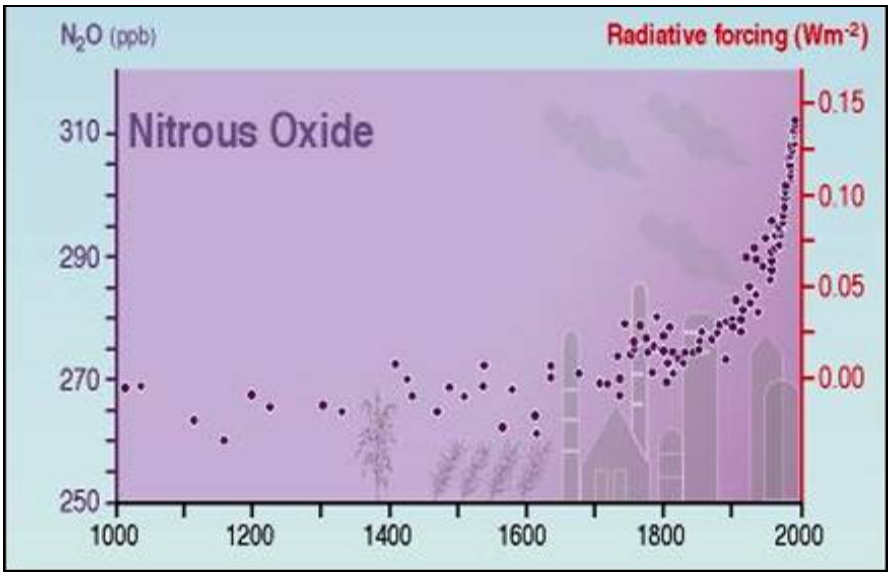


Figure 1.7 – Nitrous oxide increase in atmosphere and its radiative forcing (Wm^{-2}).

The soils are important sources of nitrous oxide and produce about 70% of N_2O emitted from biosphere to atmosphere (IPCC 1995) (Table 1.5). The bigger part of these emissions depends on anthropogenic nitrogen introduced in the natural systems, so they are considered as anthropogenic sources of N_2O.

Even if more than 20% of N_2O global emissions and 50% of total emissions ($N_2O + N_2$) depend on natural terrestrial emissions (Bowden, 1986), the observed increase is essentially due to the increased use of fertilizers and to combustion process (Bolin *et al.*, 1986). N_2O sources include natural soils (6±3 Tg yr^{-1}), oceans (1.5 Tg yr^{-1}). Biomass burning and fossil fuel combustible are important sources of N_2O, in fact, about 0.2-1 Tg of N_2O are produced each year; biomass burning contributes to more than 7% of the global source of N_2O (Cofer *et al.*, 1991).

In the past some researchers believed that the combustion of fossil fuel release N_2O as secondary product of chemical combustion of N_2 and O_2 present in the air, but today is clear that nitrous

oxide occurs only when the nitrogen is contained in the combustible itself, as in the case of the carbon and biomass but not in the gasoline or natural gas (Baird C., 2001).

Table 1.5 - Estimated sources and sinks of nitrous oxide. (IPCC, 1992)

SOURCES	**Tgyr^{-1}**
NATURAL	
Oceans	1.4-2.6
Tropical soils	
Wet forests	2.2-3.7
Dry savannas	0.5-2.0
Temperate soils	
Forest	0.05-2.0
Grasslands	?
ANTHROPOGENIC	
Cultivated soils	0.03-3.0
Biomass burning	0.2-1.0
Stationary combustion	0.1-0.3
Mobile sources	0.2-0.6
Adipic acid production	0.4-0.6
Nitric acid production	0.1-0.3
SINKS	
Removal by soils	?
Photolysis in atmosphere	7-13
ATMOSPHERE INCREASE	3-4.5

According to Skiba et al. (1993) agricultural soil emit about 6.3 $Tg\,N_2O-N$ each year and nitrification is responsible for a 10% to a 90% of total N_2O emissions in the soil. This wide margin can be partly due to the wide spatial and temporal variability of N_2O fluxes in the soil.

Atmosphere represents the biggest sinks of nitrous oxide (7-13 Tgyr^{-1}) and even if N_2O is chemically inert, it has an important role to reduce the ozone in the stratosphere and so it can increase the ozone hole (Crutzen, 1981).

1.5.1 N_2O *sinks*

In the stratosphere the molecules of N_2O can collide with excited oxygen atoms $O(^1D)$ formed during the photochemical decomposition of ozone producing nitric oxide (NO), an important regulator of the concentration and the distribution of the ozone:

$$O_3 + \text{fotone UV}\,(\lambda\, 320\,\text{nm}) \rightarrow O_2 + O(^1D)$$

$$N_2O + O(^1D) \rightarrow 2NO^{\cdot}$$

The molecules of $NO^{\cdot}$ previously produced react efficiently with the ozone subtracting to this an oxygen atoms:

$$NO^{\cdot} + O_3 \rightarrow NO_2^{\cdot} + O_2$$

Subsequently, in the regions of stratosphere where there is a high concentration of atomic oxygen, the molecules of $NO_2^{\cdot}$ react with these atoms producing O_2 and restoring $NO^{\cdot}$:

$$NO_2^{\cdot} + O \rightarrow NO^{\cdot} + O_2$$

In the stratosphere nitrous oxide is mainly removed reacting with excited oxygen atoms through the following reaction:

$$N_2O + O(^1D) \rightarrow N_2 + O_2$$

The soils generally are sources of N_2O but little amount of this gas can be assimilated in dry soils (Duxbury and Moiser, 1993) and in wet grasslands (Ryden, 1983). Also in some soils of brazilian savanna (cerrado) little but inconsistent sinks of N_2O was observed (Nobre, 1994). In the anaerobic soils, instead, we can find the best condition to reduce N_2O to N_2; in these soils N_2 is a bigger product of denitrification rather N_2O (Eriche *et al.*, 1984). The very wet soils or completely flooded can function as sinks, not only because N_2O reduction occurs but also because its diffusion from water to atmosphere is reduced. Negative fluxes were observed also in Mediterranean environments in condition of high humidity and very low N avaibility (< 1 $NO_3^- - N$ mg e 2 mg $NH_4^+ - N$ $kg^{-1}soil^{-1}$) (Rosenkranz et al., 2005).

1.5.2 Processes of N_2O *formation in the soil*

N_2O can be emitted thanks to different processes, so like little amount of NO_x, but these latter aren't fully understood yet. The main biological processes involved in N_2O production in the soil are the nitrification and the denitrification (the dissimilative reduction of nitrate in ammonia) and

the assimilative reduction of nitrate, that is the process through which the inorganic nitrogen is used by organisms to synthetize the amino acids and other organic compounds (Granli & Bøckman, 1994; Bremner, 1997; Barnard *et al.*, 2005). Little amount of N_2O e NO_x can be emitted by chemo-denitrification and pyro-denitrification, this latter process is due to the biomass burning and it occurs above all in the ecosystems as the savanna, where the fires frequently happen (Lobert *et al.*, 1990).

Among these processes, the nitrification and the denitrification represent the main sources of nitrous oxide.

1.5.2.1 Autotrophic Nitrification

The nitrification is the oxidation of reduced inorganic-N, it is a chemosynthetic and strictly aerobic process used to obtain the energy useful to fix CO_2 and to synthetize other organic compounds, this process depends on two groups of nitrifiers present in the soil and in the water.

Nitrous bacteria, like the *Nitrosomona*s e *Nitrosocystis*, which oxide the ammonia (NH_3) in nitrite (NO_2^-) through hydroxylamine NH_2OH (**nitrosation**). To *Nitrosomonas* gender belongs to one of the most diffuse species, *Nitrosomonas europea*, it is small and very mobile and has a form of a ellipsoidal stick. The bacteria that belong to this species live alone or aggregated to form short chains, they are obligated chemiolithotrophos and live either in the soil or in the water, their optimum is about 5-30 °C and pH 5.8-8.5.

Nitric bacteria, like the genders *Nitrobacter* e *Nitrococcus*, instead, oxide the NO_2^- in NO_3^- (**nitration**). The most common species of the gender *Nitrobacter* are *Nitrobacter winogradskyi* and *Nitrobacter agilis,* they generally are mobile and have a form of short stick often pyriform, their cells are rich of cytochromes, that give to the suspension a yellow colour. These bacteria are strictly aerobic, live both in the soil and in the water and their optimum is about 5-40 °C e pH 6,5-8,5.

The nitrosation and the nitration together form the nitrification and in both the stages of oxidation is produced ATP.

During the oxidation of NH_3 to hydroxylamine there is no ATP production because the reaction is endoergonic (ΔG = +3.85 Kcal/mole), while the oxidation of NH_2OH to NO_2^- has a ΔG = -69 Kcal/mole and that one of NO_2^- to NO_3^- has a ΔG = -17.5 Kcal/mole.

Because the ΔG of nitrification is very low, the amount of compounds transformed must be very considerable to compensate the requirement of the metabolism.

The ammonia, as NH_4^+, is oxidized to nitrite, transferring 6 electrons

$$NH_4^+ + 3/2\ O_2 \rightarrow 2\ H^+ + H_2O + NO_2^- (\Delta G \approx -66.5 \text{ kcal/mole})$$

The process occurs in two steps:

The first stage is due to a monooxygenase, that using O_2 as oxidizing agent, adds one O to NH_3:

$$NH_4^+ + 1/2\ O_2 \rightarrow NH_2OH + H^+ (\Delta G \approx +3.85 \text{ kcal/mole})$$

After, the hydroxylamine is oxidized thanks to a dehydrogenase, a complex cytoplasmic enzyme:

$$NH_2OH + O_2 \rightarrow H^+ + H_2O + NO_2^- (\Delta G \approx -69 \text{ kcal/mole})$$

The oxidation of nitrite in nitrate, catalyzed by the nitrite oxidase, completes the process of nitrification:

$$NO_2^- + 1/2\ O_2 \rightarrow NO_3^- (\Delta G \approx -17.5 \text{ kcal/mole})$$

During the nitrification N_2O can be formed also from the dissociation of NOH, an instable intermediate that is formed during the oxidation of the ammonia (NH_4^+) to nitrite (NO_2^-) (Hynes and Knowles 1984; Schmidt and Voerkelius 1989).

Nitrous oxide can be produced also by nitrifiers during the denitrification probably in condition of low oxygen concentration (Ritchie and Nicholas 1972; Poth and Focht 1985; Varkelius 1990; Wrage et al. 2001); this pattern, defined *nitrifier denitrification*, start from nitrite via nitric oxide (NO) (Cosciotti & Ward, 2001, Beaumont *et al*., 2002, 2004; Schmidt *et al*., 2004). In anaerobic condition *Nitrosomonas europea* can obtain the energy useful to its growth either from oxidation of NH_4^+ or from denitrification (Scmidt et al., 2004). However it is not still clear how the nitrifiers can reduce N_2O to N_2 and, although the genome has been completely sequenced, the enzyme (or enzymes) responsible for the N_2O reduction hasn't still been identified (Chain *et al.*, 2003; Schmidt *et al.*, 2004).

1.5.2.2 Denitrification

The denitrification is an aerobic process defined also dissimilative reduction of oxidizing N-compounds. All the living organism, except the chemosynthetic ones, take the necessary energy from the oxidation of organic compounds through the respiration. The denitrifiers in presence of oxygen use it as terminal acceptor of electrons, if instead there's no oxygen but there is nitrate, they use this latter as acceptor of electrons.

The denitrifiers are able to do anaerobic respiration of nitrate, using the nitrate instead of oxygen as acceptor of electrons released during the respiration; these bacteria have one respiratory chain that normally leads the electrons towards the oxygen. The electrons transfer through the respiratory

chain is coupled to the process of phosphorylation to produce ATP. These bacteria have special enzymes, the nitrate reductase and nitrite reductase, jointed to the respiratory chain that in the absence of oxygen, let flow the electrons towards the nitrate or nitrite. These enzymes form in the cellular membrane only in anaerobic condition; if there's oxygen the synthesis of reductases is repressed.

Some species belonging to the genders *Pseudomonas, Thiobacillus, Paracoccus* and *Azospirillum* are denitrifiers. The species of the gender *Pseudomonas* are chemo-organotrophs with a respiratory metabolism never fermentative, they have a single cell with a stick erect or bent form, don't produce spora and can move thanks to polar flagella. Some of them are facultative chemo-organotrophs, able to use hydrogen and carbon dioxide as source of energy, they generally use oxygen as acceptor of electrons but other ones can use nitrate. They are obligate aerobic, except the species able to use denitrification for respiration.

During the denitrification, similarly to aerobic respiration, the complete oxidation of organic substrate in CO_2 and H_2O occurs, with similar ΔG (about -686 Kcal/mole of glucose).

The denitrification permits a return of nitrogen from soil and water exchange pools to the atmospheric reservoir. This process occurs according to different reactions leading to a final product which is N_2 and to the subproducts NO and N_2O.

$$5\ C_6H_{12}O_6 + 24\ NO_3^- \rightarrow 30\ CO_2 + 24\ OH^- + 18\ H_2O + 12\ N_2$$

($\Delta G \approx$ - 3018 kcal/mole)

$$C_6H_{12}O_6 + 6\ NO_3^- \rightarrow 6\ CO_2 + 6\ OH^- + 3\ H_2O + 3\ N_2O$$

($\Delta G \approx$ - 508 kcal/mole)

$$C_6H_{12}O_6 + 12\ NO_3^- \rightarrow 6\ CO_2 + 6\ H_2O + 12\ NO_3^-$$

($\Delta G \approx$ - 496 kcal/mole)

N_2O is a subproduct that occurs from reduction of NO_3^- in N_2 during the denitrification (Payne 1981; Zumft and Kronech 1990), however not all the denitrifiers complete the reactions of reduction, generally intermediate concentration of oxygen on the interface among anaerobic and aerobic soils or microsites promote N_2O formation, both during the nitrification and the denitrification, while completely anaerobic condition favour N_2 vs. N_2O.

1.5.2.3 Eterotrophic nitrification

Nitrous oxide can be produced not only from autotrophic nitrification but also through the heterotrophic nitrification in anaerobic conditions (Umarow, 1990). This process depends on the oxidation of NH_3 / NH_4 or on the oxidation of organic-N compounds to NO_2^- and/or NO_3^- in the presence of carbon substrates under anaerobic condition (Papen and Rennenberg, 1990). The main reactions are:

$$R - NH_2 / NH_4^+ \rightarrow NH_2OH \rightarrow NOH \rightarrow NO \rightarrow NO_2^- \rightarrow (NO_2\ ?) \rightarrow NO_3^-$$
$$\downarrow$$
$$N_2O$$

Nitrous oxide can also form by chemical reactions, in particular when NO_2 and NH_2OH are decomposed in the acid soils:

$$NH_2OH + HNO_2 \rightarrow N_2O + 2H_2O$$

Overall this process represent only a minor source of N_2O (Yoshinari ., 1990).

Soils are also a significant NO and NO_2 sources (defined NO_x) (Davidson and Kingerlee 1997); tropospheric NO_x contributes to the formation of photochemical smog (Crutzen 1981) and to nitrogenous atmospheric deposition either directly or as nitrate. Nitrogen input notably influences nitrogen status in natural and semi-natural environments (Gebauer et al. 2000; Harrison et al. 2000). NO_x production in the soils is due both to the denitrification and to the chemo-denitrification, the chemical scission of HNO_2 that generally comes from NO_2^- in acid soils (Scheffer e Schachtschabel 1998).

However also nitrification has been demonstrated to be a NO_x source (Kester et al. 1997). NO_x fluxes increase with the decrease of water content in the soil (Skiba et al.1992; Yamulki et al. 1995) and nitrogen losses as NO_x can be even higher than N_2O emissions in dry soils.

A good knowledge of N_2O fluxes is essential to try to reduce its emissions, nevertheless the fluxes rate due to nitrification or to denitrification aren't well interpreted yet.

1.6 Abiotic regulating factors of greenhouse gases production and consumption

The abiotic factors have different effects on emissions of greenhouse gases, and despite the numerous studies, it is still difficult to establish an univocal relation between the different abiotic factors and gas exchanges for the several types of soil, for the different climatic conditions and for the different land uses.

The most important factor that controls NO, N_2O and CH_4 emissions is the soil water content, but also the temperature and the acidity can have relevant effects. Soil moisture and gas diffusivity strictly depend on the physical properties of soil, in particular on texture, mineralogy, organic content and its structure, the current vegetation and cultivation practices (e.g. tillage, irrigation and drainage). All these factors widely change at local, regional and global scale so as the microclimate, both in space and time (Nielsen and Bouma, 1985).

Climate for example strongly controls methane emissions in acid peats present in the subarctic mires where the cycle of freezing-thawing of the permafrost controls methane production and diffusion along soil profile (Svensson and Rosswall, 1984, Wilson *et al*., 1989).

In the rice paddies CH_4 fluxes can change seasonally in accordance to the different stages of plant growth (Schütz *et al.*, 1989; Schütz *et al.*, 1990). Also daily variations of temperature and irradiance can induce significant changes of CH_4 fluxes in rise paddies and wetlands (Aulakh *et al*., 2001).

The wide variability of the biological processes involved in the N_2O production explains why the field measures of N_2O fluxes often change markedly in the space and time. This variability can be generally attribute mostly to denitrification, because the nitrification is a less variable process. This is mainly to the necessity of occurrence of ‘’anaerobic microsites’’ for denitrification activity in aerobic soils (Firestone and Davidson, 1989).

Concerning the location of N_2O production along soil profile, the highest N_2O production occurs near soil surface, even if a significant N_2O production has also been observed in the sub-superficial soil layer (Clough *et al.*, 1999; Verchot *et al.*, 1999; Brye *et al.*, 2001; Elmi *et al.*, 2003, Müller *et al.*, 2004). N_2O produced in the topsoil can easily escape to the atmosphere without a long residence time in the soil, while N_2O formed in the lower soil layer could not reach the atmosphere (Neftel *et al.*, 2000). Seiler and Conrand (1981) reported that nitrous oxide formed in the lower soil can be consumed in the upper layer during the gas diffusion and the reduction of N_2O in N_2, these processes could increase if the soil is wet.

In the soil not completely flooded and partly anoxic the denitrification is confined principally inside the micro-aggregate defined hot-spots (Parkin, 1987) or associated with earthworm casts (Svensson

et al.,1986; Knight *et al.*, 1989). Some authors underlined that the denitrification occurred in the single aggregates can be estimated with good probability only when the spatial and temporal distribution of denitrifiers, water content, oxygen, organic decomposable compounds and nitrate can be measured and calculated (Smith 1980, Leffelaar, 1986). Denitrification variability can be also due to an uneven distribution of denitrifiers, NO_3^- , carbon, inside the aggregates (Sextone *et al.*, 1985).

1.6.1 Effects of water content and gas diffusivity

Soil water content is a key controlling factor for the rates of nitrification, denitrification, methane oxidation and methanogenesis above all for two reasons (Skopp, 1985).:

- First, it controls the availability of substrates for microbes (e.g. NH_4^+ to nitrifiers and acetate to methanogens) with their diffusion through the soil water film.
- Second, it controls soil porosity, an important control factor of gas diffusivity. It's important remember that gas diffusivity through the water is about 10000 times slower than through air.

Moreover gas diffusivity can also decrease in function of salinity, alkalinity and temperature (Heincke and Kaupenjohann, 1999).

Linn & Doran (1984) showed that lack of diffusion of organic carbon substrates in soil water films can be a limiting factor in dry soils for microbial respiration, on the contrary the oxygen can be a limiting factor in the wet soil for the opposite reason. For the same reasons chemoautotrophic nitrification could be limited by diffusion of NH_4^+ through the water films in dry soil, and by diffusion of O_2 in wet soil (Papendick & Campbell, 1981).

As mentioned previously nitrification need O_2 while denitrification is inhibited by O_2, also the concentrations of the end-products either of nitrification or of denitrification are influenced by the amount of oxygen; nitrification produces more N_2O than NO_2^- at low O_2 concentration (Goreau *et al.*, 1980). The effects of oxygen on N_2O emissions produced by nitrifiers is complex, the maximum N_2O emission generally occurs when there is enough O_2 to oxide NH_4^+ but if O_2 concentration is very low N_2O becomes an end subproduct.

Also NO, N_2O, and N_2 production obtained by denitrifying bacteria is influenced by O_2. The denitrification occurs when the partial pressure O_2 is low, but in completely anaerobic conditions N_2 represents the end-product of denitrification. In conclusion the maximum N_2O production

obtained by denitrifiers happens when partial pressure O_2 is low enough to have the reduction of NO_3^- but not so low as to have the reduction of N_2O. On the other hand water can increase the solubility of organic compounds and nitrate stimulating denitrification (Bowden, 1986).

Moreover N_2O generated in the soil can be entrapped in soil itself and it can cause an underestimation of fluxes measurement. In dry soils gas diffusivity is quicker than in the wet ones, so in wet conditions more than a week could be needed to diffuse all the produced N_2O to the atmosphere (Pathak., 1999).

An important parameter useful to understand gas diffusivity in the soil is the **w**ater-**f**illed **p**ore **s**pace (WFPS) (Fig 1.8) calculated using the bulk density and the particle density. This variable has also the advantage to be compared the soil diffusivity status among soils with different textures.

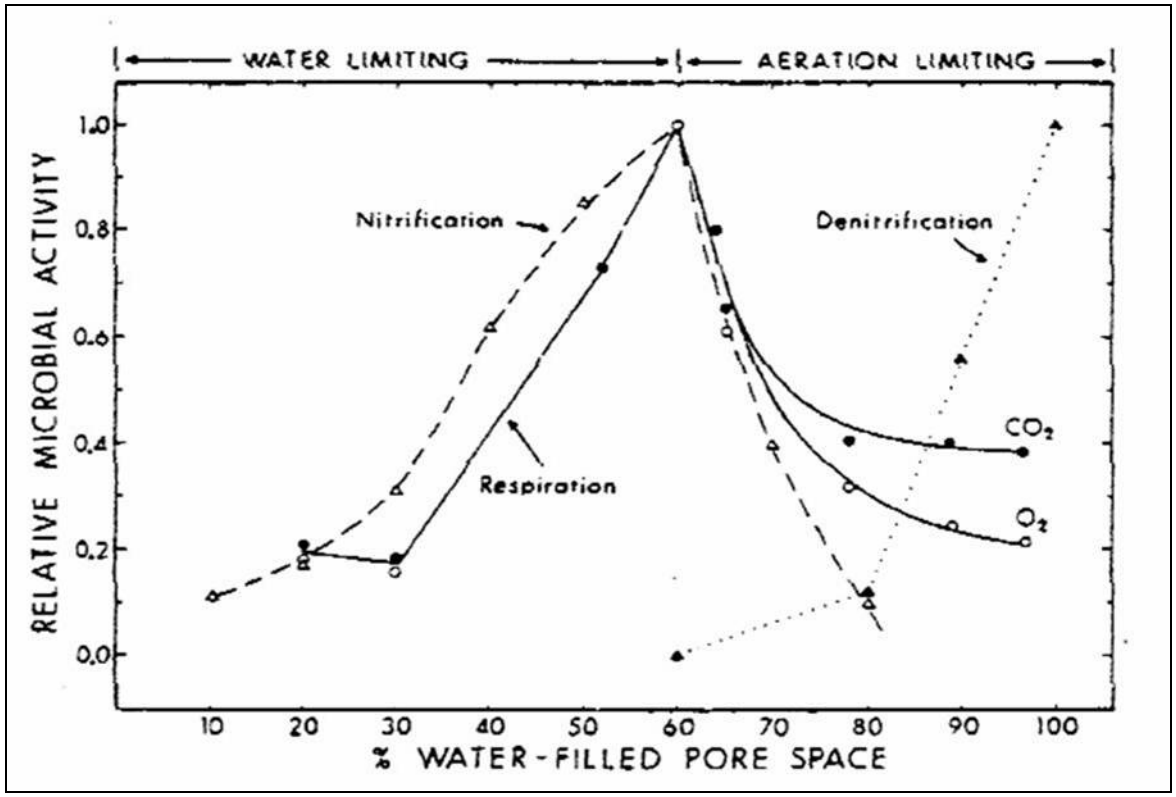


Fig 1.8 - The relation between water-filled pore space (% WFPS) and relative amounts of nitrification, denitrification, O_2 uptake by microbial respiration and CO_2 production by microbial respiration. (From Linn and Doran, 1984).

When the WFPS is lower than 60% nitrification represents the main source of NO and N_2O. For water-filled pore space higher than 60% denitirification becomes the principal N_2O source (Figure 1.8) (Davidson, 1993; Linn and Doran, 1984). The optimal WFPS value for the aerobic processes such as nitrification and microbial respiration is about 60% and it also depends on the availability of organic compounds and inorganic nitrogen (Doran et al., 1990).

It is also shown that the ratio N_2O/NO is lower than 1 when WFPS<60% while it is higher than 1 when WFPS>60%, hence it follows that nitrification produces more NO than N_2O and denitrification produces more N_2O than NO (Davidson, 1993).

1.6.2 Effects of drainage

Water content and O_2 concentration in the permeable soils depend also on drainage which hence represents an indirect controlling factor for denitrification, the two parameters being inversely related Colbourn and Harper (1987). This relationship is however not always so obvious in organic soils (Hystosols) (Laine et al., 1996, Maljanen et al., 2003, Schulte-Bisping et al., 2003).

Drainage influences also methane emissions because aerates the soil and can reduce or inactive methanogenesis reactions. Recurrent drainages in flooded rice paddies considerably decrease CH_4 emission rate (Yagi and Minami, 1990; Sass *et al*. 1992).

1.6.3 Effects of temperature

Temperature is a key controlling factor of microbial activity. Methane emissions for example increase with rising temperature not only because this stimulates directly methanogenic bacteria activity but also because it accelerates decomposition of organic compounds which create substrates useful to methanogens (Chin and Conrad, 1995). The methanogenic reactions have an optimum to about 30 C°(Pacey and De Gier, 1986). The lower limit for methane emissions instead is 10-15 C° even if in very cold environment as the tundra, where temperature reaches 2-4 C° and the permafrost occur 25 cm under the surface, methane fluxes similar to those present in temperate regions has been recorded (Sebacher *et al*., 1986).

Also methane oxidation increases in accordance to temperature. It can occur also at low temperature; in Alaskan tundra soils methane oxidation has been observed at temperature of about 7 C° thanks to special bacteria able to oxide methane at concentrations 10 times lower than ambient atmospheric methane concentration (Wahlen and Reedburgh, 1990).

The relations between the temperature and nitrification or denitrification activity are not always straight forward because of the interactions between microbial activity, water filled pore space, oxygen solubility and organic substrates availability.

The optimum temperature for nitrification ranges between 30-35 C° while temperatures below 5 C° and above 40C° are generally considered limiting (Alexander, 1977); for the denitrification the

optimum temperature ranges between 30 and 60 C° and ceases at temperature of about 70 C° (Keeney *et al*., 1979; Malhi *et al*., 1990).

Increasing of soil temperature positively influences microbial activity and gas diffusion but have negative effect on N_2O solubility (Heincke & Kaupenjohann, 1999).

The temperature dependence commonly is expressed in terms of Q_{10} value:

$$Q_{10} = \text{activity rate}\,(T+10)/\text{activity rate at T}$$

Q_{10} values observed for N_2O emissions are generally higher than 10, these values are enough high if compared with the general range of 2-3 for most biochemical process.

An explanation to this effect is due to the presence of some anaerobic zone developed in the soil; in fact, an increasing temperature provokes an increase of respiration, hence more oxygen is consumed and develop new and bigger anaerobic microsites (Smith 1997; Li *et al*., 2000) (Fig 1.9).

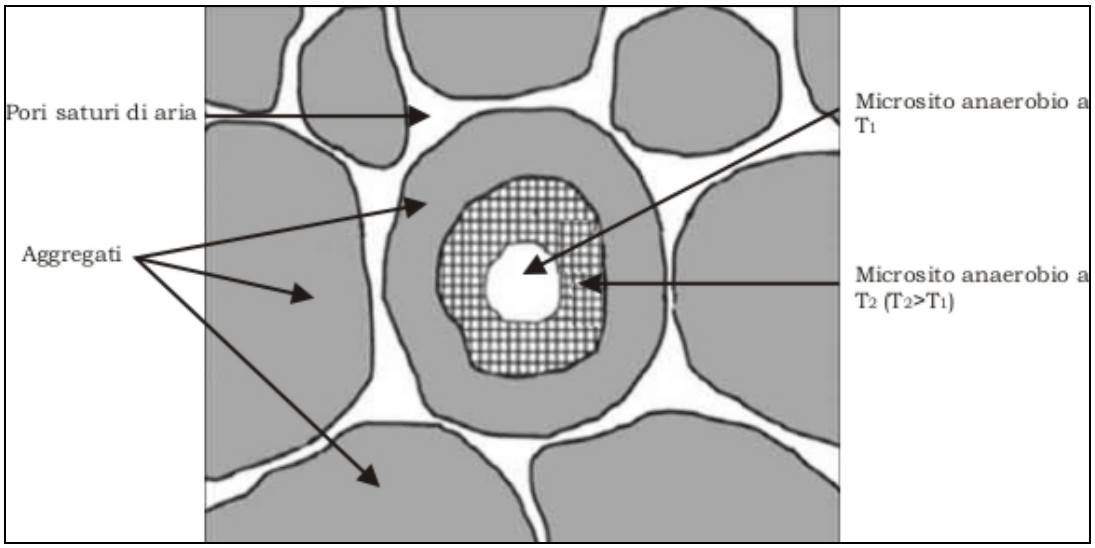


Fig. 1.9 – Relation between the size ana naerobic microsite and the soil temperature(Smith *et al*., 2003 modified).

1.6.4 Effects of acidity

Soil acidity influences all microbial processes hence also nitrification and denitrification that can be altered even if the other factors are suitable. At the same time soil acidity can depend on nitrification and denitrification end products.

In culture, chemoautotrophic nitrifiers achieve their optimum at neutral or slightly alkaline pH; the same occurs for denitrifiers even if some of them are acid-adapted and can achieve pH optimum at very low value too (e.g. pH=3.9) (Parkin *et al*., 1985).

Soil acidity can influence N_2O emissions through different mechanisms. For example the decreasing of acidity indirectly can affect N_2O fluxes decreasing decomposition rate hence reducing the availability of N substrate useful for N_2O production.

The decreasing pH directly can reduce nitrification and denitrification; acidification can inhibit N_2O reductase with the result that denitrification yields more N_2O than N_2 but can decrease also molybdenum that in turn may reduce the synthesis of nitrate reductase. Moreover with the decreasing pH NO_2 formed by NO_3 reduction would become toxic and the solubilization of aluminium or manganese might cause toxicity effects (Pathak, 1999).

Even if soil acidity is far from pH optimum of microorganisms, nitrification and denitrification can, in some cases, efficiently occur because the microsites inside of which microorganisms live and grow can have a different pH respect to the soil one.

Acidity, but also other factors, can alter one or more phases of a process bringing to the store of intermediate as N_2O. This acid effect could explain why N_2O is a common end product of denitrification in acid forest soil, but this hypothesis has not been fully tested (Melillo *et al*., 1983).

Methane production and emissions occur only when soil pH range in a strict gap between 6.4-7.8 (Williams and Crawford, 1984; Delaune et al., 1986) with an optimum that depend on soil characteristics, temperature and on the amount of methanogens (Moore and Knowles, 1987; Kimura, 1992). Flood effect can increase pH in acid soil, while in alkaline soil it generally decreases.

The increasing pH in acid soil is due principally to Fe^{3+} reduction in Fe^{2+} that simultaneously decreases redox potential. The add of nitrate, so as Fe^{3+}, Mn^{4+} and sulphate, acts as terminal electrons acceptor during the anaerobic respiration.

1.6.5 Effects of redox condition

Another variable, related to the soil water content, useful to understand CH_4 and N_2O production is the redox potential (Eh), normally measured with a platinum electrode. As methanogenesis is a strictly anaerobic process, it only can occur at low redox potential, in fact, CH_4 production often begins at redox potential comprise among -100 mV and -200mV but the maximum efficiency occurs at redox potenzial below -150 mV (Patrick, 1981). Some suggested that containing greater amounts of readily decomposable organic substrates (acetate, formate, methanol, methylated amines, etc.) and low amounts of electron acceptors (Fe^{3+}, MN_4^+, NO_3^-, SO_4^-) are likely to show high production of CH_4.

In some soil samples in which it was cultivated rice the methanogenesis happens when the redox potential range among -250 mV and -500 mV until the value of 150 mV (Masscheleyn *et al.*, 1993) while in well drained soil efficiently aerated the redox potential can achieve 300-600 mV promoting methane oxidation (Moorman and Van Breemen, 1978). N_2O production generally occurs among +200 mV and +500 mV instead N_2O reduction happens when redox potential achieves value lesser than +250 mV (Smith *et al*., 1983).

1.6.6 Effects of soil organic matter

Denitrifiers as well as nitrifiers use organic C compounds as electron donors for energy and synthesis of cellular constituents. Plant residues, green manure and farmyard manure have been reported to increase rates of denitrification (Aulakh *et al.*, 1992; Beauchamp et al., 1989). However, if the soil is N limited, denitrification losses may be reduced due to immobilization of N caused by the decomposition of residues having wider C/N ratios (Fredrickson *et al.*, 1981).

Some researchers observed that maximum rates of N_2O emission occur within the first 4 days after poultry litter application (Cabrera *et al*. 1994). The highest emission rate occurred with fine poultry litter particle followed by pelletized litter. Rolston *et al*. (1978) estimated the emission of N_2O from a field which received cow dung manure and reported that maximum emission occurred with manure application.

However, the ratio C/N of the organic compounds is important. In forest soil, where the soil organic matter is abundant, nitrification rates increase with reductions in the CN ratio of the organic matter (Ollinger et al., 2002). Klemedtsson *et al.* (2005) showed that the mean annual N_2O emissions can be described as decaying exponentially with increasing CN ratios (figure 1.10).

Fresh organic matter and water content in the soil stimulate microbial respiration, inducing O_2 consumption leading to anaerobic microsites where normal denitrification and methanogenesis could have taken place. The availability of organic carbon in soils was correlated with the production of N_2 in soil core studies, hinting at more anaerobic conditions (Mathieu et al., 2006).

As mentioned previously nitous oxide have a large spatial and temporal variability but in hystosols, N_2O emissions should theoretically be less affected by hotspots than mineral soils, as their organic content is high and their moisture status less variable over the year, giving more stable oxygen conditions.

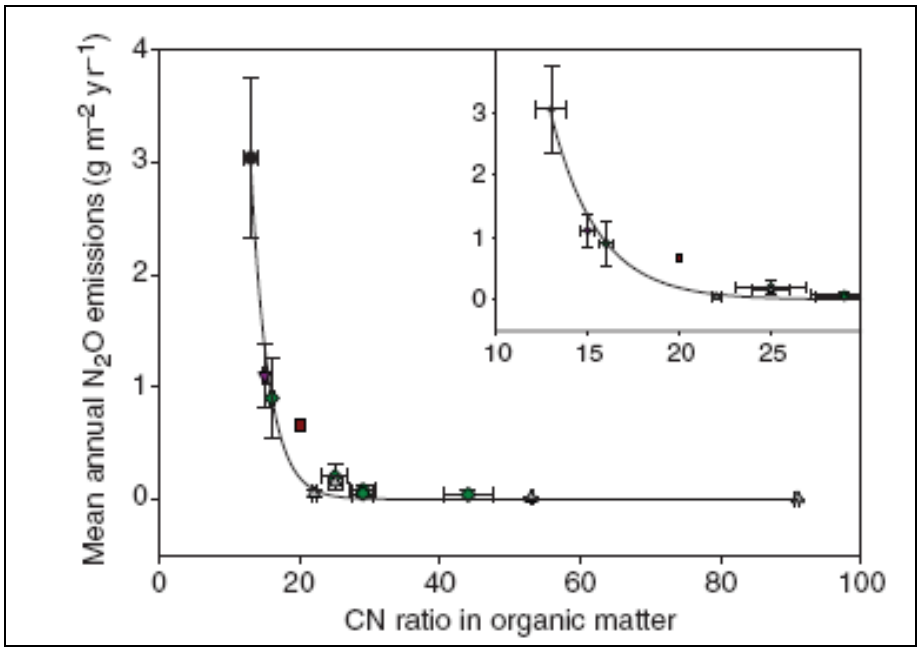


Figure 1.10 - Correlation between CN ratio in the upper layer of the soil and mean annual nitrous oxide emissions. The bars represent ± 1 standard error (Klemedtsson et al., 2005).

At low contents of fresh organic material nitrification is almost absent because heterotrophic microorganisms could compete better than nitrifiers for NH_4^+ present in the soil. At very high relative N availability, plant and heterotroph competition for NH_4^+ becomes low enough to allow nitrifiers to flourish and the N economy of the system becomes progressively more NO_3^- dominated. Nitrifiers would likely live in close-enough association with mineralizers so that NO_3^- becomes the dominant N form moving through the soil (Chen and Stark, 2000). Such ecosystems might include agricultural systems and N-rich tropical forests (Hall and Matson, 1999b).

The surplus of nitrate can inhibit methane production because NO_3^- delays the formation of methane through its effect on the redox conditions and it may have a toxic effect on methanogenesis (Jacobsen *et al.*, 1981).

The amendment of organic matter (cattle manure, pig manure, chicken manure, etc.) to a flooded rice field, increases CH_4, production. It reduces the soil Eh and provides carbon to methanogens. Organic materials influence the CH_4 formation through change in qualitative and quantitative properties of soil.

In the flooded soil fermentation is one of the major biochemical processes responsible for organic matter degradation; the main products of the fermentation are ethanol, acetate, lactate, propionate, butyrate, H_2, N_2, CH_4 and CO_2. The latter three gases usually constitute the largest portion of the gas phase of flooded soils. The major pathways of CH_4 production in flooded soil are the reduction of CO_2 with

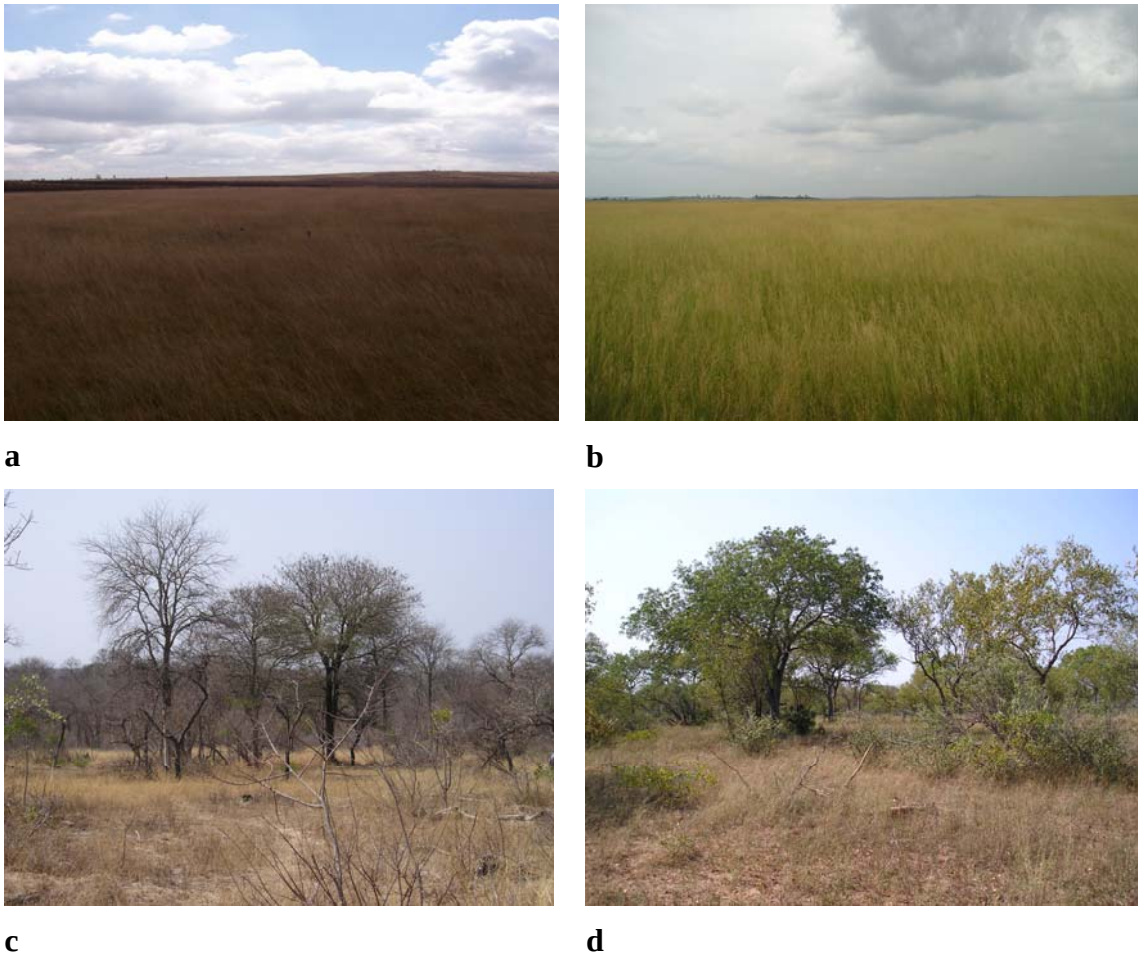
H_2, with fatty acids or alcohols as the hydrogen donor, and the transmethylation of acetic acid or methyl alcohol by CH_4 producing bacteria (Takai, 1970).

1.6.7 Effects of vegetation

The vegetation can influence soil water content and the biological processes by root activity which consumes water, and oxygen through respiration. Moreover the plants affect the emission of N_2O by influencing nitrate and carbon content of the soil and partial pressure of oxygen. Plants can directly influence nitrate availability through uptake and assimilation making it unavailable to denitrification. An indirect effect of nitrate levels arises from the supply of organic matter of root origin. Mineralization and nitrification of this material can potentially provide more nitrate for denitrification and conversely immobilization can reduce nitrate levels. Another indirect effect is the ability of few plants, e.g. rice to supply O_2 at the rhizosphere, which can enhance the nitrate content by promoting nitrification. Plant species might differ in their effect on denitrification. Higher denitrification rates in soils grown with a legume rather than with a monocotyledonous plant have been observed (Bertelsen *et al.*, 1992).

1.7 Savanna ecosystem

High temperatures, heavy rainfall, and oppressive humidity characterize equatorial and subtropical regions, except at high altitudes. At low altitudes, temperature variation is seldom less than 10 C° and is often more than 35 C°. At altitudes over 1500 meters, frozing processes can be observed during the night. The rain has a cooling effect, but when it stops, the temperature increases. Rainfall is heavy, often with thunder and lightning. Rain can arrive suddenly beating on the tree canopy and turning trickles into raging torrents and causing rivers to rising. Just as suddenly, the rain stops. Violent storms may occur, usually toward the end of the summer months.



a **b**

c **d**

Figure 1.11 -Two different kinds of seasonally dry savanna: a congolese grass savana (a dry season; b wet season) and south african broadleaf savanna (c dry season; d wet season)

Tropical seasonal forests are dominated by semi-evergreen or partially or totally deciduous woody species that shed their leaves in the dry period; the percentage of deciduous species increasing with drier conditions. At low annual average rainfall, tropical seasonal forests are replaced by tropical

woodlands with small evergreen trees. Such communities dominated by evergreen and semi-evergreen species are 5-7 m tall, and can create a dense canopy in more favorable conditions, turning into open woodland or shrubland where the soil is shallow or disturbed (coppicing or fire). These communities cover a wider area than tropical rain forest.

The savanna, indeed, is represented in tropical and subtropical areas by plant communities dominated by grasslands, occasionally interrupted by shrubs and trees (Boulier and Hadley, 1970); it can be observed in geographical areas of Africa, Asia, Latin America and Australia where a rotation of wet and dry seasons is present (Figure 1.11). Growing period mainly occurs during the wet season (from September to February) while the dry season extends from March to August during which the fire, either of natural origin or anthropic one, can occur.

According to Penninghton et al. (2000) savannas have a xeromorphic fire-tolerant grass layer different from tropical dry forests, that is formed by continuous canopy and grasses as a minor component, while Scholes and Walker (1993) in their definition of savannas exclude grassland without tree and denser woodlands (crown cover > 80%) and fire effects so the main criteria that they use are:

1. Monthly mean temperature 10° C throughout the year.
2. At least 60 consecutive days per year with insufficient water for plant growth.
3. At least 60 days per year when there is sufficient water for plant growth.
4. The wet period warmer than the dry one.
5. Woody canopy above 2 m.
6. Grasses and trees not spatially separated.
7. Aerial cover of woody plants > 5 % but < 80%.
8. Grass aerial cover at least 5% in a year with average rainfall and grazing.

Many savannas can be considered ecotones between plant communities dominated by woody species (shrub-steppe, desert scrub, woodland or forest) and grassland. Different savannas types can be recognize according to the stature (shrub vs. tree), functional form (evergreen vs. deciduous; broad-leaved vs. needle-leaved; shallow vs. deeply rooted), canopy cover (e.g. 5% to 80%) and spatial distribution (random, regular or clumped) of the woody individuals that characterize them. Likewise, the grass layer can be characterized by short vs. tall-statured species, bunch vs. rhizomatous growth forms, and C3, C4 or mixed C3/C4 photosynthetic pathway assemblages. This

variation in structural/functional characteristics depends on the different interactions between climate (especially the amount and seasonality of rainfall), soils (notably depth and texture), and disturbance (particularly grazing, browsing and fire) (Walker, 1987; Backéus, 1992).

The savanna extension is uncertain and that is due above all to lack of data, however it was estimated that the savanna has an extension that ranges among 1300-1900 million hectares of which about 60% are humid savannas, where the annual rainfall generally is about 700 mm or more; the remaining 40% are dry savannas with an annual rainfall lower of 700 mm (Bolin et al., 1979) (Fig. 1.12)

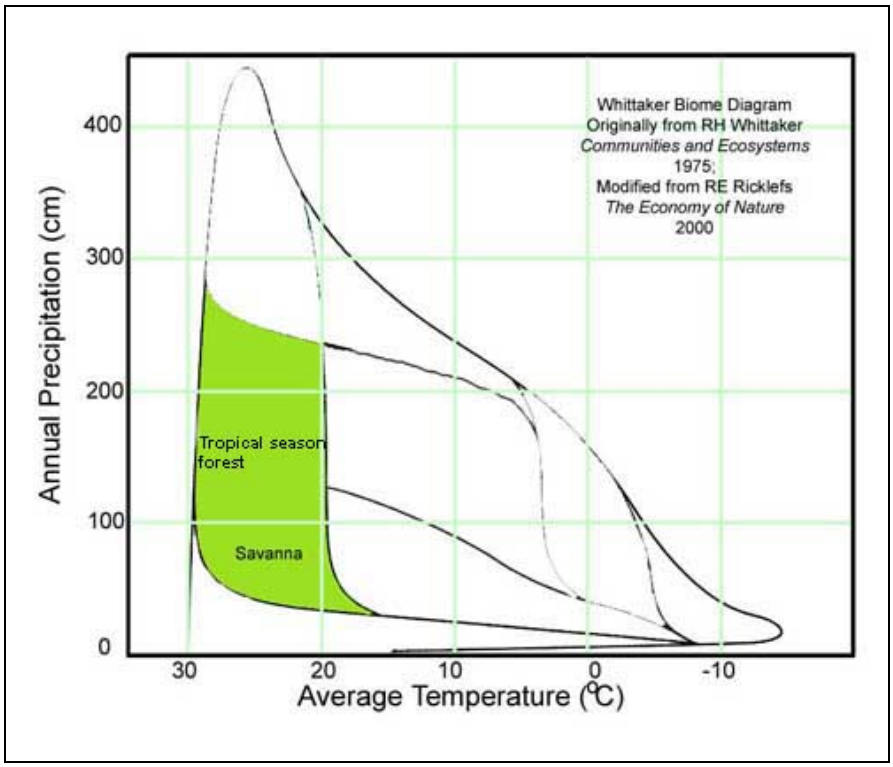


Figure 1.12 – Annual precipitation and average temperature of savanna and tropical ecosystem.

1.7.1 Man's disturbances

Vegetation distribution in Africa is due either to climatic conditions or to disturb produced by human activities, which have been going in these regions fot at least one million of years, in particular burning and grazing (Brain and Sillen, 1988).

Fire is a very important ecological factor in nature (Fox & Fox, 1987); it can be produced from natural causes as volcanic eruption, lightnings, accidental falling boulders, but it occurs thanks to anthropic factors too: today humans represent the first cause of fire. To get a fire there is the need of

abundant substrate either dead (necromass) or alive (biomass) organic materials. Moreover the combustible must have a low water content. The alternance of wet season with growth, and dry season with drying of combustible creates favourable conditions for fire risk (Figure 1.13).

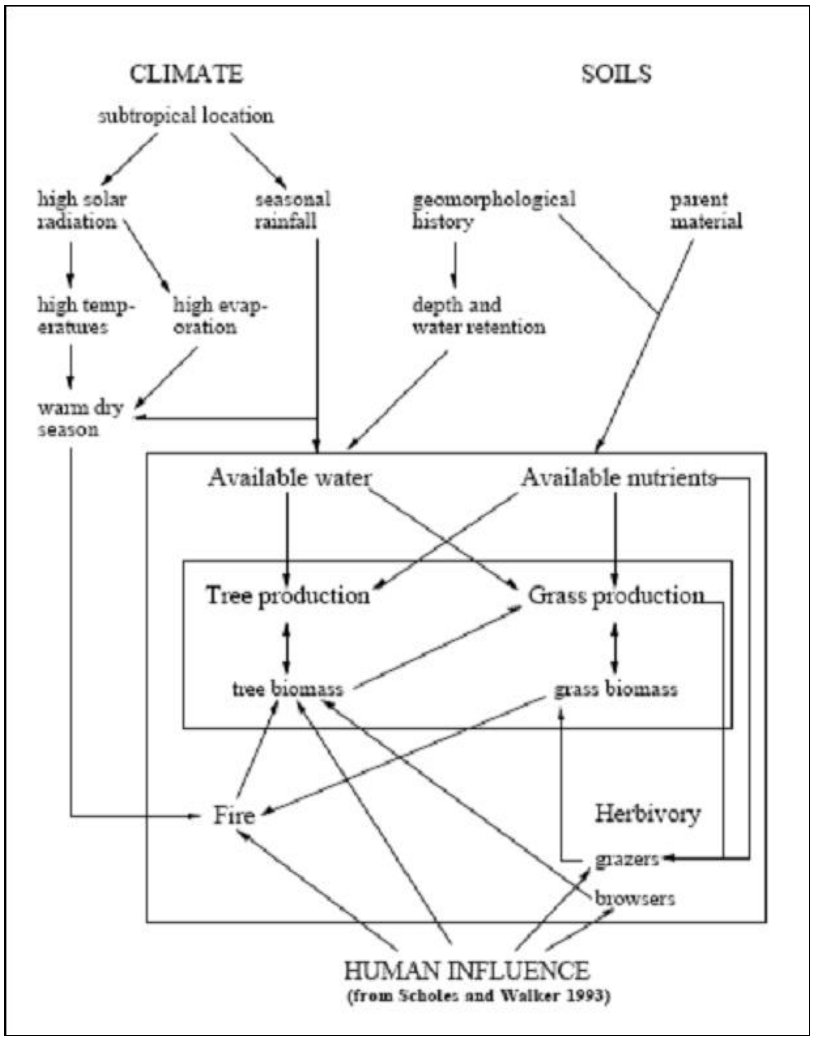


Figure 1.13 - Numerous factors interact to affect the abundance of grasses and woody vegetation in drylands (from Scholes and Walker, 1993).

Man's fire discovery and its use has determined a strong impact on vegetation; when he was still picker and hunter fire was used to burn forests in order to facilitate hunting. Fire was also useful to empty spaces from woody vegetation and to make glades in which herbivorous concentrated and where it was easier to follow and catch them (West, 1965).

Later man knew plants that produced eatables fruits, others with a high nutritional value, as the Leguminosae, so from picker and hunter he became principally a farmer. This transformation implied an increase of fire, because in this way human easily obtained new areas to farm. In subsequent centuries the woods was more and more used as fuel and to build.

While in industrialized countries, after the dismissal of agricultural and pastoral activities, forest cover is recovering, in Africa, where the forest has always represented a source of susteinance, the demographic increase and grazing continue to be a disturb for the principal ecosystems, that currently show a continuous decrease of their extension. Today the removal of wood for commercial sale and as fuelwood is still proceeding at an increasing rate (Anderson 1986; van Gils and Aongola 1988). Increasing demand for land has sped up the rotation cycle of ash fertilization agriculture (known as *chitemene*, Stromgaard 1985), resulting in decreased soil fertility and insufficient time for woodland regeneration. Savanna is purposely burned during the dry season for agricultural purpose in particular to remove grassland of weeds and pests promoting nutrient cycling, and increasing the growth of new grasses for animal grazing.

1.7.2 Fire in savanna

It's important to define fire regime to better understand its effects on vegetation through different parameters: fire intensity, frequency and timing of burning. Fire intensity represents the energy released during the fire and it can be determined through the Byram's equation (Fox & Fox, 1987):

$$E = CMV$$

Where E is the intensity of thermal power, C the specific heat of combustible, M the amount of combustible, V the propagation speed. The frequency denotes the numbers of fires in a place that occur in a determinate period while the time of burning represents the period of the year in which most fires concentrate. Fire frequency can be very variable: there's a direct relation between the interval, that is the real time between the present fire and the former, and intensity because the wider is the interval the larger will be the amount of accumulated combustible and therefore the fire will be more intense. There's, indeed, an opposite relation between the intensity and frequency of fire. On the other hand the frequency is directly related to the increase of biomass so a community that grows rapidly produces more combustible and the fires can be more frequent. Considering the season, during the dry season for the same volume of combustible there's a higher intensity than the wet season because bigger is the amount of dry combustible.

The plants react to the fire in different way, some burn with difficulty because they have specific protections (passive pyrophitism), other species indeed recovery after the fire (active pyrophitism) somentimes from seedlings but more commonly by regeneration from surviving root-crown buds,

this woody rootstock is often extensive and much branched to form a virtual subterranean tree. Such structure is typical of both African woodlands and wooded grasslands (White, 1977).

Fire is an important determinant of vegetation structure in African savannas (Frost & Robertson 1985). Its effects are most often cited as being directly responsible for observed tree/grass ratios in savannas where human induced fires have long been a feature of the system (figure 1.14). Heat may enhance germination of buried seeds (Keeley & Fotheringham 1997), but it may also kill both seeds (buried or not) and young seedlings (San José & Farinas 1983; Hassan & West 1986; Hoffman 1996; Gignoux *et al*. 1997).

Figure 1.14 – A typical fire in congolose grass savanna during the dry season.

Fire can also affect plant recruitment, growth and survival by decreasing aboveground biomass, thus reducing competition and increasing light penetration to the soil surface (Frost & Robertson 1985). Savannas generally evolve towards woodlands when fire is excluded (Menaut 1977; San José & Farinas 1983) but burning often represents a common forest management (Richards *et al*. 1999) also for use in cattle rearing (Gillon 1983). If coupled with overgrazing and variable rainfall (O'Connor 1993) this can lead to the extinction of some long-lived grass species that are obligate seeders because they lack stoloniferous or rhizomatous growth.

Experiments have shown that at least the wetter types of woodland change in structure and species composition if fire frequency is reduced or if the timing of burning is shifted from late dry season (hot fires) to early dry season (cooler, less complete fires) (Trapnell, 1959; Charter and Keay, 1960;

Chidumayo, 1988; Lock 1998). Some of these experiments were set up in what was regularly burned woodland, and, with time, fire-protected and early burned plots have changed in the direction of dry forest. The main changes include the decline of fire-resistant grasses and the appeareance of lianas and thin-barked, fire-sensitive tree species. It is possible that dry forests are less extensive in Africa because the long history of human settlement and fire use, and consequent greater fire frequency, have led to a wider loss of dry forests.

Fire is also reported to be a major mechanism for trace gas emissions from savannas. Significant quantities of CO, CO_2 NO_x , N_2O, CH_4, aerosols and and NMVOCs (**N**on-**M**ethane **V**olatile **O**rganic **C**ompounds) are released into the atmosphere during savanna fires. The burning of biomass in the savanna can be distinguished from the fires that occur in the other ecosystems, as the net CO_2 released to the atmosphere is essentially zero because biomass burned is generally replaced by other plants during the years thanks to vegetative regrowth. During the secondary succession of vegetation it was estimated that an equivalent amount of CO_2, emitted during the combustion, is removed from atmosphere thanks to photosynthesis.

The estimates of the global emissions of other greenhouse gases due to savanna burning can be based on estimates of the annual instantaneous gross release of CO_2 and on ratios of this with other trace gases released from burning.

Modification of the soil environment by fire through the conversion of nutrients present in biomass and necromass from microbial-unavailable organic forms to microbial-available inorganic forms also leads to a stimulation of biogenic trace gas emission. This influence is particularly noticeable when nutrients release by fires occurs before the onset of the rainy season

Unfortunately, many fire studies in Africa tend to be short term and small scale, while in practice fire policies in protected areas tend to be applied to larger areas for longer periods. Moreover, many fire studies use only a limited range of fire regimes to test community responses and greenhouse fluxes, use fire regimes that bear no resemblance to naturally occurring fires, or focus on responses to single fires (Parr & Brockett 1999; Parr & Chown 2003).

1.7.3 Grazing

African ecosystems are influenced not only by fire but also by grazing due to cattle, sheep, goats, donkeys and camels and all activities related to them: movements due to nomadism and transhumance, building of refuge, production of milk and cheeses, fire; this latter one is used to

open the area useful for grazing. Moreover Africa has a rich big mammalian fauna and even if it has decreased in the past 200-300 years still remains a potent force for vegetation change in Africa, in particular the ‘’the bulldozer herbivores’’: elephant, hippopotamus and the two rhinoceros species (Kortland, 1984). The timing, frequency and intensity of fires is not uniform across all savanna types because fires and grazing are often closely linked: more intense is grazing lesser the amount of combustible will be, so in the same way fire intensity; therefore grazing can have a negative feedback towards fire.

Herbivores, which tend to be attracted to the post-fire green flush of vegetation may, however, reinforce the effect of fire in preventing the dominance of trees in savannas. While mature trees can survive frequent fires in African savannas, utilization by elephants and other animals can damage trees and allow fires to burn exposed areas of wood (Yeaton, 1988). These scars tend to become larger with successive fires, and the trees eventually become structurally weakened and collapse.

Bell (1982) suggested that vegetation structure across Africa (in regions receiving >400 mm of rainfall) is largely controlled by the density of herbivores, which in turn is a function of soil fertility. He postulates that the removal of trees by elephants (*Loxodonta africana*) can open up woodlands and allow smaller herbivores such as impala (*Aepyceros melampus*) to maintain open grasslands (Figure 1.15). Impala are more abundant than any other herbivore (>15 kg) and could conceivably affect the mortality of tree seedlings given that they both browse and graze (Skinner & Smithers 1990). Subsequent studies have shown that elephants reduce tree height and density (Trollope *et al*. 1998) and that browsing by small antelope can reduce tree sapling growth (Belsky 1984).

Moreover if the fire is coupled with overgrazing and variable rainfall (O’Connor 1993) this can lead to the extinction of some long-lived grass species that are obligate seeders because they lack stoloniferous or rhizomatous growth. Some researchers believe that grazing reduce biomass so less CO_2 can be assimilated through photosynthesis, in this case grazing can be considered as a source of CO_2. Herbivores, which tend to be attracted to the post-fire green flush of vegetation may, however, reinforce the effect of fire in preventing the dominance of trees in savannas.

a

b

Figure 1.15 – A typical south African grazing made by elephant (a) and impala (b).

1.8 Available studies of N_2O and CH_4 fluxes associated to the main bioclimatic zones of SubSaharian Africa.

1.8.1 Fluxes of N_2O reported for tropical and African ecosystems

1.8.1.1 Tropical rain forest

N_2O emissions from tropical rain forest ecosystems are still poorly characterized, the majority of N_2O measurements having been made in the Amazonian and Central American region (e.g., Matson et al., 1990; Steudler et al., 1991; Verchot et all. 1999). The only data set of N_2O emissions from tropical rain forest soils of the African continent available are from Serca et al. (1994), who worked in the Congo region and Werner et al. (2007) who worked in Kenya. The latter reported a 3-month period (dry-wet season transition) at the Kakamega Rain forest, Kenya, Africa, using an automated measurement system. The mean N_2O emission was 42.9 ± 0.7 mg N m^{-2} h^{-1} and considering the duration of dry and wet season the annual N_2O emission was estimated at 2.6 ± 1.2 kg N ha^{-1} yr^{-1}. Soils predominantly acted as sinks for CH_4 (56.4 ± 0.8 mg C m^{-2} h^{-1}). For some chamber positions, episodes of net CH_4 release were observed, which was attribute to high WFPS and/or termite activity.

1.8.1.2 Seasonally-dry ecosystems: deciduous forest and woodland savanna (seasonally-dry), grassland savanna

In an extensive literature review Castaldi et al. (2006) analysed N_2O and CH_4 fluxes associated to tropical seasonally-dry ecosystems, worldwide, although the majority of data were derived from studies in South America and fewer in Africa. Analysed ecosystems varied from seasonally-dry tropical forests to open savannas to managed savannas. Fifty percent of N_2O flux data from all the analysed ecosystems were below 0.32 mg N_2O m^{-2} day^{-1}. Fluxes were so low that no statistically significant difference was observed among N_2O fluxes in control sites and burned sites or managed sites. Equally, no effect of season on N_2O fluxes was observed, although, at site level, an increase of N_2O fluxes during the rainy season was often evident. The main point related to rain seasonality concerns the critical moment when rain starts. When this occurs pulses of N_2O and NO might be measured and might last for about a week after the beginning on the rain, fading away thereafter (Werner et al. 2007). The rate of the first rainfall would be a critical factor in dry tropics in determining the rate and the species of gaseous N emitted. Clearly only a continuous monitoring

would allow to catch this critical emission moment. Overall, however, the amount of N_2O lost in beginning of the rainy season would not had critical loads to N_2O emissions in this ecosystems.

Castaldi et al. (2006) also analysed the magnitude of the measured N_2O fluxes in function of main soil types and characteristics. The results evidenced that only in soils with general good physical and chemical properties (example Andisols and Entisol), high nutrient status, high air-filled porosity and water retention, and sub neutral or neutral pH, fluxes were comparable to what normally measured in temperate forests. In pastures, managed savannas and forests located on Ultisols and Oxisols, generally characterized by a low nutrient status, pH between 4.5 and 6.5, medium water retention, coarse to loamy texture, with low organic matter content, N_2O fluxes were extremely low. Considering the distribution of soils in Africa, most of Ultisols and Oxisols are located in the areas of more intense rain input, thus although the input of water to the soil is high, the soil might not provide good water retention. This together with the relatively low nutrient status would not allow to get high N_2O fluxes. On the other hand, the soils which would create a more favorable environment to N_2O production such as Alfisols and Entisols are generally located in areas having from seasonally-dry to semi-semiarid climate. This would reduce the days of the year when significant fluxes can be produced, i.e. when the soil is near the water saturation or at least above the 60% of water filled pore space.

Specific studies on greenhouse gas fluxes in African are scanty. Andersson et al. (2002) reported N_2O fluxes measured in seasonal dry savannas in Ethiopia and Ghana, with emissions measured by an enclosure technique at natural vegetation and fallow sites. The mean soil emission rate was 0.5 μg N_2O-N m^{-2} h^{-1} in the dry season and increased during the rainy season from 1.7 to 5.5 μg N_2O-N m^{-2} h^{-1}. Levine et al. (1996) reported no fluxes (below detection limit) of N_2O in South African savannas, even when wetted with artificial rain. Scholes et al. (1997) reported emissions of NOx and N_2O from the soil during the early part of the wet season for nutrient-rich and nutrient-poor sandy soils of South Africa. Only emissions following the first wetting event of the season were quite high (76 ng N-NO m^{-2} s^{-1}, N_2O emissions averaged 8% of NOx emissions). No significant differences in the peak or subsequent emission rates was observed for either NO or N_2O between two sites of differing mineralization potentials. The total gas balance indicated that the emissions of NOx which follow the first wetting after a prolonged dry period do not make a very large contribution to the annual gaseous N emission budget, but could be a significant contributor to the high tropospheric ozone levels observed over southern Africa in springtime. Rees et al. evidenced, in a year study in a miombo woodland savanna in Zimbabwe, that the highest fluxes of N_2O occurred within the first 18 days of the onset of the first rains in November, with fluxes up to 42 μg

N m^{-2} h^{-1}. During the dry season fluxes were significantly lower. Burning did not alter significantly the fluxes on an year base, whereas deforestation reduced the amount of N lost as N_2O. This is related to the ecosystem characteristics (semi-arid climate, poor nutrient status, well drained soil) which do not allow to get significant N pulses following deforestation as observed in temperate and rain forest ecosystems.

1.8.1.3 Semiarid ecosystems

In semi-arid regions, most of the year N_2O emissions are negligible due to insufficient soil moisture, and NO might be the only relevant nitrogen gas emitted after rain pulses (Dick et al. 2006). When the rain season starts some pulses of N_2O might be measured and might last for about a week after the beginning on the rain, fading away thereafter. The rate of the first rainfall would be a critical factor in dry tropics in determining the rate and the species of gaseous N emitted.

1.8.2 Fluxes of CH_4 reported for African ecosystems

Among the few data on CH_4 fluxes in the tropical rain forest soils of the African continent Werner et al. (2007) reported for the Kakamega Rain forest, Kenya, a mean CH_4 uptake rate of 56.4 ± 796 0.8 mg C m^{-2} h^{-1} (n = 1458) and maximum CH_4 uptake rate of 146.9 mg C m^{-2} h^{-1}. From their and other studies in other tropical rain regions of the world, they concluded that being the water logging rarely encountered in upland soils, tropical upland forest soils predominantly act as sinks for atmospheric CH_4.

Soil CH_4 fluxes emissions in tropical seasonally-dry ecosystems have been analysed in a literature review by Castaldi et al. (2006) who reported that seasonally-dry ecosystems generally appeared to be a sink for methane. With an average annual daily flux in undisturbed ecosystems of - 0.48 ± 0.96 mg CH_4 m^{-2} day^{-1}, which include data from South America, Africa and Australia. The burned sites showed similar or higher CH_4 oxidation rates compared with their respective control sites (Anderson & Poth, 1988; Poth *et al*., 1995; Priemé & Christensen, 1999) suggesting no direct effect of fire on methanotrophic activity, as also demonstrated by Castaldi & Aragosa (2002a) in laboratory incubations. Higher oxidation rates in burned savanna soils, with respect to nearby control sites, have been hypothesised to depend on a temporary absence of termite activity in response to fire (Poth *et al*., 1995).

Land use dramatically modified the CH_4 flux from seasonally-dry forests and savannas, as also observed in temperate ecosystems (Smith *et al*., 2000). In the dry season, net CH_4 oxidation in the

managed sites was only 40% of net CH_4 oxidation in control sites; in the wet season, managed ecosystems became a net source of CH_4, thus with a reduction of 100% of the net CH_4 sink in the soil. The shift from CH_4 sink in the dry season to CH_4 source in the wet season was particularly evident for pastures, which appeared the highest source of CH_4 among the managed ecosystems. Pastures are generally characterized by a higher compaction and a lower macro- and micro-porosity of the soil, compared to the original forest sites (Reiners *et al*., 1994; Verchot *et al*., 1999, 2000). This could be the result of both mechanical action of herbivores and lower input of organic matter into the soil (San José & Montes, 2001). Air permeability of soil, which is mostly dependent on the largest conducting soil pores, has been shown to be positively correlated with CH_4 oxidation rates (Ball *et al*., 1997b). Therefore, a significant reduction of soil macro-porosity can be expected to reduce soil CH_4 oxidation capacity. At the same time, a lower gas diffusivity, also related to soil porosity and bulk density (Ball *et al*., 1997), favors the formation of anaerobic microsites where CH_4 production could occur. In well-aerated soils, the formation of such macrosites is associated with "hot spots" of microbial activity (Smith, 1990) where oxygen consumption, due to respiration, is faster than oxygen diffusion from adjacent pores. An increase of soil water content further reduces oxygen diffusion through soil pores, enhancing the development of anaerobic microsites. In fact, Verchot *et al*. (2000) demonstrated that a multilinear combination of soil respiration and water filled pore space explained most of the variability of CH_4 production in tropical forests and pastures, and assumed that the above mentioned mechanism, which has been postulated for N_2O production in mainly aerobic soils (Smith, 1990), was responsible for CH_4 production in the studied environments.

Less clear and more variable is the effect of other land management such as ploughing, tillage, and fertilization on reported CH_4 fluxes. As discussed for N_2O emissions, agricultural practices in these environments might generally limit the development of anaerobic conditions, thus favouring CH_4 consumption versus CH_4 production. However soil management and fertilization might significantly reduce CH_4 oxidation activity. Thus, cultivated ecosystems might be a weak sink and a weak source of CH_4.

1.9 Considerations on greenhouse gases in African agroecosystems

There are very few measurements of N_2O and CH_4 done in upland tropical and sub-tropical agricultural ecosystems in Africa so that it is not easy to define the main controlling factors on these gases in such ecosystems. As a general trend agriculture in Africa is not expected to lead to intensive management, which in terms of greenhouse emissions is the most impacting one. Also, considered the scarce water resources and the land distribution, several agricultural areas might be found in seasonally-dry climate regions, often located on well drained soil, both conditions which limit N_2O production at least during part of the year, even in presence of fertilizers. Considering fertilized and managed grasslands and pastures, this type of land management is mostly diffused in South America, whereas most of African pastoral ecosystems are located in very poor areas, in climatic conditions which goe from hyper-arid to semi-arid, less intensively used and characterized by low quality forage herbivores (Reid et al. 2004). In these conditions an increase of greenhouse gases burden might be associated to CH_4 emitted by ruminanants and by N_2O and CH_4 derived from burning, which is a constant component of these ecosystems, rather than to derived from soil biological processes. The future intensification in the use of tropical humid areas such as rice paddies, as expected for the Far East region, which would lead to a significant increase of N_2O and CH_4 production, is not expected in Africa. Deforestation of primary forests (rain forest and seasonally-dry forests) also reported as a cause of greenhouse gases production increase (Fearnside 2000, Castaldi et al. 2006), could be expected to be a minor source of GHG in Africa as deforestation in this continent proceeds at a much lower rate compared with South American ecosystems. To encourage sustainable agriculture, especially in the tropics, the use of N-fixing crops and tress has increased in the last years (Peoples et al. 1995). However, the impact of such land use on N_2O emissions is quite uncertain. Reported N_2O emissions below N-fixing trees varies depending on the ecosystem. Dick et al. (2006) have reported fluxes between 0.18 and 1.65 kg N_2O-N ha^{-1} $year^{-1}$ in Senegalese soils under *Acacia raddiana* trees. Pérez et al. (2004) have reported fluxes of 15.3 N_2O-N ha^{-1} $year^{-1}$ in mexican savanna covered by mesquite (*Prosopis laevigata*). For the same N-fixing plant Virginia et al. (1982) have reported significant variations of fluxes if they were measured under the canopy of mesquite (101.5 kg N_2O-N ha^{-1} $year^{-1}$) or outside canopy (1.7 kg N_2O-N ha^{-1} $year^{-1}$), underlying the importance of canopy density in determine fluxes of N_2O per hectare of land, which means that if the plant cover is not close to 100% we cannot apply a simple emission factor for the total land surface. Lower fluxes (1.2 kg N_2O-N ha^{-1} $year^{-1}$) have been reported for cultivations of *Acacia schaffneri* in mexican savannas (Pérez et al. 2004). A final consideration regards the type of management for the cultivated woodland. In order to improve soil

quality fallow is generally used to incorporate the plant residues into the soil, thus enriching the soil in C and N. This practice, when applied to temperate soils, generally leads to high N_2O production (Baggs et al. 2000). However, in arid ecosystems, which are often nutrient limited, the incorporation might not necessarily result in high N_2O fluxes. This not only depends on the aridity, as discussed previously, which limits decomposition and hence mineral N availability, and N_2O microbial production, but, also, in a nitrogen limited environment the surplus of nitrogen might be used in a very competitive way by the plants as soon as the rain starts. Different results might be obtained in the humid tropics as demonstrated in western Kenya by Millar et al. (2004), who measured N_2O fluxes from improved fallow agroforestry-systems, incorporating leguminous and non leguminous residues. In the first case 4.1 kg N_2O-N ha^{-1} were emitted over 84 days, and the flux was higher than in the latter treatment. These fluxes are significantly higher than fluxes reported by Chikowo et al. (2004) in an improved fallow maize cultivation in Zimbabwe (seasonally-dry climate), where the highest fluxes obtained in leguminous treated plots were 12.3 g N_2O-N ha^{-1} day^{-1}. This, as evidenced in the previous examples, is related to the different dynamics in the cycle of N and pedoclimatic conditions which characterize the seasonally-dry tropics compared with the humid tropics.

1.10 Objective of the research

Despite it enormous extension, a small amount of data on GHGs is available for the African continent compared to other tropical and temperate ecosystems. If undisturbed, these ecosystems should present environmental conditions favouring CH_4 uptake vs. CH_4 production, and limiting N_2O production. However spational and seasonal variability of GHG fluxes and the main determinant of this variability is still not completely clear in these ecosystems. Moreover, unluckily, the seasonally dry ecosystems are subject to a strong anthropic pressure mainly due to the fire, grazing and to the conversion of primary ecosystems in grasslands and croplands. The effects of this conversion on N_2O and CH_4 fluxes could increase nitrous oxide production, reduce soil capacity to oxide atmospheric methane and increase methane production as result of physic chemical and biological changes of ecosystems. In a future scenario the continuous changes of land use in the seasonally dry ecosystems significantly could increase greenhouse gases concentration.

The present study aims at increasing our understanding of the physical and physiological controls on savanna GHG fluxes, both to improve fundamental ecological understanding and to improve management and conservation strategies of these important ecosystems.

The work is part of a wide project CARBOAFRICA (EU STREP- 2007-2009) which aims at creating a GHG fluxes monitoring network of Africa, in order to quantify, understand and predict, by a multi-disciplinary integrated approach, greenhouse gas emissions in Sub-Saharan Africa and its associated spatial and temporal variability.

The numbers of land cover, land use, disturbance case studies which require investigation is very high so for the present study two cases were chosen:

1) The impact of fire on GHG fluxes in grassland savannas of Congo Brazzaville.

The region is extensively covered by grassland which are managed with fire by the local population. Fire hence represents a constant disturbance in the area. Our aim was to determine the effect that burning has on CH_4, N_2O and CO_2 emissions in these savannas.

2) Evaluation of the influence of plant cover on GHG fluxes in open woodland savannas of South Africa

Woodland savannas are often characterized by a quite sparse arboreous cover separated by open areas dominated by grass. In order to upscale fluxes form plot to site and eventually region it is simportant to understand if in terms of GHG emissions these two environments can be considerate as two different and separate ecosystems or the variability is minimal due to limiting factors. Thus

GHGs were studied from two representative woodland communities of savannas in the Kruger National Park. In each site the areas under canopy and between canopies were analysed in a comparative manner. Fluxes were related to spatial distribution of plant cover and soil type, and hence characteristics

The data obtained in the study will also provide a base to implement models of ecosystem nitrogen and carbon dynamics in savannas within the context of the project.

2 MATERIALS AND METHODS

2.1-Gas fluxes measurements

2.1.1 Chamber structure

CH_4 and N_2O exchanges from soil to atmosphere are generally measured directly estimating gas increase or decrease inside the chamber deployed on the soil surface, this method is defined static system. As H_2O and CO_2 fluxes can be measured, with good results, through micrometeorological method or eddy towers, CH_4 and N_2O can't be analyzed thanks to these techniques for the absence of a vertical concentration gradients above the canopy. The micrometeorological technique generally require a very expensive equipment and more difficult sampling and measurement procedures than the closed chamber method.

Soil-atmosphere exchanges of greenhouse gases using static chambers can be determined periodically collecting gas samples from the chambers and measuring the change in concentration of the gas with time during the period of linear concentration change.

The static chamber method is widely used for its good efficiency in fact, very small fluxes can be measured, the chambers can be easily moved giving the opportunity to measure at different locations, they are cheap and easy to build, they don't require electronic equipment and don't have a big impact on the environment where they are deployed (at the end of sampling they can be easily removed).

Even if the static chambers could seem simple upset bins on the soil, in which it's possible to store the gases, they must be opportunely designed. The dimensions of the chamber depend on the vegetation, the soil and fluxes intensity. During the designing of the chamber it's important to value the volume/basal area ratio (V/S); the chambers with small V/S have a more rapid concentration increase; instead, chambers with a large volume/basal area ratio show a more constant rate of concentration but they need of a longer sampling intervals to estimate gas variations stored in the chamber (Matthias *et al.*, 1978). The static chamber should have a V/S ratio small enough to have a rapid response of gas exchange, yet large enough to minimize disturbance of the enclosed surface.

In general flux measurements on sites with high fluxes should be done with chamber with a large V/S ratio while in sites with low fluxes it's opportune to use chamber with a small V/S ratio.

The chambers can have any geometry even if the most used ones in the field have a rectangular or cylindrical cross-section to facilitate gas mixing; the basal area can be between 175 cm^2 and 1 m^2 even if those ones most used have a basal section between 500 cm^2 and 900 cm^2.

CH_4 and N_2O emissions are very variable spatially, even over a distance of a few centimetres. Some studies showed that N_2O fluxes measured through chambers with an area more than 0.5 m^2 have a variability between replicates smaller than ones with a basal area of 0.008 m^2 (Ambus *et al*., 1992), instead other studies have indicate no effect.

Also chamber volume plays a fundamental role, chambers with a small volume are more susceptible to temperature, that influences microbial activity, and humidity that can increase gas dilution provoking an underestimate of fluxes.

Generally during the design and scantling of chambers further to the parameters due to the fluxes it should take in account also the cost of materials and the logistic problems.

The chamber have to be built with inert materials so impermeable, non-reactive and not a source or sink for the gas species of interest. For CH_4 and N_2O emissions normally are used some materials, as PVC, aluminium, polypropylene, plexiglass while for other gases as NO_x, NH_3 and sulphur gases it is used the glass or Teflon.

The static or closed chamber system is defined also steady-state systems, not to confound with the dynamic or open chamber system (defined non-steady-state systems), in it the concentration gradient controlling molecular diffusion across the soil-atmosphere surface is assumed constant after an initial period of adjustment. Trace gas concentration gradient in the chamber decrease stably for the continuous gas concentration changes.

Some researchers showed that the static chamber provokes little changes of concentration gradient, having in this way not only smaller bias in observed gas transport rate but also a more rapid recovery to the pre-disturb condition between consecutive measurement periods (Denmead, 1979).

The use, instead, of a dynamic system needs a sufficient time stabilizing gas gradient to the new condition in the chamber, however this period is difficult to established and can change with the different site conditions (Jury *et al.*, 1982).

Sometimes the chambers present a vent which dimensions depend on chamber volume and wind speed, it generally has a diameter of about 4-5 mm and 70 mm in length and it's useful not only to transmit pressure fluctuates to the gas closed in the chamber, but also to compensate volume decrease in the chamber due to sampling process (Hutchinson and Moiser, 1981) (Figure 2.1).

According to Conen & Smith (1998) the vent isn't useful, in this way it can be avoid the depressurizing in the chamber that could provoke an increase of fluxes from soil to atmosphere. The position of the vent must be far from the stopcock useful for the gas sampling otherwise during the aspiration the gases presented in atmosphere can be withdrawn. Conen & Smith (1998) suggested that the vent tube should be mounted as near the ground surface rather than the top of the chamber, on the lateral wall of the chamber in horizontal position and downwind to minimize wind speed.

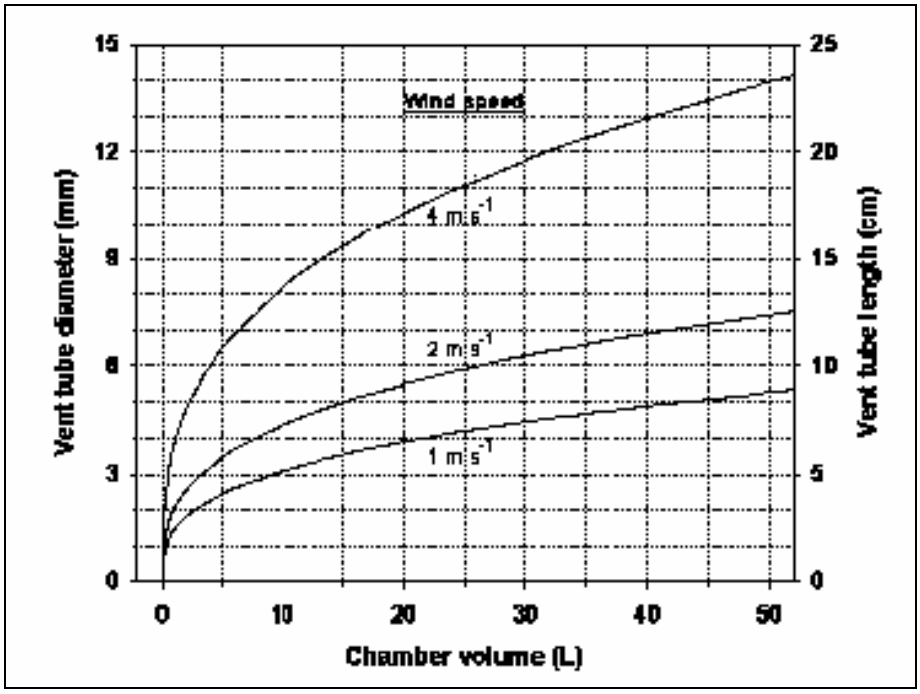


Figure 2.2 - Optimal dimensions of vent tube according to chamber volume and wind speed (Hutchinson & Mosier, 1981).

The chamber with a vent are particularly used where the soil is very permeable or when the greenhouse gases sources or sinks are located near the soil-atmosphere interface. When the mass flow is significant, the exchange rate can be underestimated if it isn't used a static chamber with a vent, instead, in condition of low mass flow the use of a vent is indifferent.

The static chamber can be single-component or formed by multiple-components. The single-component chambers are very cheap and easy to install but they notably influence gas fluxes above all during the phase of installation. The multiple-components chambers are more expensive but as they are installed in two steps reduce the perturbations. In fact during the first step is inserted the collar in the soil then, after a phase of chemical-physical stabilization, the chamber is connected to the collar, in this way it's also possible to sample in different points in the same site (Figure 2.2).

Another advantage of leaving the collars in place is that the soil disturbance and damaged roots are minimized. However a very long period can change the microclimate within the collars and the

changes in humidity or shading can cause algal growth, furthermore in clay or compacted soil accumulation of rainwater can occur.

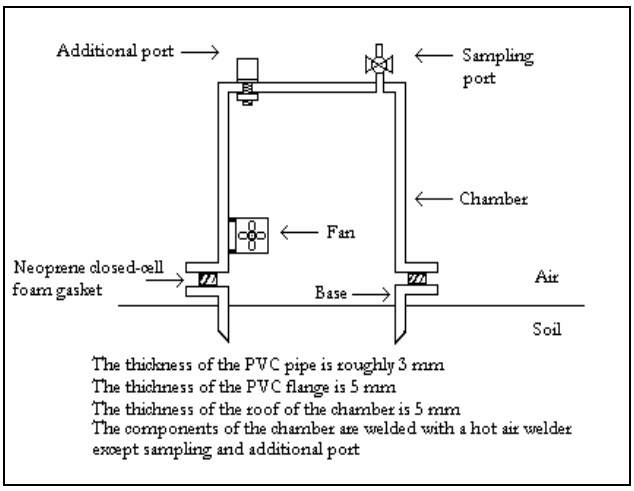


Figure 2.2 – Multi component static chamber used in for GHGs fluxes

During the phase of installation it is important to establish how the chamber or the collar must penetrate into the soil to reduce the errors due to the lateral diffusion (Healy *et al.*, 1996). In coarse-textured, dry or highly porous soils the chamber or the collar should penetrate 5-10 cm, specially for deployment times of 20 minutes or more (Hutchinson & Livingston, 2001). The chamber or the different components of it must be sealed even if some researchers showed that the gas losses due to the lateral diffusion and to the leaking seal are smaller than to the vent ones.

The static chamber can have different problems for example the boundary layer resistance at the soil-atmosphere interface can increase inside the chamber causing a decrease of the flux rate of the gas in it, besides the emissions can decrease for the gas concentration increase inside the chamber, this problem could be avoid reducing the time between two samplings (Jury *et al.*, 1982; Hutchinson and Moiser 1981). The pressure applied during the chamber installation can provoke pressure changes in the soil causing an enhance of gas emission, this negative effect can be overcome by installing first the collars, that normally are open, and after a short time the chamber. With single-component chamber is useful insert before the chamber, making a groove in the soil, after the chamber can be removed for a brief time to allow dissipation of any gas released during the disturbance and then replaced, in this way gas emissions due to the pressure don't take in account during flux calculation.

The closed chamber can remove or alter the atmospheric pressure fluctuations due to natural turbulence that normally occur near the soil surface and produce a pumping effect on the surface layer of soil which increase soil air movement. Inside the chamber these turbulence could not occur

so it's possible underestimate gas flux that would have occurred without the chamber in place, to reduce this effect it is opportune to build an appropriately vent (Hutchinson and Moiser 1981). Temperature changes in the soil and the gas under the chamber can influence gas emissions, little temperature changes within and outside the chamber generally don't disturb gas exchange rate but despite of high temperature variations; to limit this effect the chamber can be covered with reflective or opaque material (Matthias et *al.*, 1980).

2.1.2 Sampling gas

To measure trace gases flux it needs to sample gases accumulated in the chambers and analyze them or directly in the filed or in the lab, the gases should be taken at regular interval during sampling period and this latter shouldn't be over 60 minutes. Sampling period generally should be the shortest possible to avoid eventual perturbation, but not too short to observe no increase or decrease of gas concentration; moreover 3 points are useful at least to calculate the flux: time 0 and other two points at regular intervals (for example 0, 20, 40 min.).

The gases are sampled thanks to a polypropylene syringe inserted in a stopckoc place on the chamber or in a septa put on the top of the same and slowly withdrawn. During the gas sampling for the pumping effect outside gases can be introduced through the vent tube inside the chamber provoking a gas dilution. The error due to the dilution depends on gas volume withdrawn and on the V/S ratio (figure 2.3) if the volume sampled is less than 30 ml and V/S ratio is higher than 10, dilution effect is negligible.

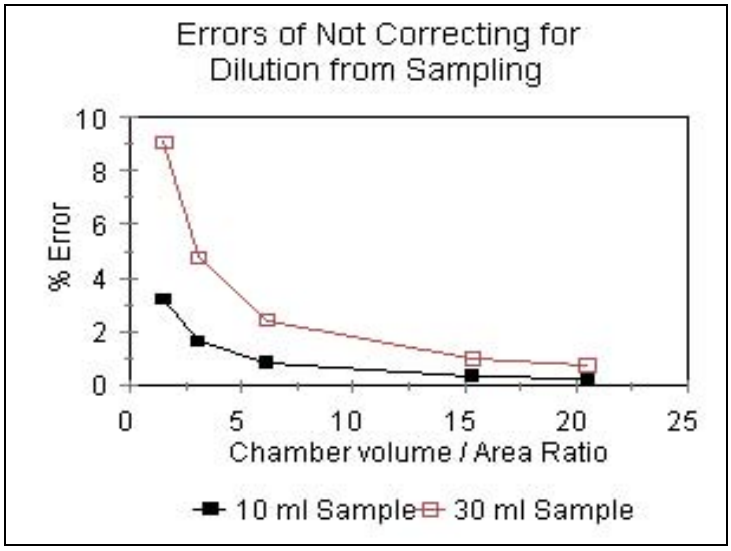


Figure 2.3 - Error due to dilution during gas samplig considering gas volume withdrawn and V/S ratio.

The volume of gas to sample depends on analysis technique to use, they are generally withdrawn 5-30 ml of gas which are stored inside glass vials of equal volume, previously sealed thanks to butyl rubber septa and crimps, where the void is created using a vacuum pump. Eventually a slight over pressure inside the vials is advisable. Holes, created by the needle used to inject the samples through the rubber, can be temporarily sealed thanks to a thermo fusible glue and stored in a refrigerator at 2-4 °C, which decreases the pressure within the vials and gas leaks. In this way the system can store gas for more than 45 days. In any case the best option is to analyse gas samples quickly, as anyway a slight variation of concentration of ±2% is possible after 15 days.

2.1.3 Gas chromatographic technique

Since the end of 1950s, with the introduction of gas chromatographic methods, it has become possible to analyze gases and volatile compounds which were previously measured with traditional chemical methods that were much less specific for most of the compounds, less sensitive and more time consuming.

Gas chromatography is a technique for separating volatile substances by passing a gas stream over a stationary phase. The components to be separated are carried through a column containing the stationary phase, by an inert gas (carrier gas). If the stationary phase is solid we speak of Gas-Solid Chromatography; the column is packed with an adsorbent of small particle size, or the inner surface of a capillary column is coated with a thin adsorbent layer. If the stationary phase is an unvolatile liquid coated either on an inert support material or on the internal surface of a capillary column, then we speak of Gas-Liquid Chromatography.

The carrier gas must be inert, pure, suitable for the detector and preferably not too expensive. Commonly used gases are hydrogen, helium, nitrogen and argon. Column efficiency in separating the volatile compounds depends also upon choosing a suitable linear gas velocity. The optimum flow rate can be determined by making a Van Deemter plot of HETP (Height Equivalent to a Theoretical Plate) vs linear gas velocity. The most efficient flow-rate is at the minimum HETP (for more details see McNair and Bonelli, 1969). The simplest way to measure gas flow rate is with a soap-bubble flowmeter and a stopwatch.

Gases are introduced instantaneously into the column by gas-tight syringes, introducing the needle through a self-sealing septum (reproducibility around 2% relative), or via by-pass sample loops (reproducibility better than 0.5%). A better resolution is provided by automated GC injector systems (Smith and Harris, 1970; Parkin, 1985; Smith and Arah, 1991), which are also labour-saving, than by manual injection.

The columns used are stainless steel tubes, 6 mm in diameter, filled with Porapak Q, a porous polymer composed of ethylvinylbenzene cross-linked with divinylbenzene to form a uniform structure of a distinct pore size. The columns contain only the porous polymer beads screened to a defined mesh range, no liquid support is present. The solute molecules partition directly from the gas phase into the amorphous polymer. There are several kinds of Porapak, P, Q, R, S, T and N. Porapak Q is non polar and widely used for separating CO_2 and N_2O from N_2 and O_2 samples.

The chromatographic detector is a device which measures the amount of separated components in the carrier gas, producing an electrical signal proportional to the concentration of the component. Some of the detectors in current used for the determination of gaseous products are listed in Table 2.1.

Table 2.1 - Detectors used for gas chromatographic analysis.

Detector	Application
Thermal conductivity (TCD)	all gases (major constituents of mixtures)
Flame ionization (FID)	hydrocarbons (trace concentrations)
Helium ionization	all gases (trace concentrations)
Electron capture (ECD)	N_2O; O_2; CO_2; halogenated compounds
Ultrasonic	all gases (wide concentration range)
Flame photometric	sulfur and phosphorus compounds

In the ECD detector, β-radiation from a radioactive source (usually nickel-63) ionizes the carrier gas (nitrogen or argon or argon-methane), and an applied potential causes current to flow. Electrophilic compounds capture the electrons produced by the ionization and the current is reduced. This provides the basis for a highly sensitive and selective detector. The limit of detection for N_2O can be of the order of 1.0 ppbv N_2O.

The TCD detector employs a tungsten filament which is heated by passing a constant current through it. Carrier gas flows continuously over the heated filament and dissipates heat at a constant rate. When sample molecules mixed with the carrier gas pass over the hot filament, the rate of heat loss is reduced and the resistance of the filament increases. The resistance change is measured by a Wheatstone bridge and the signal fed to a recorder.

In the FID detector, hydrogen and air are used to produce a flame. A collector electrode with a DC potential applied is placed above the flame and its conductivity measured. As organic products are combusted, the low conductivity produced by the hydrogen increases and the current which flows can be amplified and fed to a recorder.

For the present study we used a GC systems equipped to analyse greenhouse gases CH_4, CO_2 and N_2O on one single air sample as described by Loftfield et al. (1997). Gas is loaded on a 1 ml loop connected to a 10-ports valve (Valco Europe, Switzerland). A precolumn of 1 m (O.D. 1/8", 0.08" I.D.), filled with Porapak 80-100 Q and maintained at 60°C, is connected to the 10-port valve in order to operate frontflush and backflush. From the pre-column, the gas passes into the main column (T Porapak 80-100 Q, O.D. 1/8", 0.08" I.D., 2 m length), also held at 60°C. Then it is directed, via a 4-ports valve (Valco Europe, Switzerland) firstly to a flame ionization detector (FID, for CH_4 measurement) and, after 86 seconds, to an electron capture detector (ECD, for CO_2 and N_2O measurements), held at 280°C. Pure nitrogen is used as carrier gas at a flow rate of 40 cm^3 min^{-1}. Calibrated standards are used for N_2O and CH_4 concentration determination, and are injected on duplicate every 20 samples.

2.1.4 Photoacoustic technique

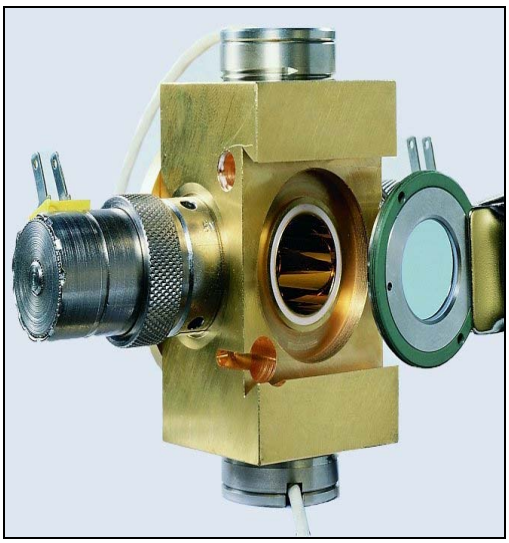
Gas concentration in the chamber can be analyzed directly in field using a photoacoustic analyzer joining both the inlet and outlet of the instrument to static chamber. Its use in field needs 12 V DC source and DC/AC (600 W) inverter moreover before starting the analysis a warm up period (at least ½ hour) is necessary to stabilize the conditions inside the cell of the analyzer. Photoacoustic spectroscopy has some advantages in particular it allows to measure gas concentration in remote areas by using a relative simple technique which does not require the involvement of gas cylinders, high energy power, voluminous instrumentation such as field GC techniques of even more sophisticated laser techniques.

Photoacustic Spectroscopy (P.A.S. Photo Acoustic Spectroscopy) is based on phenomenon known as Photoacoustic Effect which corresponds to acoustic emissions produced by gas sample close in limited space and interested by a radiation released from intermittent light source. This phenomenon was discovered and studied by Alexander Bell at the end of 1800 but it was revalued only from 1970 when there was a renewed interest due to the development of lasers and very sensitive detection techniques.

The instruments based on the photoacoustic principles are used to analyze different chemical gaseous compounds presented in the environment, in the civil and industrial waste and in the troposphere.

a) Nichrome infrared source and ellipsoidal mirror useful to facalize electromagnetic radiation.

b) The filters mounted in a rotating carousel.



c) germanium window and the photoacoustic gas cell.

d) Brüel & Kjaer condenser microphone.

Figure 2.4 – Principal components of photoacoustic analyzer.

The infrared radiation source of Innova's Photoacoustic Field Gas-Monitor is composed by heated nichrome wire (alloy of nichel and bichromate potassium) heated until 800°C (figure 2.4a), the electromagnetic radiation from source is focused by an ellipsoidal mirror and modulated with a mechanical chopper through which it's emitted with intermittence. As the infrared source produces radiation with different frequency, the analyzer have optical filters inserted in a carousel (until 5 except water filter) that works as frequency selector (figure 2.4b). The selected radiation passes through a germanium (figure 2.4c) window before entering in the photoacoustic gas cell covered by reflective surface useful to redouble radiation intensity and provide of a valve system; here it is analyzed the gas sample previously withdrawn thanks a pump.

When a gas is irradiated with the light of a frequency that corresponds to a resonant vibration frequency of the gas, some of the light will be absorbed. This will cause some of the molecules of the gas to be excited to a higher vibration energy state. These molecules will subsequently relax back to the initial vibration state through a combination of radiate and non-radiate processes. For vibration excitation, the primary relaxation process is non-radiate vibration to translation energy transfer. This results in increased heat energy of the gas molecules and therefore a temperature and pressure increase in the gas. If the irradiating light is modulated, then the temperature and pressure will be as well. The modulated pressure will result in an acoustic wave, which can be detected with a sound measuring device, such as a microphone (figure 2.4d).

The amplitude of the acoustic wave will depend upon such factors as the geometry of the gas cell, incident light intensity, absorbing gas concentration, absorption coefficient, and the background

gas. For a non-resonant spherical gas cell under steady state conditions, the amplitude of the acoustic wave can be found from the following equation:

$$P=K((Cp/Cv)-1)Io\ c(1/f)$$

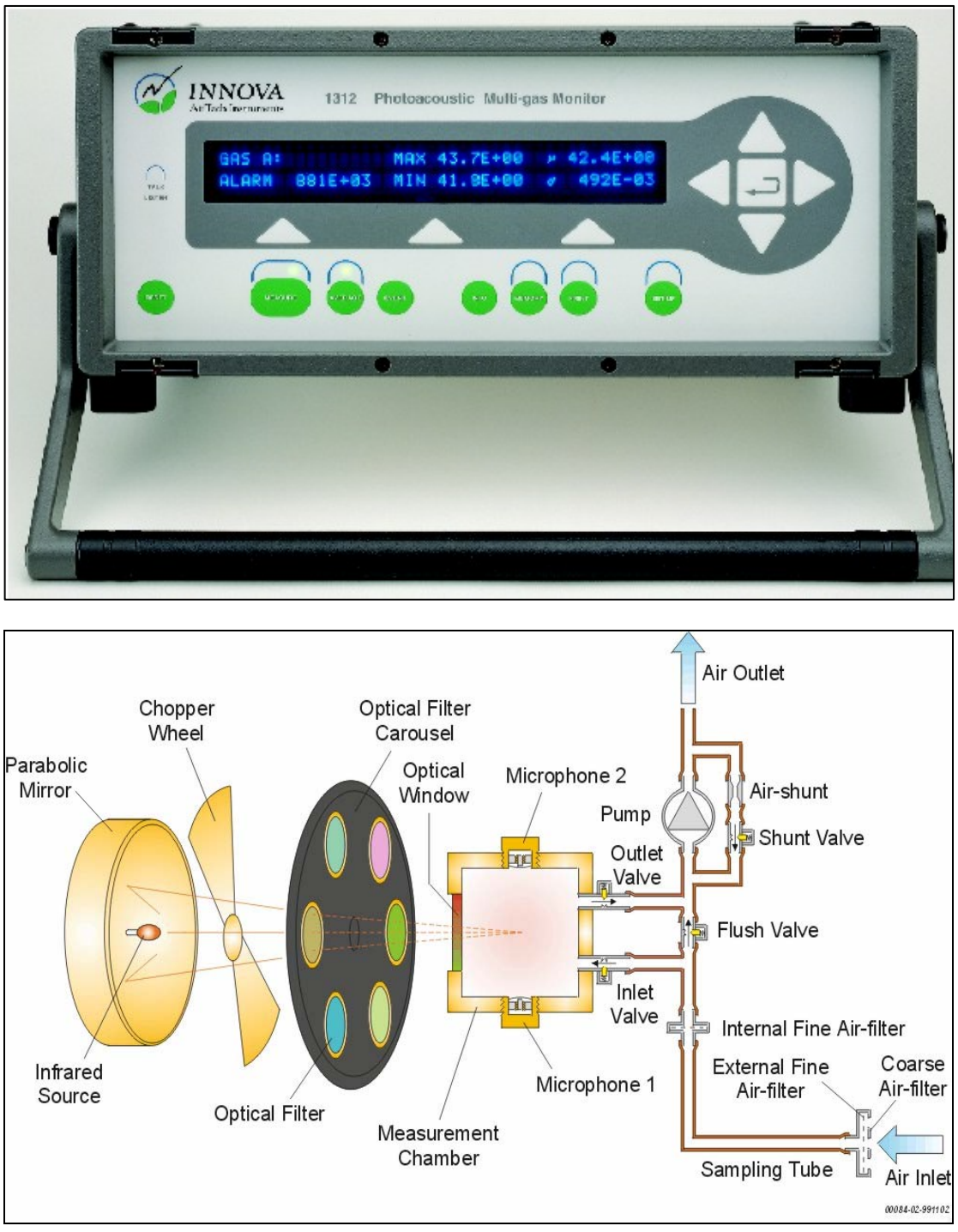


Figure 2.5 – Photoacustic analyzer and its principle of functioning.

In the above, *P* is the sound pressure, Io is the incident light intensity, *c* is the absorbing gas concentration, *f* is the modulation frequency (Chopper frequency), *Cp* and *Cv* are heat capacities (C_p/C_v vary from 1.7 Argon to 1.1 Butane) and *K* is a cell and gas dependent constant.

The acoustic signal is detected with a pair of specially designed, Brüel & Kjaer condenser microphones. The electrical signals from the microphones are amplified by pre-amplifiers mounted directly on the backside of the microphone and added together in a summation amplifier before being sent to an analogue-to-digital converter for further processing. The digitised signal is then converted to a concentration reading using the proper calibration factor stored in the instrument (figure 2.5).

Some gases absorb infrared light at similar wavelengths and this can result in not knowing if the concentration displayed is from one gas, another gas or both. This is called cross-interference, and is a known problem when using traditional transmission spectrometers. Innova's Photoacoustic Field Gas-Monitor compensates for this by being calibrated for all the gases one wants to measure. In other words, first filter A, filter B, filter C etc. are calibrated with gas 1, then gas 2, gas 3 etc., until the contribution from all the gases for all the filters are known. All the cross-compensation factors are calibrated and stored in the instrument during the calibration. Later, under normal measurement conditions, the monitor will automatically compensate for these gases when they are present. This is known as automatic cross-compensation.

The interference due to the presence of the other gases in the sample is also a limit, in fact the photoacoustic instrument has only one algorithm used to compensate gases interference present in the middle region of infrared so the other compounds could give wrong results.

Photoacoustic spectroscopy has some advantages in particular:

1. Cell volume is very little (3 cm^3) and permits to use small gas sample both during the analysis and during calibration, moreover, time response is short because of reduced dimensions of the cell.
2. Microphones give a high stability to instrument, microphones are some of the most stable trasducers with output drifts <10% over 100 years.
3. Nichrome wires as infrared source has showed a detection limit between ppb and ppm range getting a high sensitivity.
4. Dynamic range can be 100000 times bigger than detection limit for a determinate gas that is the instrument can measure different concentrations without calibrating.

Photoacoustic analyzer (Innova AirTech 1312) has filters to measure CO, CO_2, NH_3, N_2O, CH_4 and H_2O, each filter has a specific wavelength and dynamic range; their characteristics are resumed in the table 2.2:

Table 2.2 – Technical characteristics of the filters used to analyze greenhouse gases.

GAS	CODE	λ[1]	DYNAMIC RANGE (SIT 5s)	DETECTION LIMIT
CO	UA0984	4.7 um	0.2 - 20000 ppm	0.2 ppm
CO_2	UA0982	14.1 um	1.5 - 150000 ppm	1.5 ppm
NH_3	UA0973	9.1 um	0.2 - 20000 ppm	0.2 ppm
N_2O	UA0985	4.5 um	0.03 - 3000 ppm	0.03 ppm
CH_4	UA0969	8 um	0.4 - 40000 ppm	0.4 ppm

Instrument accuracy can be increased through integration time SIT (**S**ample **I**ntegretion **T**ime) that changes between 0.5s/50s and its selection depends on sample period and the measure number, so it should be found the right compromise among sensibility, sample period and measure number useful for next statistic analysis.

The monitoring period ***MP*** is the time between the start of the first measurement cycle and the finish of the final measurement cycle in a monitoring task; it can be selected by the users, in this case the analyzer stops the monitoring automatically at the end of pre-set period time, or not selected in this case the instrument will go on the monitoring until hand-operated stop.

To know measure number during the monitoring period it's important to know response time and sampling interval too.

Time response t_r is the time instrument takes to give a response (gas concentration), depends on SIT, on time useful to wash the cell and teflon tube, and on the time taken to aspire the sample, it's calculated as:

$$t_{r(tubeon)} \approx 8 + t_{chamber} + t_{tube} + \sum_{i=1}^{n} (5 + SIT_i)$$

n = number of filter

SIT_i = Integration time for filter i-esimo

[1] λ is the wavelength of electromagnetic radiation

Sample interval ***SI*** represents the time between the start of one measurement cycle and the start of the following measurement cycle. The sample can be continuous, in this case there is no pause between the start of a measurement cycle and the following one or not continuous, that is, there is a pause (calculable) between the start of a measurement cycle and the following one.

Programming a determined monitoring period and the type of sampling the photoacoustic analyzer performs, during this time interval, different measures number:

$n_a \approx \frac{MP}{t_r}$ if the sample interval is continuous

$n_a \approx 1 + \frac{MP}{SI}$ in the case it's fixed a sample interval

n_a = measure number expected

2.1.4 Flux estimation

To measure gas fluxes, the rate of gas exchange per surface unit, we need to determine the variation of concentration (ppmv) inside the chamber, possibly in the phase of linear increase over time, as depending on the time of closure, internal volume and flux strength the increase of concentration might loose its linearity after a certain time length, due to diffusion problems. If the dimensions of static chamber and deployment period have been opportunely chosen according to the rate of gas exchange and the characteristics of sampling area, a linear regression can be used (time vs. concentrations) and the slope *a* will represent the variation of concentration over time dC/dt. Because the concentration can change among the different samplings events, mainly due to temperature variations inside the chamber, it's advisable to transform the ppm (v/v) measured in the gas samples, firstly in a measure of concentration ($\mu g/l$) and then to compute the rate of concentration change over time $\frac{dC}{dt}$. The concentration C of the gas sample can be obtained using the following law:

$$PV = nRT \Rightarrow \frac{n}{V} = \frac{P}{RT} = C$$

So we will calculate the concentration of the different samples taken inside the chamber (C_1, C_2, etc).

$C_1\left(\frac{\mu g}{l}\right) = ppm_1 \frac{P_1 MW}{RT_1}$ where MW (molecular weight of the gas), P_1 e T_1 are respectively the concentration, pressure and temperature at the time t_1.

$C_2\left(\frac{\mu g}{l}\right) = ppm_2 \frac{P_2 MW}{RT_2}$ where MW (molecular weight of the gas), P_2 e T_2 are the concentration, pressure and temperature at the time t_2.

Then we will plot the concentrations over time and from the slope of the liner regression line we will get $\frac{dC}{dt}$.

The flux (mg m^{-2} day^{-1}) gas is given by:

$$F = \frac{dC}{dt} \times \frac{V}{S} \times \frac{1440\,\min}{1day}$$

Where:

V = chamber volume (m^3)

S = chamber area (m^2)

Often field fluxes are low so it's important to know the minimum detection limit (MDL), it is the minimum flux that can be calculated using a specific analyzer. MDL for the photoacustic analyzer depend on detection limit, chamber characteristics (V and S) and the monitoring period (MP).

$$MDL = \frac{detection\,limit}{MP} \times \frac{V}{S} = \frac{mg}{m^2 s}$$

In the classical methodology (GC) the minimum detection limit is given by the statistic error, obtained computing the standard deviation of different samples of standard measured by GC (n>30), chamber characteristics and deployment period. As the precision is generally a function of concentration, the standards used should have concentration near to atmospheric one.

$$MDL = \frac{2\,st.dev}{total\,deployment\,time} \times \frac{V}{S} = \frac{mg}{m^2 s}$$

2.2 Comparison of PAS and GC techniques for N_2O, CO_2 and CH_4 gas measurements

Very different performances of the PAS system were evidenced for three gases of interest CH_4, N_2O and CO_2. In order to verify the PAS performance the same gas samples were analysed by a standardized gas chromatographic technique using a set up as described in the previous paragraph.

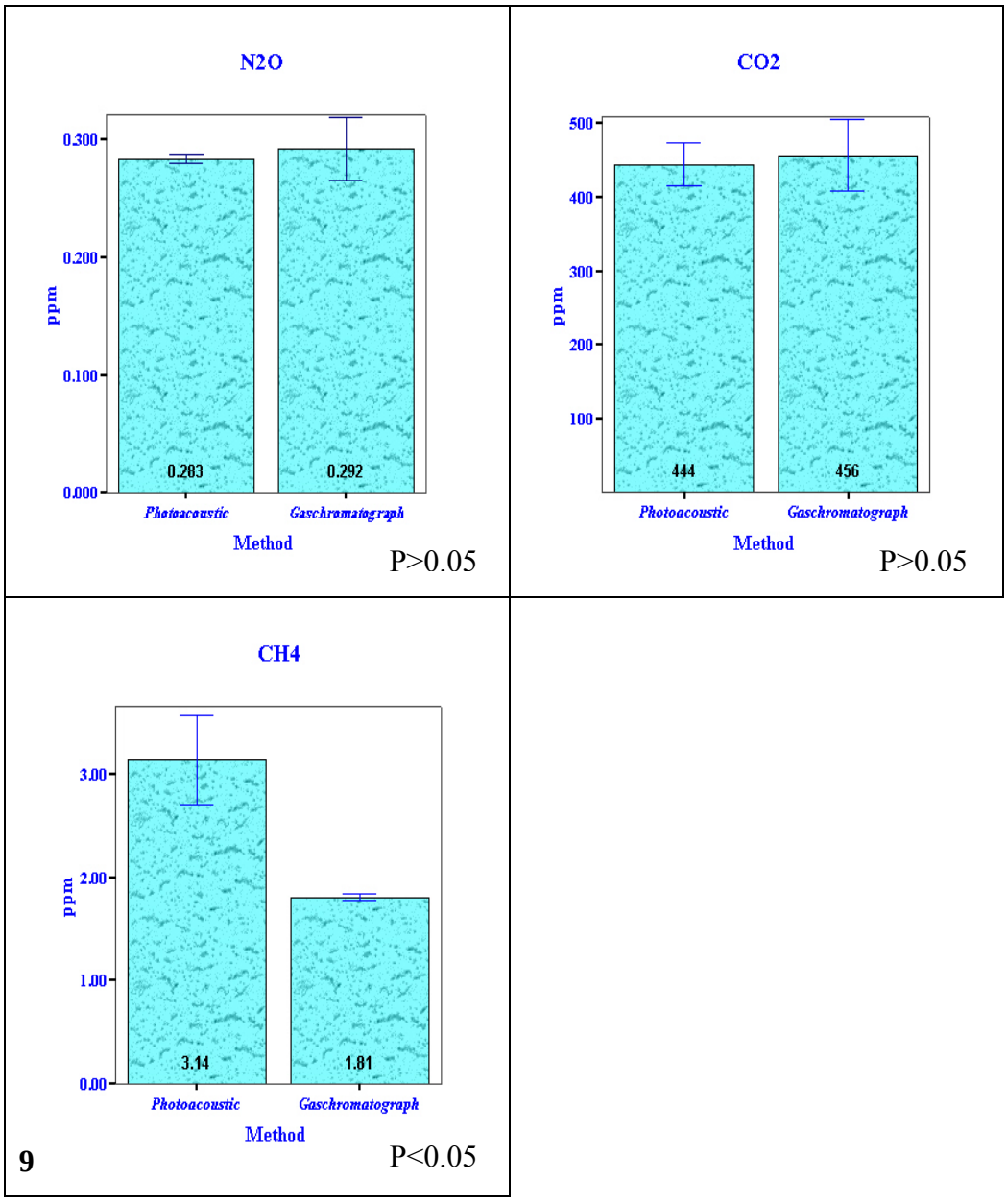


Figure 2.6 - Air concentration of N_2O, CO_2 and CH_4 measured using PAS and GC techniques.

In Figure 2.6 the measured concentrations of air samples are compared using the PAS and GC technique for CH_4, N_2O and CO_2. The measurements of N_2O and CO_2 concentrations give a comparable result (no significant difference one-way ANOVA, $P>0.05$) with the two techniques, although the comparison between the two methodologies showed that a greater accuracy in the

measure can be achieved by photoacoustic technique, and this is particularly true for the N_2O. For this gas the reported measures (Figure 2.6) are 283 ± 3.7 (st.dev) ppb by PAS and 292 ± 26.9 (st.dev) ppb by GC technique. Thus, the former technique is 10 times more precise than the latter. This improves the sensitivity of the technique, allowing to detect small fluxes where the increase of N_2O concentration over time is very slow and could fall within the error associated to the GC analysis. The results of CH_4 measurement show, instead, a very bad performance of the PAS technique, both in terms of precision, as it give a completely wrong atmospheric concentration, and in terms of error associated to the technique (Figure 2.6).

Concentration of CH_4 superior to the expected ones have been associated to pressure variations and above all to water content interference (Miklos *et al.*, 2002). In fact, it has been reported that the interference due to water vapour strongly affects CH_4 reading by PAS technique, even at very low concentrations of water vapour.

In Figure 2.7 we report the result of a fertilization experiment where water vapour, CH_4 and N_2O fluxes were measured immediately after fertilization using a closed chambers inserted in the fertilized soil. It can be noticed that there is a close relationship between CH_4 concentration variations and water vapour. Moreover, the concentration of CH_4 in the chamber air is absolutely out of any acceptable range of atmospheric concentration, which should be around 1.8 ppm.

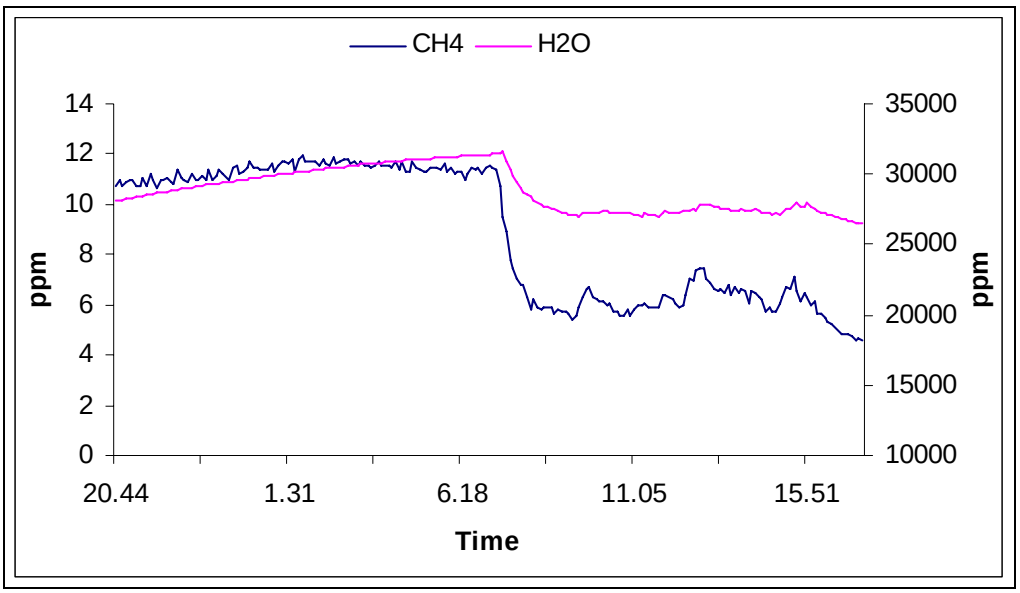


Figure 2.7 – Concentration of CH4 and water vapour inside a closed chamber inserted into a fertilized (ammonium sulphate) soil.

Nevertheless, the use of a water trap put before the inlet of the instrument does not improve the reading of methane concentration. In table 2.3 the concentration of different gases sampled in atmosphere are reported using different filters both for water (drierite, silica gel, magnesium perchlorate) and for hydrocarbons (active carbon), the latter has been used to verify if the presence of other hydrocarbons could influence the analysis of methane concentration. It can be noticed that the filter which most reduces water vapour (anidrone) does not reduce CH_4 concentration, while it makes impossible to read N_2O. This test was used also to verify which water filter can be used to reduce water excess in the samples without having an absorbing effect on the gas which have to be measured. Drierite seemed to have a good performance, thus it was subsequently used for CO_2 and N_2O flux measurements.

Table 2.3 – Measured concentration of several gases measured by PAS technique using different filters before the inlet of PAS.

		N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
C: Carbon dioxide [ppm]	DRIERITE	69	397.90	16.68	2.01	355.65	436.45
	CARBONE ATTIVO	39	423.75	13.12	2.10	396.28	450.46
	GEL DI SILICE	661	393.72	3.41	.13	384.38	445.22
	ANIDRONE	91	394.02	7.84	.82	387.59	460.31
	Total	860	395.45	9.18	.31	355.65	460.31
D: Dinitrogen oxide [ppm]	DRIERITE	69	.294	.007	.001	.282	.313
	CARBONE ATTIVO	39	.291	.007	.001	.279	.308
	GEL DI SILICE	661	.266	.008	.000	.240	.312
	ANIDRONE	91	-.001	.026	.003	-.025	.190
	Total	860	.241	.085	.003	-.025	.313
E: Methane [ppm]	DRIERITE	69	3.48	.60	.07	2.39	6.78
	CARBONE ATTIVO	39	3.04	.40	.06	2.56	4.24
	GEL DI SILICE	661	1.40	.12	.00	1.11	2.19
	ANIDRONE	91	2.32	.23	.02	.97	2.57
	Total	860	1.74	.71	.02	.97	6.78
W: Water Vapour [ppm]	DRIERITE	69	5371.78	3409.56	410.46	2809.93	14945.7
	CARBONE ATTIVO	39	11257.6	390.90	62.59	10552.5	11855.9
	GEL DI SILICE	661	2133.95	143.32	5.57	1747.76	2992.39
	ANIDRONE	91	201.39	124.06	13.00	147.14	1125.48
	Total	860	2602.99	2396.08	81.71	147.14	14945.7

Some researchers have reported interference between CO_2 and N_2O species during the PAS analyses (Flechard et al., 2005). In order to test this hypothesis we measured both gases in two different soil, the first (Castel Volturno) where from previous GC analyses, coupled to closed static chamber technique, we know that soil emits both CO_2 and N_2O; the second soil (Tolfa) where we know that soil does not emit N_2O in any condition due to the lack of nitrification and NO_3^- production at the site. In figure 2.8a we have the increase of CO_2 and N_2O in the closed chamber in

Castel Volturno, where it can be seen that both gases are increasing over time. In Figure 2.8b we have the same gases measured in Tolfa site. Here only CO_2 increases while N_2O concentration remains a flat lines corresponding to atmospheric N_2O concentration. This comparison provides evidence that CO_2 concentration does not influence the PAS reading of N_2O creating as a consequence a false flux of N_2O.

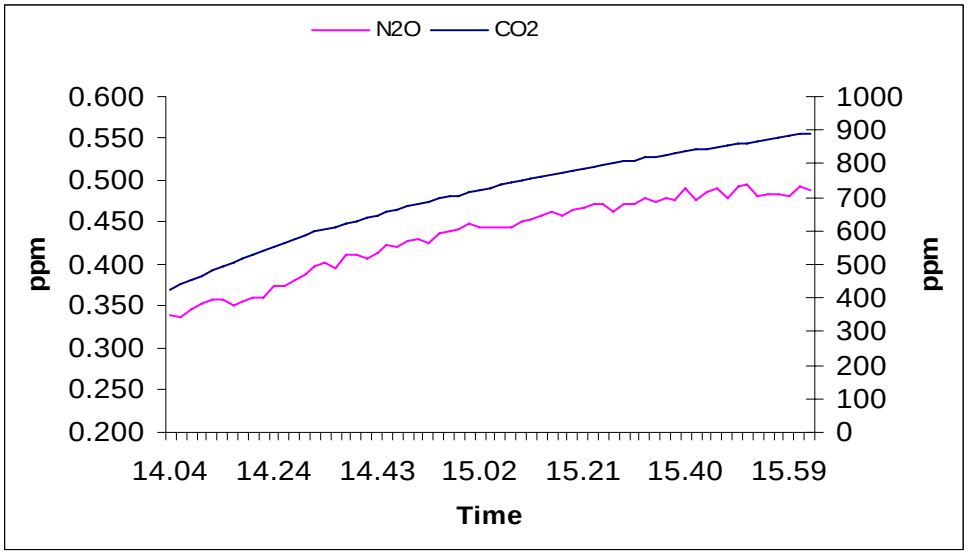


Figure 2.8 a – CO_2 and N_2O concentrations measured by PAS at Castel Volturno site in a closed chambers inserted in the soil.

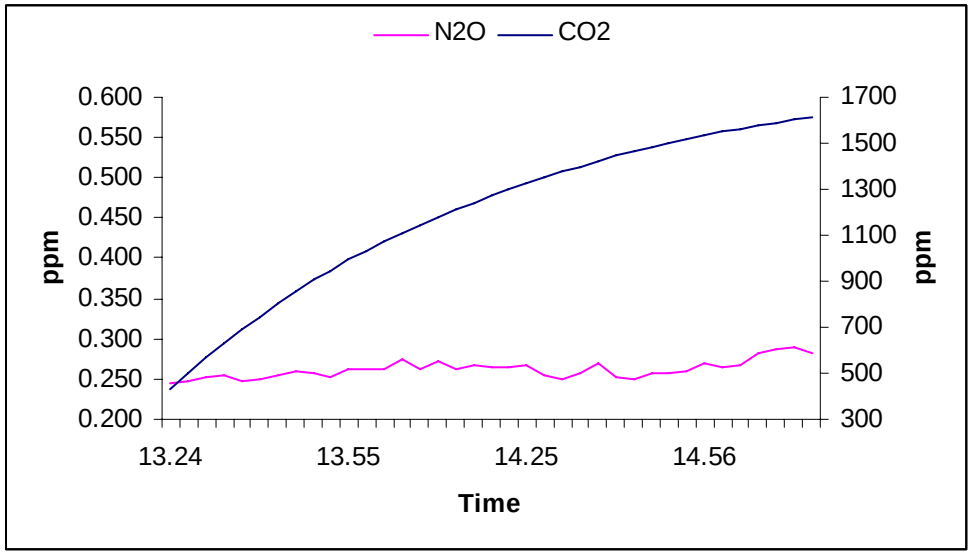


Figure 2.8 b – CO_2 and N_2O concentrations measured by PAS at Tolfa in a closed chambers inserted in the soil.

2.2.1 Comparison of PAS and GC measurements of certified standards

In Table 2.4 are reported the concentrations of greenhouse gases in calibration cylinders as certified by the manufacturer, with the available error and the measured concentrations using the PAS. N_2O readings are correct at high concentrations, whereas a systematic underestimation is obtained at low concentrations of N_2O. This systematic underestimation can be used to calculated a correction factor to obtain the real absolute concentration in a gas sample in the same range of concentration. A possible explanation for this result might be the type of calibration which is applied to the PAS instrument which requires a two point calibration with the high point which is generally much above the atmospheric concentration of N_2O. This might introduce an error when determining samples at low concentrations. The same is true for CO_2. Concerning CH_4, the reading at high concentration are quite correct, whereas at low concentrations the instrument is very unstable and imprecise as shown by the graph in Figure 2.9.

Table 2.4 – Measured concentration of several gases measured by PAS technique using different filters before the inlet of PAS.

	PAS	Cylinder
N_20 ppm	0.314±0.002	0.340*
	0.709±0.018	0.785±0.079
	1.317±0.025	1.530±0.077
	7.07±0.03	7.00*
	10.11±0.02	10.00*
CO_2 ppm	336.3±2.8	358.0±7.2
	667.3±8.8	719.0±14.4
	1057.9±6.4	1102.10*
CH_4 ppm	9.53±0.96	10*
	31.4±1.09	30*

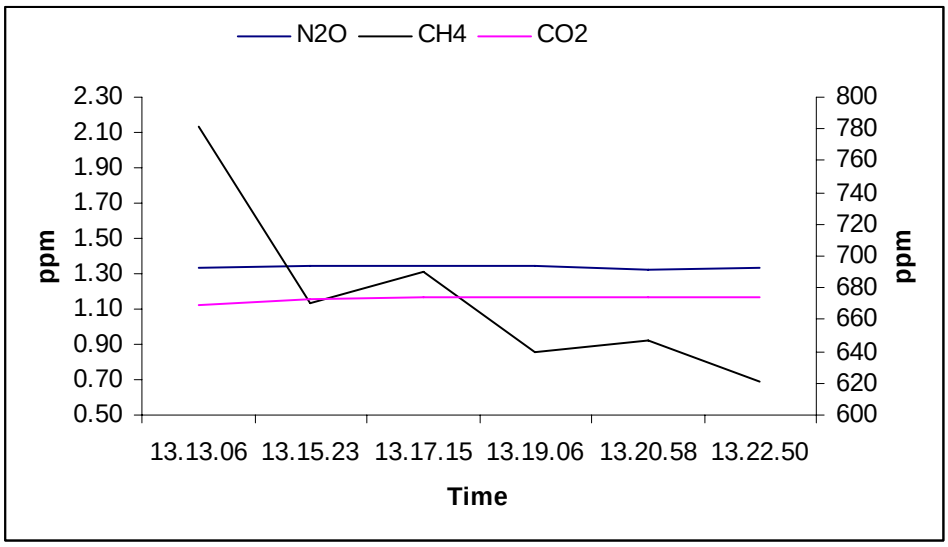


Figure 2.9 – Concentration of the three greenhouse gases measured by PAS from cylinders.

2.2.2 Comparison GC versus PAS techniques using closed chambers

Measurements of CO_2 and N_2O fluxes have been compared in the field by using both techniques associated to closed chambers.

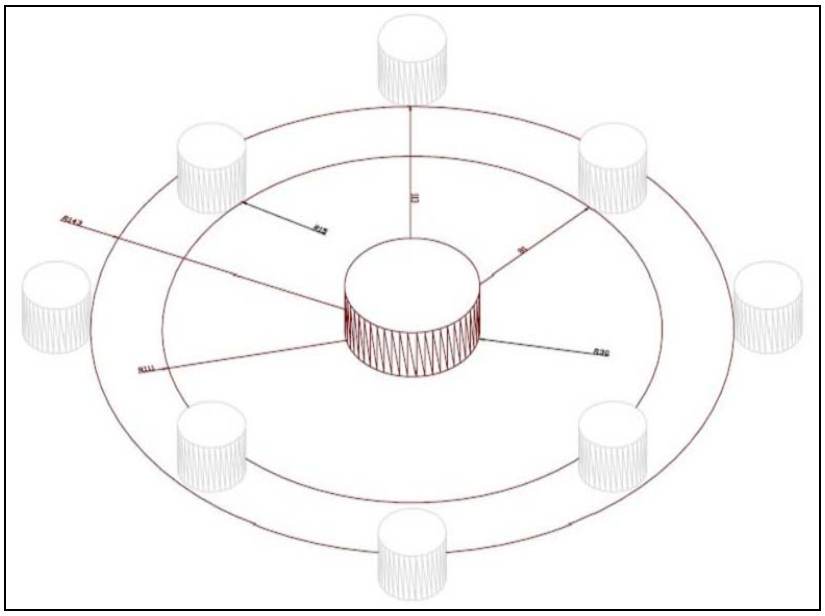


Figure 2.10 – Central closed chamber connected on line with the PAS analyser surrounded by closed chambers sampled manually and analysed by GC for the determination of CO_2 and N_2O concentration.

The fluxes were measured in a meadow located in an open maquis environment, and chambers were distributed as shown in Figure 2.10. Fluxes of CO_2 and N_2O measured by PAS were 7.5 g m^{-2} day^{-1} and 0.48 mg m^{-2} day^{-1},respectively. Fluxes of CO_2 and N_2O measured by GC technique were 6.1 ± 2.1 g m^{-2} day^{-1} and 0.43 ± 0.2 mg m^{-2} day^{-1},respectively. Thus, fluxes measured by the two techniques appeared perfectly comparable. The same experiment was repeated using more chambers connected to PAS and results were again comparable. Interestingly, in Figure 2.11 is reported the increase of CO_2 measured inside the closed chamber reported in the middle of the scheme of Figure 8, and it can be seen that the increase of concentration over time is linear over 50 minutes of chamber closure. The concentration is measured by PAS analyser, connected online to the chamber.

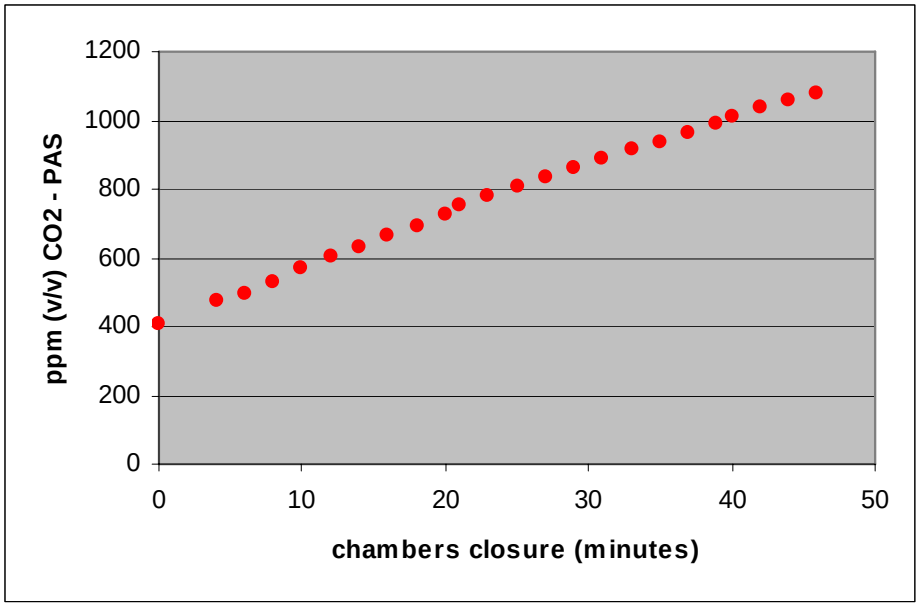


Figure 2.11 – Increase of CO_2 concentration over time inside the closed chamber connected to PAS via inlet and outlet tubes in a dynamic mode, as measured by PAS analyser.

2.3 Soil analysis

2.3.1 Extractable NH_4^+-N and NO_3^--N

Inorganic nitrogen primarily exists in soil as the ammonium ion (NH_4^+) and nitrate ion (NO_3^-) and represents less than 2% of the total nitrogen in a typical soil. The bulk of the nitrogen in soils is "fixed' to organic molecules and is not readily available to plants. To estimate the amount of nitrogen that is available for plant growth extraction procedures have been developed and the amount of nitrogen determined under the conditions of the extraction have been termed Exchangeable Nitrogen.

Mineral N was determined on extracts by potensiometric analysis using two different electrodes: the Orion ammonia electrode 95-12 and the Orion nitrate electrode 9307. Soil was extracted with a 0.5 M K_2SO_4 solution (1:4 soil extractant, v/v) the molarity used generally depends on the quantity of mineral N present in the soil, the cation exchange capacity of the soil and the soil composition so the concentration of K_2SO_4 was previously tested to get the minimum concentration which gives the maximum extraction of the soil. KCl generally is used for this purpose but Cl^- strongly interferes with NO_3^- electrode while SO_4^{2-} cause less disturbance.

For each replicates 15g of homogenized soil were placed in 100 ml specimen cup where is added 75 ml 0.5 M K_2SO_4, all samples was shaken for about 1 hours on a shaking machine and the suspension filtered through a paper filter (Whatman n°42). The extracts can be stored for several months at -18 °C before analysis, though a maximum of 2 weeks is advisable to avoid slight changes in ammonium concentration.

The ammonia in each extract was determined through an ion-selective electrode (ISE) that is a gas-detecting electrode able to sense the level of dissolved ammonia (not ammonium) in aqueous solution. Nevertheless, it is used as if it were a specific ion electrode. The electrode can be used to measure ammonium by addition of strong base to convert ammonium to ammonia according to the equation:

$$NH_4^+ + OH^- \Leftrightarrow NH_3 + H_2O$$

The ammonia electrode is free of interferences by cations, anions and other dissolved species, except volatile amines. Sample color and turbidity do not affect the measurement. The calibration is performed in a series of standards (0.1 ppm, 1ppm, 10 ppm) in which previously is added ISA solution (ionic strength adjustment solution) to ensure that samples and standards have similar ion

strength, proper pH, and to reduce the effect of interfering ions. A minimum of 3 point calibration is recommended to compensate for the electrode's non-linear response, moreover samples and standards should be at the same temperature to avoid temperature effects on the reproducibility. The concentration of the samples is determined by comparison to the standards.

Although the ammonia electrode is functionally simple, the mechanism of its operation is complex. Like other electrodes it follows Nernst's Law and responds logarithmically to the activity of dissolved ammonia in a solution. The activity is proportional to the ammonia concentration, which, in turn, is proportional to the concentration of ammonium ion that was originally present. The electrode contains a hydrophobic membrane which is permeable to ammonia, but not to any ionic species. Dissolved ammonia in the sample solution diffuses into the electrode until the activity of ammonia is the same on both sides of the membrane. Ammonia which passes through the membrane dissolves in solution contained within the electrode according to the equation:

$$NH_3 + H_2O \Leftrightarrow NH_4^+ + OH^- \quad (1)$$

The potential of an internal sensing element varies in a Nernstian manner with changes in the hydroxide level:

$$E = E_0 - S\log[OH^-] \quad (2)$$

where E is the measured electrode potential, E_0 is the reference potential and S is the slope (-59.2 mv/decade) of the electrode response:

The relationship between ammonia, and ammonium and hydroxide ions is given by the equation:

$$\frac{[NH_4^+][OH^-]}{[NH_3]} = \text{cos tan t} \quad (3)$$

The internal filling solution contains ammonium chloride at a sufficiently high concentration so that $[NH_4^+]$ can be considered fixed. Equation (3) then becomes:

$$[OH^-] = [NH_3] = \text{constant equation (4)}$$

Combination of (2 and 4) shows that the electrode response to ammonia is also Nernstian. Each ten-fold increase in ammonia concentration gives rise to a minus 58.5 mV change in electrode potential at room temperature. The potential developed under standard conditions is reproducible to better than ± 0.5mV, or ± 2 per cent according to the Orion manual (Anonymous, 1972). The ammonia electrode can accurately measure concentrations as low as 10 parts per billion.

Solubilized nitrate is also determined potentiometrically using a nitrate ion-selective electrode (ISE) in conjunction with a double-junction reference electrode and a pH.

Standards and samples are mixed with an ionic strength adjustment solution (Orion 930711) in a 50:1 ratio; the nitrate ISE was calibrated using standards that narrowly bracket the expected sample concentration, as the sample concentration was unknown it was calibrate with samples between 0.1 ppm and 10.0 ppm nitrate standards. Calibration is performed by analyzing a series of standards and plotting mV vs. nitrate-nitrogen concentration on semilog paper with a slope of about -54 to -60 mV per decade. Temperature changes affect electrode potentials therefore, standards and samples was equilibrated at the same temperature (± 1 °C).

The electrode is filled with a solution (Orion 900046 to approximately ¼ of its body) and is equipped with a organophillic membrane contains a nitrate selective ion exchanger. Dissolved nitrate in the sample produces an electric potential across the membrane measured against a constant reference with a pH/mv meter or specific ion meter. The potential varies according the Nernst equation with changes in the nitrate level and under standard conditions is reproducible to better ± 2%:

$$E = E_0 - S\log[A]$$

Where A is the nitrate ion level in the solution.

2.3.2 Microbial biomass

The active fraction of organic matter consisted of amino acids, groups of proteins and carbohydrates, represents small, but dynamic portion of the vast and slowly changing background of stabile organic matter. This labile pool is readily available for microbial use and is mostly stored by soil microorganisms. A portion of such kind of organic substances can be quantified as an indicator of actual amount of microbial populations. Thus, there has been increasing interest to definite measurements of the soil microbial biomass and several methods have been attempted to improve more accurate and useful procedure for microbial biomass measurements over the last two decades.

To estimate soil microbial biomass N in the soil was used the extraction method after a 24 h $CHCL_3$-fumigation in the dark to kill the micro-organisms, at last the fumigated samples was compared with non fumigated ones. For each replicates 5 g of homogenized soil were placed in a glass capsule and moved inside a desiccator containing about 25 ml of chloroform as fumigant (the

chloroform has to be purified from ethanol before use it) in a small beaker with some boiling ship useful subsequently to see the boiling. The desiccator thanks to a vacuum pump is vacued until the $CHCL_3$ has boiled and then covered with a black plastic sheet to create dark condition. After 24 h the beaker with chloroform is removed and repeated evacuation are applied to remove the residual $CHCL_3$ vapour in the soil.

Through the fumigation a fraction of the cell constituents becomes soluble and can be extracted from the soil putting the samples in 50 ml plastic corns where in each of them is added 20 ml 0.5 M K_2SO_4; they was shaken for about 1 hours on a shaking machine and the suspension filtered through a paper filter (Whatman n°42), in this way the N is solubilized as amino acids and ammonium.

When an aqueous solution of an α-amino acid is treated with ninhydrin (triketohydrindene hydrate), a violet colour is produced. In the first stage of the reaction, the amino acid is oxidized to give an α-amino acid, this is further hydrolyzed to an α-keto acid and ammonia. The ammonia reacts further to give the violet pigment and the α-keto acid decarboxylates to give an aldehyde. The violet solution shows a significant absorption at 570 nm that can be analyzed using a spectrophotometer previously calibrated hence the intensity of absorption is proportional to the α-amino acid present.

2.3.3 Denitrification enzyme activitiy (DEA)

Quantification of actual denitrification rates is hindered by high spatial and temporal variation, and therefore, in some cases, assay of denitrifier biomass are a good index of denitrification potential since they integrate multiple factors that influence denitrification activity (Groffman, 1987). In this study we measured denitrification enzyme activity (DEA) using a simple acetylene-block denitrification enzyme assay as an index of denitrifier biomass in different savanna soil and an integrated measure of the denitrification potential (Smith and Tiedje, 1979).

On each sampling date, was collected three replicate cores (5.8 cm diameter) of soil to a 10-cm depth; before sampling the soil with a PVC soil sampling device, the aboveground plant material was removed with clippers in the field. For each core three replicates of 5 g of homogenized dry soil were placed in 50 ml gas-tight jars fitted with a sampling port. The DEA was measured using the acetylene-based anaerobic assay as described by Smith and Tiedje 1979 with some adjustments. In each flask is added 5 ml solution containing NO_3^- (100 μg N g^{-1} dry soil) and glucose (C/N, 4/1;

Castaldi and Smith 1998a) after preparing the solution it was degassed through the sonication under vacuum for about 20 minutes; than each vial was vacued and filled up helium and 10% (v/v), so 5 ml, of acetylene was used to inhibit nitrous oxide reductase activities (Yoshinary and Knowles 1976). Samples were then incubated at 25°C for 2 h in the dark; the incubation time is previously chosen to measure DEA without enzyme synthesis, in this way is avoided the use of antibiotic chloramphenicol which inhibit de novo protein synthesis by bacteria but can act as substrate and having non-target effects (Badalucco et al. 1994; Castaldi and Smith 1998b). After incubation 5-ml gas sample was withdrawn with an air-tight syringe and analyzed by means of a gas cromatograph (Fison series 800) equipped with an electron capture detector (ECD) maintained at 280°C (if it's intended to measure CO_2 too the ECD temperature have to be decrease to make CO_2 detectable but in this way decrease ECD sensitivity for N_2O). The injection port was provided with a 1-ml port. A precolumn filled with Poropak Q was held at the same temperature and used in combination with a six-way valve (Valco) to operate front flush and a backflush systems to avoid N_2O peak interference from O_2 and C_2H_2. The N_2 was used as the sole carrier gas (flow rate 40 cm^3 min^{-1}) as the system was set up to direct elute gases first to flam ionization detector and then to the ECD, using a four-way valve (Valco).

The denitrification enzyme activity is calculated as:

$$DEA = \frac{C \cdot V \cdot 1.14 \cdot t}{SW}$$

C = N_2O concentration expressed in ppm

V = volume of N_2O in the flask

1.14 = factor to convert N_2O in N_2O-N

t = incubation time (2 h)

SW = dry soil weight (5 g)

2.3.4 Soil pH

Soil pH was determined introducing 10 g of air-dried soil sieved at 2 mm in 50 ml gas-tight jars shaken with 25 ml of deionized water for about 2 hours on a shaking machine; the jars with the suspension need to be left to sediment to separate the solution from soil. After this stage the pH of the solution is measured inserting a glass electrode in the solution and recorded the value after its

stabilization. The pH meter is calibrated at ambient temperature before the measurement with buffer solution in the range of our purpose so at pH 4.0 and 7.0.

2.3.5 Soil moisture and soil temperature

Soil moisture was measured in the field through a soil moisture sensor (ThetaProbe ML2x) that measures volumetric soil moisture θ_V, the volume of water per unit volume of soil typically cm^3 H_2O cm^{-3}, by the well established method of responding to changes in the apparent dielectric constant. These changes was converted into DC voltage (virtually proportional to soil moisture) and the meter converts the mV reading into soil moisture units using a linearization table and soil specific parameters pre-installed for the sensor.

The temperature during the sampling was measured using a HI93510 thermometer (Hanna Instruments Canada Inc., Laval, Quebec) constituted by thermocouple where the dissimilar metal creates a circuit in which the two legs generate different voltages, leaving a small difference in voltage available for measurement. That difference increases with temperature, and can typically be between one and seventy microvolts per degree Celsius (μV/°C) for the modern range of available metal combinations.

2.3.6 Soil water content

Water content of soil is generally expressed as "gravimetric water content (θ_g)", the mass of water per unit mass of oven-dry soil, or as "volumetric water content (θ_v)", the volume of water per unit volume of soil. For routine purposes the chosen method was the former. About 30 g of dry soil were weighed in small cups and placed in a pvc cylinder arranged with a membrane on its top and previously weighed. The water through the capillarity mechanism move in the cylinder through the membrane; the next morning the cylinder with the soil and the water is weighed. The mass of the water is:

Mass of water = (cylinder weight + soil weight + water weight) – (cylinder weight + soil weight)

while the gravimetric water content, expressed as g g^{-1} or as a percentage, is:

$$\theta_g = \frac{\text{Mass of water}}{\text{Mass of dry soil}}$$

2.3.7 Soil bulk density and water filled pore space

Soil bulk density is a measure of how dense and tightly packed a sample of soil is. It is determined by measuring the mass of dry soil per unit of volume (g/mL or g/cm3). The bulk density of soil depends on the structure (shape) of the soil peds, how tightly they are packed, the number of spaces (pores), and the composition of the soil particles; it is used to convert between mass and volume of a soil sample. Soils made of minerals will have a different bulk density than soils made of organic material. In general, soil bulk density can range from 0.5 g/mL or less in organic soils with many pore spaces, to as high as 2.0 g/mL or greater in very compact mineral horizons. The bulk density of a soil sample should be adjusted for any rocks or coarse fragments it contains but in the savanna soils it unlikely occurs.

Bulk density is calculated from the mass of a given volume of dry soil, including the air spaces, but excluding materials larger than soil, such as rocks or materials with dimensions greater than 2.0 mm.

Soil bulk density = mass dry soil / bulk volume of soil

The bulk volume of soil depends on the volume of soil occupied in the pvc cylinder it was calculated as:

Bullk volume of soil = $\pi r^2 h$

where h is the high of soil inside the cylinder

The fraction of pore space in a soil, its porosity, is calculated as one minus the ratio of bulk density to particle density:

ε = 1 - [bulk density/particle density]

Some typical values of particle density and porosity of soil are reported in Table 2.5.

Table 2.5 - Reproduced from D. L. Rowell (1993).

	Particle density(g cm^{-3})	*Bulk density(g cm^{-3})*	*Porosity(cm^3 cm^{-3})*
Cultivated mineral soils, plough horizons	2.60	- 1.4	0.46
medium-heavy textured, light texture	2.60	- 1.7	- 0.35
Subsoils and parent materials	2.65	- 1.8	- 0.32
Grassland and woodland, A horizons	2.4	0.8 - 1.2	- 0.50
Peats	1.4	0.1 - 0.3	0.93 - 0.79

The water-filled pore space WFPS (also known as degree of saturation Hillel, 1980b) is an easily measured soil parameter, it is the volume of water present in the soil relative to total volume of pores. It often expressed as a percentage, is the ratio of volumetric soil water content (θ_v) to total porosity of the soil (ε):

$$\mathbf{WFPS} = [100 \times \theta_v]/ \varepsilon$$

where $\varepsilon = cm^3$ pore space / cm^3 soil.

The volumetric water content can be easily derived from the gravimetric water content as it is equal to:

$$\boldsymbol{\theta_v} = \theta_g \times \text{bulk density/ density of water}$$

The WFPS has the advantage of being largely comparable among soils of different texture as it takes into account the total porosity of the soil and the compaction, and is consequently directly related to gas diffusivity while θ_v and θ_g parameters cannot do it.

2.4 Statistical analysis

For each site daily mean GHG fluxes, soil temperature, and soil moisture for each treatment were calculated by averaging the replicates for each sampling day. Kolmogorov Smirnov test has been applied to test the normality. One way or two way of Analysis of variance (ANOVA) were performed to test the statistical significant differences between the different factors considering as sources of variation gas fluxes; if null hypotheses is rejected ($P<0.05$) Tukey post-hoc test has been used (SPSS 13).

To value the relationship between the different variables was calculated Pearson correlation and than verified the significance (Sokal & Rohlf, 1995). Pearson correlation was calculated considering:

1) the environments to value the relationship between the variables according to the different environmental condition.

2) the treatments belong to a specific environment to verify the correlation due to the presence/absence of water.

In case of statistically significant correlation was valued the relationships between GHG fluxes and the variables using model fitting.

3 GHGs FLUXES FROM UNBURNED AND BURNED CONGOLESE GRASS SAVANNA

3.1 Premise

Africa is known as the "Fire Continent" (Komarek 1965) and prescribed burning is a widely recognized and essential ecological factor in for managing its grassland and savanna ecosystems. In African grassland and savanna areas used for nature conservation and game ranching there is general consensus that fire has occurred naturally since time immemorial and that it is often essential for the ecological well-being of these ecosystems (Bothma 1996; Thomson 1992; Trollope 1990). Nevertheless, views on the most appropriate burning system for wildlife areas vary widely. Initially, ideas on the use of fire were based on ecological equilibrium theory and burning was applied at a fixed return period. However, with the development of non-equilibrium theory of savanna dynamics, prescribed burning is now applied under more diverse conditions (van Wilgen et. al. 2003). Even within this new paradigm burning systems vary from so-called "natural" systems based entirely on lightning as the ignition source, to actively applied burning systems based on the condition of the rangelands. The literature indicates that fire management is best developed in southern Africa. African savannas are mostly characterized by grasslands with scattered trees and shrubs supporting high biodiversity. They occur in areas with a clear dry season, followed by a rainy season. Generally, density of trees and shrubs is dependent on dry season duration, soil fertility and fire occurrence. A significant part of these areas are used in Africa as grazing lands (about 13 million km^2), although 80% of pastoral land are hyper arid, arid and semi-arid ecosystems (savanna and steppe). Sub-humid and humid pastoral lands are mostly restricted to central western and north central Africa, with some small highland pastures in Ethiopia and South Africa (Reid et al. 2004). Seasonal fires play a vital role in the savannas biodiversity. In October, a series of violent thunderstorms, followed by a strong drying wind, signals the beginning of the dry season. Fire is prevalent at the height of the dry season. Fires in savannas are often caused by poachers who want to clear away dead grass to make it easier to see their prey. The fires do not devastate the community. Most of the animals killed by the fires are insects with short life spans. Underground holes and crevices provide a safe refuge for small creatures. Larger animals are usually able to run fast enough to escape the fire. Although the dry stems and leaves of grasses are consumed by fire, the grasses deep roots remain unharmed. These roots, with all their starch reserves, are ready to send up new growth when the soil becomes more moist. The scattered shrubs

can also subsist on food reserves in their roots while they await the time to venture above the soil again. Unlike grasses and shrubs, trees survive a fire by retaining some moisture in all their above-ground parts throughout the dry season. Sometimes they have a corky bark or semisucculent trunk covered with smooth resinous bark, both being fire resistant. A fire leaves scorched earth covered with a fine layer of powdery black ash in its wake. Violent thunderstorms occur again heralding the rainy season. When the rains come, savanna bunch grasses grow vigorously. Some of the larger grasses grow an inch or more in 24 hours.

Being fire such a common land use practice in African savannas it could be a relevant influencing factor on GHGs emissions at regional scale. However, the literature on fire impact on GHGs in tropical savanna is quite scarce so that more research is needed. The aim of this study wa hence to evaluate the impact of fire on a tropical herbaceous savanna in Congo Brazzaville. The study was articulated in 4 main 3 objectives. First, to quantify GHGs fluxes (CO_2, N_2O e CH_4) in unburned and burned areas and to correlated them to the main environmental characteristics. For this purpose 2 field campaigns were organized at different distance from fire event, 1 month and 7 months after, one in the dry season when fire oocurs and one at the end of growing season. Second, to simulate the "flushing" induced effect of rain on GHGs when soil is dry, as this phenomenon is considered in this extreme environments to contribute to a significant portion of the total GHGs emissions. A irrigation experiment, which simulated rain and different conditions of soil water content was set up in each sampling campaign. Third, to simulate in laboratory incubation the N dynamics and N_2O emissions in function of soil water and temperature to better interpret field data. Fourth, to quantify the contribution of termites nests to the overall CH_4 production by measuring CH_4 emissions directly from nests and at increasing distance from the nest.

3.2 Site description

The research site is located in the littoral region of Congo, close to the little village of Tchizalamou (4° 17'20.61"13 S and 11°39'22.78" E), in the Kouilou district, near the Atlantic Ocean at an altitude of about 82 m above sea level. (Figure 3.1). The site is dominated by humid tropical forests (c. 65%) and humid savannas (c. 35%) (CARPE, 2003). The Kouilou region is covered by savannas lying between the coastline and the Mayombe forest. In this area thanks to the comparison of aerial photographs it was possible to show that during the last decades forest has progressed over savannas at a rate of about 100 m per century Fabing (2000).

Analogous situations occur in the near regions where Congolese Mayombe forest advance at a rate of between 20 and 50 m per century over savannas (Schwartz et al., 1996), and around 100 m per century in coastal Gabon (Delègue et al., 2001).

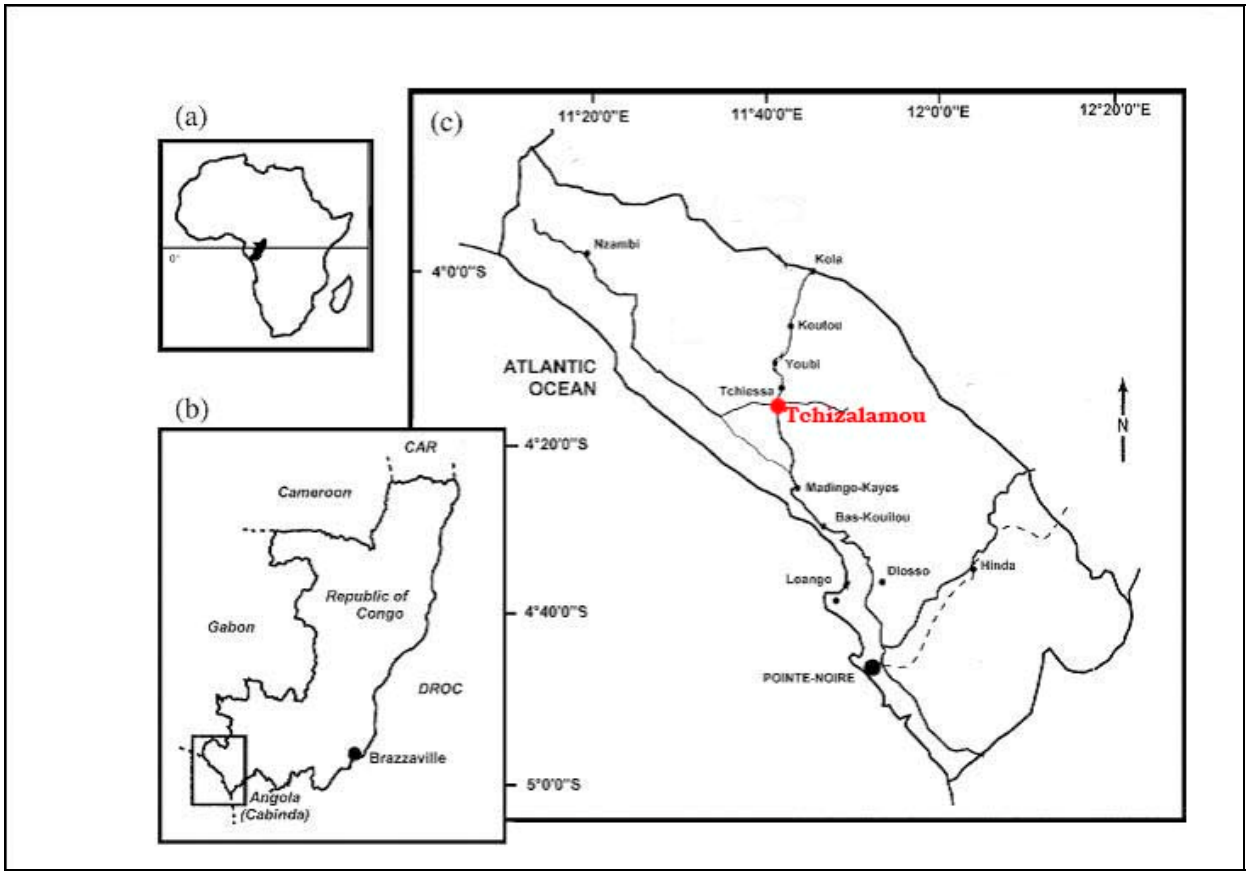


Figure 3.1 – Tchizalamou site station in the Republic of Congo.

Many source consistently indicate that herbaceous savannas have occupied the Pointe-Noire region (Congo) since the Upper Holocene (Trouvé, 1992; Schwartz et al., 1995). The present grass and shrub savannas result from two cumulative factors: drying climatic, which occurred ca. 3,000 B.P. abruptly, and the arrival and expansion of populations practising savanna burning to a great extent. Before Upper Holocene, the open vegetation during dry climatic periods was open forest or at least tree savannas (Schwartz et al., 1995).

The climate of the Tchizalamou site is a two-season transition equatorial type, characterized by a long cool dry but cloudy season from May or June to September, followed by a rainy season from October to April or May with about 1200 mm precipitation (Madingo-Kayes meteorological station 1982–2001). The annual temperature is about 25° C, with seasonal variations of ca. 5° C (Laclau et al., 2002; Loumeto, 2002). The vegetation in the study region is a mosaic of forest and grove-spread savannas.

The herbaceous layer in savannas is low (Laclau et al., 2002), being dominated by short Gramineae and Cyperaceae (e.g., *Bulbostylis laniceps, Ctenium newtonii* and *Rhynchelytrum repens*) with locally tall Gramineae such as *Hyparrhenia diplandra, Loudetia arundinacea, Imperata cylindrica* or *Panicum maximum*. The graminaceae *Loudetia arundinacea* stend make up more than 80% of the aerial biomass of this savanna, which reached about 5 Mg ha^{-1} of dry matter at the end of the rainy season (Laclau *et al.*, 2002). Some shrubs of 1–2 m height are present: *Annona arenaria, Bridelia ferruginea and Psorospermum febrifugum*.

Many of these savannas are today colonized by forest trees. Locally, tree or shrub clusters of various sizes are present in these savannas, some of which are natural while others have an anthropogenic origin and correspond to abandoned villages. For these latter, human interference is easily deduced from the presence of plants such as oil-palms (*Elaeis guineensis*), avocados (*Persea americana*) and mango trees (*Mangifera indica*) (Vennetier, 1968; Hecketsweiler & Mokoko-Ikonga, 1991). On the other hand, natural tree clusters are characterized by the lack of these fruit-trees and are rich in pioneer forest species such as *Anthocleista schweinfurthii, Aucoumea klaineana, Barteria nigritiana, Macaranga spinosa, M. barteri and Xylopia aethiopica*. Other tree species are found in these clumps, such as *Klainedoxa gabonensis* and *Sacoglottis gabonensis*, typical of humid forests, and *Chrysobalanus icaco*, usually found in littoral groves.

b

a

Figure 3.2 – Congolese savanna before (a) and after (b) the fire.

The geological bedrock of the littoral savannas of Congo is composed of Plio- Pleistocene thick detrital formations of continental origin (Jamet, 1975). 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) and field capacity is around 20% of volumetric humidity. The soils in these savannas have a low clay content, are poor in nutrients and therefore unsuitable for agricultural purposes.

The studied area is interested by wide superficial fires during the mid-june but other little fires often occur during all the dry season; in the figure 3.2 is showed the savanna before and after the fire. As in this region the savanna is almost completely constituted by a grass layer, the fires generally are very rapid and superficial.

In this site are also presented many temites nests. They generally don't have an height over the 50 cm (figure 3.3), but despite of their size they are very densely distributed, about 1200 termites' nests/ hectare (De Groundcourt Pers Comm); for these reasons in this area there probably are important sources of methane. The termites' species observed are essentially two: *Cubitermes sp*. (86% of nests) and *Trinervitermes sp.* (21% of nests) (burrower); while another little amount is represented by others humivorous (9% of nests).

Figure 3.3 – Termites nest found in Tcizalamou station.

3.3 Experimental design

Two intensive campaigns were organized in Congo for the measurements of gas fluxes. One campaign was one month after burning, from the end of June until the first day of July 2007, during the dry season. The second was 7 months after fire, in February 2008, towards the end of the wet season. Fire was induced over a large area of several hectars where, however, an area had been isolated, burning the surrounding vegetation, to keep it safe from fire passage. This area was used as an unburned control and was about 1/2 hectar wide. In order to simulate rain events and to obtain a gradient of soil water contents, which could provide insite of GHGs fluxes variability in function of soil water content, a big plot was protected from the rain, in both unburned and unburned areas, by covering it with a tent. The area under the tent was divided into 5 squared sub-plots (2.25 m^2 each), all cointaining 4 chambers. A control plot was set outside the tent, and one inside so to have an unirrigated plot where however the effect of the tent itself on soil parameters could be tested by comparing the outside-inside controls (figure 3.4). The control outside the tent was representative of the savanna environment at normal environmental conditions. Plots inside the tent were watered at different times before the campaign in order to get a gradient of water contents (figure 3.4).

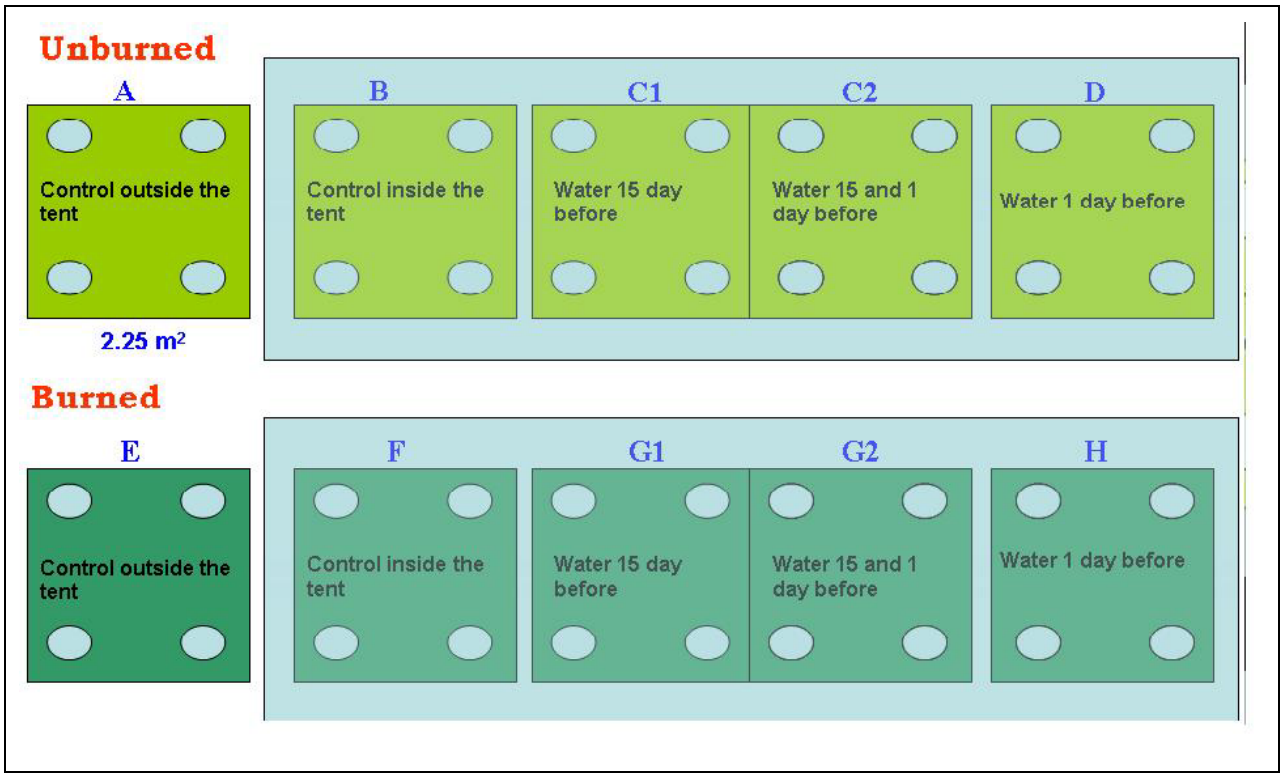


Figure 3.4. Experimental design used in Congo for the measurements of gas fluxes.

The amount of water for the experiment was decided on the base of meteo data recorded from the flux tower installed in Tchizalamou from 07/07/2006 to 30/04/2007. The rain water fallen each day (from noon to noon) was collected and divided into frequency classes (0-2mm, 2-5mm, 5-10mm, 10-20mm, 20-30mm, 30-40mm, 40-60mm, 60-80mm, 80-100mm, 100-120mm, 120-140mm) and the probability for a rain of each class was calculated, data are summarized in table 3.1.

Table 3.1 Frequencies and probabilities of rain during the dry and wet season.

	dry season	july7-oct14	wet season	oct15-april30
rain/24h	frequency	P %	frequency	P %
0	62	59.6	59	30.1
0-2	37	35.6	53	27.0
2-5	5	4.8	16	8.2
5-10	0	0.0	16	8.2
10-20	0	0.0	26	13.3
20-30	0	0.0	11	5.6
30-40	0	0.0	3	1.5
40-60	0	0.0	5	2.6
60-80	0	0.0	3	1.5
80-100	0	0.0	1	0.5
100-120	0	0.0	3	1.5
120-140	0	0	1	0.5

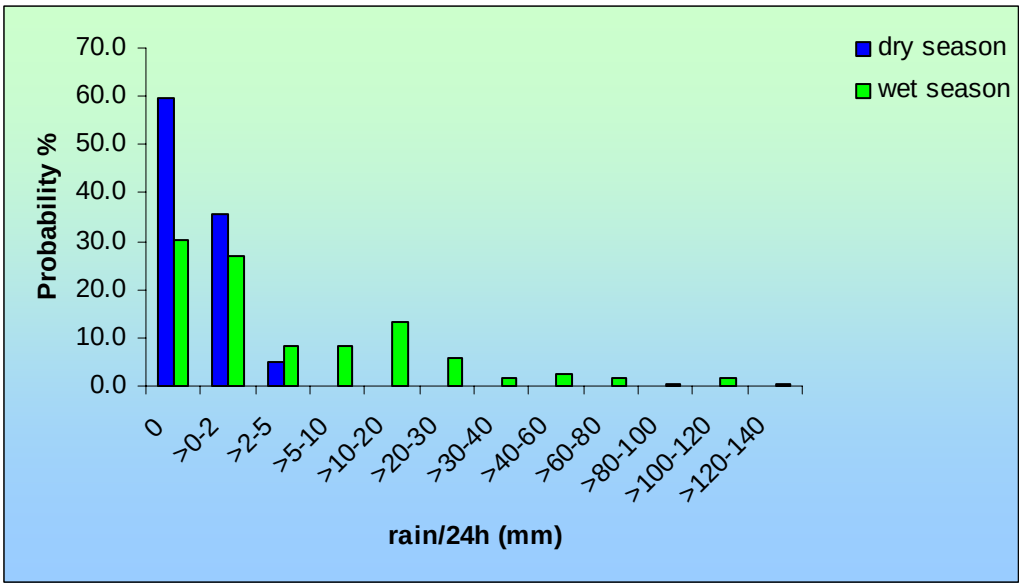


Figure 3.5 Probabilities of rain according to the classes.

In the figure 3.5 it is possible to note that during the wet season the probability of a precipitation event between 30 and 40 mm is very low, generally the larger amount of total precipitation is due to frequent small rains. For this research we choose a watering from 20 mm to 30 mm that is enough representative of a normal "big" rain during the rainy season. Considering the area, each sub-plot of 2.25 m^2 was watered with 67.5 liters of water. In this way a heavy precipitation of 30 mm was simulated.

The analysis of the greenhouse gases was made using both the gascromatograph and photoacustic techniques; the first was used to analyze methane and nitrous oxide gases (figure 3.6a) while the second one was used to analyze directly only nitrous oxide because the instrument is not suited for CH_4 (figure 3.6b). The first method employed to sample the gases is the classical method using multi-component static chambers (Chapter 2). The static chamber consisted of PVC collars (7 cm high for 31.5 cm diameter) and the chamber lids (20 cm high for 31.5 cm diameter), which were provided with a 2 way stopckok sampling port, in which the polypropylene syringe could be inserted. The collars were inserted in the soil to about 5 cm depth, in a slot previously formed by a metal cutting ring and were joined to the lids by 4 clips. Having 4 chambers per subplot, we had overall 20 chambers in the unburned and 20 chambers in the burned area.

a **b**

Figure 3.6 – Chambers used for fluxes measurements through gas chromatographic technique (a) and photoacoustic analyzer (b).

The change in gas concentration in the chamber with time, was determined by taking replicate gas samples (20 ml) from the chamber headspace by syringe equipped with a 2 way stopckok and transferring them to glass vials (20 ml). Gas samples were taken normally at time 0-20-40 minutes, to avoid excessive overheating of the air inside the chambers.The vials were sealed using a thermo fusible glue and were sent to Environmental Science Department labs (Italy) for gas chromatographic analysis, which occurred within a month. The other method used to estimate gas exchanges from soil to atmosphere was the photoacustic technique; thanks to it the gas can be analyzed directly in the field using the analyser on line via an inlet and an outlet connection between analyzer and a static chamber that has the same structure and dimensions of the previous one used for the classical method (Figure 3.6b). A continuous sampling interval (***SI***) and a monitoring period (***MP***) of 40 minutes were chosen, while to have an high accuracy it was chosen an integration time of 50 seconds (***SIT***). An example is reported in figure 3.7. The flux rate was obtained by a linear fitting of row data.

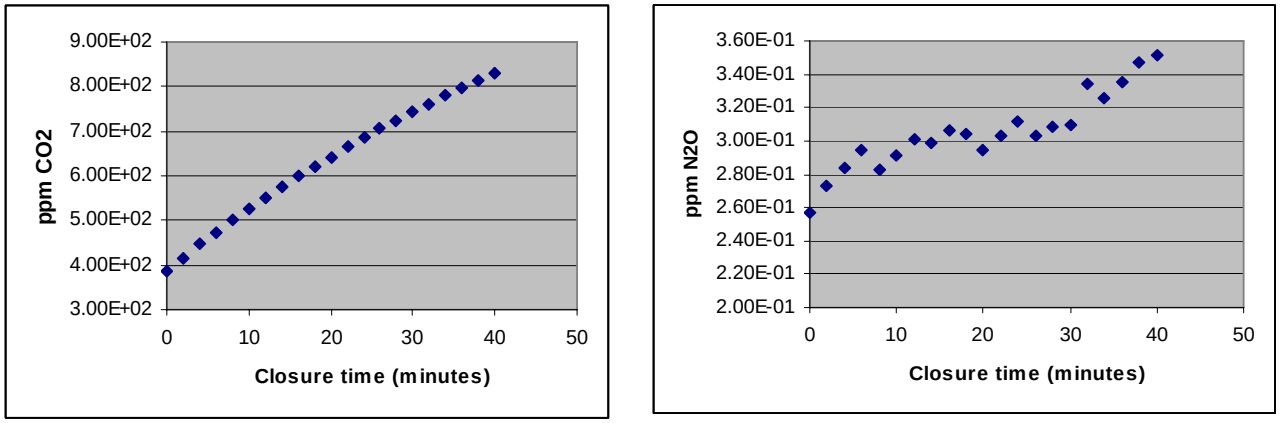


Fig 3.7 – Increase of CO2 and N2O concentration in the chamber over time measured by PAS every 2 minutes.

At the end of each sampling soil humidity and soil temperature were recorded inside the collar.

3.4 Laboratory incubations

Soil was sampled at 0-10 cm depth during the first campaign form unburned and burned control plots, was sieved (2 mm mesh) and air dried. It was then sent to the Environmental Science Department labs (SUN-Italy) were the experiment was carried on. Soils, 50 grams, were incubated in half liter conic flasks. The incubation was organised in order to have soil incubated at different water filled pore space (0, 25%, 50%, 100%) and at 2 temperatures (25°C and 37°C). For each single treatment we had 3 replicates. Immediately after water addition soil were incubated in thermostated cells. Gas samples were measured on days 1, 3, 10 and 15, by closing the flask with a rubber bang air tight lid and sampling the gas at time zero and after 24 hours. The remaining time flask were kept open and the amount of water adjusted by gravimetrical determination. At the beginning and at the end of the incubation soil mineral N was determined to quantify mineralization and nitrification net rates over 15 days of incubation. Also microbial biomass and α-amino-N was quantified on the soil at the end of the experiment. For the description of methods see chapter 2.

3.5 Results

3.5.1 Soil Parameters

In Table 3.2 are reported the main chimico-physical characteristic measured in the burned and unburned savanna plots, one month after burning (1st campaign) and 7 months after burning (2nd field campaign).

The soils present a quite acid pH and a very low content of soil C and N, as often found in savannas ecosystems. It can be clearly seen, that in the second campaign which occurred at the end of the grass growing season, the input of new organic matter increased soil C, whereas on the contrary a reduction of total and mineral N was observe in the unburned plots (table 3.2), probably as a consequence of grass exploitation of this resource. Where soil burned also total and mineral N were higher in the second campaign, although the difference was minimal. Only in the case of α-amino-N the burning induce statistically significant effect, being the burned soil richer of low molecular weight N compounds (containing aminoacids, protein, enzymes) (table 3.2).

Table 3.2 – Some chemical characteristics of the unburned and burned savanna plots measured during the first and second field campaign on the top 10 cm of soil.

	pH	Total soil C (%)	Total soil N (%)	NH_4^+-N μg N g^{-1} d.s.	NO_3-N μg N g^{-1} d.s.	α-amino-N μg N g^{-1} d.s.
1st camp.*						
Unburned	$3.65^{a} \pm 0.03$	$0.95^{a} \pm 0.07$	$0.06^{b} \pm 0.01$	$2.72^{b} \pm 0.01$	$0.34^{ab} \pm 0.07$	$13.50^{a} \pm 5.92$
Burned	$3.73^{a} \pm 0.01$	$1.17^{abc} \pm 0.99$	$0.08^{b} \pm 0.01$	$3.44^{b} \pm 0.70$	$0.38^{bc} \pm 0.04$	$26.42^{c} \pm 1.64$
2nd camp.**						
Unburned		$1.53^{b} \pm 0.22$	$0.02^{a} \pm 0.03$	$0.14^{a} \pm 0.19$	$0.18^{a} \pm 0.10$	$21.71^{b} \pm 0.28$
Burned		$2.03^{c} \pm 0.30$	$0.26^{c} \pm 0.11$	$5.41^{c} \pm 0.23$	$0.58^{c} \pm 0.16$	$27.68^{c} \pm 0.92$

* one month after burning; ** 7 months after burning, end of wet season

Table 3.3- Average values of soil water content and soil temperature recorded in the control outside (A, E) and inside (B, F) tent during the 2 field campaigns in burned and unbuned plots. Different numbers in apex show statistically significant differences ($P<0.05$) between the different plots.

	Unburned		**Burned**	
First campaign	**A**	**B**	**E**	**F**
Volumetric water content (%)	3.8 ± 0.9^{1}	4.6 ± 1.7^{1}	4.1 ± 1.7^{1}	3.9 ± 1.2^{1}
Temperature °C	34.8 ± 7.1^{1}	33.3 ± 3.8^{1}	34.2 ± 8.6^{1}	35.1 ± 5.3^{1}
Second campaign				
% water content	1.7 ± 0.7^{1}	1.2 ± 0.8^{1}	0.9 ± 0.6^{1}	1.0 ± 0.4^{1}
Temperature	27.6 ± 0.7^{1}	28.0 ± 1.3^{1}	28.4 ± 0.9^{1}	28.9 ± 1.8^{1}

As soil water content and temperature are two key controlling factors for GHGs fluxes, the first thing we wanted to verify was the impact of tent cover on these parameters. In table 3.3 are reported the average soil water contents and temperatures measured in all the control plots during the two campaigns. Firstly, in both campaign the results of two way ANOVA made on data showed no significant difference between the controls under and outside the tent. For this reason in the next statistical analysis data from controls inside and outside the tent were merged and analysed as one single statistical population, keeping separate the sole treatments burned vs. unburned. A second observation is that both soil properties resulted not significantly different in the two treatments (unburned vs. burned) in both seasons. Third, soil water content was slightly lower in the second

campaign, despite the period could still be classified as wet season. However, it was at the end of the period when rains become less frequent, so that after few days of intense evaporation and evapotraspiration, this light textured soil was quickly going back to its initial, very low soil water content.

In figure 3.8 is reported the soil water content variation in the irrigated plots along the following days, and it can be noticed that it drops very fast already within the fiest week back to background values.

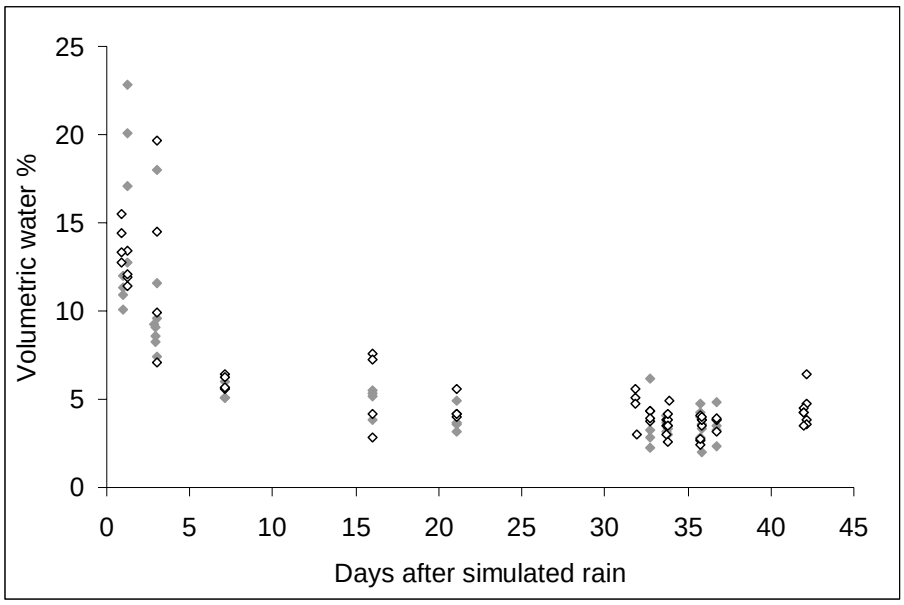


Figure 3.8 - Volumetric soil water content in burned (dark) and unburned (white) plots starting from the day of water addition.

3.5.2 Methane soil-atmosphere gas exchange

Methane fluxes were analyzed in both campaigns using static chambers and analysing gas sample by GC later on at the Second University of Naples. SUN-DSA. In figure 3.9 are reported the fluxes measured in the control sites in the 2 campaigns.

Data show that in the first month after burning a net CH_4 production is observed in the unburned plots, whereas the burned plots are a methane sink. In the second campaign the site acts on average as a sink, showing however a big variability that makes the two treatments not significantly different (figure 3.9). Overall burned plots always show lower or more negative net fluxes than the respective unburned plots in the two seasons (figure 3.9).

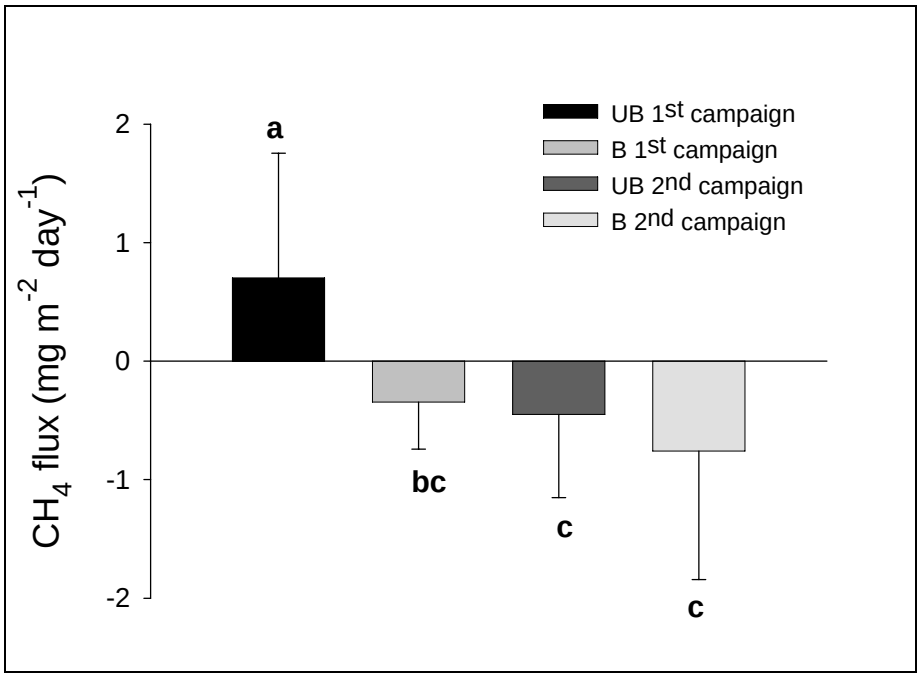


Figure -.3.9- CH_4 fluxes measured in the control plots in the 2 campaign.

The observed difference found both in the control and burned sites cannot hence be attribute to the sole time elapsed after burning, as the effect should be noted on the sole burned sites, so that controls should indeed remain a source of CH_4. Analysing the relationship between water and CH_4 fluxes (figure 3.10) for the sole control plots it can be observed that: 1) the 2nd campaign (bullet) which presents more negative fluxes than the 1st campaign (triangles) has drier soils than the first (figure 3.10). A drier soil means higher diffusivity of methane in soil, and higher consumption rates; 2) within each sampling season on average white simbols (unburned) are above the dark symbols (burned), i.e. in the unburned plots net fluxes are higher; 3) the separation between unburned and burned plots (white vs dark) is clearer in the first campaign (triangles) where almost all the white triangles are above the dark ones, whereas in the second campaign the situation is more mix. This suggest that fire might disturbe a possible existing source of methane, which we can hypothesise to be termites, and after 7 months this source might start to riconstitude more diffusely making less visible the fire effect on them. The fact that in the second campaign we find net negative average fluxes might depend then not on termites being disturbed by fire but on the lower water content which favour consumption vs. production. Fitting the data of Fig. 3.10, keeping separated unburned and burned plots, unburned data are fitted by a linear relationship, thus CH_4 flux grows with increasing soil water content, whereas burned plots are fitted by an asynthotic exponential curve which tends to zero. This means that increasing water content in soil in unburned site reduces CH_4 oxidation activity leaving untouched the source (termites) so that the source gets more and more

predominant swopping fluxes from negative to positive at about 3% soil water content. In the burned sites the ground source (termites) is affected so that when water content increases the CH_4 oxidation capacity decrease but the source is scarce so the net flux keeps always below zero. Thus reconstitution of ground termites activity occurs but after 7 months it has not yet completely recovered.

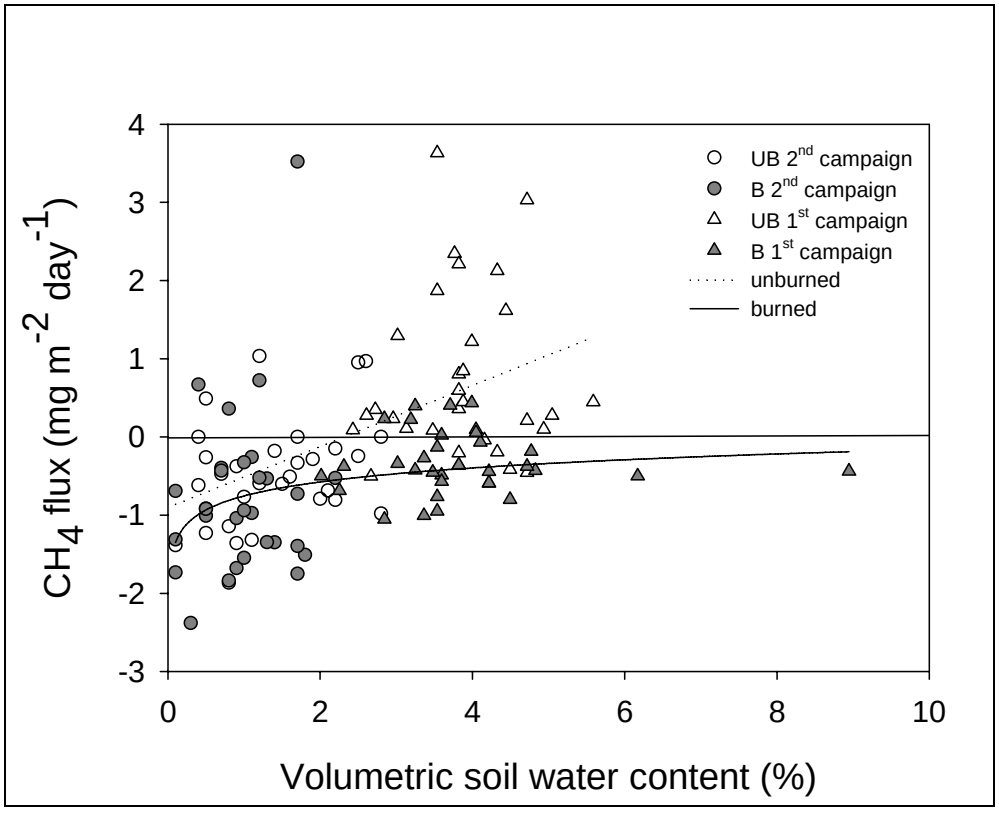


Fig. 3.10 - Relationship between volumetric soil water content and CH_4 fluxes in unburned and burned savanna in the 1st (A) and 2nd (B) sampling campaigns, in the sole control plots (unwatered).

In the following graph (figure 3.11) we have all the data (also irrigated) in the 2 campaigns. The graph shows that: 1) again it is clear that in the 1st campaign CH_4 flux in unburned plots (white simbols) are above burned ones (dark symbols), while in the second campaign the difference is less marked; 2) in the first campaign higher water contents correspond to higher emissions (or positive net fluxes) than in the second campaign.

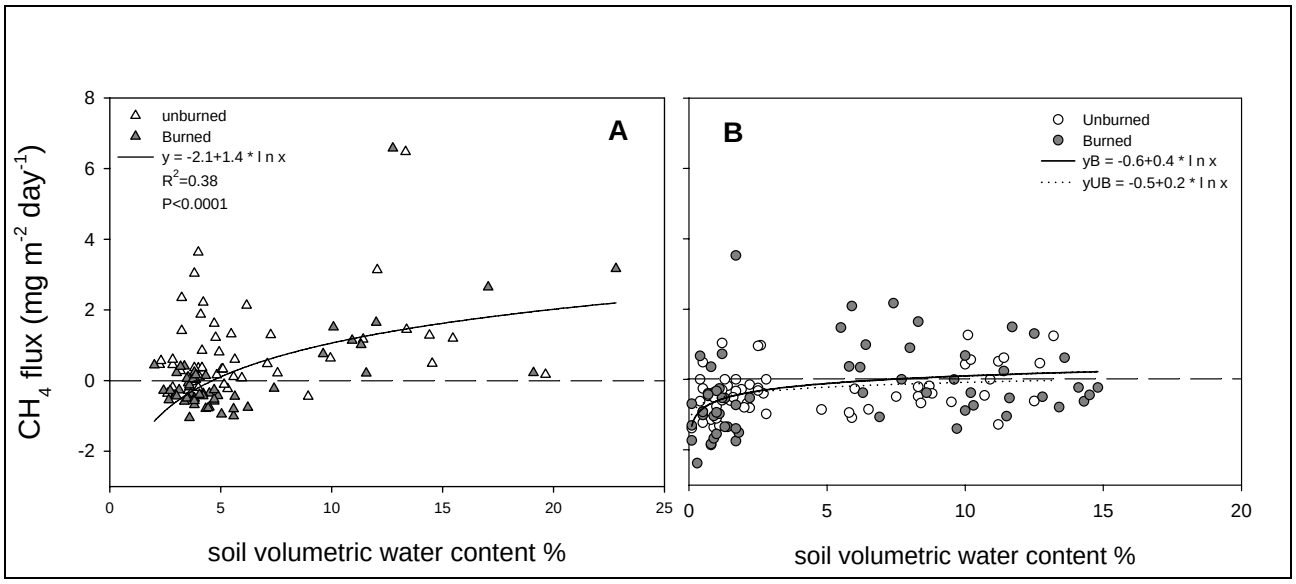


Figure 3.11 - Relationship between volumetric soil water content and CH_4 fluxes in unburned and burned savanna plots in the 1st (A) and 2nd (B) sampling campaigns, in control and watered treatments.

3.5.3 CH_4 emissions from termites nests

Given the enormous presence of termites nests and activity at the site, and in general in savannas environments we also tried to quantify: a) the amount of CH_4 emitted from the nests; b) how far from the above ground part of the nest net CH_4 emissions can be observed. In fact termites nest have a significant part of it developing underground (figure 3.12)

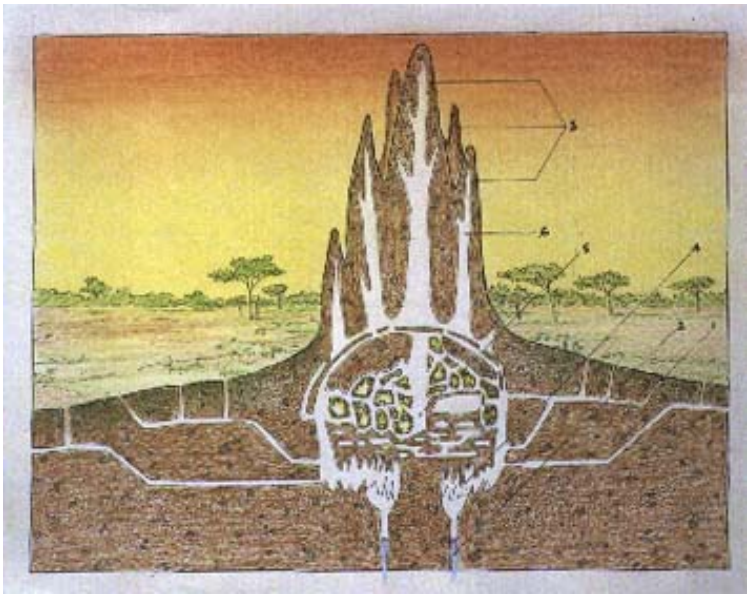


Figure 3.12- Distribution of galleries and channels in the termite nests above and below ground.

These informations would help both to describe CH_4 budget for the site and to interpret the spacial variability of measured CH_4 fluxes. For this reason we set one chamber on the nest, one at 1 meter and one at 3 meters from the nest (figure 3.13).

Figure 3.13 - Experimental design used to quantify the contribute of termites to CH_4 emissions.

The study was carried out from January 2008 until June 2008 in the unburned plots, in order to avoid to confuse the real termite potential distribution with fire effect. Flux measurements were made on five dates on 4 mounds of which 3 belonged to Cubiterms specie that represent about 86% of the total termites at site (Grandcourt Pers. Comm) and the last one, less frequent, belonged to Nasotitermes specie; the volume of each mounds was variable but it was estimated assuming the shape of each mounds as a cylinder with upon a semi-sphere body. The flux on each nest was measured using a two overimposed chambers with a diameter of 50 cm and a total eight of 60 cm (figure 3.13).

The results of analysis conduced on termites are resumed in table 3.4.

Table 3.4 - Descriptive analysis of methane fluxes according to the distance and the sampling day.

Descriptive Statistics

Dependent Variable: CH4 flux mg m-2 day-1

DISTANCE	DATE	Mean	Std. Deviation	N
on nest	21-JAN-08	43,32	27,72	4
	24-FEB-08	30,42	12,38	4
	14-APR-08	25,81	11,77	4
	24-MAY-08	23,40	20,08	4
	03-JUN-08	5,45	,79	4
	Total	25,68	19,71	20
<1 m	21-JAN-08	-,43	,71	4
	24-FEB-08	-1,70	1,99	4
	14-APR-08	-1,56	1,23	4
	24-MAY-08	-,18	1,40	4
	03-JUN-08	,20	,65	4
	Total	-,73	1,39	20
3 m	21-JAN-08	-,79	2,24	4
	24-FEB-08	-1,26	,89	4
	14-APR-08	-,74	1,07	4
	24-MAY-08	,26	1,42	4
	03-JUN-08	-,21	,68	4
	Total	-,55	1,34	20
Total	21-JAN-08	14,03	26,05	12
	24-FEB-08	9,15	17,02	12
	14-APR-08	7,84	14,66	12
	24-MAY-08	7,83	15,60	12
	03-JUN-08	1,82	2,77	12
	Total	8,13	16,82	60

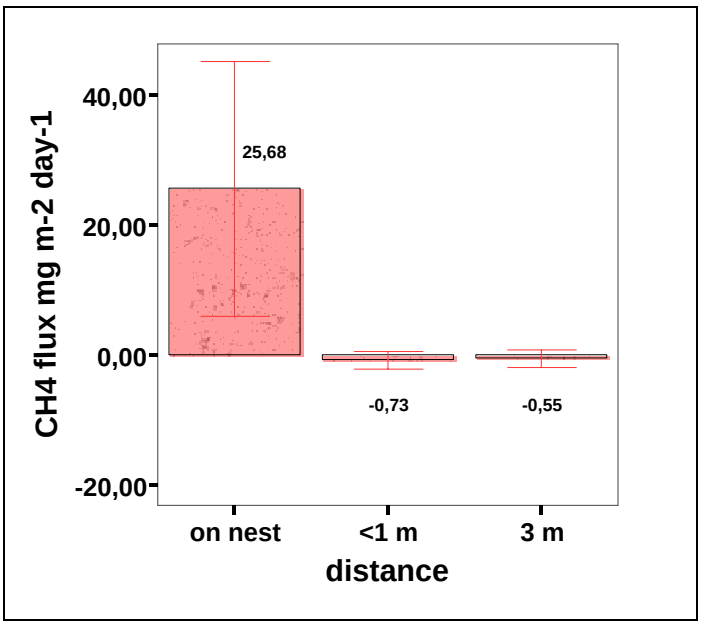


Fig 3.14 - Temporal average of methane fluxes according to the distance from the nest.

Considering all the 5 field samplings, nests produce on average 25.68 ± 19.71 mg CH_4 m^{-2} day^{-1}. Soil at 1 and 3 meters goes from being a slight sink to a slight source (Fig 3.14).

Considering the single field campaigns (Fig 3.15) it can be noticed that in all the sampling dates CH4 production was observed from nests. This however was very variable depending on the date. As a general trend the flux from nests seems to decrease from the 21 of January to third of June (Fig 3.16). This trend happears inversely related to the data of total standing biomass and NDVI (data not shown, Grandcourt pers. Comm).

In general the relative activity of the different nests was always similar in the different sampling dates, the Nasotitermes having always the highest activity. More variable are the fluxes at 1 and 3 meters from the nest, althought much lower (100 folds) or even negative.

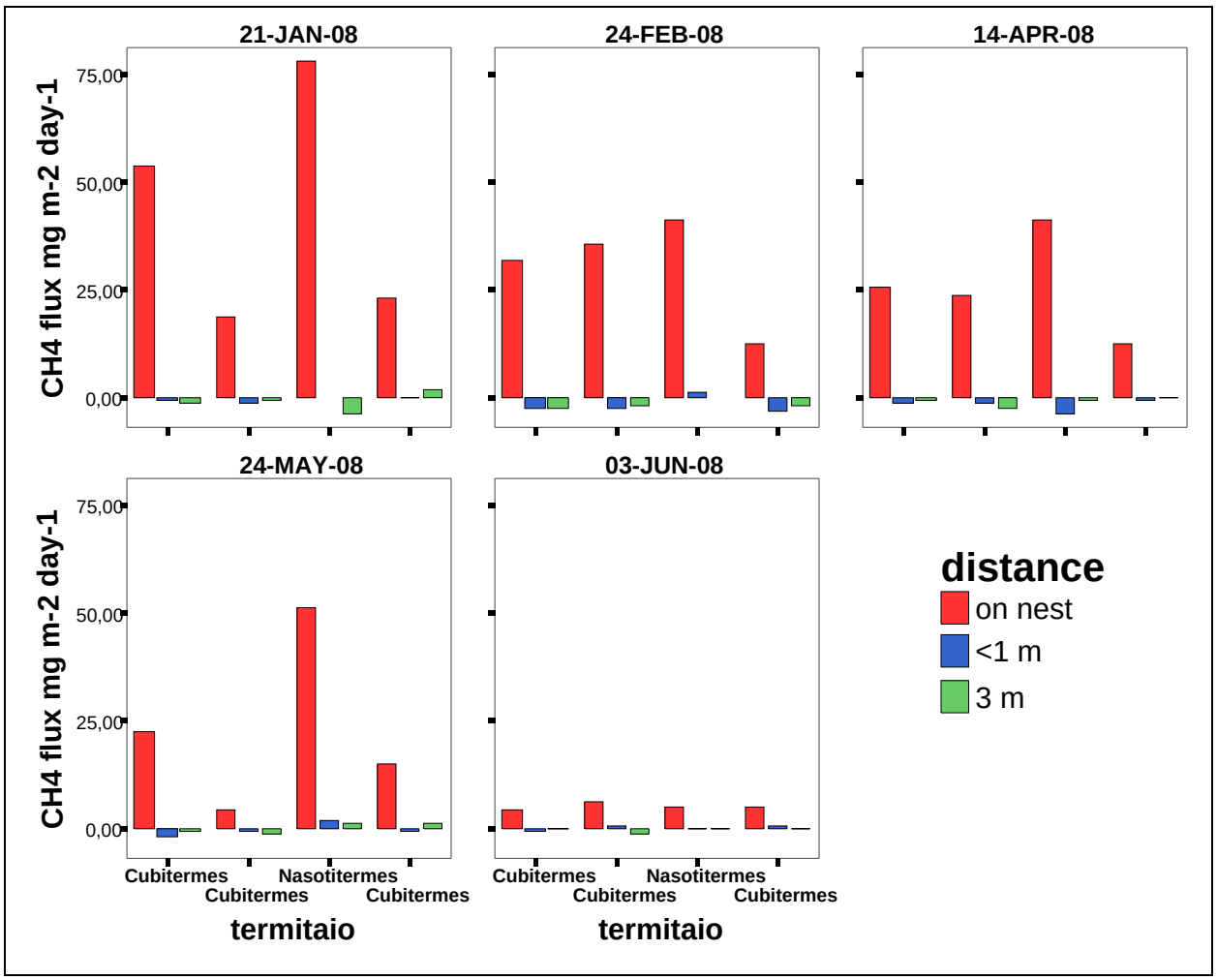


Figure 3.15 - Methane fluxes analyzed in each field campaign at different distances.

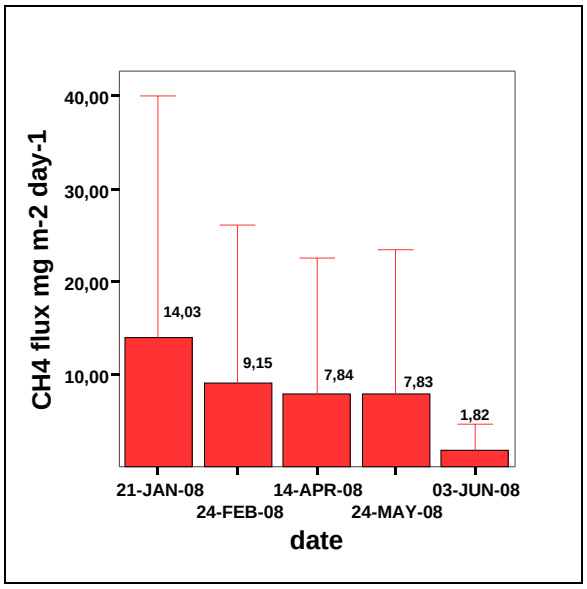


Figure 3.16 - Spatial average of methane fluxes according to the different sampling dates

From the flux data measured at each date and nest volume the following emissions factors of CH_4 per cm^3 of nest per day were derived as showed in Table 3.5.

Table 3.5 - Methane emitted per each cm^3 of termite mound.

DATE	Cubitermes 1 mg CH_4 $cm^{-3}day^{-1}$	Cubitermes 2 mg CH_4 $cm^{-3}day^{-1}$	Nasotitermes mg CH_4 $cm^{-3}day^{-1}$	Cubitermes 3 mg CH_4 $cm^{-3}day^{-1}$
21/01/2008	3.17E-04	9.27E-05	6.97E-04	7.01E-05
24/02/2008	1.89E-04	1.78E-04	3.70E-04	3.82E-05
14/04/2008	1.53E-04	1.18E-04	3.69E-04	3.78E-05
24/05/2008	1.34E-04	2.26E-05	4.61E-04	4.52E-05
03/07/2008	2.77E-05	3.28E-05	4.74E-05	1.58E-05

De Grandcourt et al. (pers com) measured a density of termite mounds within the studied savanna of 1200 nests ha^{-1}. Using this value and data from Table 5 the CH_4 production due to termites at site ranged from 0.11 Kg CH_4 ha^{-1} y^{-1} to 4.84 CH_4 Kg ha^{-1} y^{-1} these values were calculated considering the maximum and minimum emissions per unit of nest volume.

3.4.4 Nitrous oxide fluxes

Fluxes of N_2O were analysed in the first campaign by both fotoacustic and gas cromatographic technique, while in the second campaign only by the second technique. Fluxes resulted very low (Fig 3.17).

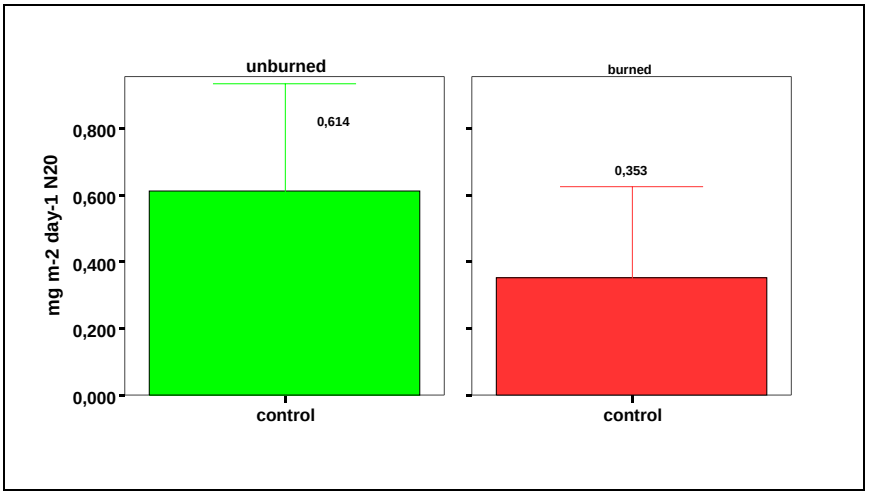


Figure 3.17 - N_2O fluxes analyzed during the first field campaign in the unburned and burned controls by photoacustic technique.

They range from zero to one event of 1.37 mg N_2O m^{-2} day^{-1} and no real difference could be evidence between burned and unburned plots (Table 3.6). The GC technique resulted not sufficiently sensitive to check these low fluxes over the same time interval of chamber closure. However given the very high temperatures at site, a longer closure time was not considered acceptable.

Table 3.6 - Descriptive statistic of N_2O fluxes analyzed by gas photoacoustic technique.

Descriptives

FLUX_N2O

	N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
unburned	12	,614	,319	,092	,251	1,368
burned	11	,353	,272	,082	,000	,729
Total	23	,489	,319	,067	,000	1,368

Addition of water only slightly increased the N_2O emissions which anyway were never higher than 2.5 mg N_2O m^{-2} day^{-1}, even when soil was quite wet (Fig 3.18). Laboratory data presented in the next paragraph show that at the soil water content measured in the control situation fluxes were to be expected to be very low.

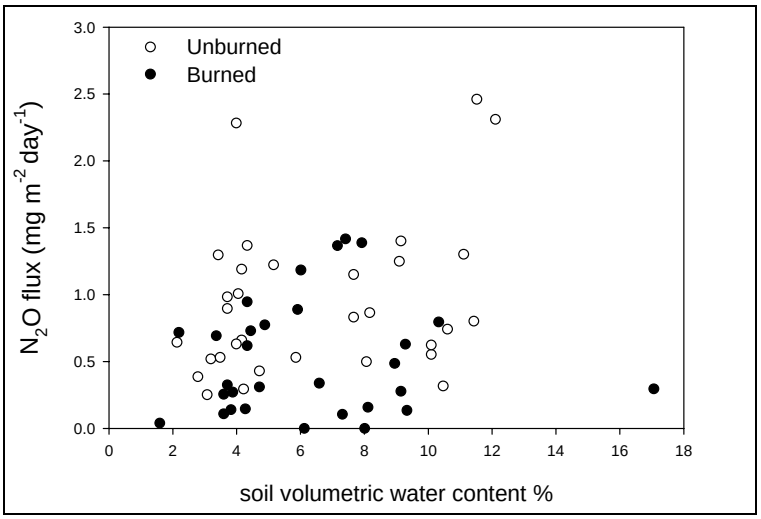


Figure 3.18 - Relationship between N_2O emissions measured by PAS analyser and soil water content in the control and burned plots, untreated and irrigated.

3.5.5 Soil respiration

Data of soil respiration were obtained by GC technique. This is not the commonly used technique as the online fast responding IR analyser are those usually used for this purpose. There is evidence that fluxes of CO_2 measured using static chambers and GC significantly underestimate the CO_2 emissions. So the present data were only used to define a treatment trend rather than an absolute measure of respiration in the field and to compare these data with laboratory rates measured also by GC. GC data were also compared with those obtained by the PAS technique (Fig 3.19a).

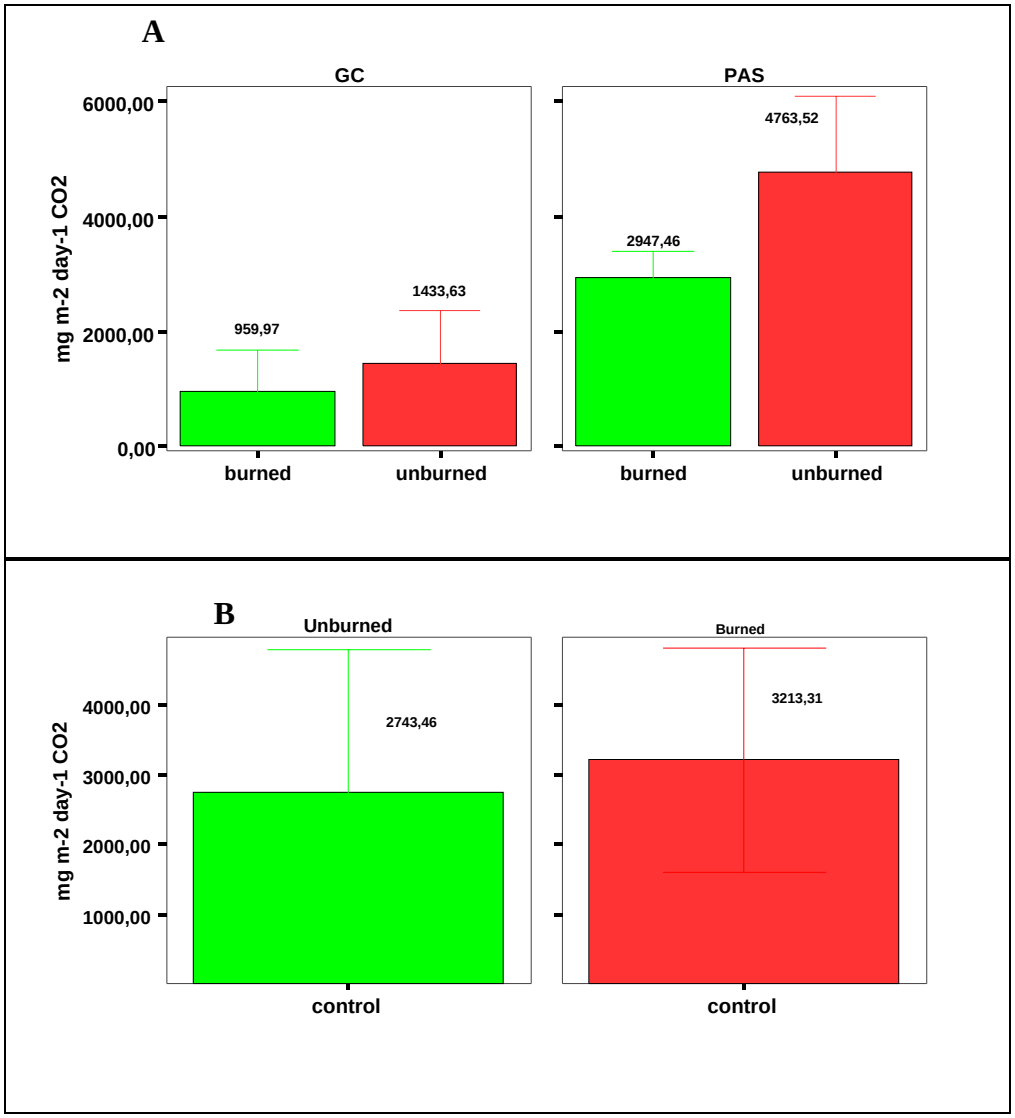


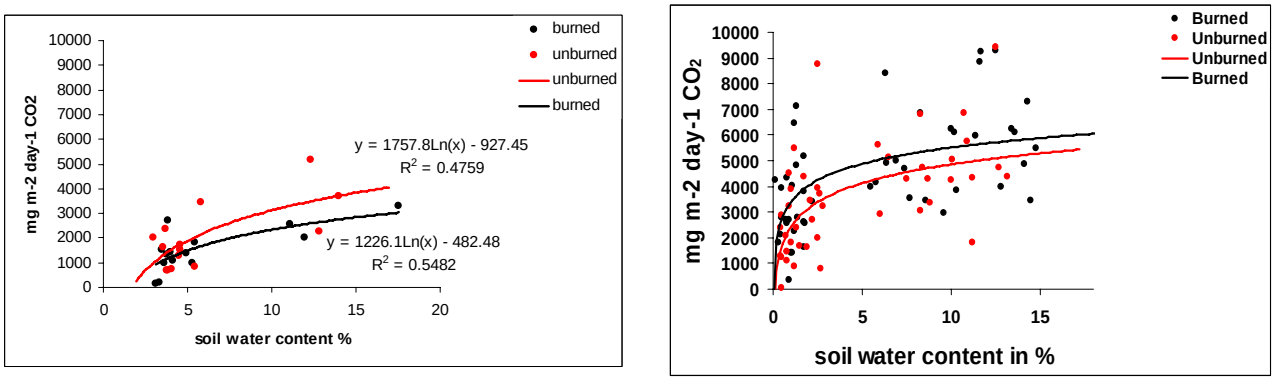
Figure 3.19 - A) CO_2 fluxes analyzed during the first field campaign in the unburned and burned controls by GC and PAS techniques; B) and in the second field campaign only by GC technique.

Despite the different absolute values data indicate that fire induce on average a higher emission of CO_2 from soil 1 month after fire (Fig 3.19A) although the result were statistically significant only

when PAS was used. The difference seems to desappear in the second campaign at the end of the growing season when however soil respiration is higher in both unburned and burned plots, probably as a result of grass growth which adds autotrophic respiration and stimulates heterotrophic repiration (Comparison of GC data from Fig 3.19 A and B graphs).

The Pearson correlation coefficient between soil water content or temperatures and CO2 was calculated for the two treatments including data from control and irrigated plots. In both unburned and burned areas there was a statistically significant positive correlation between soil water content and CO_2 fluxes, whereas no correlation was found with temperature mainly because this parameter varied very little between campaigns and treatments.

In Figure 3.20 data of soil water contents are plotted against data of CO_2 fluxes.



a **b**

Figure 3.20 - Fluxes of soil respiration plotted versus soil water content for burned and unburned plots following the simulated rain in the first (A) and second (B) field campaigns measured by GC.

A steeper increase of soil respiration in function of increasing soil water content can be noted in the second campaign, when the grass is present. In the first campaign burned plots seems to respond faster to water addition, whereas in the second campaign there is not real difference and data are much more scattered so that relation is not statistically significant. In figure 3.21 we have replotted the data of soil respiration measured by our colleague in Congo using a IRGA system. It can be seen a good agreement of trends, bringing to the same basic conclusions although the range of fluxes measured is higher with this second technique.

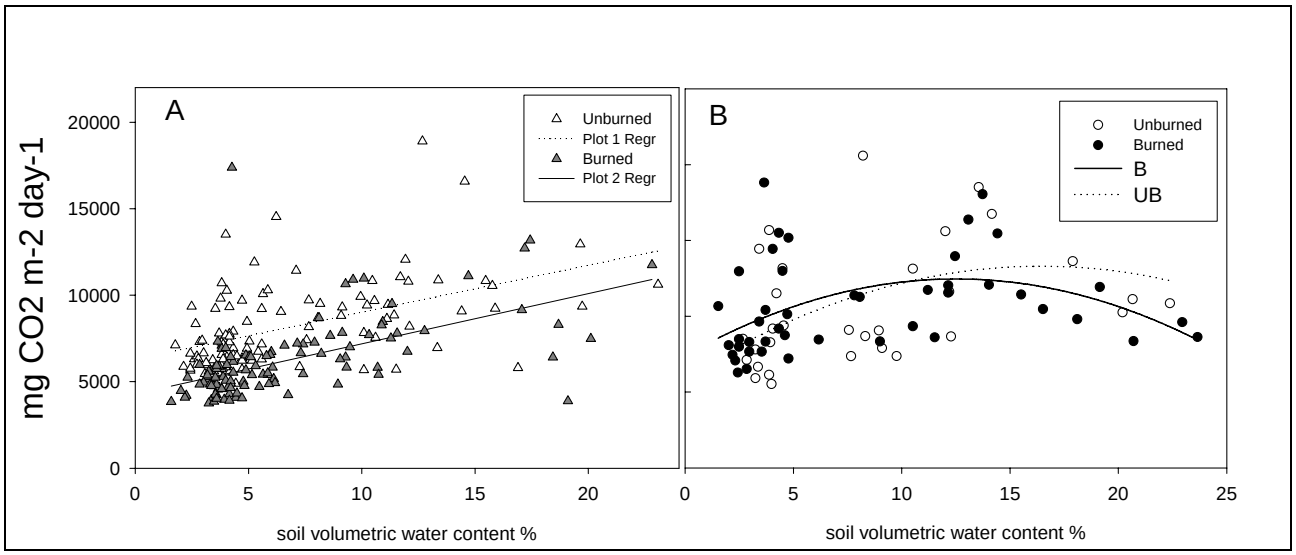


Figure 3.21 Fluxes of soil respiration measured by IRGA technique in the first (A) and second (B) campaigns by the French group (raw data from De Grandcourt et al. unpublished).

3.6 Laboratory experiment: determination of N_2O and CO_2 emissions and related factors from water and temperature in soil from burned and unburned sites

3.6.1 Gas fluxes

N_2O emissions were overall quite low. The highest fluxes were generally measured the first day after water addition, although this was not true in all cases. In general at 37°C N_2O emission decreased significantly after one day (Fig 3.22).

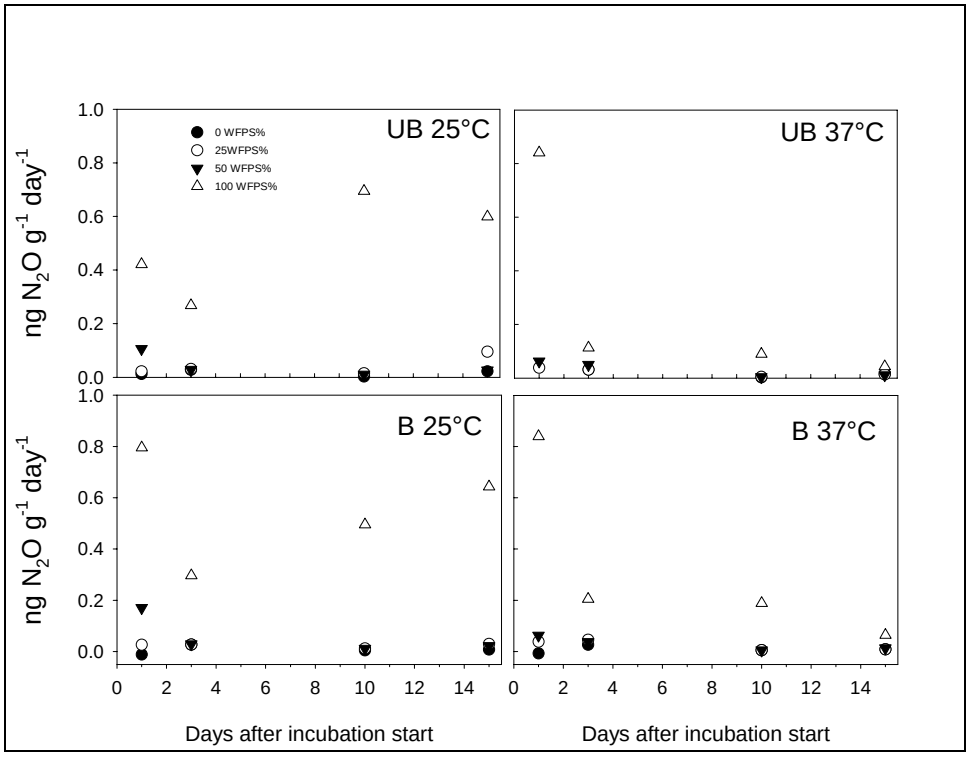


Figure 3.22- N_2O emissions measured along 14 days after wetting the soil.

To compared the treatments we then used only data from the 1st day when fluxes were generally higher. Laboratory data showed that only above 50% of water filled pore space (about 38% volumetric soil water content) N_2O emissions increased significantly ($P<0.05$, two way ANOVA). The emissions were significantly higher in the burned soil but this difference was clear only at 100% WFPS, when fluxes were higher (figure 3.23). Fluxes measured at 25°c and 37°C were instead not significantly different.

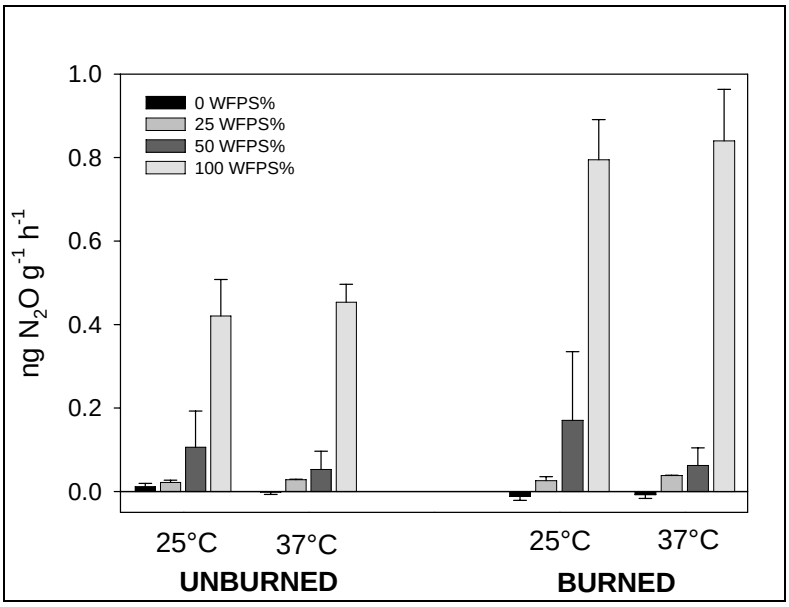


Figure 3.23 - N_2O emission in both the environmental conditions according to the temperature and WFPS

In Figure 3.24 are represented the variations of N_2O emission in fucntion of the WFPS %. The relationship is exponential in all cases ($y = y_0 + a \cdot e^{b \cdot x}$) and while there is no difference for the same treatment at different temperatures, there is a significant difference between treatments (burned and unburned) indipendently from the temperature. Equation parameters are reported in table 3.7.

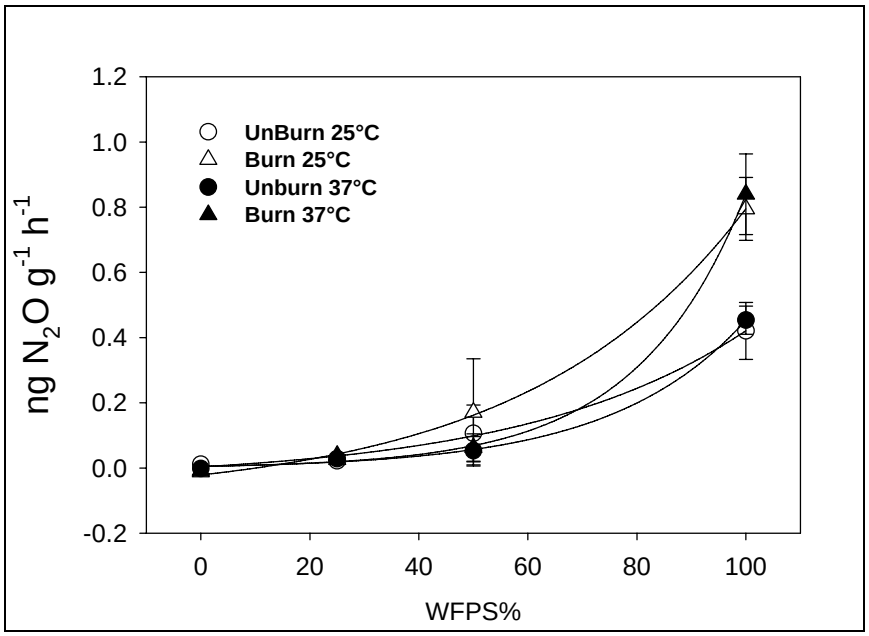


Figure 3.24 - N_2O data plotted versus WFPS % .

Table 3.7 - Parameters obtained for the equations represented in Fig 3.24 ($y = y_0 + a \cdot e^{b \cdot x}$).

	Y0	a	b	R^2
Unburned 25°C	-0.0361	0.0396	0.0245	0.9982
Burned 25°C	-0.0959	0.0746	0.0248	0.9993
Unburned 37°C	-0.0033	0.0079	0.0406	0.9993
Burned 37°C	-0.0005	0.0057	0.0500	0.9992

The microbial response to the experimental conditions is shown in figure 3.25-3.26 a,b. Microbial biomass (Fig 3.25) was always slightly higher at 25°C than at 37°C, whereas no real difference was showed by microbial activity measured as N mineralization and nitrification rates. The optimal values of microbial growth were reached at 50 WFPS%, as typically know from literature. The growth was also higher in the burned sites, although not in all cases. In any case the burning did not seem to have affected the microbial potential for growth. Even in the dry soil (0% WFPS) microbial biomass alive was found in significant quantities and two weeks of incubation at optimal water content only increase the biomass of 1/3. At high water contents no increase was observed compared with the control.

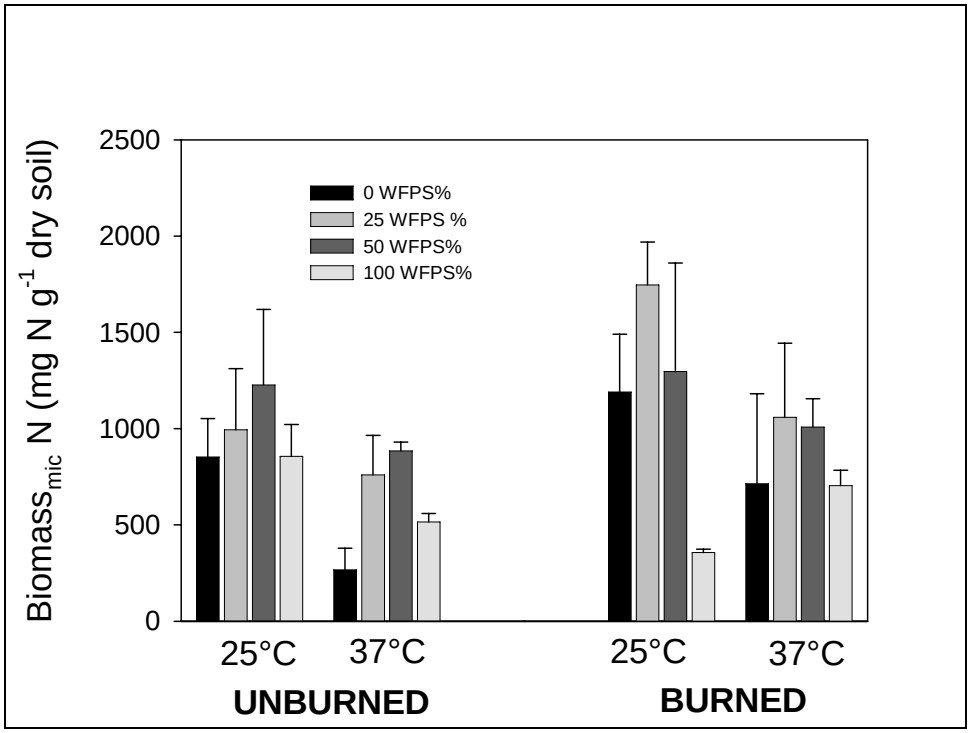
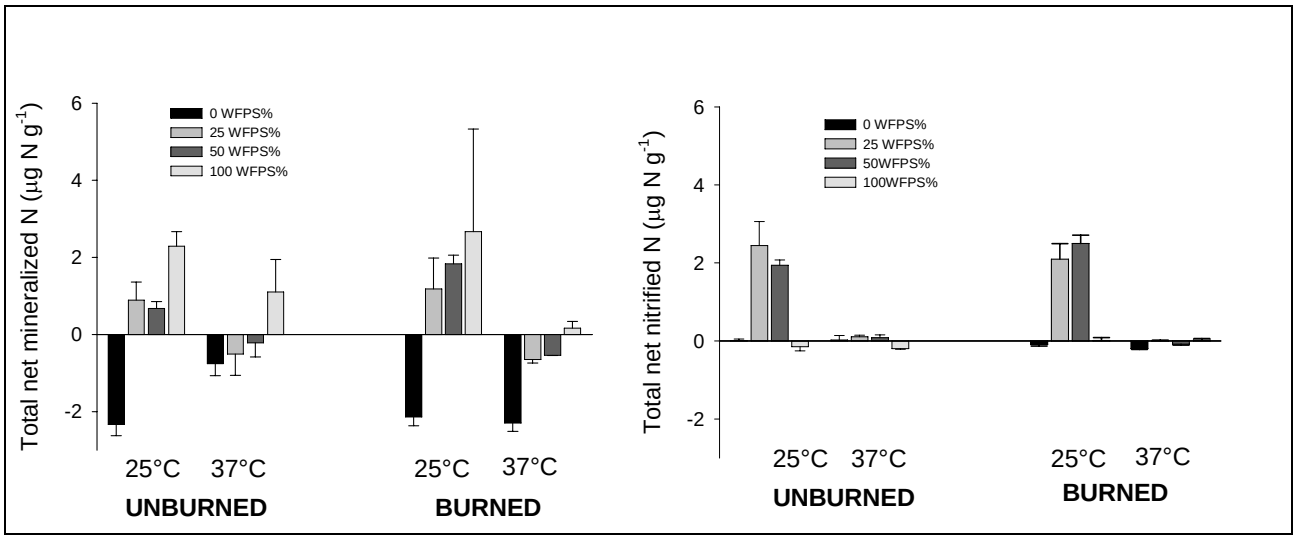


Figure 3.25 - Microbial biomass analysed at different WFPS and temperature in the unburned/burned plots.



a **b**

Figure 3.26 - Mineralization (a) and nitrification rates (b) analysed at different WFPS and temperature in the unburned/burned plots.

Mineralization showed net maximal values at 100% WFPS. At 37°C immobilization prevailed over net release of mineral N. Nitrification absent at 0% and at 100% WFPS. Also it was almost completely blocked at 37°C . Alfa-amino-N in soil was stimulated by water increase but not by temperature increase. It was slightly higher although not in all occasions in the burned site (figure 3.27).

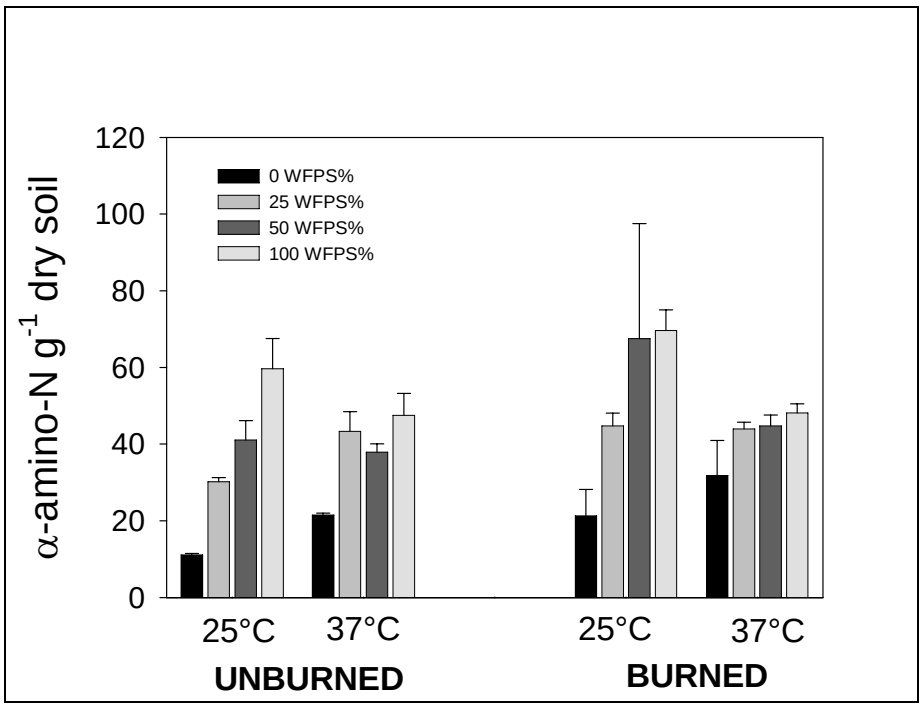


Figure 3.27 – Alfa amino N analysed at different WFPS and temperature in the unburned/burned plots.

Figure 3.28 shows modeled data of N_2O fluxes obtained using the derived equations of figure 3.24 and climatic data available for the site (volumetric water content). Basically the curves at 25°C were used because the difference of N_2O emissions between the 25 and 37 °C were not found significant. Equally the temperature was not use to produce the temporal variation of N_2O emissions. To derive N_2O emissions in the field a bulk density of 1.3 was used to pass from grams of soil to cm^3 and from volumetric water content to WFPS. An emitting depth of 10 cm was also assumed. The obtained fluxes are in the range of values recorded with GC technique and lower than PAS data. However, the soil used in the laboratory was dry and so maybe less reactive than in the field. Thus we can consider this graph as an approximation of field values

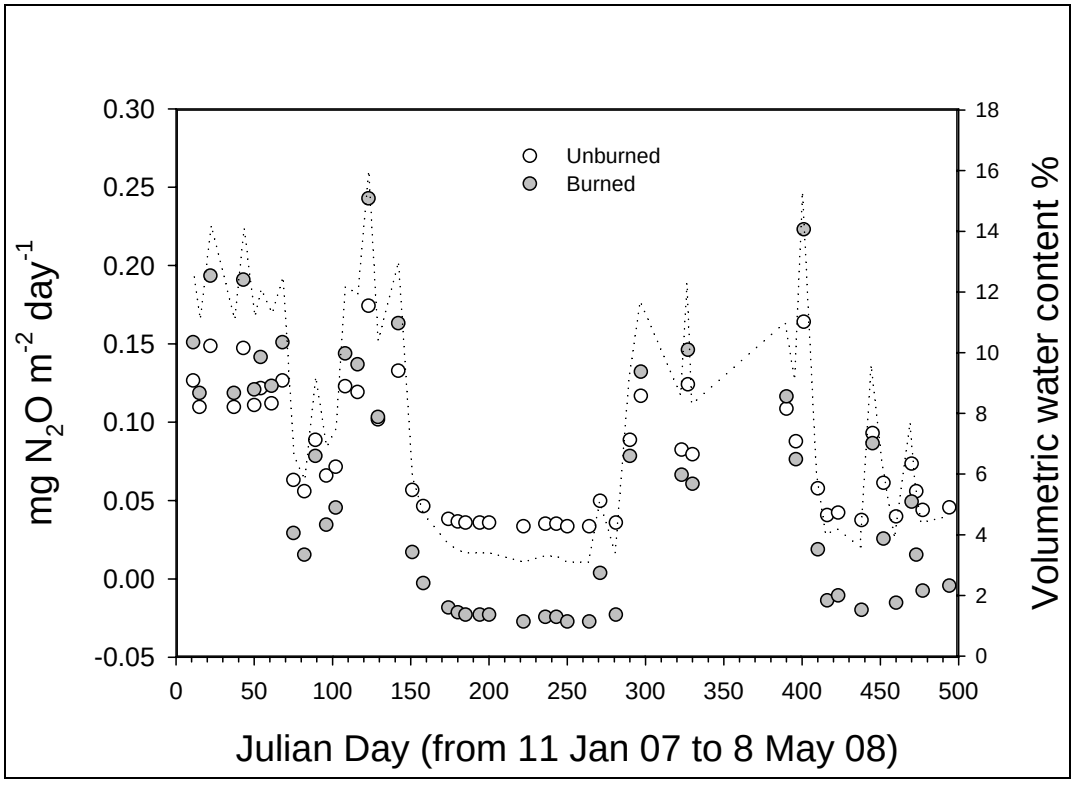


Figure 3.28 – Modeled data of N_2O fluxes in function of the time and volumetric water content.

3.7 Discussion

3.7.1 Nitrous oxide emissions

The analysed savanna ecosystem showed a very different potential for emissions of the 3 different greenhouse gases. As also found in previous studies in savannas the N_2O fluxes were extremely low. And were in the range of fluxes previously measured in savanna ecosystems worldwide (range from - 0,21 to 2.16 mg N_2O-N m^{-2} day^{-1}, median flux 0.32 mg N_2O m^{-2} day^{-1} Castaldi et al. 2004; Castaldi et al. 2006). In other studies in savanna environments N_2O fluxes had been found never to exceede 0.7 mg N_2O-N m^{-2} $day^{-1}$when soils had not been experimentally treated (fertilized, simulated rain) and higher fluxes from savannas have been only measured in isolated patches of nutrient rich savannas in nutrient poor soils (Otter and Scholes 2000) or savannas located in a valley characterized by higher soil water retention and accumulation of organic matter (Sanhueza *et al*. 1990). Higher N_2O fluxes in seasonally dry tropical environemnt are reported only for forests (Sanhueza *et al*. 1990; Verchot *et al*. 1999, Castaldi et al. 2006). A combination of environmental factors concurr to keep N_2O fluxes low: good soil dreinage, low pH and low nutrient status, as reported in the present study (table 3.2). Thus during the dry season the strong controlling factor is represented by the low soil water content which limits the possibility to develop anaerobic microsites, where N_2O production could take place (Firestone and Davidson 1989; Smith 1990). In the studied sites, the water filled pore space (WFPS %) was always below 42%, thus 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; Castaldi 2000). In fact, the rain simulation on the field produced very low emissions of N_2O in most chambers, with few exceptions. This confirmed by the laboratory incubations which demonstrated that only above 50 of WFPS% the increase of N_2O production was more evident. However, even at 100% of WFPS the lab soil did not produce high fluxes. The main reason for this can be found in the low available N and C in these soils which both in the laboratory and in the field did not allow for a significant increaes of N_2O production even at more favourable water content. This is confirmed both by field soil analyses (Table 3.2). The lab data show that even when soil is completely dry a significant portion of microbial biomass is still alive, maybe in a quiescent form, but its potential of growth over 15 days is quite low (30% increase over 15 days) probably moslty limited by C but most of all by N in soil, which this soils is very low both in the

form of total and and mineral N (Table 3.2). In fact soils tend to immobilize rather than to release soil mineral N, expecially at higher temperatures.

Thus the effect of burning on N_2O emissions can be very difficulty detected. In the field no difference can be observed between unburned and burned areas. However, Carfora et al. (pers. Comm) demonstrated that the same soils (analysed in a parallel experiment at Edinburgh) produce significant amounts of NO after wetting (up to 11.4 mg NO m^{-2} day^{-1}) and that burning triplicates the emission measured in the control. The flush, as evidence in other studies (refs) is much higher at water contents below 10%, and lasts more or less 2-4 days. Thus at the observed conditions in the field NO is the prevalent emitted gas. Even this case the measured emission if much lower than those reported for cultivated ecosystems were N is in excess (Bowma, 1990). Thus even in the case of NO, the N represent a limiting factor, despite the favourable water content. The maximum flush of N-NO (5.3 mg NO-N m^{-2} day^{-1} burned and 3 mg NO-N m^{-2} day^{-1} unburned) and N-N_2O (1.6 mg N-N_2O m^{-2} day^{-1}) predicted in the field from laboratory data might be supported by the small amount of mineral N present in the soil (compare with values in Table 3.2). On the contrary, microbes don't seem able to immediately exploit the available farction of a-amino-N present in soil, which is much in excess to the gas flush hypotesised. This might be due to protection of these compunds by fast mineralization, or to low activity of microbes in the field due to microbial biomass limitation or to adverse conditions of pH or other factors.

Graph 3.23 show that burning reduces N_2O emission potential in the soil at low water content over the control, whereas at high water contents the opposite occurs. This might be due to the fact that the higher mineral N made available by fire is only exploitable at high water content by survivors. Whereas, at low water content the adverse effect of fire on microbial activity, both direct by fire high temperature killing of microflora, and indirect, via increased irradiation, darkness, temperature, evaporation, etc, in the soil, could not be balance by the possibility to use increased N in the soil. Thus, considering the values of soil water content in the field from graph 15, burning might result in lower total annual N2O emissions (28.7 ± 0.9 mg N2O m^{-2} y^{-1} in unburned and 21.6 ± 1.8 m^{-2} y^{-1} in burned savannas).

3.7.2 Methane fluxes

The effect of burning on CH_4 fluxes was much more evident, but this was true particularly in the first campaign. In fact soil CH_4 emissions were significantly different in unburned and burned plots after 1 month form burning. Unburned soils behaved as CH_4 source whereas burned soils as sink. When we measure fluxes with chambers the flux we see is a net flux, i.e. production-consumption.

Production can derive from anaerobic microbial processes and termites, consumption is due to methanotrophs activity, world wide common in aerobic soils. Thus, what we see in the unburned condition is a prevalence of the source over the sink. In burned plots we might assumed either that the sink is increase or the source is decreased. An increase of the sink might occur if CH4 can diffuse more quickly to microbial sites of consumption. This might require a lower water content in the burned site, which however was not the case. Then with might only conclude that fire decreased the source. In this case we might expect that the source was termite activity, as at these very low water content anaerobic CH_4 production by microbial activity cannot be expected. Moreoevr if this was the case then we also should measure some significant N_2O production, which was not. Thus fire affects termites in some way. As below ground channels should not necessarily might be altered by fire, we can imagine that termites mostly leave in the nest above ground. This nest is more exposed to fire, so that fire can kill part of the population. This a lower number of termites can transit in the below ground galleries, releasing less methane per unit of ground than in unburned conditions. This released methane is normally consumed by the methanotrophs present in the soil around galleries. Thus the balance source-sink controls the net CH_4 emission/uptake.

When the soil water content increases (simulated rain) the soil very quickly shift from being a sink to a source in the unburned plots, already at 2% of soil water content, whereas in the burned soil the sink is reduced but the shift does not occur in the first month after burning, thus the termites disturbance by fire is such that soil remains a sink. Calculating the difference of average CH_4 flux between burned and unburned site in the first month we can say that at least 1 mg of CH_4 m^2 day^{-1} was due to termite activity, to which we must add an unkown quantity which is still present in the soil but balanced by the sink. Other authors have evidence that clearance of the savanna soil surface (grasses and litter) by burning also produces a significant reduction of the methane production from the soil-grass system (Poth et al., 1995; Zepp et al., 1996). An other explanation might be that burning reduces that amount of palatable and usable substrate that termites can use. However, the most abundant group at site is composed by *Cubitermes* sp. which are soil feeders. As such they feed on the upper mineral soil. The vast majority of species in this group ingest topsoil rich in organic matter (Sleaford *et al.*, 1996). They are normally distributed in the soil profile, in the organic litter layer (leaves and twigs) and/or in epigeal mounds (Bignell *et al*., 1997; Eggleton *et al*., 1995;1996;1997). Compared to litter feeding termites the soil feeders should than be less sensitive to the reduction of above ground biomass due to fire. Fires should be very strong to affect the to organic layer of the soil.

After 7 months from burning, the distinction of burned and unburned site in terms of CH_4 emissions was not anymore so strongly visible and was not statistically significant. In both cases net average

flux was negative, but this could be attribute to the lower content that favours consumption activity , as explained above. Thus, seven months might have been sufficient for termite population to recover.

A rough estimate of total contribution to CH_4 balance in the Congo savanna can be made by using for the unburned site the CH_4 vs. soil water relationship derived in figure 3.11 (y = -2.1+1.4*ln X) and using the available soil water content for the sites. This provide about 50 days of flux data equally distributed over the two seasons from which a daily average and then a total annual flux can be calculated. The result is that the site emits from the sole soil 2,2 ± 0.7 kg CH_4 ha^{-1} $year^{-1}$. To this we can add the calculated CH_4 emission from the nests (25.68 ± 19.71 mg CH_4 m^{-2} day^{-1}) which correspond to 93.7 ± 71.9 kg CH_4 ha^{-1} $year^{-1}$. Thus on the extreme end we might overall go from a production of 23.3 to 169,3 kg CH_4 ha^{-1} $year^{-1}$. Mostly to be attributed to termites activity. Burning seems to bring to zero the soil source but it is not clear the effect on nests immediately after burning so that a precise balance of CH_4 emissions after burning on a year base is more difficult.

Interestingly, Ndiaye et all. (2004) found that, compared to the reference savanna soil, the mound was a hot spot of mineral nitrogen (100-times NH_4^+ and 50-times NO_3^-). This high level of mineral nitrogen was associated with a higher density of denitrifying bacteria and increased denitrification and ammonification potentials (3 and 4 times, respectively) in the mound compartments compared with the reference soil. However, the specific activity of the denitrifying bacteria was lower in the internal wall of the mound than in the reference soil. Also, no potential nitrification was observed in the mound, demonstrating a serious perturbation of the nitrogen cycle induced by the soil-feeding termite *C. niokoloensis* in the sandy savanna soil, with, as a consequence, an enrichment in organic matter and nutrients for plants (Ndiaye et all. 2004). This enrichment in mineral nitrogen could be particularly important in the savanna ecosystems where nitrogen is considered to be a limiting factor for plant productivity (Medina, 1996). This might lead to think that mounds might be also a source of N_2O. However, the lack of potential nitrification and low specific activity of the denitrifying bacteria on the mound suggest that the this mineral N cannot exploited. In fact, we also measured N_2O messions from the mound together with N_2O, but these were always zero.

3.7.3 Soil Respiration

Burning induced an increase in CO_2 emissions compared to unburned plots only one month after fire. This was true for control plots, but when water was added the unburned soil respired more. The difference however was not very much pronounced. In the second campaign the grass had regrown in both treatments and soil respiration was higher, as expected and not different in the two

treatments. More frequent data sampling of soil respiration from our colleagues in Congo indicate that a significant flush of CO_2 accompanies water addition (Birch, 1961) but decrements faster than water soil content in time. The peak seems equal in the burned and unburned soil. Data on burning effect on soil respiration in tropical savannas are quite controversial. Poth et al. (1995) found a higher soil emission of CO_2 in burned than unburned plots in Brazilian tropical savannah after wetting soils. Conversely, in situ soil respiration was not different between burned and unburned plots after wetting in savannahs in Nigeria, Venezuela and South Africa (Adedeji, 1983; Hao et al., 1988; Zepp et al., 1996). Zepp et al. (1996) proposed that most of the microbial activity in savannah on well-drained soils takes place deeper in the soil than in soil layers reached by the effects of fire, while thus reduce the effect of fire on microbial activity.

Overall a more detailed study on CO_2 flux exchange at site is conducted by the INRA French group at the site using Eddy Covariance towers so to check all the CO_2 exchanges in this ecosystem.

3.8 Conclusions

In conclusion the studied savanna site seem a very low source of N_2O and a high source of CH_4, rather than a sink, as instead often found in seasonally-dry ecosystems, even in the tropics, when undisturbed (Castaldi et al., 2004; Castaldi et al. 2006). However, positive fluxes have been frequently reported in these ecosystems in the wet season (Hao *et al*. 1988; Scharffe *et al*. 1990; Sanhueza *et al*. 1994; Poth *et al*. 1995; MacDonald *et al*. 1998; Otter and Scholes 2000; Verchot et al. 2000, Castaldi et al., 2004; Castaldi et al. 2006). Fire stimulate NOx and to a much lesser extent N_2O production being the soil limited by water and substrate. On the contrary, for a transient period fire decrease the source of CH_4 in the site, which could reduce the CH_4 production at the site of at least 1/3. Thus the impact of fire on GHGs non CO_2 seems minimal of even toward a mitigating effect. It effect on C emissions is instead more complex. Apparently some slight reduction of respiration could be obatined in the dry season but this might be counterbalanced by C lost during biomass burning.

4 THE INFLUENCE OF PLANT COVER ON SPATIAL VARIABILITY OF GHGs IN SOUTH AFRICAN OPEN WOODLAND SAVANNAS

4.1 Premise

As outlined in Scholes and Hall (1996), South African tree savanna are those with 10 to 50 percent of cover by woody plants and a developed grass layer, wehereas woodland savannas with 50 to 100 percent of tree canopy cover and a sparse gramineous layer. A 50% of woody plants over allow for the coexistence of two different environments: under canopy and within canopy. In the sub tropical environment, which is interested by extreme conditions for microbial activity, i.e. strong aridity which limit decomposition and mineralization, poor soils (often of acid sandy types), low pH, high irradiance and evaporation, the two environments might be much more critically different than in temperate ecosystems. Trees offer higher soil shading, higher input of litter and hence of C, N and other nutrients, which also affect soil water retention, all factors which strongly affect GHGs production and soil-atmosphere exchange. Thus a high variability of GHG fluxes might be expected at site level and hence at landscape level.

Thus an experiment was set up in order to understand:

1. the magnitude of GHGs fluxes at this tropical site,
2. if there is a real significant difference of GHGs fluxes in the two types of environment such that the relative percentage of woody vs grass cover must be taken into account when scaling up fluxes,
3. if site characteristics such as the soil type might influence GHG fluxes more than the variability associated to different plant cover within each soil type, or instead the difference.

4.2 Site description

The site in South Africa is located in Skukuza within the east side of the southern Kruger National Park (31°29.813' E 25°01.184' S). It's 10 km south of the Sabie river that represents the nearest park boundary and it's at an altitude of about 365 m above sea level (Fig. 4.1). The landscape has a typical undulating form with drainage lines about 3 km apart and ridge tops about 40 m above valley floors. This leads to a catenal pattern of soils and vegetation, with sandy nutrient-poor soil (Clovelley, Hutton and Glenrosa forms are characteristic; luvisols in the FAO classification and a

typic ustalf in USDA terms) on the crests, and clay and fertile soil (Estcourt and Sterkspruit forms; solonetz in FAO, natrustalfs in USDA) on the lower parts of the slope (Schoels *et al.*, 2001).

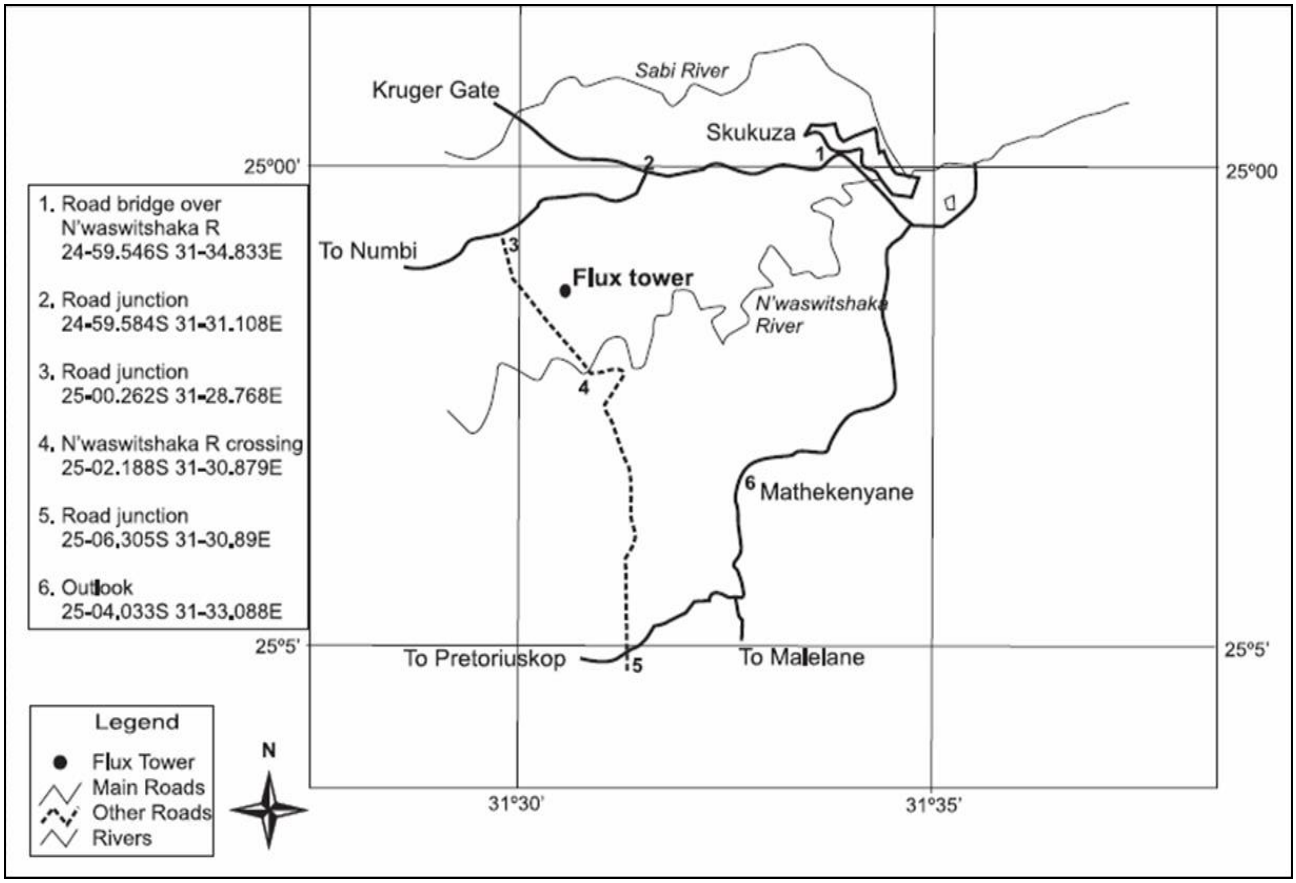


Fig. 4.1 Geographical location of the Skukuza research site at Kruger national Park.

The climate is strongly seasonal. Based on along climatic series available for the period 1960 – 1999, the mean annual temperature range is in the range 14.3-29.5 °C (Table 4.1); the coldest month is June with a mean monthly temperature of 15.1 °C and the warmest month is January with a mean monthly temperature of 26.6 °C. However, during the summer months temperature can reach values up to 40°C. Precipitation is mostly concentrated in summer. The average annual precipitation is 547.1 mm with a maximum in January (92.9 mm) and a minimum in August (6.2 mm). The climogram (Fig. 4.2) (Walter & Lieth, 1960) of the site shows the very large period of aridity concentrated from the end of March until the end of October, with maximum values in the months of June, July and August, despite the temperatures which are lower than in other months.

Table 4.1 - Climate data of Skukuza for the period 1960 to 1999 (Schoels et al., 2001 modified).

Temperature (°C)				Precipitation
mouth	min.	max.	mean	mm
Jan	20.6	32.6	26.6	92.9
Feb	20.4	32.0	26.2	87.4
Mar	19.1	31.2	25.2	73.2
Apr	15.4	29.4	22.4	33.2
May	10.0	27.9	19.0	13.5
Jun	5.6	25.9	15.8	9.5
Jul	5.7	25.9	15.8	10.4
Aug	8.7	27.3	18.0	6.2
Sep	12.8	29.3	21.1	25.7
Oct	16.0	29.8	22.9	34.9
Nov	18.1	30.6	24.4	75.9
Dec	19.7	31.9	25.8	84.3
Year	14.3	29.5	21.9	547.1

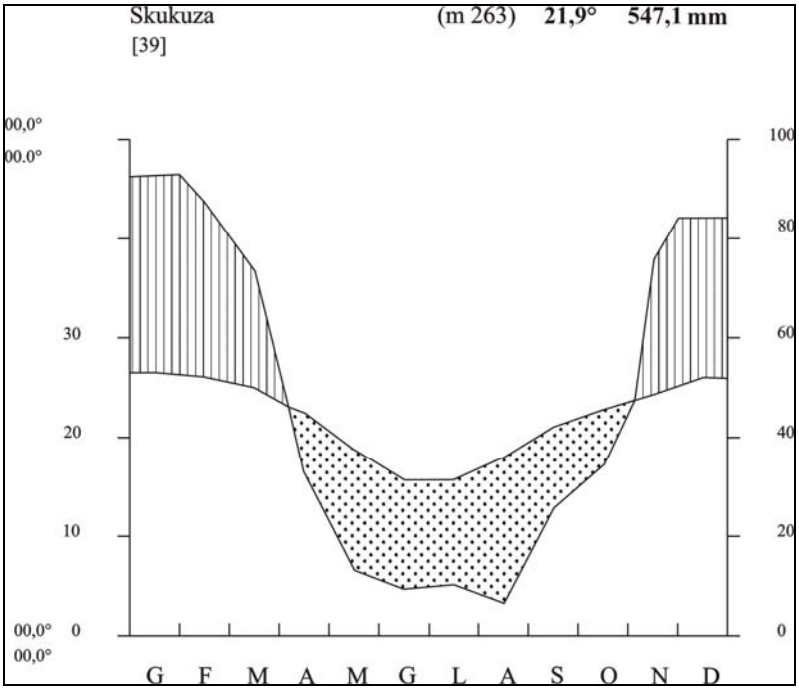


Fig. 4.2 The climogram of the site Skukuza site based on data from Table 4.1

The plant community which characterize the site is an open woodland savanna where arboreus species are organized in sparse groups of few individuals intermingled with a grassland community (Fig. 4.3). The *Combretum* and *Acacia* species (mostly *Acacia nigrescens* and *Combretum apiculatum*) dominate the tree component. The sandy uplands are dominated by *Combretum apiculatum*, with fibrous, low palatability grasses such as *Pogonarthria squarosa* and *Perotis patens* common in the herbaceous layer. This vegetation is representative of the 'broad-leafed' savannas occurring in relatively dry, infertile situations throughout southern Africa. On the other hand, the clay soil, containing more nutrients such as nitrogen and phosphorous, is dominated By *Acacia nigrescens*, *A. nilotica*, the former being more diffused at landscape levelthan the latter, and the grass layer is dominated by palatable species such as *Panicum maximum* and *Urochloa mossambicensis*. This community represents the arid, fertile 'fine-leafed' savanna type, widespread in southern Africa (Scholes., 1997).

The fauna of the park is diverse, including 147 species of mammals and 492 birds. Important herbivores include elephant (Loxodonta africana Blumenbach, 1797), buffalo (Syncerus caffer Sparrman, 1779), impala (Aepyceros melampus Lichtenstein, 1812), zebra (Equus burchelli Gray, 1824), wildebeest (Connochaetes taurinus Burchell, 1823), waterbuck (Kobus ellipsiprymnus Ogilby, 1833), kudu (Tragelaphus strepsiceros Pallas, 1766), giraffe (Giraffa camelopardalis Linnaeus, 1758), white and black rhinoceros (Ceratotherium simum Burchell, 1817 and Diceros bicornis Linnaeus, 1758) and hippopotamus (Hippopotamus amphibius Linnaeus, 1758). Herbivory, particularly by grazers, has an important impact on fires through the consumption of grass fuels. In addition, browsing (particularly by elephants) has a strong impact on subsequent tree mortality in fires. In addition, grazing (particularly by elephants) has a strong impact on savanna structure by pushing over trees.

Fig. 4.3 – Details of the savanna investigated site showing the alternation of covered areas and open ones.

4.3 Field work and data collection

At Skukuza site two different study areas were chosen according to the soil characteristics and the vegetation, which predominated in the areas, as described in the previous paragraph. The first was represented by a poor sandy soil dominated by Combretum apiculatum, while the second was located on a clay and fertile soil covered by Acacia nigrescens.

Within each site the same experimental design was set up in order to compare GHG fluxes in (Fig. 4.4, 4.5):

- open areas vs. under canopy areas
- untreated vs. watered savanna (once or twice)

In each area 3 plots per each plant cover type were selected (Under canopy, between canopy) and in each plot 3 chambers were used (Fig. 4.4) to measure GHG fluxes. One chamber was always used as control and was left untreated. A second chambers had been watered one week before the measurements, and together with the third one was watered also one day before sampling. The added water simulated a rain of about 25 mm, which represents a quite heavy rain event for the area. Hence for each treatment and each environment we had 3 replicates. Watering was done in order to simulate the effect of rain on flushes of GHG gases, as also tested in the Congo site, which are typically observed in seasonally-dry ecosystems, and which might represent a singnificant part of the overall GHG emission on a yearly base, especially for N_2O. The double watering was done with two aims: 1) to create a gradient of water contents at the moment of gas sampling; 2) to have an areas watered twice which might respond differently in terms of gaseous emissions either because the first water stimulates the microbial biomass present in the dry soil which hence responds faster to the second watering, or inversely the first burst consumes available substrates thus resulting in a lower emission at the second watering event.

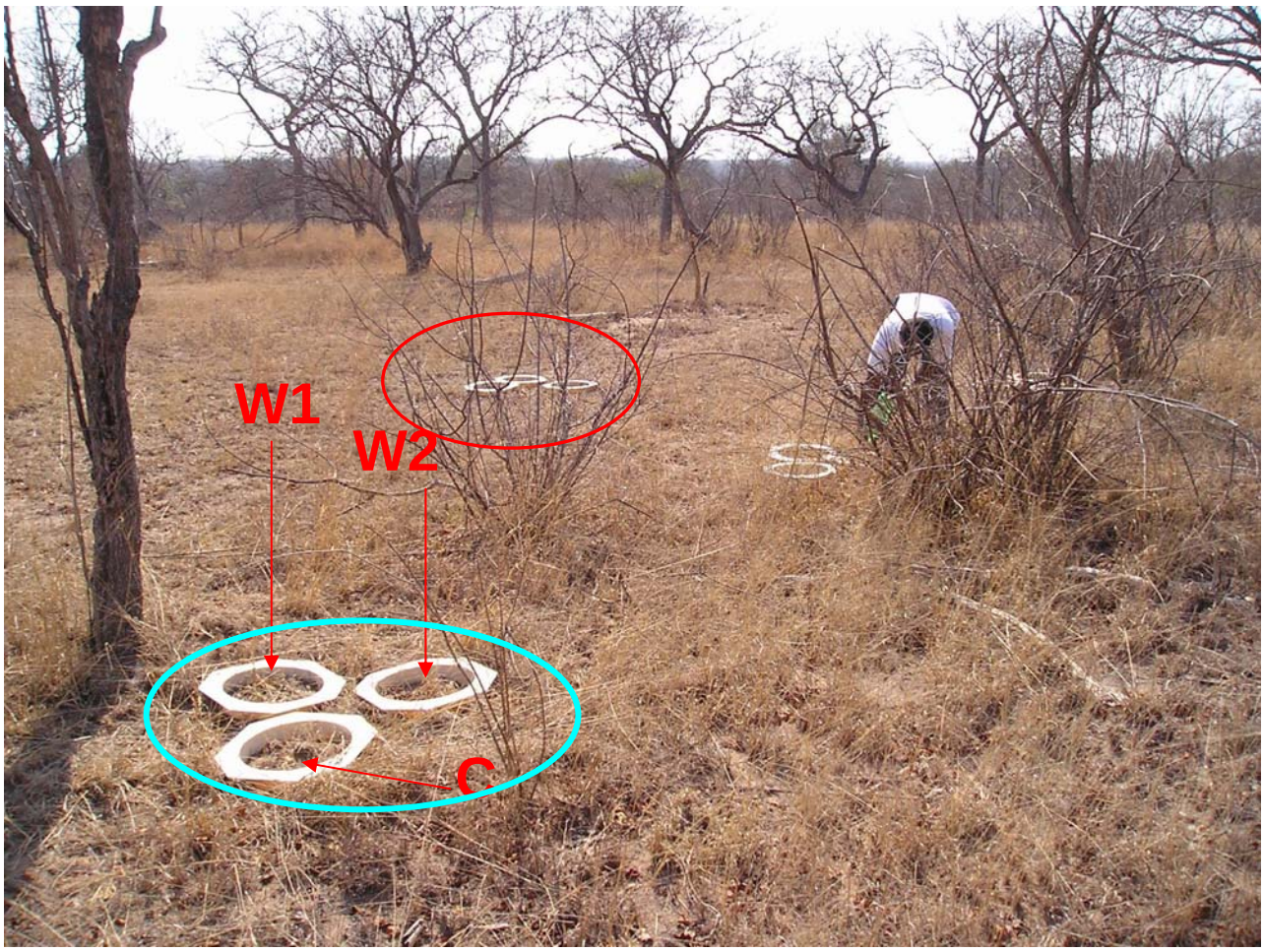


Fig. 4.4 – Example of chambers distribution under canopy (blue) and in open areas (red) in the *Combretum* site, untreated (C), watered once (W1), watered twice (W2).

Fig. 4.5 – Example of chambers distribution under canopy (blue) and in open areas (red) in the *Acacia* site.

Two campaigns were carried on at the sites, one in the plain dry season (from the end of August to September 2007) and an other at the end of the wet season (from the end of March to April 2008).

In the first campaign only N_2O and CO_2 were monitored using the photoacustic instrument which was connected via an inlet and an outlet port to the PVC chambers (Fig. 4.6).

Fig. 4.6 - The fotoacustic analyser used at Skukuza connected to the lid of the PVC chamber.

In the second campaign both fotoacustic and GC technique were used, however the high temperatures reached in this period probably induced a failure in the instrument performance so that GHGs (CO_2, N_2O and CH_4) gases could be analysed only by GC technique.

The static chamber consisted of a PVC collar (7 cm high for 31.5 cm diameter) and a chamber lid (20 cm high for 31.5 cm diameter). The collar is inserted in the soil, 2-3 days before sampling, to about 5 cm depth in a slot previously formed by a metal cutting ring and it's joined to the chamber by 4 clips made from short lengths of square-section. Gas samples for GC analysis were withdrawn at 0, 30 and 60 minutes, whereas when the PAS analyser was used the lid was kept closed for 40 minutes, sampling gas for online analysis each 2 minutes, with an integration time of 50 seconds.

Fig. 4.7 - Soil temperature and humidity were measured by probes in the chambers after gas sampling. Soil cores were taken in the same location of collars at the end of the experiment.

During each campaign soil temperature and soil water content were monitored daily form each plot and treatment. Three soil cores were sampled the last day of field campaign using a PVC soil sampling device from the top 10 cm inside the collars (Fig. 4.7). The samples were labelled and stored in plastic bags and brought back to the laboratory were they were dried and sieved for shipping to DSA-SUN for further analyses.

4.4. Results

4.4.1 Nitrous oxide emissions

Nitrous oxide (N_2O) emissions were measured only in the first campaign. Fluxes of N_2O during the dry season campaign were always zero, with the exception of 2 hotspots of 1.0 and 0.77 mg N_2O m^{-2} day^{-1} over 15 measurements in the sub-canopy environment in the Acacia site and one hotspots of 0.66 mg N_2O m^{-2} day^{-1} in the between-canopy environment in the Combretum site. Thus on average the following data can be reported for the analysed control environments over the 4 days (Table 4.2).

Table 4.2- Average N_2O fluxes measured in control plots in the different environments in the two campaigns.

Cobretum		Acacia	
Sub-canopy	Between canopy	Sub-canopy	Between canopy
mg N_2O m^{-2} day^{-1}	mg N_2O m^{-2} day^{-1}	mg N_2O m^{-2} day^{-1}	mg N_2O m^{-2} day^{-1}
0.07 ± 0.21	0.00 ± 0.00	0.12 ± 0.31	0.00 ± 0.00

When water was added to the soil to simulate a rain event N_2O fluxes increased, although only above 7% of soil water content singificant emissions might be detected. In the Cumbretum site there was no significant difference in the N_2O production in response to soil wetting in the two environments (sub canopy and between canopy), whereas in the Acacia site N_2O production was comparable to those measured in the Cumbretum, whereas in the sub-canopy area some significant increment could be detected above 20% of soil water content. This value was never reached in the sandy soil of Cumbretum. Only for the sub-canopy Acacia plots it was then possible to find a significant relationship between variation of N_2O production and soil water content (Fig 4.8) which was exponential in the range of water content measured at the site.

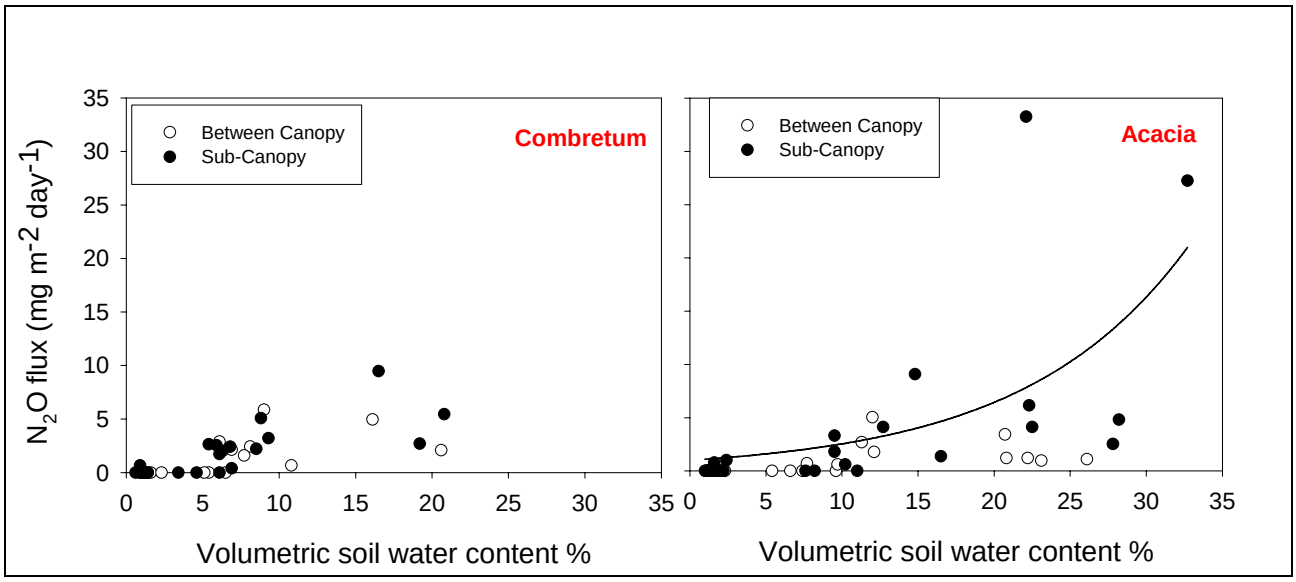


Figure 4.8 - N_2O production plotted vs. soil volumetric water content using dry season campaign data.

Considering the sole acacia sub-canopy site, which presented the highest fluxes, separating the plots which had been watered one week before and at the beginning of the experiment (twice) and the plots watered only once at the beginning (once) no significant difference can be appreciated in the response to water addition between the two events (Fig. 4.9).

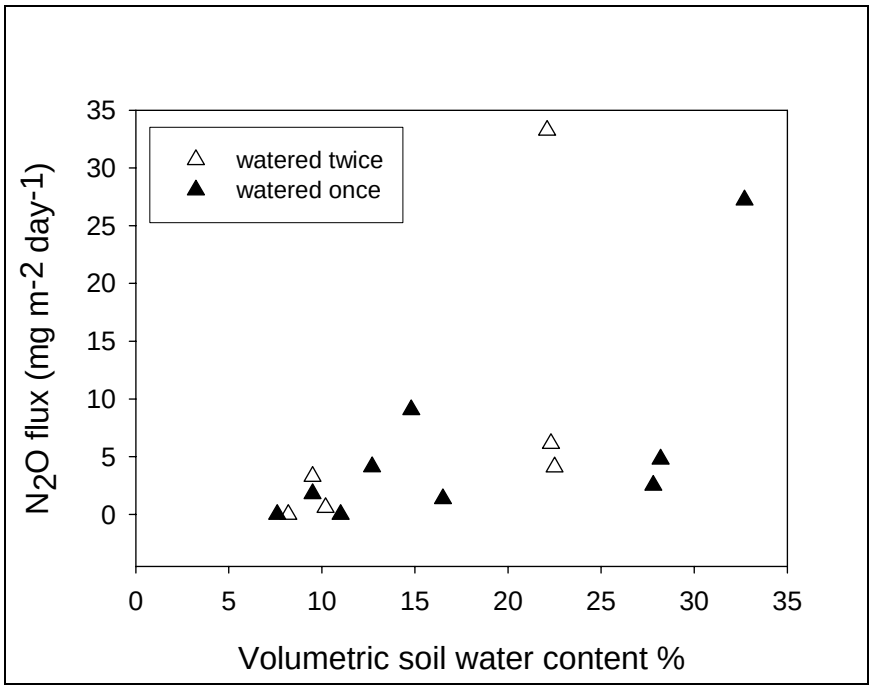


Fig. 4.9 - N_2O production plotted vs. soil volumetric water content at the sub-canopy plots in the Acacia site.

The flush of N_2O induced by water addition lasted for no more than 3 days, where it was possible to detect a significant production, both in the Acacia (Fig 4.10) and in the Combretum site (Fig. 4.11).

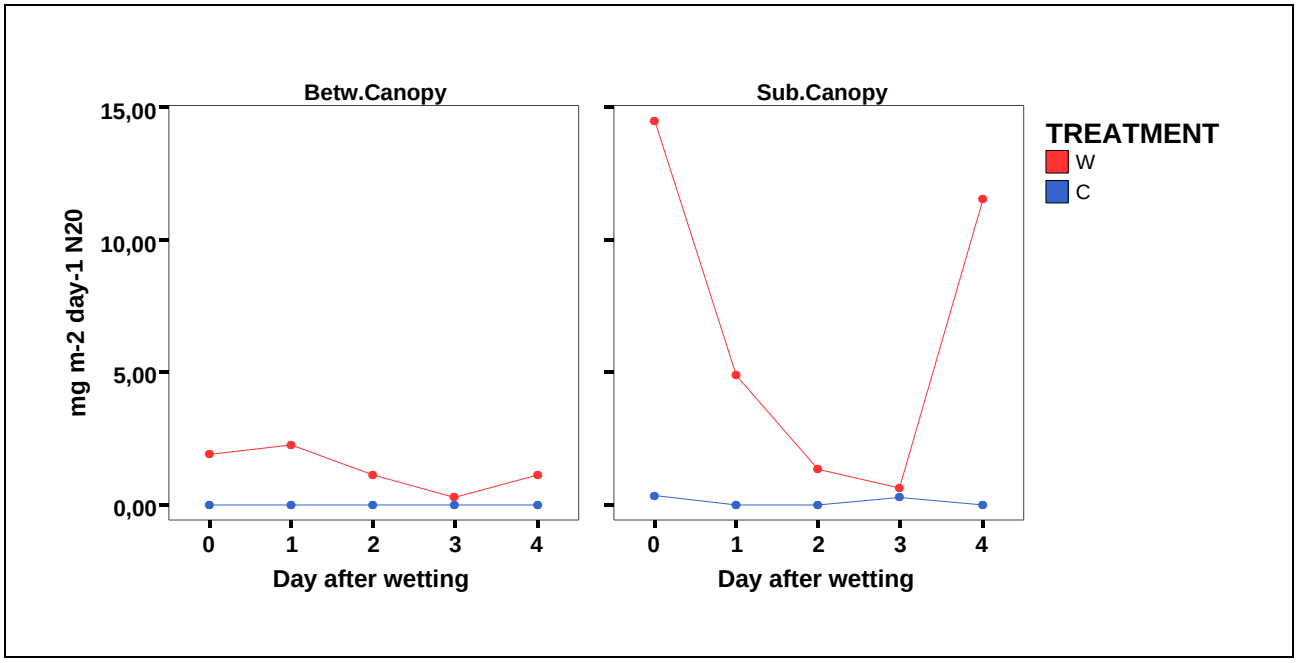


Fig. 4.10 - N_2O fluxes recorded in the open and covered area dominated by Acacia

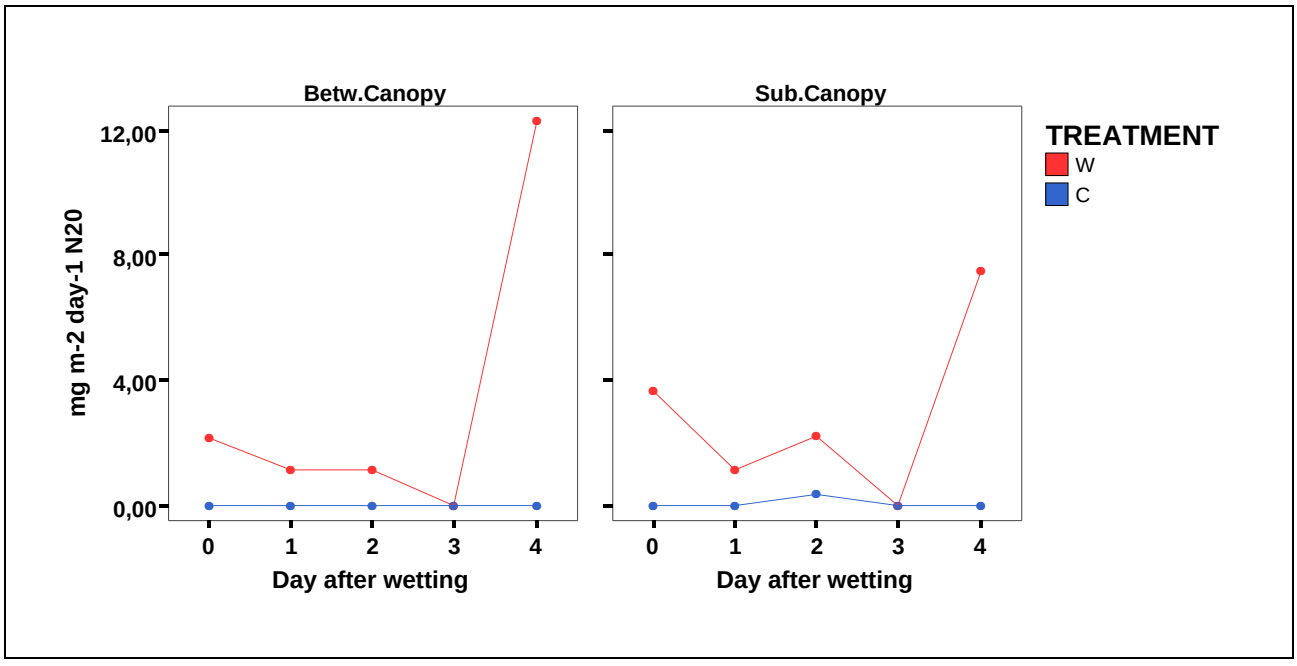


Fig 4.11 N_2O fluxes recorded in the open and covered area dominated by Combretum.

4.4.2 Methane emissions

Methane emissions were measured only during the second field campaign thanks to gas chromatograph techniques. CH_4 fluxes during the wet season campaign were generally negative showing a net methane consumption, ranging between -0.63/0.58 mg CH_4 m^{-2} day^{-1} over 12 measurements in the between-canopy environment in the Acacia site and -0.76/0.30 mg CH_4 m^{-2} day^{-1} in the sub-canopy environment in the Combretum site. In table are resumed the analysed control environments over the 4 days (Table 4.3).

Table 4.3- Average CH_4 fluxes measured in control plots in the different environments in the second campaigns.

Cobretum		Acacia	
Sub-canopy	Between canopy	Sub-canopy	Between canopy
mg CH_4 m^{-2} day^{-1}	mg CH_4 m^{-2} day^{-1}	mg CH_4 m^{-2} day^{-1}	mg CH_4 m^{-2} day^{-1}
-0.34 ± 0.38	-0.38 ± 0.32	-0.32 ± 0.38	-0.37 ± 0.16

In both the areas the fluxes were averaged over four days and divided according to the treatments and the two environments (sub canopy and between canopy) to see if the watering affect methane fluxes (4.12-4.13). In the Acacia site occurred a net consumption of methane differently from Combretum site where there was a net methane production in the plots watered at beginning of the experiment. In the sandy soil of Combretum there was no significant difference in the CH_4 fluxes in response to soil wetting in the two environments (sub canopy and between canopy), whereas in the Acacia site CH_4 consumption in the controls was comparable to those measured in the Cumbretum.

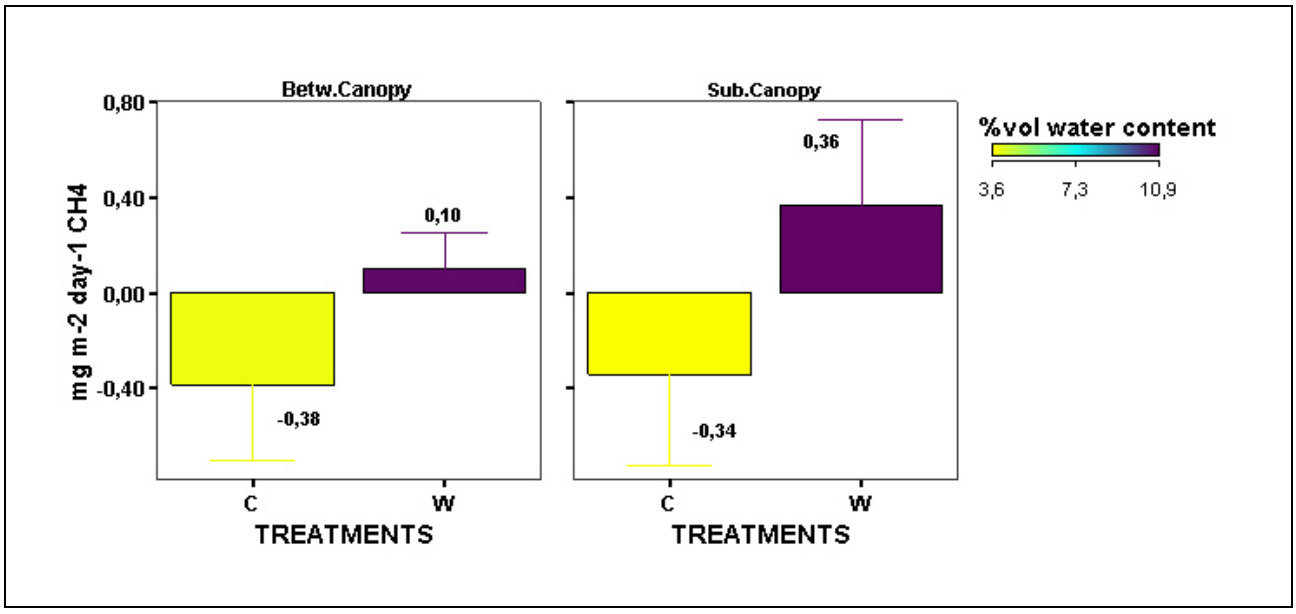


Fig 4.12 - CH_4 bar diagram relative Combretum in function of the treatments and environmental conditions (Between canopy/Sub canopy). Soil water content is represented as a colour scale.

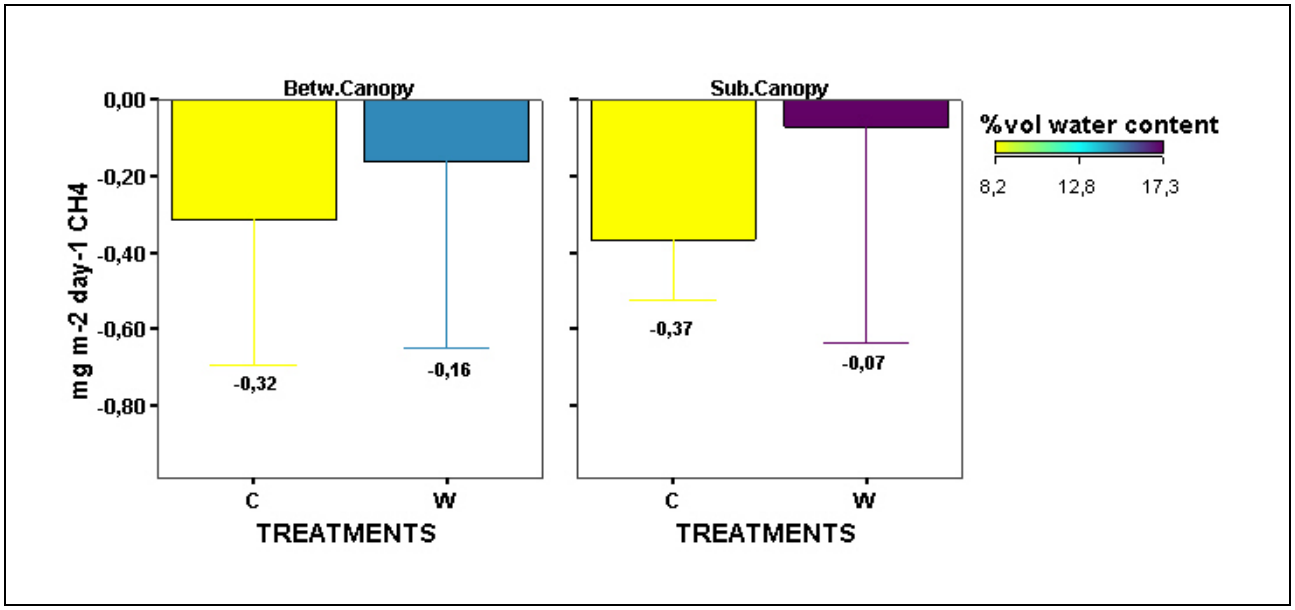


Fig 4.13 - CH_4 bar diagram relative to Acacia in function of the treatments and environmental conditions (Between canopy/Sub canopy). Soil water content is represented as a colour scale.

Considering the Combretum site, which presented a distinct consumption and production of methane, and separating the controls and the plots which had been watered at the beginning of the experiment appear a linear methane trend according to soil water content. (Figure 4.14) When water was added to the soil to simulate a rain event CH_4 fluxes increased, although only above 6% of soil water content singificant emissions might be detected.

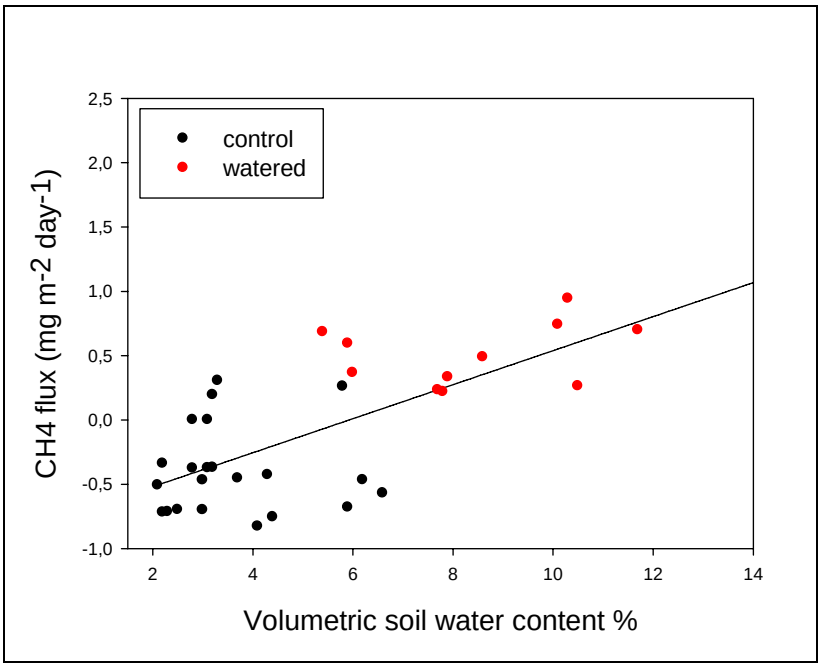


Figure 4.14 - CH_4 fluxes plotted vs. soil volumetric water content according to the treatments in the Combretum site.

In figure 4.15 is represented methane fluxes vs soil water content according to the different environmental conditions. The fluxes have a linear positive trend and the highest flux occured in the sub-canopy site whereas in the area between canopy a little methane production could be detected above 6% of soil water content.

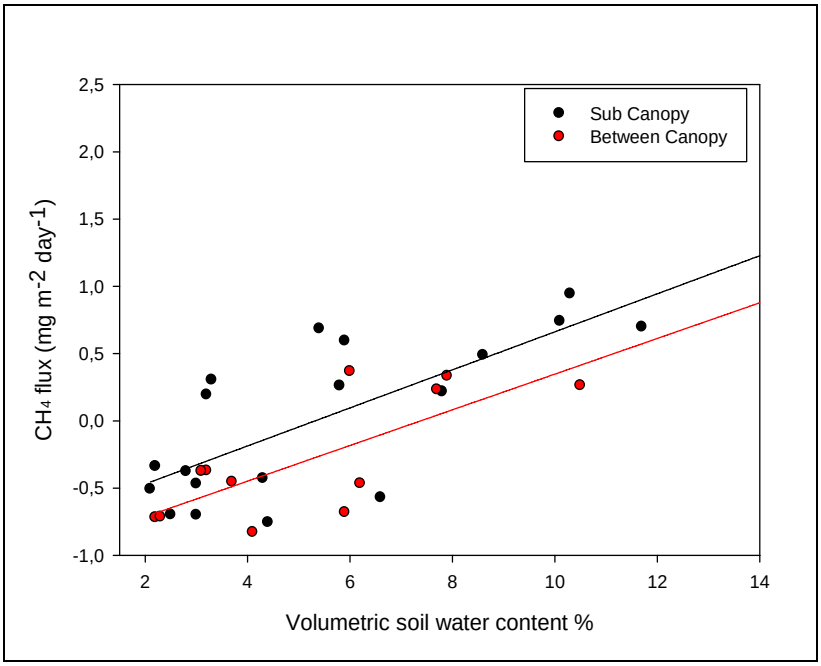


Figure 4.15 - CH_4 fluxes plotted vs. soil volumetric water content according to environmental conditions in the Combretum site.

4.4.3 Carbon dioxide emissions

CO_2 emissions were measured in both the field campaign, in particular during the first field campaign the gases was analyzed through the photoacoustic methodology whereas during the wet season, as the rainfall could compromise the results of analysis, the gases was analyzed thanks to gas chromatographic technique.

In Acacia site during the dry season campaign there were differences between the environments (Sub-canopy/Between canopy) and also between the controls and the plots watered at the beginning of the experiment where it was recorded the highest fluxes. In the area covered by Combretum there weren't differences between the covered area and the open ones while was occurred differences between the different treatments. During the second field campaign considering each site at time there were statistically differences either between the environments either between the treatments and as the previous season in the watered area occurred the highest CO_2.

As the controls reflect the real environmental conditions of the season in table 4.4 are reported an average for the analysed control over the 4 days.

Table 4.4 - Average CO_2 fluxes measured in control plots in the different environments in the two field campaigns.

	Cobretum		Acacia	
	Sub-canopy	Between canopy	Sub-canopy	Between canopy
	mg CO_2 m^{-2} day^{-1}	mg CO_2 m^{-2} day^{-1}	mg CO_2 m^{-2} day^{-1}	mg CO_2 m^{-2} day^{-1}
Dry	907.02 ± 92.49	1063.10 ± 261.21	981.42 ± 515.33	781.02 ± 152.58
Wet	2187.31± 1418.48	1184.54 ± 1716.83	1634.73 ±2762.83	879.54 ± 1620.23

During the first field campaign there weren't significant statistically differences ($P<0.05$) between the Combretum site where it was produced about 2733.81 mg CO_2 m^{-2} day^{-1} and Acacia site where CO_2 production was about 4084.72 mg CO_2 m^{-2} day^{-1} while significant differences occurred between the different environmental conditions (Sub-canopy/Between canopy), in particular during the filed campaign in the open areas was produced about 2514.15 mg CO_2 m^{-2} day^{-1} indeed in the other environment about 4429.48 mg CO_2 m^{-2} day^{-1}. In the second field campaign as the previous season there were statistically differences between the environments due to the highest fluxes recorded in the areas below a tree canopy about 5138.27 mg CO_2 m^{-2} day^{-1}(averaged value) than the open areas about 2892.83 mg CO_2 m^{-2} day^{-1} (averaged value). The Acacia site showed the highest CO_2 production 4302.23 CO_2 m^{-2} day^{-1} but it didn't differ from as produced in the Combretum site 3728.87 CO_2 m^{-2} day^{-1}.

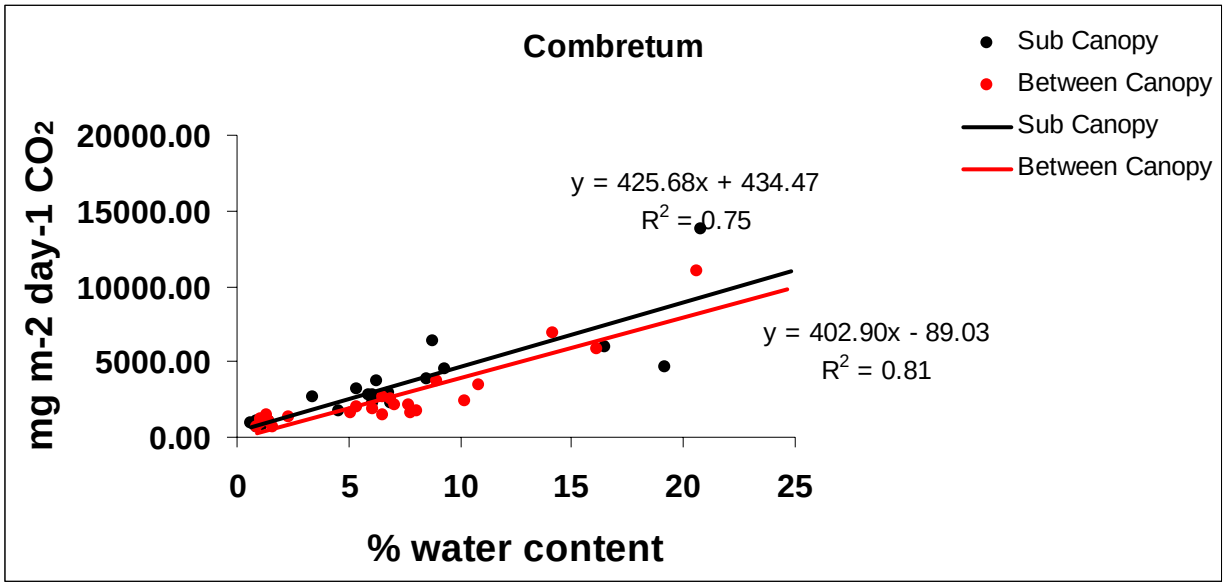


Figure 4.16 - CO_2 production plotted vs. soil volumetric water content in Combretum site during the dry season.

During the dry season when water was added to the soil to simulate a rain event CO_2 fluxes increased in both the sites; at same percentage of soil water content the fluxes were higher in the areas below a tree canopy than the open areas (Figure 4.16-4.17). In the Combretum site there was no significant difference in the CO_2 production in response to soil wetting in the two environments (sub canopy and between canopy), the fluxes seem to increase at the same rate.

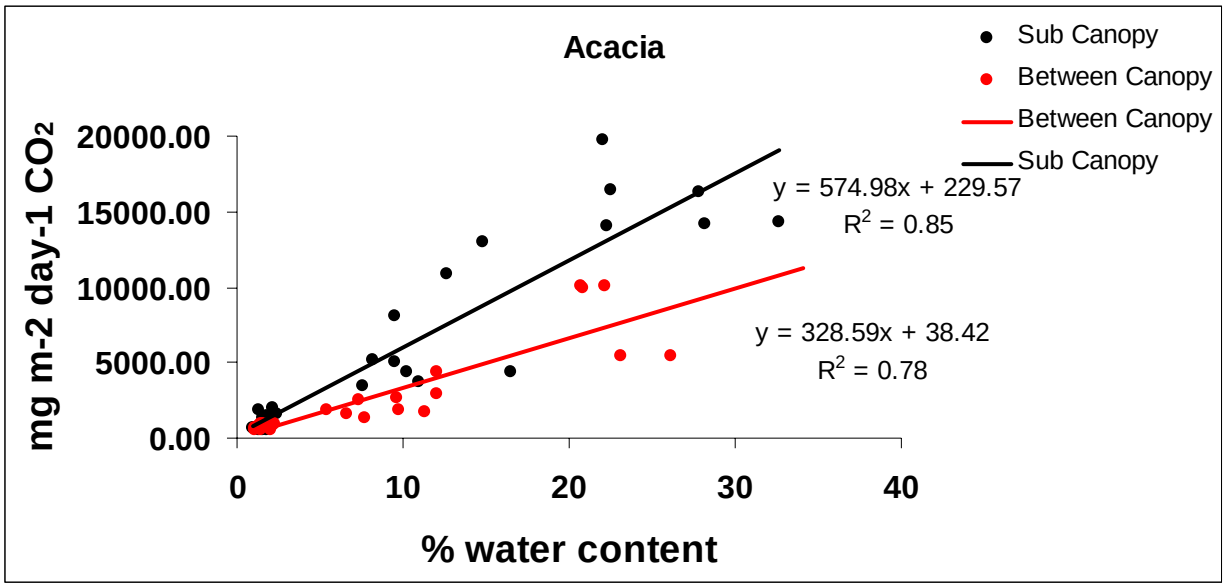


Figure 4.17 - CO_2 production plotted vs. soil volumetric water content in Acacia site during the dry season.

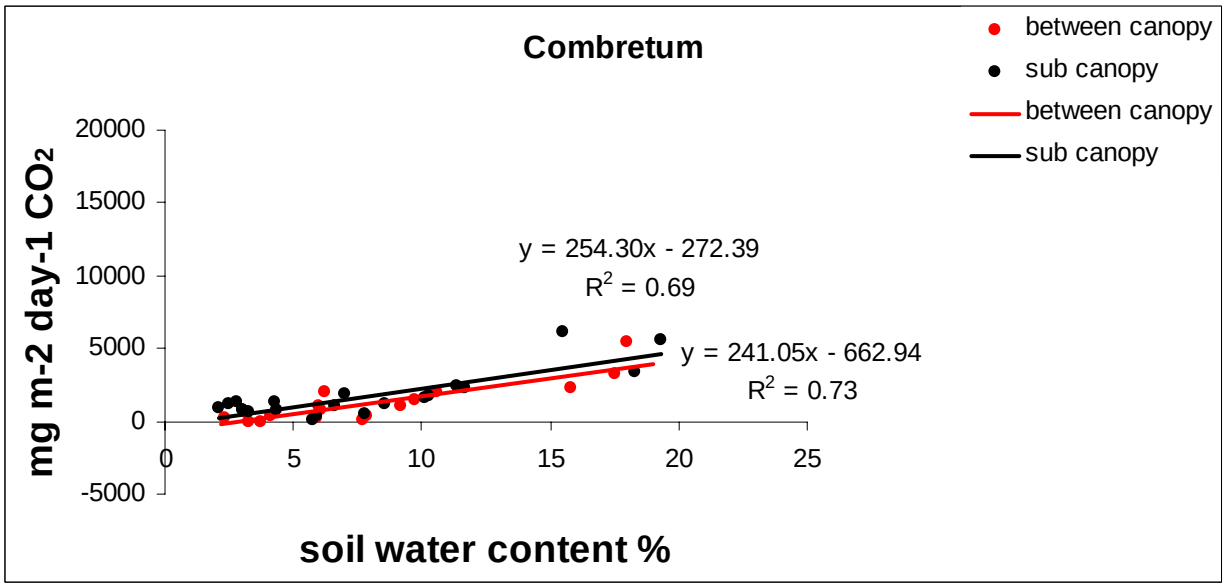


Figure 4.18 - CO_2 production plotted vs. soil volumetric water content using Combretum site in wet season campaign data.

In the second field campaign in the Combretum site CO_2 fluxes had the same linear trend than the dry season (figure 4.18) but the rate of CO_2 production is lower. In this season CO_2 fluxes were

always below 10000 mg CO_2 m^{-2} day^{-1} while during the previous season the fluxes was higher specially in the areas below a tree canopy.

As in Acacia site was observed no statistically correlation between CO_2 fluxes and soil water content there wasn't represented the scatterplot with relative regression

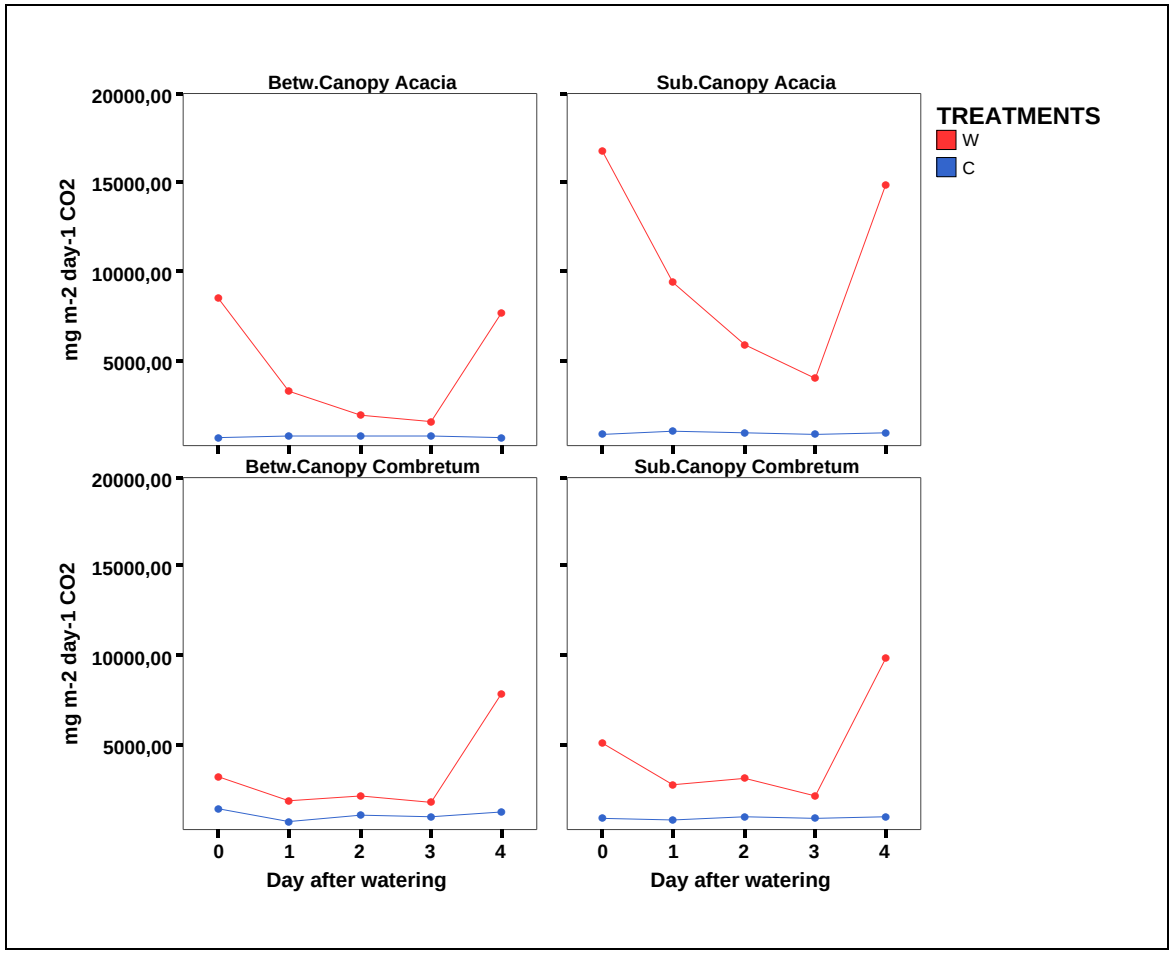


Figure 4.19 - CO_2 fluxes recorded in the open and covered areas during the dry season

In dry season either in the Acacia either in the Combretum site the flush of CO_2 induced by water addition lasted for no more than 2 days (Fig. 4.19); the highest fluxes occurred in areas below a tree canopy in both the sites. Also during the second field campaign in both the site water addition generated a flush higher in the covered areas than the open ones (figure 4.20) and as the previous season the rewetting provoked a second peak generally lower than the first. The flushes during the wet season was bigger than the first field campaign, and the highest values recorded after the first watering occurred in the covered areas, the results are resumed in table 4.5

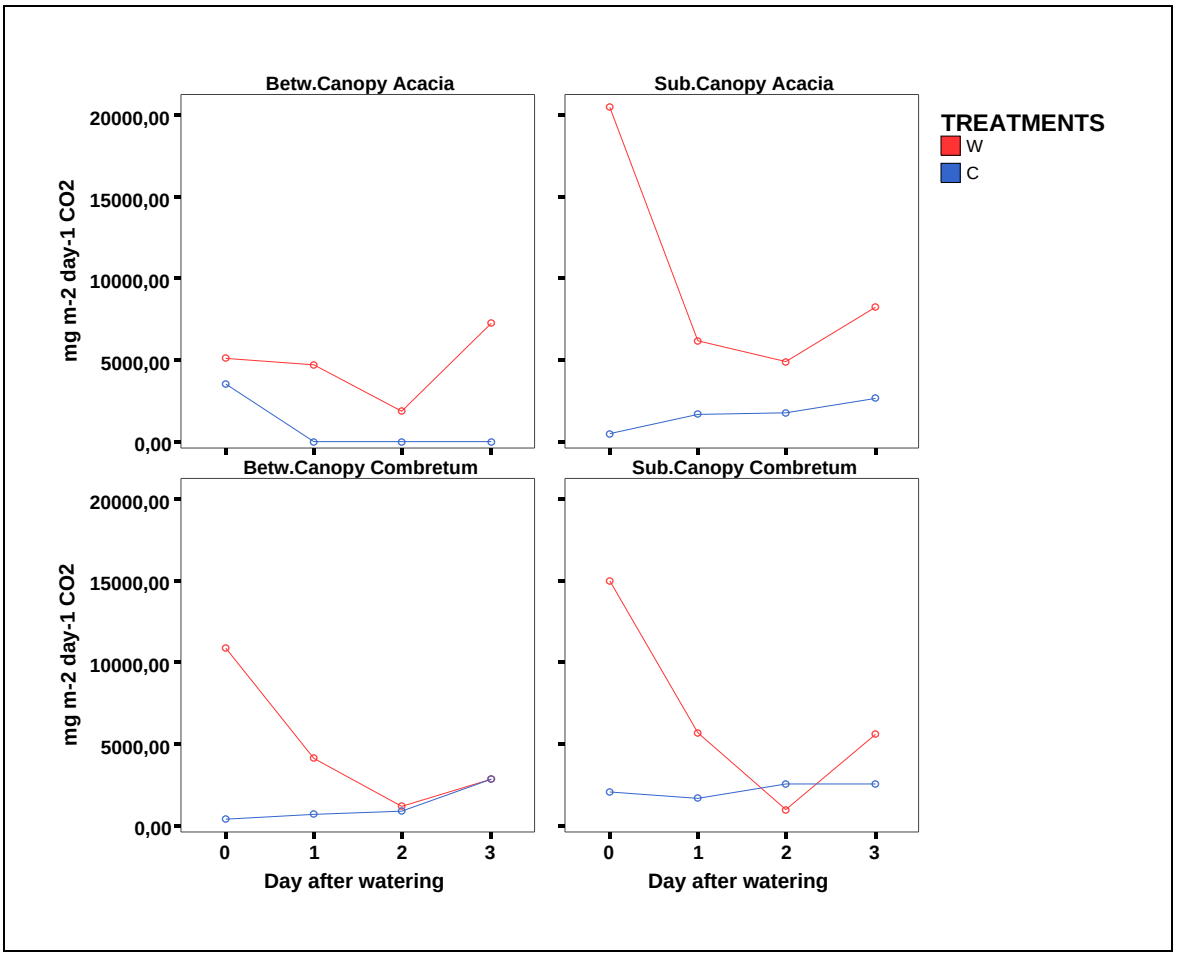


Figure 4.20 - CO_2 fluxes recorded in the open and covered areas during the wet season

Table 4.5 - CO_2 fluxes soon after the addition of water in both the seasons and the environmental conditions.

Season	Acacia mg CO_2 m^{-2} day^{-1}	Combretum mg CO_2 m^{-2} day^{-1}
Dry	16697.43	5146.17
Wet	20472.24	15023.64

4.5 Discussion

4.5.1 Nitrous oxide fluxes

The analysed savanna ecosystem showed a very low fluxes in all the areas investigated and there was no significant differences between the Acacia site and the area covered by Combretum. The absence of differences could be due to the low fluxes and to the high spatial and temporal variability.

N_2O emissions from savanna and from other agroecosystems are notoriously variable in space and time, and the uncertainty in the annual, field-scale N_2O budget is high, especially when fluxes are measured using manually operated chambers. The literature abounds with studies reporting, or even dedicated to, the small-scale spatial variability of fluxes (Ambus & Christensen, 1994), and their temporal variability has been extensively described at the diurnal (Denmead et al., 1979; Blackmer et al., 1982; Christensen, 1983; Smith et al., 1998), seasonal (Christensen, 1983; Velthof et al., 1996; Smith & Dobbie, 2001) and interannual (Velthof et al., 1996; Clayton et al., 1997) time scales.

As N_2O flushes depends on soil water content, so on onset rainfall, the main point related to rain seasonality concerns the critical moment when rain starts. In this study some plot was watered to simulate the effects of rainfall and other was left untreated to reflect the real environmental condition present in savanna.

Soon after the watering in both the areas investigated occurred a peak of N_2O bigger in areas below a tree canopy than in the open ones, the flush of N_2O induced by water addition lasted for no more than 3 days. These results coincide with as observed by Werner et al. 2007 which showed N_2O pulses lasted for about a week after the beginning on the rain, fading aways thereafter. Groffman and Tiedje (1989) reported an hysteresis effect as a result of which respiration and denitrification gradually decrease, without peaking, when soils dry. Several explanations have been given for the fact that oxygen represses microbial denitrification. Oxygen may either repress nitrate reductase (Knowles, 1982), interfere with the thermodynamics (Thauer *et al*., 1977) or inhibit nitrate uptake in cells (Hernandez and Rowe, 1987). The rate of the first rainfall would be a critical factor in dry tropics in determining the rate and the species of gaseous N emitted.

The primary effect of water on N_2O production in aerobic and partially aerobic soils is to restrict O_2 levels by reducing the air–water interfacial area within air-filled pores, thus producing an anaerobic

condition (Davidson E.A., 1992). Generally, an increase in denitrification rates following irrigation and precipitation is commonly observed (Ryden J.C & Lund L.J,1980).

The analysis of variance applied to the Acacia site showed statistically significance differences either between the environments either between the watered plot and the control.

In the areas deployed in the Sub canopy was observed a thicker litter layer and it was recognized that the higher organic matter input on the forest floor increases the content of nutrients and improves soil structure (Kay & Angers, 2000). Moreover, the shading of soil surfaces, due to the higher vegetation cover, reduces the tendency of the surface to dry. This could explain the observed higher of N_2O fluxes from areas localized below tree canopy and the differences between the environments. In the Combretum site didn't occur differences between the two environmental conditions probably because the vegetation was lower and the litter layer thinner than the area covered by Acacia, so the effect of the sun that influence the soil water content was the same in both the environments; this consideration is supported by analysis of variance that didn't show statistically differences in the Combretum site between the open areas and those ones covered by vegetation as regard soil water content.

In both the sites in the wetted plots was found the highest flux because the wetting enhances mineralisation of organic matter with resultant pulses in nitrification, and creates (local) anaerobic conditions that favour denitrification. Under these conditions, the production of N_2O may exceed the reduction of N_2O to N_2. However, a very low water content observed in the controls also limits the overall activity of soil microorganisms, reducing the amount of N cycled in the ecosystem (Bate, 1981; Castaldi & Aragosa, 2002b).

In figure 4.10-4.11 was represented the fluxes in function of the treatments and the environments considering the rewetting too; in this graphs is clearer the dependence of N_2O fluxes from water, in fact the third days soil water content decrease until to became a limiting factor to N_2O production but after the second watering occur a N_2O peak, it happens because in savanna ecosystems, mineralization and available mineral N generally increase at onset rainfall that coincide with beginning of the wet season (Bate, 1981).

In the area covered by Combretum the second N_2O peak is lower than the first one and it is in accordance with as observed by other authors who showed that the peaks in N_2O decrease with subsequent rainfall events (Letey *et al.*, 1981). In Acacia site, indeed, the second peak is lower than the first one, it could be due to clay soil characteristics that reduce the diffusion processes.

4.5.2 Methane fluxes

The areas investigated in this research appear to be a sink for methane, only in the Acacia site occurred a very little production with a maximum of 1.17 CH_4 m^{-2} day^{-1} recorded in the watered and covered area.

Soil CH_4 fluxes emissions in tropical seasonally-dry ecosystems have been analysed in a literature review by Castaldi et al. (2006) who reported that seasonally-dry ecosystems generally appeared to be a sink for methane. With an average annual daily flux in undisturbed ecosystems of - 0.48 ± 0.96 mg CH_4 m^{-2} day^{-1}, which include data from South America, Africa and Australia.

Among the few data on CH_4 fluxes in the tropical rain forest soils of the African continent Werner et al. (2007) reported for the Kakamega Rain forest, Kenya, a mean CH_4 uptake rate of 56.4 ± 796 0.8 mg C m^{-2} h^{-1} (n = 1458) and maximum CH_4 uptake rate of 146.9 mg C m^{-2} h^{-1}. From their and other studies in other tropical rain regions of the world, they concluded that being the waterlogging rarely encountered in upland soils, tropical upland forest soils predominantly act as sinks for atmospheric CH_4.

In the Acacia site methane consume appear higher in the plots left untreated than the watered ones probably because the watering in addiction to the clay soil characteristic produce anaerobic condition indispensable to methane formation; the production is higher in the area below a tree canopy where the litter layer is abundant and methanogenesis is favourite. Even if the production is higher in the covered and watered area the balance was a net methane consumption (-0.07 CH_4 m^{-2} day^{-1}).

The clay soil in Acacia site was an important factor of methane production. Air permeability of soil, which is mostly dependent on the largest conducting soil pores, has been shown to be positively correlated with CH_4 oxidation rates (Ball et al., 1997b). Therefore, a significant reduction of soil macro-porosity can be expected to reduce soil CH_4 oxidation capacity. At the same time, a lower gas diffusivity, also related to soil porosity and bulk density (Ball et al., 1997), favours the formation of anaerobic microsites where CH_4 production could occur. In well-aerated soils, the formation of such macrosites is associated with “hot spots” of microbial activity (Smith, 1990) where oxygen consumption, due to respiration, is faster than oxygen diffusion from adjacent pores. An increase of soil water content further reduces oxygen diffusion through soil pores, enhancing the development of anaerobic microsites. In fact, Verchot et al. (2000) demonstrated that a multilinear combination of soil respiration and water filled pore space explained most of the variability of CH_4 production in tropical forests and pastures.

In the area covered by Acacia the fluxes was so low that is impossible to distinguish methane production or consumption. One way ANOVA resumed in table 4.5 didn't show statistically differences between the same treatment in both the environments, also considering the same environmental condition the analysis of variance didn't verify statistically differences between the different treatments.

Table 4.5 - The number in apex show statistically significant differences P<0.05 between the different treatments considering the same raw, while the letters show statistically significant differences between the same treatment keeping unchanged the environmental condition (values in the same column).

	C	**W**
	mg m-2 day-1 CH_4	**mg m-2 day-1 CH_4**
Between Canopy	$-0.32^{1,a}\pm0.38$	$-0.16^{1,a}\pm0.49$
Sub Canopy	$-0.37^{1,a}\pm0.16$	$-0.07^{1,a}\pm0.56$

Also in the Combretum site there was a net methane consumption in the untreated plots but in the wetted areas occurred CH_4 emission higher in area below tree canopy (figure 4.12). This results weren't verified in the clay soil covered by Acacia and it seems controversial because the sandy soil are well drained so methane production generally are reduced because there aren't anaerobic condition useful to methanogenesis. In the area covered by Combretum CH_4 production probably is due to the termites, that was observed during the fields campaign, or to the sandy characteristics of soil, in fact some authors found that rates of CH_4 production increased when the aggregate size of the soil increased (Jackel *et al.*, 2001). A low CH_4 production in the clay soil is reported by Sass and Fisher (1997) that found a negative co-relationship between CH_4 emission and clay content.

Table 4.6 - The number in apex show statistically significant differences P<0.05 between the different treatments considering the same raw, while the letters show statistically significant differences between the same treatment keeping unchanged the environmental condition (values in the same column).

	C	**W**
	mg m-2 day-1 CH_4	**mg m-2 day-1 CH_4**
Between Canopy	$-0.38^{1,a}\pm0.32$	$0.10^{2,a}\pm0.15$
Sub Canopy	$-0.34^{1,a}\pm0.38$	$0.36^{2,b}\pm0.36$

In table 4.6 is resumed the analysis of variance, there were statistically differences between the treatments in both the environmental conditions, while considering the same environment between

the controls didn't exist statistically significance differences indeed between the wetted area occur statistically differences due to the higher CH_4 production in the covered area.

4.5.3 Soil Respiration

CO_2 fluxes are higher in the areas with a dense vegetation cover, in particular where it was applied the watering because a bigger amount of organic compounds and water useful to respiration is present. Overall in the Acacia soil respired more even if between the two site take in account (Acacia/Combretum) didn't occur statistically differences. During the second field campaign that coincide with the wet season there were a vegetative regrowth so a bigger thick litter layer is available and soil respiration was higher. As the first field campaign CO_2 fluxes was higher in site covered by Acacia but the differences between the sites weren't so much pronounced. Soon after the watering occurred a flush of CO_2; several factors can contribute to this CO_2 pulse. A considerable proportion of soil microorganisms die during drought (van Gestel et al., 1991), and the dead cells can be decomposed quickly during a rewetting. In addition, availability of organic substrates can increase through desorption from the soil matrix (Seneviratne and Wild, 1985) and through increased exposure of organic surfaces to microorganisms (Birch, 1959).

More studies on CO_2 fluxes are conduced by CSIR South African group using either Eddy Covariance towers either static chamber to measure CO_2 exchange in this ecosystem.

4.6 Comaprison of GHGs between different savanna ecosystems

In their natural environment, savanna aerated soils continuously change from "wet" and "dry" conditions, which in turn conditions soil and near surface temperatures. Therefore, in spite of the differences between the two periods, all the data were averaged to obtain a representative value for the natural savanna broad-leaf system. In that way, the averaged flux for the clay soil covered by Acacia shows a net consumption of methane of -0.23 mgm^{-2} day^{-1}, and a standard deviation of 0.43 mgm^{-2} day^{-1} (n=48) while occurs a net production of nitrous oxide of 2.01 mgm^{-2} day^{-1} and a standard deviation of 5.67 mgm^{-2} day^{-1} (n=59); this result is in agreement with published data summarized in Table 4.8. On the other hand, the sandy soil covered by Combretum showed a clear consumption of CH_4 with an average of -0.07 mgm^{-2} day^{-1} and a standard deviation of 0.44 mg m^{-2} day^{-1} (n=48) while occurs a net production of N_2O 1.89 mgm^{-2} day^{-1} and a standard deviation of 4.54 mgm^{-2} day^{-1} (n=49). A t-test analysis of the data indicates that the two sets (clay soil and

sandy) aren't statistically different P>0.05 (95% significance) a summary of the statistical results is given in Table 4.7.

Table 4.7 T-Test (α=0.05) to compare clay soil and sandy soil, the different number in apex show statistically significant differences P<0.05 between the di soil characteristics.

	Clay soil (Acacia)	Sandy soil (Combretum)
CH_4 (mgm^{-2} day^{-1})	-0.23±0.43[1]	-0.07±0.44[1]
N_2O (mgm^{-2} day^{-1})	2.01±5.67[1]	1.89±4.54[1]

Table 4.8 Average daily fluxes of N_2O and CH_4 from savanna ecosystems. Fluxes have been recalculated from the original units and expressed as mg N2O m^{-2} day^{-1} and mg CH4 m^{-2} day^{-1} (Castaldi *et al.*, 2006 modified)

			N_2O (mgm^{-2} day^{-1})	CH_4 (mgm^{-2} day^{-1})
References	**Country**	**Ecosystem**		
Donoso et al (1993)	Venezuela	Savanna	-0.16	
Levine et al.	South Africa	Savanna	0.00	
	South Africa	Burned savanna	0.00	
MacDonald et al. (2001)	Cameroon	Savanna fallow		-0.40
	Cameroon	Savanna young plantation		-0.47
	Cameroon	Savanna old plantation		-0.20
Otter & Scholes (2000)	South Africa	Savanna nutrient poor		-0.79
	South Africa	Savanna nutrient poor		-0.59
Poth et al. (1995)	Ghana	Cultivated savanna		-0.41
Priemé & Christensen (1999)	Ghana	Savanna+18 years fallow		-0.47
	Ghana	Savanna+25 years fallow		-0.22
	Ghana	Unburned savanna		-0.22
	Ghana	Burned savanna		-0.12
Sanhueza et al. (1994)	Venezuela	Savanna	0.27	
Sanhueza et al. (1990)	Venezuela	Savanna ridge	0.11	
	Venezuela	Savanna bottom	0.63	
Sharffe et al. (1990)	Venezuela	Savanna		0.80
Singh et al (1997)	India	Savanna		-13.8

4.7 Conclusions

The analysed savanna ecosystem showed a very low N_2O fluxes and behaved as methane sink in all the areas investigated, between the Acacia site and the area covered by Combretum didn't occur differences. The absence of differences could be due to the low fluxes and to the high spatial and temporal variability. The wide variability of the biological processes involved in the N_2O production explains why the field measures of N_2O fluxes often change markedly in the space and time. Significative N_2O fluxes generally was observed in Acacia site localized on a clay soil and in the wetted areas below a tree canopy where there was a thick litter layer. The effect of soil texture on N_2O emission likely results from physical variations in air and water proportions. Water infiltration rate and gas diffusion rates are greatly influenced by soil texture and hence N_2O emission (Aulakh *et al.*, 1995). Chaterpaul *et al*. (1980) reported greater rates of N2O emission in finely textured soils. Methane generally was consumed but little emissions occurred in the area below a tree canopy with an high soil water content. Fresh organic matter and water content in the soil stimulate microbial respiration, inducing O_2 consumption leading to anaerobic microsites where normal denitrification and methanogenesis could have taken place. As the previous gases also CO_2 fluxes was higher in the wetted area with a dense vegetative cover (Sub canopy) where a bigger amount of organic compounds useful to respiration is present.

REFERENCES

Adedeji, F.O., 1983. Effect of fire on soil microbial activity in Nigerian southern Guinea savanna. Revue d'E´ cologie et de Biologie du Sol 20, 483–492.

Ailakh M.S., Wassman R., Reenberg H., 2001. Methane transport capacity of rice plants. II. Variations among different rice cultivars and relationship with morphological characteristics. Nutrient Cycling in Agroecosystem. 58, 367-375.

Alexander M., 1977. *Introduction to soil microbiology* (2^{nd} edition). John Wiley and Sons, New York.

Ambus P., Clayton H., Smith K.A. and Cristensen J. 1992. Similar field N_2O flux from soil measured with different chamber techniques. Atm. Environm. (submitted for publication).

Ambus P, Christensen S (1994) Measurement of N_2O emission from a fertilized grassland – an analysis of spatial variability. *Journal of Geophysical Research*, 99, 16549–16555.

Anderson, I.C. & Poth, M.A. 1998. Controls on fluxes of trace gases from Brazilian Cerrado soils. *Journal of Environmental Quality*, **27**, 1117-1124.

Andersson, M.; Kjoller, A.; Struwe, S. 2002. Soil emissions of nitrous oxide from savannahs in Africa: estimating annual rates. In Editor(s): Van Ham, J. Non-CO2 Greenhouse Gases: Scientific Understanding, Control Options and Policy Aspects, Proceedings of the International Symposium, 3rd, Maastricht, Netherlands, Jan. 21-23, 2002 (2002), 139-144.

Anonymous (1972) Instructional Manual: Ammonia Electrode Model 95-10: Orion Research Inc., Cambridge, Mass. 24 pp.

Arrhenius, S. 1896. On the influence of carbonic acid in the air upon the temperature of the ground. *The Philosophical Magazine*, Series 5, volume **41**, No 251. (Reprinted in Tisglow 1992, **3**: 3-26)

Aulakh, M. S., Doran, J. W. and Mosier, A. R., *Adv. Soil Sci*. 1992, **18**, 2–57.

Aulakh, M.S., Wassmann, R., Reenberg, H., (2001): Methane emissions from rice fields quantification, mechanisms, role of management and mitigation options. − Advances in Agronomy 70, 193-260.

Baird C., 2001. *Chimica Ambientale*. Zanichelli, Bologna.

Badalucco L., Pomaré F., Grgo S., Landi L., Nannipieri P. 1994. Activity and degraddation of streptomycin and cycloheximide in soil. Biol Fertil Soils 18: 334-340.

Ball, B.C., Dobbie, K.E., Parker J.P. & Smith, K.A. 1997. The influence of gas transport and porosity on methane oxidation in soils. *Journal of Geophysical Research*, 102, 23301-23308.

Baggs EM, Rees RM, Smith KA, Vinten AJA 2000. Nitrous oxide emission from soils after incorporatingcrop residues. Soil Use and Management 16, 82-87.

Barnard R., Leadley P., Hungate B.A. 2005. Global change, nitrification, and denitrification: a review. *Global Biogeochemical Cycles*, **19**, GN1007, doi: 10.1029/2004GB002282.

Bartlett et al., 1988. Methane fluxes from the central Amazonian floodplain. *Journal of Geophysical Research* **93**: 1571-1582.

Bate, G.C. (1981) Nitrogen cycling in savanna ecosystems. Terrestrial nitrogen cycles, Vol. 33 (ed. by F.E. Clark and T. Rosswall), pp. 463–475. Ecological Bulletin, Stockholm.

Beauchamp, E. G., Trevors, J. T. and Paul, J. W., *Adv. Soil Sci.*, 1989, **10**, 113–142.

Beaumont HJE, Hommes NG, Sayavedra-Soto LA *et al*. 2002. Nitrite reductase of Nitrosomonas europea is not essential for production of gaseous nitrogen oxides and confers tolerance to nitrite. *Journal of Bacteriology*, **184**, 2557-2560.

Beaumont HJE, van Schooten B, Lens SI *et al*. 2004. *Nitrosomonas europea* expresses a nitric oxide reductase during nitrification. *Journal of Bacteriology*, **186**, 4417-4421.

Bender, M. & Conrad, R. (1995) Effect of CH4 concentrations and soil conditions on the induction of CH4 oxidation activity. Soil Biology and Biochemistry, 27, 1517–1527.

Benstead, J. & King, G.M. (2001) The effect of soil acidification on atmospheric methane uptake by a Main forest soil. FEMS Microbiology Ecology, 34, 207–212

Bertelsen, f. and Jensen, E. S., *Plant Soil*, 1992, **142**, 287-295

Birch, H.F. 1959. Further observations on humus decomposition and nitrification. Plant Soil 9:262–286.

Bignell DE, Eggleto P, Nunes L, Thomas KA (1997) Termites as mediators of carbon fluxes in tropical forest. In: *Forests and Insects* (eds Watt A, Stork NE, Hunter MD), pp 109-134. Chapman & Hall, London

Blake D.L. and F.S. Rowland, 1988. Continuing worldwide increase in tropospheric methane. *Science* **239**: 1129-1131.

Blackmer AM, Robbins SG, Bremner JM (1982) Diurnal variability in rate of emission of nitrous-oxide from soils. *Soil Science Society of America Journal*, 46, 937–942.

Bollen, G.J., 1969. The selective effect of heat treatment on the microflora of a greenhouse soil. Netherlands Journal of Plant Pathology 75, 157–163.

Bolin, B., B.R. Böös, J. Jäger and R.A. Warrick (eds), 1986. *The greenhouse effect, climatic change and ecosystems*. SCOPE 29, John Wiley and Sons, Chichester.

Bowden W.A., 1986. Gaseous nitrogen emissions from undisturbated terrestrial ecosystem: An assessment of their impacts on local and global nitrogen budgets. Biogechemistry **2**: 249-279.

Bowman A.F. (1990) Exchange of greenhouse gases between terrestrial ecosystems and the atmoshpere. pp.61-127. In Soil and the Greenhouse Effect. Bowman A.S. (eds). John Wiley and Sons Ltd. Chichester.

Bremner J.M., 1997. Sources of nitrous oxide in the soils. *Nutrient Cycling in Agroecosystems*, **49**, 7-16.

Brye K.R., Norman J.M., Bundy L.G. *et al*. 2001. Nitrogen and carbon leaching in agroecosystems and their role in denitrification potential. *Journal of Environmental quality*, **30**, 58-70.

Brumme, R. & Borken, W. 1999. Site variation in methane oxidation as affected by atmospheric deposition and type of temperate forest ecosystem. *Global Biogeochemical Cycles*, 13, 493–501.

Burton D.L., Beauchamp E.G., *Soil Sci. Soc. Am. J.*, 1994, **58**, 115-122.

Cabrera, M. L., Chiang, S. C., Merka, W. C., Pancorbo, O. C. and Thompson, O. C., *Soil Sci. Soc. Am. J*., 1994, **58**, 807–811.

Casciotti K.L., Ward B.E. 2001. Dissimilatory nitrite reductase genes from autotrophic ammonia-oxidizing bacteria. *Applied and Environmental Microbiology*, **67**, 2213-2221.

Castaldi S, KA Smith (1998a). The effect of different N substrates on biological N_2O production from forest and agricultural light-textured soils. Plant Soil 199:229-238.

Castaldi S, KA Smith (1998b). Effect of cycloheximide on N_2O and NO_3^- production in a forest and an agricultural soil. Biol Fertil Soils 27:27-34.

Castaldi, S. 2000. Responses of nitrous oxide, dinitrogen and carbon dioxide production and oxygen consumption to temperature in forest and agricultural light-textured soils determined by model experiment. *Biology and Fertility of Soils*, **32**, 67-72.

Castaldi, S. & Aragosa, D. (2002a) Factors influencing nitrification and denitrification variability in a natural and fire disturbed Mediterranean shrubland. Biology and Fertility of Soils, 36, 418–425.

Castaldi S, Ermice A. and Strumia S. 2006. Fluxes of N_2O and CH_4 from soils of savannas and seasonally-dry ecosystems. *Journal of Biogeography* 33, 401-415.

Castro, M. S., Steudler, P. A., Melillo, J. M., Aber, J. D., and Bowden, R. D.: Factors controlling atmospheric methane consumption by temperate forest soils, Global Biogeochem. Cycles, 9, 1–10, 1995.

Chain P., Lamerdin J., Larimer F. et al. 2003. Complete genome sequence of the ammonia-oxidizing bacterium and obligate chemolithoautotroph *Nitrosomonas europea. Journal of Bacteriology*, **185,** 2759-2773.

Chaterpaul, L., Paul, E. A. and Calaco, W., Abstracts of the 80th Annual Meeting, American Society of Microbiology, Florida, 11–16 May, 1980.

Chen J., and Stark J. 2000. Plant species effects and carbon and nitrogen cycling in a sagebrush-crested wheatgrass soil. Soil Biology and Biochemistry **32**: 47-57.

Chin K.J.,Conrad R., 1995. Intermediary metabolism in methanogenic paddy soils and influence of temperature. FMS Microbiology and Ecology 18, 85-102.

Christensen S (1983) Nitrous-oxide emission from a soil under permanent grass – seasonal and diurnal fluctuations as influenced by manuring and fertilization. *Soil Biology and Biochemistry*, 15, 531–536.

Clayton H, McTaggart IP, Parker J et al. (1997) Nitrous oxide emissions from fertilised grassland: a 2-year study of the effects of N fertiliser form and environmental conditions. *Biology and Fertility of Soils*, 25, 252–260.

Clough T.J., Jarvis S.C., Dixon E.R. et al. 1999. Carbon induced subsoil denitrification of ^{15}N-labelled nitrate in 1 m deep soil columns. *Soil Biology and Biochemistry*, **31**, 31-41.

Colbourn P. and Harper I.W., 1987. Denitrification in drained end undrained arable clay soil. *Journal of Soil Science* **38**: 531-539.

Conen F. & Smith K.A. 1998. A re-examination of closed flux chamber methods for the measurement of trace gas emissions from soils to atmosphere. *European Journal of Soil Science*, **49**, 701-707.

Conrad, R. 1989. Control of methane production in terrestrial ecosystems. *Exchange of trace gases between terrestrial ecosystems and the atmosphere* (eds M.O Andreae and Schimel D.S.), pp.39-58. John Wiley & Sons, New York, USA.

Crutzen P.J., 1981. Atmospheric chemical processes of the oxides of nitrogen, includine nitrous oxide. In: Delwiche C.C. (ed.), Denitrifcation, nitrification and Atmospheric Nitrous Oxide. John Wiley and Sons, Chichester, UK, pp. 17-44.

Crutzen P.J., 1991. Methane's sinks and sources. Nature **350**: 380-381.

Davidson, E.A. 1991. Fluxes of nitrous oxide and nitric oxide from terrestrial ecosystems. *Microbial production and consumption of greenhouse gases: Methane, nitrogen oxides and halomethanes* (eds J.E. Rogers and W.B. Whitman), pp. 219-235. American Society for Microbiology, Washington, D.C.

Davidson E.A. (1993) Soil water content and the ratio of nitrous oxide to nitric oxide emitted from soil. In: Oremland R.S. (ed) The Biogeochemistry of Global Change: *Radiatively Active Trace Gases*, pp. 369-386. Chapman & Hall, New York.

Davidson E.A. and Kingerlee W. 1997. A global inventory of nitric oxide emissions from soils. Nutr. Cycl. Agroecosyst. 48: 37-50.

DeLaune R.D., Smith C.J. and Patrick W.H. 1986. Methane production in Mississipi River deltaic peat plain, *Org. Geochem*., 9, 193-197.

Dele`gue, M.A., Fuhr, M., Schwartz, D., Mariotti, A. & Nasi, R. (2001) Recent origin of a large part of the forest cover in the Gabon coastal area based on stable carbon isotope data. *Oecologia*, 129, 106–113.

Denmead O.D. 1979. Chamber system for measuring nitrous oxide emission from soils in the field. *Soil Science Society of America Journal*, **43**, 89-95.

Deka, H.K., Mishra, R.R., 1983. The effect of slash burning on soil microflora. Plant and Soil 73, 167–175.

Denier Van Der Gon H.A.C., Neue H.U., 1995. Influence of organic matter incorporation on the methane emission from wetland rice field. Global Biogeochemical Cycles 11, 11-22.

Dick J, Skiba UM, Munro R and Deans D (2006) Effects of N-fixing and non-fixing trees and crops on NO and N2O emissions from Senegalese soils. J. of Biogeography 33, 416-423.

Donoso L., Santana R., Sanhueza E. 1993. Seasonal variation of N_2O fluxes at tropical savannah sita: soil consumption of N_2O during the dry seasons. *Geophysical Research Letters*, **20**, 1379-1382.

Doran J.W., Mielke L.N. & Power J.F. (1990) Microbial activity as regulated by soil water-filled pore space. In. Transactions of the 14th International Congress of Soil Science. Symposium III-3; Ecology of Soil Microorganism in the Microhabital Environments. August 12-18, 1990, Kyoto, Japan

Dunn, P.H., Barro, S.C., Poth, M., 1985. Soil moisture affects survival of microorganisms in heated chaparral soil. Soil Biology & Biochemistry 17, 143–148.

Duxbury J.M. and Moiser A.R. 1993. Status and issues concerning agricultural emissions of greenhouse gases. In: *Agricultural Dimensions of Global Climate Change.* H.M. Kaiser and T.E. Drennen (eds.). St. Lucie Press, Delray Beach, Florida, U.S.A., pp. 229-258.

Eggleton, P.E., Bignell, D.E., Sands, W.A., Waite B., Wood, T.G., & Lawton, J.H. 1995. The species richness of termites (Isoptera) under differing levels of forest disturbance in the Mbalmayo Forest Reserve, southern Cameroon. *Journal of Tropical Ecology*. **11**: 85-98.

Eggleton, P., Bignell, D.E., Sands, W.A., Mawdsley, N.A., Lawton J.H., Wood, T.G. and Bignell, N.C. 1996. The diversity, abundance and biomass of termites under differing levels of disturbance in the Mbalmayo Forest Reserve, southern Cameroon. *Phil. Trans. R. Soc. London. B.* **351**: 51-68.

Eggleton, P., Homathevi, R., Jeeva, D., Jones, D.T., Davies, R.G. & Maryati, M. 1997. The species richness and composition of termites (Isoptera) in primary and regenerating lowland dipterocarp forest in Sabah, East Malaysia. *Ecotropica*. **3**: 119-128.

El-Swaify, S.A. 1980. Physical and Mechanical characteristics of Oxisols. *Soils with variable charge*. (ed. B.K.G. Theng), pp. 325-352. New Zealand Society of Soil Science, C/-Soil Bureau, Department of Scientific and Industrial Research, private bag, Lower Hutt.

Elmi A.A., Chandra M., Chantal H. *et al*. 2003. Denitrification and nitrous oxide to nitrous oxide plus dinitrogen ratios in the soil profile under three tillage systems. *Biology and Fertility of Soils*, **38**, 340-348.

Erich M.S., Bererie A. and Duxbury J.M. 1994. Activities of denitrifying enzymes in freshly sampled soils. *Soil Sci*. 38: 25-32.

Fabing, A. (2000) Bilan spatial et structurel de l'antagonisme 'pression anthropique / dynamique forestie`re naturelle' en zone de forte croissance urbaine. Unpublished Thesis, University Louis Pasteur, Strasbourg.

Fearnside PM. 2000. Global warming and tropical land use change: greenhouse gas emissions from biomass burning, decomposition, and soils in forest conversion, shifting cultivation and secondary vegetation. Climatic Change 46: 115 -158.

Firestone M.K. and Davidson E.A., 1989. Microbiological basis of NO and N_2O production and consuption in soil. In: *Exchange of trace gases between terrestrial ecosystem and the atmosphere* (eds M.O. Andrea and D.S. Schimel). Dahlem workshop Report, John Wiley and Sons, Chichester. p. 7-21.

Flechard C.R., Neftel A., Jocker M., Amman C., Fuhrer J. 2005. Bi-directional soil/atmosphere N_2O exchange over two mown grassland system with contrasting management practices. *Global Change Biology*, **11**, 2114-2127.

Fox M.D., Fox B.J., 1987. The role of fire in the scleromorphic forest and shrublands of eastern Australia. In: *The role of fire in Ecological Sistems,* Trabaud L. (ed.), SPB Academic Publishing, The Hague, pp. 23-48.

Fraser PJ, Ramussen RA, Creffield JW, French JR, Khail MAK (1986) Termites and global CH4-another assestment. *Journal of Atmospheric Chemistry*, **4**, 295-310.

Fredrickson, J. K., Koehler, F. E. and Cheng, H. H., *Agron. Abstr.*, Am. Soc. Agron., Madison, Wisconsin, 1981, p. 176.

Gebauer G., Zeller B., Schmidt G., May C., Buchmann N., Colin-Belgrad M. et al. 2000. The fate of ^{15}N -labelled nitrogen inputs to coniferous and broadleaf forests. IN: Schulze (ed.), Carbon nitrogen Cycling in European forest Ecosystems. Ecological studies 142, Springer-Verlag, Berlin-Heidelberg, Germany, pp. 144-170.

Goreau T.J., Kaplan W.A., Wofsy S.C., McEltroy N.B., Valios F.W. & Watson S.W. (1980). Production Of NO_2^- and N_2O by nitrying bacteria at reduced concentrations of oxygen. *Applied Environmental Microbiology*, **40**, 526-532.

Granli T., & Bøckman O.C. 1994. Nitrous oxide from agriculture. N*orwegian Journal of Agriculture Sciences*, **12** (Suppl.), 7-128.

Groffman, P.M. 1987. Nitrification and denitrification in soil: A comparison of enzyme assay, incubation and enumeration methods. Plant Soil 97:445–450.

Grofmann, P.M. and J.M. Tiedje, 1989. Denitrification in northern temperate forest soils: Relationships between denitrification and environmental factors at the landscape scale. *Soil Biology and Biochemistry* **21**: 621-626.

Hanson, R. S. and Hanson, T. E.: Methanotrophic bacteria, Microbiol. Rev., 60, 439–471, 1996.

Harrison A.F., Schulze E.D. (ed.), Carbon and Nitrogen Cycling in European Forest Ecosystems Ecological Studies 142. Springer-Verlag, Berlin-Heidelberg, Germany, pp. 171-188.

Hall S.J., and Matson P.A. 1999b. Nutrient status of tropical rain forests influences soil N dynamics after n additions. Ecological Monographs **73**:107-129

Hao, W.M., Scharffe, D., Crutzen, P.J., Sanhueza, E., 1988. Production of N2O, CH4, and CO2 from soils in tropical savanna during the dry season. Journal of Atmospheric Chemistry 7, 93–105.

Healy R.W., Striegl R.G., Russell T.F., Hutchinson G.L. & Livingston G.P. 1996. Numerical evaluation of static-chamber measurement of soil-atmosphere gas exchange: identification of physical processes. *Soil Science Society of America Journal*, **60**, 740-747.

Heincke M., Kaupenjohann M. 1999. Effects of soil solution on the dynamics of N_2O emissions a rewiew. *Nutrient Cycling in Agroecosystem*, **55**, 133-157.

Hernandez, D. and J.J. Rowe, 1987. Oxygen regulation of nitrate uptake in denitrifying *Pseudomonas aeruginosa*. *Applied and Environmental Microbiology* **53**: 745-750.

Hillel D. 1980b. Fundamentals of soil physics. Academic Press, New York.

Houghton JT, Ding Y, Griggs DJ, Noguer M, van der Linden PJ, Dai X et al. *Climate change 2001: The scientific Basis. Intergovernmental Panel on Climate Change*. IPCC. Cambridge: Cambridge University Press, p.39

Hutchinson G.L. & Moiser A.R. 1981. Improved soil cover method for field measurement of nitrous oxide fluxes. *Soil Science Society of America Journal*, **45**, 311-316.

Hutchinson G.L. & Livingston G.P. 2001. Vents and seals in non-steady-state chambers used for measuring gas exchange between soil and the atmosphere. *European Journal of Soil Science*, **52**, 675-682

Hynes R.K. and Knowles R. 1984. Production of nitrous oxide by *Nitrosomonas europea*: effects of acetylene, pH, and oxygen. Can J. Microbiol. 30: 1397-1404.

Intergovernmental Panel on Climate Change. 2001. Climate change 2001: the scientific basis. Contribution of Working Group 1 to the third assessment report of the Intergovernmental Panel on Climate Change. J.T. Houghton, Y. Ding, D.J. Griggs, M. Noguer, P.J. van der Linden, X. Dai, K. Maskell and C.A. Johnson, editors. Cambridge University Press, Cambridge, UK.

IPCC 1992. *Climate Change 1992*, the Supplementary Report to the IPCC Scientific Assessment (eds B.A. Callander and S.K.Varney). Cambridge University Press. Cambridge.

IPCC-Intergovernmental Panel on Climate Change 1995. Climate change 1994-radiative forcing of climate change and an evaluation of the IPCC IS92 emission scenarios. In: Houghton J.T., Meira Filho M.G., BruceJ., Hoesung Lee J., callander B.A., Haites E. et al. (eds). Cambridge University Press, Cambridge, UK.

IPCC 2000. *Emissions Scenarios. 2000*. Special Report on the International Panel on Climate Change (eds N. Nakicenovic and R.Swart), pp. 570.Cambridge University Press, UK.

Jacobsen, P., W.H. Patrick Jr. and B.G. Williams, 1981. Sulphide and methane formation in soils and sediments. *Soil Science* **132**: 279-287.

Jackel, U., Schnell, S., Conrad, R., (2001): Effects of moisture, texture and aggregate size of paddy soil on production and consumption of CH4. − Soil Biology and Biochemistry 33, 965-971.

JEA/EPA, 1990. *Methane emissions and opportunities for control.* Workshop results of Intergovernmental Panel on Climate Change. Coordinated by Japan Environmental Agency and United States Environmental Protection Agency.

Jamet, R., 1975. Evolution des principales caractéristiques des sols des reboisements de Pointe-Noire, MC 189. ORSTOM, Brazzaville.

Jones W.J., Nagle D.P. and Whitman W.B. 1987. Methanogens and diversity of archaebacteria. Microbiol. Rev. **51** 135-177.

Jury W.A., Letey J. & Collins T. 1982. Analysis of chamber methods used for measuring nitrous oxide production in the field. *Soil Science Society of America Journal*, **46**, 250-256.

Kaiser, P., 1983. The role of soil micro-organisms in savanna ecosystems. In: Bourlie`re, F., (Ed.), Tropical Savannas, Elsevier, Amsterdam, pp. 541–557.

Kay, B.D. & Angers, D.A. (2000) Soil structure. Handbook of soil science (ed. by M.E. Sumner), pp. A229–A278. CRC Press LLC392, Boca Raton, London, New York, Washington, DC.

Keeney D.R., Fillery I.R. and Marx G.P., 1979. Effect of temperature on the gaseous nitrogen products of denitrification in a silty loam soil. *Soil Sci.Soc.Am.J.* **43**, 1124-1128.

Keppler, F., Hamilton, J. T. G., Brass, M., and Roeckmann, T.: Methane emissions from terrestrial plants under aerobic conditions, Nature, 439, 187–191, 2006.

Kester R.A., Meijer M.E., Libochant J.A., de Boer W. and Laanbroek H.J. 1997. Contribution of nitrification and denitrification to the NO and N_2O emissions of an acid forest soil, a river sediment and a fertilized grassland soil. Soil Biol. Biochem. 29: 1655-1664.

Khalil, M.A.K. and R.A. Rasmussen, 1983. Sources, sinks and seasonal cycles of atmospheric methane. *Journal of Geophysical Research* **88**: 5131-5144.

Khahil MAK, Ramussen RA (1990) Constraints on the global sources of CH4 and an analysis of recent budgets. *Tellus*, 42B, 229-236.

Kimura M. 1992. Methane emissions from paddy soil in Japan and Thailand, in Proceedings of an International Workshop Organized in the Framework of the Dutch National Research Programme on Global Air Pollution and Climate Change (Wageningen, 24-27 August 1992), edited By N.H. Batjes and E.M. Bridges, *WISE Rep*. 2, pp. 43-79, Int. Soil Ref. and Inf. Cent., Wageningen, Netherlands.

Klemedtsson L., Von Arnold K., Weslien P., Gundersen P. 2005. Soil CN ratio as a scalar parameter to predict nitrous oxide emissions. *Global Change Biology*, **11**, 1142-1147.

Knight D., Elliot P.W. and Andersen 1989. Effects of earthworms upon transformations and movement of nitrogen from organic matter applied to agricultural soils. In: J.A.A. Hansen and K. Henriksen (eds). *Nitrogen in inorganic wastes applied to soils* Academic Press, London p. 59-80

Knowles, R., 1982. Denitrification. *Microbiology Review* **46**: 43-70.

Komarek, E. V. 1965. Fire ecology: Grasslands and man. *Proceedings, Tall Timbers Fire Ecology Conference* 4:169-220.

Laclau, J.P., Sama-Poumba, W., de Dieu Nzila, J., Bouillet, J.P. & Ranger, J. (2002) Biomass and nutrient dynamics in a littoral savanna subject to annual fires in Congo. *Acta Oecologica*, 23, 41–50.

Laine J, Silvola J, Tolonen K et al. (1996) Effect of water-level drawdown on global climatic warming: Northern peatlands. *Ambio*, 25, 179–184.

Lal, L. 1980. Physical and Mechanical characteristics of Alfisols and Ultisols, with particular reference to soils in the tropics. *Soils with variable charge*. (ed. B.K.G. Theng), pp.253-280. New Zealand Society of Soil Science, C/-Soil Bureau, Department of Scientific and Industrial Research, private bag, Lower Hutt.

Leffelaar P.A., 1986. Dynamics of partial anaerobiosis, denitrification, and water in a soil aggregate: Experimental. *Soil Science* **142**: 352-366.

Levine, Joel S., Winstead, Edward L., Parsons, Dirk A. B., Scholes, Mary C., Scholes, Robert J., Cofer, Wesley R., III, Cahoon, Donald R., Jr., Sebacher, Daniel I. 1996. Biogenic soils emissions of nitric oxide (NO) and nitrous oxide (N2O) from savannas in South Africa: the impact of wetting and burning. Journal of Geophysical Research, [Atmospheres] 101(D19), 23689-23697.

Letey, J., N. Valoras, D.D. Focht and J.C. Ryden, 1981. Nitrous oxide production and reduction during denitrification as affected by redox potential. *Soil Science Society of America Journal* **45**: 727-730.

Li C.S., 2000. Modelling trace gas emission from agricultural ecosystem. Nutrient Cycling in Agroecosystem 58, 259-267.

Li C., Aber J., Stange F., Butterbach-Bahl K., Papen H., 2000. A process-oriented model of N_2O e NO emissions from forest soils: 1. Model development. *Journal of Geophysical Research*, **105**, 4369-4384.

Linn D.M., and J.W. Doran, 1984. Effect of water filled pore space on carbon dioxide and nitrous oxide production in tilled and non-tilled soils. *Soil Science Society America Journal* **48**: 1267-1271

Lobert J.M., Scharffe D.M., Hao W.H. and Crützen, 1990.Importance of biomass burning in the atmospheric budgets of nitrogen-containing gases. *Nature* **346**: 552-554

Loftfield N, Flessa H, Augustin J, Beese F (1997) Automated gas chromatographic system for rapid analysis of the atmospheric trace gases methane, carbon dioxide and nitrous oxide. *Journal of Environmental Quality*, **26** (2), 560-564.

Loumeto, J.J. (2002) Le syste`me litie`re des foreˆts a` peuplements d'okoume´s (*Aucoumea klaineana*): Exemple de deux sites congolais, la foreˆt du Chaillu et la foreˆt du littoral. Unpublished Thesis, University Paris VI.

MacDonald, J.A., Eggleton, P., Bignell, D.E, Forzi, F. & Fowler, D. (1998) Methane emissions by termites and oxidation by soils, across a forest disturbance gradient in the Mbalmayo Forest reserve, Cameroon. *Global Change Biology,* **4**, 409-418.

MacDonald, J.A., Fowler, D., Hargreaves, K.J., Skiba, U., Leith, I.D. & Murray, M.B. 1998. Methane emission rates from a northern wetland: response to temperature, water table and transport. *Atmospheric Environment*, 32, 3219–3227.

Mahli S.S., McGill W.B. and Nyborg M**.** (1990) Nitrate losses in soils: effect of temperature, moisture and substrate concentration. *Soil Biol. Biochem.* **22**, 733-737.

Maljanen M, Liikanen A, Silvola J et al. (2003) Nitrous oxide emissions from boreal organic soil under different land-use. *Soil Biology and Biochemistry*, **35**, 689–700.

Martius C, Wassaman R, Thein U, Bandeira A, Rennenberg H, Junk W, Seiler W (1993) CH4 emission from wood feeding termites in rain forests of Amazonia. Chemosphere, 26, 623-632.

Massacheleyn P.H., Delaune R.D., Patrick W.H., 1993. Methane and nitrous oxide emission from laboratory measurements of rice soil suspension. Effect of soil oxidation – reduction status. Chemosphere 26, 251-260.

Mathieu O, Leveque J, Henault C et al. (2006) Emissions and spatial variability of N_2O, N_2 and nitrous oxide mole fraction at the field scale, revealed with ^{15}N isotopic techniques. *Soil Biology and Biochemistry*, 38, 941–951.

Matson, P., P. Vitousek, G. Livingston, and N. Swanberg. 1990. Sources of variation in nitrous oxide flux from Amazonian ecosystems, J. Geophys. Res., 95(D10), 16,789– 16,798.

Matthias A.D., Yarger D.N., Weinbeck R.S. 1978. A numerical evaluation of chamber methods for determining gas fluxes. *Geophysical Research Letters*, **5**, 765-768.

Matthias A.D., Blackmer A.M. & Bremner J.M. 1980. A simple chamber for field measurement of emissions of nitrous oxide from soil. *Journal of Environmental Quality*, **9**, 251-256

Miklos A; Lim CH; Hsiang WW; Liang GC; Kung AH; et al. (2002) Photoacoustic measurement of methane concentrations with a compact pulsed optical parametric oscillator. *Applied Optics* **41** (15): 2985-2993

Moiser A.R., Mohanty S.K., Bhadrachalam A. and Chakravorti S.P., *Biol. Fert. Soils*, 1990, **9**, 61-67.

Moore T.R. and Knowles R. 1987. Methane and carbon dioxide evolution from subartic fens, *Can. J. soil Sci.*, 67, 77-81

Moorman F.R. and van Breemen N., 1978. *Rice: soil, water, land*. International Rice Research Institute, Los Banos.

Müller C., Stevens R.J., Laughin R.J. *et al.* 2004. Microbial processes and the site of N_2O production in a temperate grassland soil. *Soil Biology and Biochemistry*, **36**, 453-461.

Ndiaye D. Lensi R Lepage M Brauman A. 2004 The effect of the soil-feeding termite *Cubitermes niokoloensis* on soil microbial activity in a semi-arid savanna in West Africa Plant and Soil 10, 277-286.

Neftel A., Blatter A., Scmid M. *et al*. 2000. An experimental determination of the scale lenght of N_2O in the soil of a grassland. *Journal of Geophysical Research Atmospheres*, **105**, 12095-12103.

Nielsen D.R. and Bouma J. (eds), 1985. *Soil spatial variability*. Proceedings Workshop ISSS and SSSA, Las Vegas. Pudoc, Wageningen.

Nobre A.D., 1994. Nitrous oxide emissions from tropical soils. Thesis. Univ. New Hampshire, Durham, NH., p. 141.

Ollinger SV, Smith ML, Martin ME et al. (2002) Regional variation in foliar chemistry and N cycling among forests of diverse history and composition. *Ecology*, **83**, 339–355.

Oremland R.M., 1988. Biogeochemistry of methanogenic bacteria. In: *Biology of anaerobic microorganism* (ed. A.J.B. Zehnder). John Wiley and Sons, New York. P. 641-706.

Öquisit M.G., Nilsson M., Sorensson F., et al. 2004. Nitrous oxide production in a forest soil at low temperatures – processes and environmental controls. *FEMS Microbiology Ecology*, **49**, 371-378.

Otter, L.B. & Scholes, M.C. (2000) Methane sources and sinks in a periodically flooded South African savanna. *Global Biogeochemical Cycles*, **14,** 97-111.

Pacey J.G., De Gier J.P., 1986. The factors influencing landfill gas production. In: *Energy from landfill gas*. Proceedings of a conference jointly sponsored by the United Kingdom Department of Energy and the United States Department of Energy (October 1986). P.51-59.

Papen H. and Renneberg H., 1990. Microbial processes involved in emissions of radiatively important trace gases. In: *Transactions 14th International Congress of Soil Science, Kyoto* **II**: 232-237.

Papendick R.I. & Campbell G.S., 1981. Theory and measurement of water potential. In: Parr J.F., Gardner W.R. & Elliot L.F. (eds) *Water Potential Relations in Soil Microbiology*, pp. 1-22. Soil Science Society of America Special Publications Number 9, Madison Wisconsin, USA.

Parkin T.B. (1985) Automated analysis of nitrous oxide. *Soil Sci. Soc. Am. J.* 49, 273-276.

Parkin T.B., 1987. Soil microsites as a source of denitrifcation variability. *Soil Science Society of America Journal* **51**: 1194-1199.

Patrick W.H. Jr, 1981. The role of inorganic redox system in controlling reduction paddy soils. In: *Proceedings of the Symposium on Paddy Soils*. Institute of Soil Science, Academia Sinica, Science Press, Beijing. P. 107-117.

Pathak, H.: 1999, 'Emissions of nitrous oxide from soil', Curr. Sci. 77, 359−369.

Payne W.J. 1981. The status of nitric oxide as intermediates in denitrification. In. Delwiche C.C. (ed.), Denitrification, Nitrification and Atmospheric Nitrous Oxide. John Wiley and Sons, Chichester, UK, pp. 85-103.

Pérez MVA, Castañeda JG, Frias-Hernàndez JT, Franco- Hernàndez O, van Cleemput O, Dendooven L, Olalde V (2004) trace gas emissions from soil of the central highlands of mexico as affected by natural vegetation: a laboratory study. Biology and Fertility of Soil 40, 252-259.

Poth M. and Focht D.D. 1985. ^{15}N kinetic ananlysis of N_2O production by *Nitrosomonas europea*: an exanimation of nitrifier denitrification. Appl. Environ. Microbiol. 49: 1134-1141.

Poth., M., Anderson, I.C., Miranda, H.S., Miranda, A.C. & Riggan, P.G. 1995. The magnitude and persistence of soil NO, N_2O, CH_4 and CO_2 fluxes from burned tropical savanna in Brasil. *Global Biogeochemical Cycles* , **9,** 503-513.

Potter, C.S., Davidson, E.A. & Verchot, L.V. (1996a) Estimation of global biogeochemical controls and seasonality in soil methane consumption. Chemosphere, 32, 2219–2246.

Priemé, A. & Christensen, S. 1999. Methane uptake by a selection of soils in Ghana with different land use. *Journal of Geophysical Research*, **104 (D19)**, 23,617- 23,622.

Prentice, I.C. 2001. The carbon cycle and atmospheric carbon dioxide. Pages 183-238 *in* J.T. Houghton, Y. Ding, D.J. Griggs, M. Noguer, P.J. van der Linden, X. Dai, K. Maskell and C.A. Johnson, editors. Climate change 2001: the scientific basis. Contribution of Working Group 1 to the third assessment report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK.

Raison, R.J., 1979. Modification of the soil environment by vegetation fires, with particular reference to nitrogen transformations: a review. Plant and Soil 51, 73–108.

Reiners, W.A., Bouwman, A.F., Parson, W.F.J. & Keller, M. 1994. Tropical rain-forest conversion to pasture – changes in vegetation and soil properties. *Ecological Applications*, **4**, 363-377.

Ridgwell, A.J., Marshall, S.J. & Gregson, K. (1999) Consumption of atmospheric methane by soils: a process-based model. Global Biogeochemical Cycles, 13, 59–70.

Ritchie G.A.F. and Nicholas D.J.D. Identification of nitrous oxide produced by oxidative and reductive processes in *Nitrosomonas europea.* Biochem. 19. 187-193.

Rolston, D. E., Hoffman, D. L. and Toy, D. W., *Soil Sci. Soc. Am. J.*, 1978, **42**, 813–819.

Rosenkranz P., Brüggerman N., Papen H. *et al*. 2005. NO_2 NO CH_4 exchange, and microbial N turnover over Mediterranean pine pine forest soil. *Biogeosciences Discussions*, **2**, 673-702.

Ryden, J. C. and Lund, L. J., *J. Environ. Qual.*, 1980, **9**, 387–393.

Ryden J.C., 1983. Denitrification loss from a grassland soil in the field receiving different rates of nitrogen as ammonium nitrate. *J. Soil Sci*. 34: 355-365.

San José, J.J. & Montes, R. 2001. Management effects on carbon stocks and fluxes across the Orinoco Savannas. *Forest Ecology and Management*, **150**, 293-311.

Sanhueza, E., Hao, W.M., Scharffe, D., Donoso, L. & Crutzen, P.J. (1990) N_2O and NO emissions from soils of the Northern Part of the Guayana Shield, Venezuela. *Journal of Geophysical Research*, **95** (D13), 22,481-22,488.

Sanhueza, E., Càrdenas, L., Donoso, L. & Santana, M. (1994) Effect of plowing on CO_2, CO, CH_4, N_2O and NO fluxes from tropical savannah soils. *Journal of Geophysical Research*, **99** (D8), 16,429-16,434.

Sass R.L., Ficsher F.M., Yang Y.B., Turner F.T. and Jund M.F., 1992. Methane emissions from rice fields: the effect of flood water management. *Global Biogeochemical Cycles* 5: 335-350.

Sass, R.L, Fisher Jr , F.M., (1997): Methane emission from rice paddies: A process studies summary. − Nutrient Cycling in Agroecosystems 49, 119-127.

Sass R.L., Fischer Jr. F.M., Huang Y., 2000. A process-based model for methane emission from irrigated rice field: experimental basis and assumption. Nutrient Cycling in Agroecosystem 58, 249-258.

Scheffer F. and Schachtschabel P. 1998. Lehrbuch der Bodenkunde. 14 th edn. Ferdinand Enke Verlag, Suttgart, Germany, pp 274-275.

Schimel, D., D. Alves, I. Enting, M. Heimann, F. Joos, D. Raynaud, T. Wigley, M. Prather, R. Derwent, D. Ehhalt, P. Fraser, E. Sanhueza, X. Zhou, P. Jonas, R. Charlson, H. Rodhe, S. Sadasivan, K.P. Shine, Y. Fouquart, V. Ramaswamy, S. Solomon, J. Srinivasan, D. Albritton, I. Isaksen, M. Lal, and D. Wuebbles. 1996. Radiative forcing of climate change. Pages 65-132 *in* J.T. Houghton, L.G. Meira Filho, B.A. Callender, N. Harris, A. Kattenberg and K. Maskell, editors. Climate change 1995: the science of climate change. Contribution of Working Group 1 to the second assessment of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK.

Schmidt H.-L. and Vorkelius S. 1989. origin and isotope effects of oxygen in compounds of the nitrogen cycle. In: Isotopes in nature 5th Working Meeting, Proceedings. Leipzing, Germany.

Schmidt I., van Spanning R.J.M., Jetten M.S.M. 2004. Denitrification and ammonia oxidation by *Nitrosomonas europea* wild-type, and NirK- and NorB-deficient mutans. *Microbiology*, **150**, 4107-4114.

Scholes, M. C., Martin, R., Scholes, R. J., Parsons, D., Winstead, E. 1997. NO and N2O emissions from savanna soils following the first simulated rains of the season. Nutrient Cycling in Agroecosystems 48(1-2), 115-122.

Scholes RJ et al. (2001) The environment and vegetation of the flux measurement site near Skukuza, Kruger National Park. Koedoe 44:73-83.

Schulte-Bisping H, Brumme R, Priesack E (2003) Nitrous oxide emission inventory of German forest soils. *Journal of Geophysical Research*, **108**, 4132.

Schütz H., Holzaptel-Pschorn A., Conrad R., Rennemberg H., Seiler W., 1989. A three-year continuous record on the influence of day time season and fertilizar treatment on methane emission rates from an Italian rice paddy. Journal of Geophysics Research 94, 16405-16416.

Schütz H. Seiler W and Conrad R., 1990. Influence of soil temperature on methane emission from rice paddy fields. *Biogeochemistry* **11**: 77-95.

Schwartz, D., Deschamps, R., Elenga, H., Lanfanchri, R., Mariotti, A., Vincens, A., 1995. Les savanes du Congo: une végétation spécifique de l'holocène supérieur. 2nd Symposium on African Palynology, Pub. Occas. CIFEG, 1995/31, Orléans. pp. 99–108.

Schwartz, D., de Foresta, H., Mariotti, A., Balesdent, J., Massimba, J.P. & Girardin, C. (1996) Present dynamics of the savanna-forest boundary in the Congolese Mayombe: a pedological, botanical and isotopic (C-13 and C-14) study. *Oecologia*, 106, 516–524.

Sebacher D.I., Harris R.C., Bartlett K.B., Sebacher S.M., Grice S.S., 1986. Atmospheric methane sources: Alaskan tundra bogs, an alpine fen, and a subartic boreal marsh. *Tellus* **38 B**: 1-10

Seiler W. and Conrad R., J. *Air Pollut. Cont. Assoc.*, 1981, 31, 767-772.

Seneviratne, R., and A. Wild. 1985. Effect of mild drying on the mineralization of soil nitrogen. Plant Soil 84:175–179.

Serca, D., R. Delmas, C. Jambert, and L. Labroue. 1994. Emissions of nitrogen-oxides from equatorial rain-forests in central Africa—Origin and regulation of NO emissions from soils, Tellus, Ser. B, 46(4), 243– 254.

Sextone A.J., Parkin T.B. and Tiedje J.M., 1985. Temporal response of soil denitrification rates to rainfall and irrigation. *Soil Science Society of America Journal* **49**: 99-103.

Shallcross, D.E., K. Wang, and C.H. Dimmer. 2003. Biogeochemical cycles and residence times. Pages 90-123 *in* C.N. Hewitt and A.V. Jackson, editors. Handbook of atmospheric science: principles and applications. Blackwell Publishing, Malden, MA, USA.

Shaw L.J., Nicol G.W., Smith Z. et al. 2006. *Nitrosopira* spp. Can produce nitrous oxide via nitrifier denitrification pathway. *Environmental Microbiology*, **8**, 214-222.

Shoji, S., Nanzyo, M. & Dahlgren, R. 1993. Volcanic ash soil. Genesis, properties and utilization. Developments in Soil Science 21, Elsevier.

Singh, J.S., Singh, S., Raghubanshi, A.S., Singh, Saranath, Kashyap, A.K. & Reddy, V.S. (1997) Effects of soil nitrogen, carbon and moisture on methane uptake by dry tropical forest soils. *Plant and Soil*, **196**, 115-121.

Skiba U., Hargreaves K.J., Fowler D. and Smith K.A. 1992. Fluxes of nitric and nitrous oxides from agricultural soils in a cool temperate climate. Atmos. Environ. 26A: 2477-2488.

Skiba U., Smith K.A. and Fowler D. 1993. Nitrification and denitrification as sources of nitric oxide in a sandy loam soil. Soil Biol. Biochem. 25: 1527-1536.

Skopp J., 1985. Oxygen uptake and transport in soil: analysis of the air-water interfacial area. *Soil Science Society of America Journal*, **54**, 1619-1625.

Smith K.A., 1980. A model of the extent of anaerobic zones in aggregate soils its potential to estimate of denitrification. *Journal of Soil Science* **31**: 263-277.

Smith, K.A. 1990. Anaerobic zones and denitrification in soil: modelling and measurements. *Denitrification in Soil and Sediment.* (eds N.P. Revsboech and J. Sørensen). Plenum Press, New York, pp. 228-240.

Smith K.A., 1997. The potential for feedback effects induced by global warming on emissions of nitrous oxide by soils. *Global Change Biology*, **3**, 327-338.

Smith K.A. and Arah J.R.M. (1991) Gas chromatographic analysis of the soil atmosphere. In *Soil Analysis; Modern Instrumental Techniques.*(K.A. Smith Ed.) Marcell Dekker, New York, pp.505-546.

Smith K.A. and Harris W. (1970) An automatic device for injection of gas samples in to a gas chromatograph. *J.Chromatogr.* 53, 358-362.

Smith, M.S., and J.M. Tiedje. 1979. Phases of denitrification following oxygen depletion in soil. Soil Biol. Biochem. 11:262–267.

Smith KA, Thomson PE, Clayton H et al. (1998) Effects of temperature, water content and nitrogen fertilization on emissions of nitrous oxide by soils. *Atmospheric Environment*, 32, 3301–3309.

Smith, K.A., Dobbie, K.E., Ball, B.C., Bakken, L.R., Sitaula, B.K., Hansen, S., Brumme, R., Borken, W.B., Christensen, S., Priemé, A., Fowler, D., MacDonald, A., Skiba, U., Klemedtsson, L., Kasimir-Klemedtsson, A., Derorska, A. & Orlanski, P. 2000. Oxidation of atmospheric methane in Northern European soils, comparison with other ecosystems, and uncertainties in the global terrestrial sink. *Global Change Biology*, **6,** 791-803.

Smith KA, Dobbie KE (2001) The impact of sampling frequency and sampling times on chamber-based measurements of N_2O emissions from fertilized soils. *Global Change Biology*, 7, 933–945.

Steudler, P. A., J. M. Melillo, R. D. Bowden, M. S. Castro, and A. E. Lugo. 1991. The effects of natural and human disturbances on soil-nitrogen dynamics and trace gas fluxes in a Puerto Rican wet forest, Biotropica, 23(4), 356–363.

Svensson B.H., Different temperature optima in methane formation when enrichments from acid peat are supplemented with acetate or hydrogen, *Appl. Environ. Microbiol*., 48, 389-394, 1984

Svensson B.H., Rosswall R., 1984. In situ methane production from acid peat in plant communities with different moisture regimes in subartic mire. *Oikos* **43** :341-350.

Svensson B.H., Boström U., Klemedtsson L., 1986. Potential for higher rates of denitrification in earthworms casts than in the surrounding soil. *Biology and Fertility of Soils* **2**: 147-149.

Thauer, R.K., K. Jungermann and K. Decker, 1977. Energy conservation in chemotrophic anaerobic bacteria. *Bacteriol.Rev.* **41**: 100-180.

Takai, Y.: 1970, 'The mechanism of methane fermentation in flooded paddy soil', *Soil Science and Plant Nutrition* **16**, 238–244.

Takai Y., Wada E. 1990. Methane formation in waterlogged paddy soil and its controlling factors. In: *Soils on a warmer Earth* (eds H.W. Scharpenseel, H.W., M. Schomaker and A. Ayoub). Devolopments in *Soil Science* 20, Elsevier Amsterdam. P. 101-107.

Umarov M.M., 1990. biotic sources of nitrous oxide in the context of the global budget of nitrous oxide. In: *Soils and the greenhouse effect* (ed. A.F. Bouwman). John Wiley and Sons, Chichester. P. 263-268.

UNFCC 2002 U.S. Global Change Research Program Carbon Cycle Program: An Interagency Partnership, www.carboncyclescience.gov.

van Gestel, M., J.N. Ladd, and M. Amato. 1991. Carbon and nitrogen mineralization from two soils of contrasting texture and microaggregate stability: Influence of sequential fumigation, drying and storage. Soil Biol. Biochem. 23:313–322.

Van Reenen, C.A., Visser, G.J., Loos, M.A., 1992. Soil microorganisms and activities in relation to season, soil factors and fire. In: Van Wilgen, B.W., Richardson, D.M., Kruger, F.J., Van Hensbergen, H.J. (Eds.), Fire in South African Mountain Fynbos. Ecosystem, Community and Species Responses at Swartboskloof, Springer, Berlin, pp. 258–272.

Velthof GL, Brader AB, Oenema O (1996) Seasonal variations in nitrous oxide losses from managed grasslands in the Netherlands. *Plant and Soil*, 181, 263–274.

Verchot, L. V., E. A. Davidson, J. H. Cattaˆnio, I. L. Ackerman, H. E. Erickson, and M. Keller. 1999. Land use change and biogeochemical controls of nitrogen oxide emissions from soils in eastern Amazonia, Global Biogeochem. Cycles, 13(1), 31– 46.

Verchot L.V., Davdson E.A., Cattânio J.H. *et al*. 1999. Land use change and biogeochemical controls of nitrogen oxide from soils in eastern Amazonia. *Global Biogeochemical Cycles*, **13**, 31-46.

Verchot, L.V., Davidson, E.A., Cattânio, J.H. & Ackerman, I.L. 2000. Land use change and biogeochemical controls of methane fluxes in soils of Eastern Amazonia. *Ecosystems*, **3**, 41-56.

Werner C., Kiese R., Butterbach-Bahl K. 2007. Soil-atmosphere exchange of N_2O, CH_4, and CO_2 and controlling environmental factors for tropical rain forest sites in western Kenya. J. Geophys. Res. (in press).

Voerkelius S. 1990. Isotopendiskriminierungen bei der Nitrifikation und Denitrifikation; Grundlagen und Anwedungen der Herkunfts-Zuordnung von Nitrat und Distickstoffmonoxid. Phd Dissertation, Technical university of Munich, Germany.

Wahlen S.C. and Reedburgh W.S.,1990. Consumption of atmospheric methane by tundra soils. *Nature* **346**: 160-162.

Walter H., Lieth H. 1960-67. *Klima-Diagramm-Welatlas.* VEB Gustav-Fischer Verlag, Jena.

Wassmann R., Martius C.S., 1997. Methane emission from Amazon flood plain. In: Junk W.J., (Ed.), The Central Amazon floodplain: Ecological Studies 126., Springer-Verlang, Berlin. Pp. 137-143.

West, L.T. & Beinroth, F.H. 2000. Ultisols. *Handbook of Soil Science* (ed. M.E. Sumner), pp. E-358-E-372. CRC Press LLC392. Boca Raton, London, New York, Washington DC.

Wilding, L.P., Smeck, N.E. & Hall, G.F. 1983. Entisols. *Pedogenesis and Soil Taxonomy. II. Soil Orders,* pp. 55-90. Developments in Soil Science. Elsevier. Amsterdam, Oxford, New York, Tokyo.

Wilding, L.P., Smeck, N.E. & Hall, G.F. 1983. Inceptisols. *Pedogenesis and Soil Taxonomy. II. Soil Orders*, pp. 355-382. Developments in Soil Science. Elsevier. Amsterdam, Oxford, New York, Tokyo.

Wilson J.O., Crill P.M., Bartlett K.B., Sebacher D.I., Harris P.C., Sass R.L., 1989. Seasonal variation of methane emission from temperate swamp. *Biogeochemisty* **8**: 55-71.

Williams R.J. and Crawford R.L. 1984. Methane production in Minesota peatlands, Appl. Environ. Microbiol., 47, 1266-1271.

World Meteorological Organization, 2006. *Annual Greenhouse Gas Bulletin*. WMO-GAW, Geneve.

Wrage N., Velthof G.L., van Beusichem M.L. and Oenema O. 2001. Role of nitrifier denitrification in the production of nitrous oxide. Soil. Biol. Biochem. 33: 1723-1732.

Yagi K. and Minami K., 1990a. Effect of organic matter applications on methane emissions from Japanese paddy fields. *Soil Sci. Plant Nutr.* 36, 599-610.

Yamane I. and Sato K., 1967. Effect of temperature on the decomposition of organic substances in flooded soil. *Soil Science and Plant Nutrition* **13**: 94-100.

Yamulki S., Goulding K.W.T., Webster C.P. and Harrison R.M. 1995. Studies on NO and N_2O fluxes from a wheat field. Atmos Environ. 29. 1627-1635.

Yavitt J.B., Lang G.E., Wieder R.K. 1987. Control of carbon mineralization to CH_4 and CO_2 in anerobic *Sphagnum*-derived peat from Big Run Bog, West Virginia, *Biogeochemistry*, **4**, 141-157.

Yavitt J.B., Lang G.E. and Downey M. 1988. Potential methane production and methane oxidation in peatland ecosystems of Appalachian mountains, United States, *Global Biogeochem. Cycles*, 2, 253-268.

Yeaton, R.I. (1988) Porcupines, fires and the dynamics of the tree layer of the *Burkea Africana* savanna. *J. Ecol.* **76,** 1017–1029.

Yoshinary T, Knowles R (1976). Acetylene inhibition of nitrous oxide reduction by denitrifying bacteria. Biochem Biophys Re Commun 69:705-710.

Yoshinari T., in *Denitrification in Soil and Sediment* (eds Revsbech, N.P. and Sorenson, J.), Plenum, New York 1990, pp. 129-149.

Zepp, R.G., Miller, W.L., Burke, R.A., Parsons, D.A.B., Scholes, M.C., 1996. Effects of moisture and burning on soil-atmosphere exchange of trace carbon gases in a southern African savanna. Journal of Geophysical Research D 101, 23699–23706.

Zimmerman P. R., et al. 1982.Termites: A Potentially Large Source of Atmospheric Methane, Carbon Dioxide, And Molecular Hydrogen. Science, 218(5):563-565.

Zumft W.G. and Kroneck M.H. 1990. metabolism of nitrous oxide. In. Revsbech N.P. and Sørensen J. (eds), Denitrification in Soil and Sediment. Plenum Press, New York, USA, pp. 37-55.