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MASTERARBEIT

Titel der Masterarbeit

Influence of fire on potential soil GHG emissions and microbial community structure in a shrubland in central Spain.

Verfasser

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Abstract

The goal of this work was to better understand the influence of fire on potential soil GHG emissions and microbial community structure in a shrubland in central Spain. The experiment was conducted in "Quintos de Mora" which is located 165 km south of Madrid and 50 km south of Toledo (39° 51` 48.40`` N; 4° 00` 31.52`` W). The experimental site was designed by the researchers of the SECCIA (Simulation of Effects of Climate Change in a Shrubland Affected by Fire) group. Samples were collected from September (2009) until February (2010).

We concluded from this work that fire reduced the emissions of carbon dioxide (CO_2) from soil; it contributed positively to an increase of ammonium (NH_4^+) and maintained this pattern 46 days after the fire; with fire there was a greater loss of NH_4^+ and nitrate (NO_3^-) via leaching over time; fire drastically increased nitric oxide (NO) and nitrous oxide (N_2O) emission rates from the soil with rising temperature and soil water content. Fire negatively affected microorganisms in the Mediterranean shrubland; fungi were controlled mainly through the influence of pH, preferring lower pH.

Zusammenfassung

Das Ziel dieser Arbeit war es, die Auswirkungen von Feuer/Brandereignissen auf potentielle Treibhausgasemissionen aus Böden und der mikrobiellen Gemeinschaft aufzuzeigen. Das Experiment wurde in "Quintos de Mora" durchgeführt. Quintos de Mora liegt 165 km südlich von Madrid und 50 km südlich von Toledo (39° 51` 48.40`` N; 4° 00` 31.52`` W). Der Versuchsstandort (eine Macchie auf seichtgründigem Boden) wurde von den ForscherInnen des SECCIA (Simulation of Effects of Climate Change in a Shrubland Affected by Fire) Projektes angelegt. Die Bodenproben wurden von September 2009 bis Februar 2010 gesammelt und später im Labor in Wien analysiert.

Die Ergebnisse dieser Arbeit zeigten, dass Brand die Bodenrespiration langfristig reduziert. Grund dafür war ein starker Rückgang der Mikroorganismen. Bodenpilze waren am stärksten davon betroffen. Der Anstieg des pH Wertes war der wahrscheinlichste Grund für diesen Rückgang. Inorganischer Stickstoff wurde nach dem Feuer stark angereichert, wodurch es auch zu einer erhöhten Auswaschung ins Grundwasser kam. Dieser Anstieg konnte über längere Zeit beobachtet werden. Das Potential, dass Bodenmikroorganismen nach dem Verbrennen der Vegetation das klimawirksame Treibhausgas Distickstoffmonoxide (N_2O) bilden, war mit steigendem Wassergehalt und steigender Temperatur besonders hoch. Auch Sticksoffmonoxid (NO) wurde unter diesen Bedingungen vermehrt produziert.

1 Introduction

The microorganisms in the soil play an important role in the decomposition of litter and organic matter, nutrient release and the availability of nutrients in the soil (Garcia and Rice, 1994; O'Lear et al., 1996). Consequently plant nutrient uptake, growth and productivity are affected (Bardgett et al., 1999; Janna et al., 2005).

Since microorganisms play a fundamental role in the quality, health and fertility of the soils, studies of soil microorganisms have increased considerably in recent years (Arias et al., 2005; Lubchencoet al., 1991). Microorganisms cause soil structures to fix and stabilize the soil aggregates (Elliott et al., 1996), freeing nutrients through mineralization, nitrification and organic matter transformation; they are considered the ecologic motors of terrestrial systems (Killham, 1994), and play a crucial role in global warming as they produce or capture GHG (IPCC, 2001). In the gas exchange processes between soil and the atmosphere, the soil has a decisive role because it is the site of biochemical changes resulting from the action of microorganisms, which are responsible for these gas exchanges. The soil is an important source of atmospheric nitrous oxide (N_2O) and methane (CH_4), and has great significance in the nitric oxide (NO) balance (Conrad, 1996). It is also in the soil where a carbon reserve larger than the aerial carbon biomass is trapped (Atjay et al., 1979), providing an important source of CO_2 (Raich et al., 2002).

For this reason, it is important to become thoroughly familiar with the populations of microorganisms, and to understand their functioning, relationships and interrelationships between each other and the environment.

Organic mineralization of nitrogen occurs in the soil, caused by the degradation of proteins, amino acids and nucleic acids into ammonium (NH_4^+)—which is the mineral form of nitrogen (Paul and Clark, 1996). Also, NH_4^+ is aerobically metabolized in the soil by microorganisms and converted into nitrites (NO_2^-) and nitrates (NO_3^-) by a process known as nitrification. In the anaerobic process known as denitrification, microorganisms use NO_3^- to oxidize organic matter. These microbiological processes produce NO, N_2O (Firestone and Davidson, 1989) and N_2 (Fig. 1). On a microscopic scale these two processes—anaerobic and aerobic—

can occur together (van Cleemput and Samater, 1996), therefore in the soil, nitrification and denitrification occur simultaneously. The net value of the soil-atmosphere-gas exchange of NO and N_2O depends on the balance between production, consumption, transport regulation and the recycling rates of the nitrogen within the ecosystem (Galbally, 1989).

NO can also be produced through abiotic processes, i.e., through the chemical decomposition of the NO_2^- ion (Galbally, 1989). This process is called chemodenitrification.

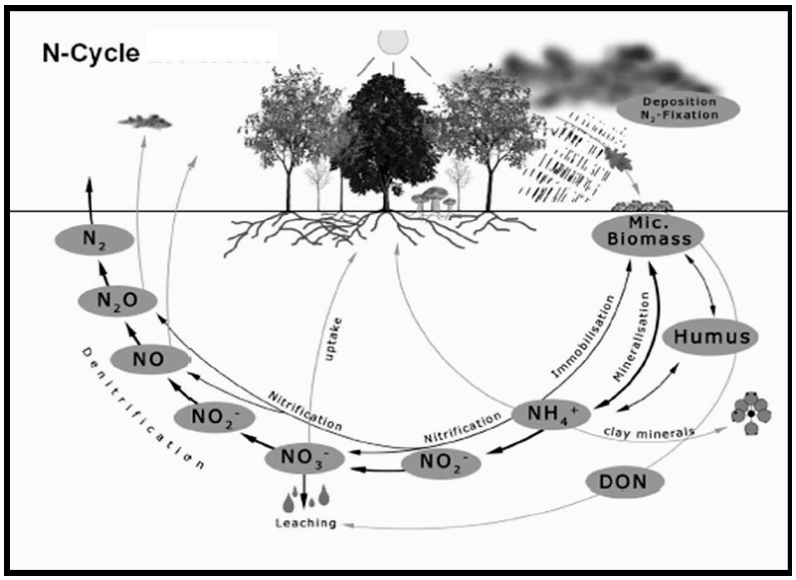

Fig. 1- Nitrogen cycle in forest ecosystems (Source: Ambus and Zechmeister-Boltenstern, 2007)

If we compare only the atmospheric concentrations of CO_2 (365 µl l^{-1}) and N_2O (0.3 µl l^{-1}), the greenhouse gas effect of N_2O would not be of striking importance in the regulation of atmospheric temperatures. But if we consider its thermal absorption capacity, which is 150 times higher than that of CO_2 (Paul and Clark, 1996), we see its real significance in temperature change.

Various ecosystems emit NO_x resulting from biogenic activities, and these biogenic emissions can be compared to the emissions which come from anthropogenic activities and sources in terms of magnitude (Levy et al., 1992). The soil-atmosphere fluxes of N_2O, NO, and CH_4 affect relevant processes related to global heating and environmental pollution (Ramanathan et al., 1987).

Another important dual role of the NO, together with nitrogen dioxide (NO_2), is the regulation of ozone (O_3) concentration and hydroxyl radicals (OH) in the troposphere (Penner et al., 1991), leading to implications on global heating. The NO and NO_2 concentration in the troposphere is the factor which will determine the production or destruction of O_3 (Crutzen, 1979; Cardenas et al., 1993).

Methane (CH_4) is another important gas to be considered, although it is not as concentrated in the atmosphere as is the CO_2. With atmospheric concentration of 1.7 µl l^{-1}, CH_4 has 30 times the thermal absorption power as does CO_2 (Paul and Clark, 1996).

Methane is formed in anaerobic conditions, where methanogenic bacteria (archaebacteria) oxidize nitrogen sources, preferentially the NH_4^+ (Paul and Clark, 1996). The presence of nitrates, on the other hand, inhibits methane production, and oxygen acts as an inhibitor of anaerobic respiration (Conrad, 1989).

The excess of soil humidity creates an anaerobic environment and contributes to the CH_4 production. Wetlands are the biggest global source of CH_4 (Prather and Ehhalt, 2001). In tropical forests the soils normally work to bring the level of CH_4 down, but with the transformation of the forests into pastures and agricultural areas, the soils become a source of CH_4, because of the compaction and reducing of gas diffusion rates which, therefore, form anaerobic environments and favor CH_4 production (Keller et al., 1990, 1993).

Diffusion is a control agent which acts on the supply of CH_4 to methanotrophic organisms (see below), and in very humid soils the oxidation rates of CH_4 are low (Striegl, 1993). Therefore, with a reduction of humidity, the rates of methane uptake rise (Paul and Clark, 1996).

The organisms which oxidize CH_4 are called methanotrophic and are, as a rule, aerobic. Nitrifying organisms can also oxidize CH_4. For this oxidation to occur, the presence of NO_3^- and NO_2^- is required, along with large amounts of NH_4^+ to inhibit oxidation. Extremely dry conditions can reduce the rate of natural biological activities, inhibiting, therefore, CH_4 oxidation (Paul and Clark, 1996).

Another important feature is the soil type. For example, well-aerated soils bring about higher CH_4 oxidation rates (e.g. Mosier et al., 1991; Bartlett et al., 1992; Strieglet al., 1992; Whalen et al., 1992) because of the higher porosity of the substrate, which favors gas transportation (Potter et al., 1996). In these soils, the main control agent of CH_4 oxidation is gas diffusion (Striegl, 1993).

In the field, environmental variations like temperature and humidity are strong factors which control the production of CH_4 as well as CO_2 (Peterjohn et al., 1994).

As previously discussed, CO_2 is an important GHG. Its atmospheric concentration was 260 $\mu l\ l^{-1}$ before 1860, whereas in 1995 the concentration had increased to 360 $\mu l\ l^{-1}$. This increase is a consequence of fossil fuel use and extensive soil use in agriculture as well as forest burning (Paul and Clark, 1996).

Pinto et al. (2002) highlights humidity as the main factor controlling emissions of CO_2 and NO. Fierro et al. (2007) observed that in the field the biggest controller of CO_2 emissions in burned plots was water availability. Other studies show that temperature, not humidity, is the main factor and acts more effectively in CO_2 emissions than in the production of NH_4^+ (Christ and David, 1996; Neff and Hooper, 2002). In general, CO_2 emissions through respiration increase exponentially with temperature in cases where soil humidity is not a limiting factor (Schaufler et al., 2010).

Another problem of fire in relation to carbon is the stabile form of carbon (coal) that the fire produces. This carbon remains out of the sphere of biologic activities, not being absorbed or transformed by the action of microorganisms, and therefore not being used as an energy and organic matter source (Albrecht et al., 1995). Another important factor is the liquid loss of CO_2, which only occurs with the reduction of the stock of biomass in the ecosystem, caused by changes in soil use (Crutzen and Andreae, 1990). Fierro et al. (2007) concludes that the efficiency of the

microorganisms in carbon conversion can be reduced by the action of fire. On the other hand, in agricultural systems fire can increase CO_2, CH_4, N_2O and NO_x emissions (Lima et al., 1999).

Fire can also increase the emission of GHG. For example, during the burning of biomass, nitrogen substances present in vegetation and soil surface seep into the atmosphere through the volatilization of nitrogen oxides caused by fire (Ewel et al., 1981; Nye and Greenland, 1964; Matson et al., 1987), resulting in a significant transference of NO from the soil to the atmosphere (Neff et al., 1995) and also the NO_x, and CO_2. These emissions have consequences on global warning.

With the occurrence of fire, there is not only a loss of gases and disturbance of the nutrient cycles, but in acidic soils erosion and leaching can also occur, with subsequent losses of nutrients (Haag, 1985). One way that these losses by leaching occur is when the rainwater washes away the nutrients below the root system of plants. The losses are related to the soil features, availability and solubility of the nutrients found in the soil, as well as the amount and distribution of rain (Myers et al., 1994; Russelle, 1997). In soils with a high cationic exchange capacity and a high degree of saturation, raining may cause substantial loss of mineral nutrients. When compared with unsaturated soils with a low capacity of cationic exchange, the nutrient losses are lower. In the first system, the loss of nutrients is proportional to the volume of draining, because the concentration of nutrients in the water remains constant. In the second system, the increase of draining dilutes the nutrients—and losses decrease according to the volume of water which leaves the system (Haag, 1985). Since soils generally have negative charges, the anion NO_3^- is not retained by the soil, being subject to leaching by rainwater.

Fire directly affects ecosystems and their entire production chain, disrupting biological cycles which maintain the ecological equilibrium. Fire also affects the climatic alterations (local or global) because of the emission of GHG as mentioned above.

The field of science which studies the climatic changes caused by human activities is relatively new. Researchers' efforts to understand the mechanisms of the

greenhouse effects have generated several scientific papers around the world in recent years.

Climate changes have occurred throughout the history of the earth, having global and/or local dimensions. The causes of these changes were natural in times before the industrial revolution (ca. 1750), but in recent centuries human activities are increasing the GHG effect in the atmosphere, triggering a continuous increase of the mean temperature on the earth's surface.

Greenhouse gases absorb short waves from the sun (which do not produce heat) and convert this energy into infra-red energy, which are long waves (which produce heat). They also emit this long wave energy, therefore increasing the local surrounding temperatures. In addition, the resulting layer of GHG makes it more difficult for the infra-red energy to return to space, therefore trapping the resultant heat in the atmosphere (Graedel and Crutzen, 1994).

There are two factors caused by human activities which are the main drivers of GHG increase into the atmosphere:

1) The burning of fossil fuels such as petroleum and its derivatives—natural gas and natural coal.

2) The second factor is the land-use change—especially the practices of deforestation, burning, and agricultural and animal husbandry activities. As a consequence of these activities, 20 to 60 million hectares are cleared and burned every year—most of this land containing tropical forests and savannas (Crutzen and Andreae, 1990).

According to some studies, one of the important effects of the climatic global change is an expected decrease of precipitation and the increase in length of the dry season (Lavorel et al., 1998; Pinol et al., 1998) in the Mediterranean are as for the upcoming years (IPCC 2007). These phenomena—coupled with the rise of the global mean temperature—will as a consequence cause an escalation in the number and magnitude of burnings. For the Mediterranean area, fire is a determinant factor in climate changes.

The Mediterranean region has been identified as a hot spot region concerning climate changes (Giorgi, 2006). In the past this region suffered great climate changes

(Luterbacher et al., 2006) which affected the entire region. Furthermore, some authors pinpoint the Mediterranean region as a region very susceptible to climate change, having great potential to add to the accumulation of GHG in the atmosphere (e.g. Lionello et al., 2006; Ulbrich et al., 2006).

Climate changes can change the carbon and nitrogen cycles, as well as interfere with the dynamics of other gases. After burning, effects in the N and C cycles in the Mediterranean ecosystems are still unclear.

The goal of this work was to intensifying the general knowledge regarding burning, its implications and consequences on the gas exchange between soil and atmosphere in the Mediterranean region.

This study is part of the *Nitroeurope* project (http://www.nitroeurope.eu/), the goal of which is to study the nitrogen cycle in different European ecosystems and analyze its implications and influences on the emission of GHG.

Within the *Nitroeurope* project (NEU), the experimental burning of a Mediterranean shrubland in *Quintos de Mora* (Fig. 2) was done in collaboration with the Universidad Politechnica de Madrid (U.D. Edafologia y Ecologia), the *Karlsruhe Institute for Meteorology and Climate Research* (IMK-IFU, Garmisch-Partenkirchen, Germany), the *Federal Research and Training Centre for Forests, Natural Hazards and Landscape* (BFW, Vienna, Austria), the *National Laboratory for Sustainable Energy* (Risø DTU, Roskilde, Denmark) and the *Castilla La Mancha University* (UCLM, Toledo).

Our goals were:

(i) To analyze the influence of fire on the gas exchange between soil and the atmosphere as a result of the action of microorganisms

(ii) To analyze the influence of fire on the populations of microorganisms and

(iii) To analyze the influence of fire on the leaching of inorganic nitrogen.

Our hypotheses were:

(i) Fire negatively affects the populations of microorganisms in the soil

(ii) Fire produces a higher loss of NO_3^- by leaching

(iii) Fire directly affects the emission and uptake potential of GHG.

Fig. 2 – General outline of Mediterranean burning experiment, participants' working groups and contributions of each group. Arrows indicate the parties involved with this thesis.

2 Material & Methods

2.1 Site

The experiment was conducted in Quintos de Mora (Fig. 3-4) which is located 165 km south of Madrid and 50 km south of Toledo (39° 51` 48.40`` N; 4° 00` 31.52`` W). The soil is sandy. The dominant vegetation is *Arbutus unedo, Erica australis, Quercus ilex subsp. ballota, Rosmarinus officinalis, Phyllirea angustifolia, Cistus ladanifer*.

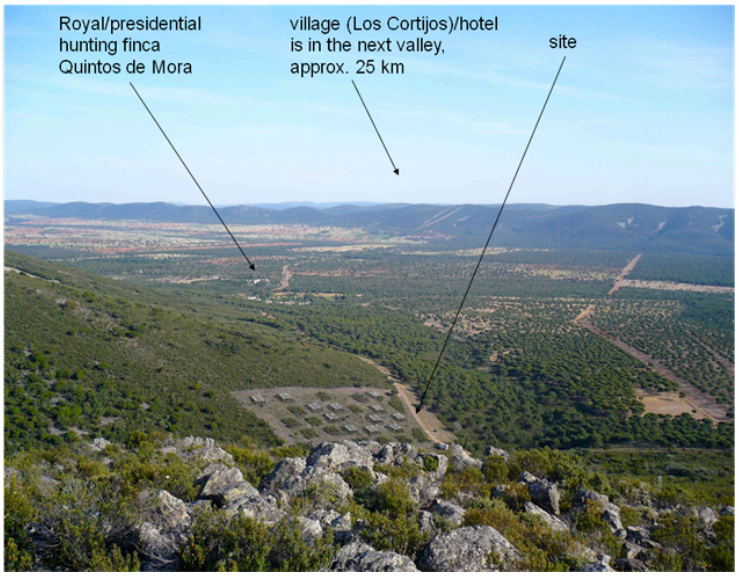

Fig. 3 –The study area with plots ready for burning (Source: Dannenmann M.)

2.2 Experimental design

The experimental site was designed by the researchers of the SECCIA (Simulation of Effects of Climate Change in a Shrubland Affected by Fire) project of the Universidad de Castilla La Mancha, Toledo. The study area was divided in 25

plots, which were organized in 5 blocks (A, B, C, D and NEU) of 5 rows (1-5). NEU 5 was divided in two plots (Fig. 5). Each plot enclosed an area of 36 m^2 (6 x 6 m), but only the inner 5 x 5 m were used for sampling to avoid the edge effect. In blocks A-D all treatments and in block NEU only burned and unburned treatments were represented (Fig. 5). Samples were collected from September (2009) until February (2010) according to the Table 1.

Fig. 4 – The study area after burning (Source: Dannenmann M.)

The following treatments were installed (Fig. 5): ambient precipitation (C) divided in burned plots = red and control plots = green; mean historical rainfall (CH): ~ - 25% of historical rainfall (T1); ~ -50% of historical rainfall (T2). The historical rainfall is based on the rainfall measured at the meteorological station Los Cortijos over the last 60 years. At treatments T1 and T2 movable roofs with an integrated automated irrigation system was installed. The roofs were closed automatically during rainfall. Samples for this study were only taken in control and burned plots of the NEU plots.

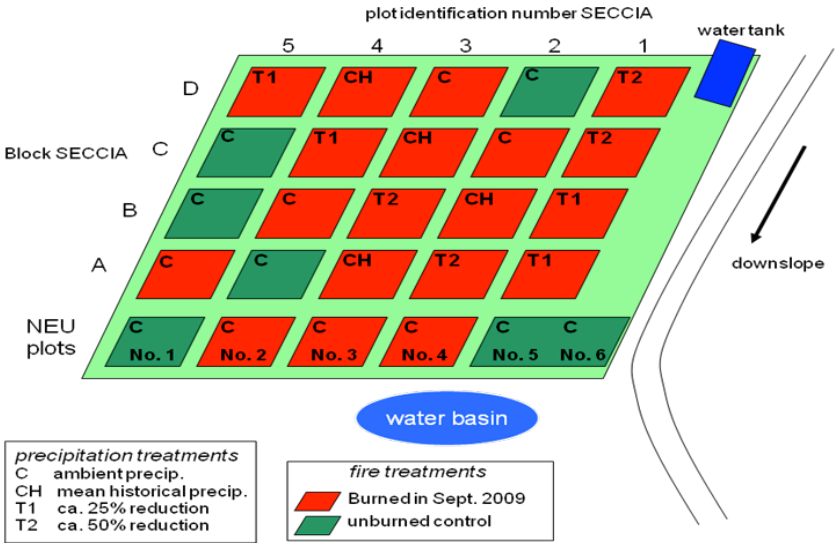

Fig. 5 – Scheme of the plots and treatments (Source: Dannenmann M.)

Table 1 – Sampling dates and analysis.

Leaf Litter, Ash and Soil Samples	Date									
	22.09.09	24.09.09	26.09.09	06.10.09	27.10.09	09.11.09	25.11.09	18.12.09	14.01.09	24.02.09
Soil inorganic N		fire								
N-mineralization										
Microbial community										
Gas fluxes										
NO_3 and NH_4 leaching										

2.3 Soil sampling

Potential soil GHG fluxes:

For this study samples were taken from the NEU plots to determine potential N_2O, NO, CH_4, CO_2 fluxes, microbial community composition (PLFA), C and N in the microbial biomass, NH_4^+, NO_3^- and N-mineralization from burned and control plots. The area was burned on 24th September 2009 (Fig. 6).

To avoid the negative effect caused by sieving (Reichstein et al, 2005) we used intact soil cores for the determination of potential N_2O, NO_x, CH_4 and CO_2 fluxes. Two days after burning 5 cm of the A-horizon were collected by means of 54 steel cylinders (diameter, 9 cm; height, 5 cm) from three NEU control plots (n=27) and three NEU burned plots (n=27) (Fig. 7). Leaf litter or ash layer were removed prior to sampling. The cores were excavated, sealed with Parafilm and then shipped to the laboratory in Austria in a cooling box with ice cartridges.

Soil analysis from sieved soils:

For PFLA, microbial biomass C and N, NH_4^+, NO_3^- and N-mineralization analysis, litter/ash and soil samples were collected at the six NEU plots two days before burning. For the litter/ash sampling 3 x 900 cm^2 were collected randomly over each plot. Litter height was measured. The 3 samples were pooled per plot. Due to the spatial heterogeneity of the plots (observed in a pre-experiment) samples were taken in a grid design (50 cm x 30 cm) (Fig. 7). The soil samples were collected by means of a small steel corer with a diameter of 2 cm and a depth of 5 cm (Fig. 8). After burning, samples were collected 2, 12 and 46 days, as described above, but with ~ 5 cm offset of the previous sample spot to avoid recollection of previously disturbed soil. Soil samples were sieved after sampling (5 mm mesh size) and stored at 5°C in PVC bags. The litter and ash samples were ground in the laboratory.

Fig. 6 – Study area during and after burning on 24th September, 2009. Photo: Tejedor

Nitrogen leaching:

Resin bags were used to determine inorganic nitrogen leaching in the SECCIA Plots. They were made of a polyester fabric 120.35 (120 = Mesh, 35 micro resistance wire) and included an area of 78.5 cm^2 (Fig. 9). Each bag was filled with 6g of Amberlite, IR 120 (Na^+- ion exchanger resin), and 6g Dowex 1 x 8 (Cl^- - ion exchanger resin). In total 24 resin bags were buried in 12 plots (n=2/plot) (6 control, 6 burned). Two resin bags were placed in 30 cm soil depth under intact soil to avoid sampling from leachate from destroyed soil. A central hole was buried in each plot, which was stabilized with a wire mesh (Fig. 10). In 25 cm depth two tunnels (2 cm height, 15 cm length) were excavated uphill in an angle of ~ 45°. The first set of samples were inserted on 29th of September and changed in a monthly interval (27.10.2009, 25.11.2009, 18.12 2009, 14.01.2010 and 24.02.2010). Before the placement of the resin bags into the tunnels they were moistened with distilled water. During incubation the holes were closed with soil filled plastic pots (Fig. 10). After exposure of one month, resin bags were changed and stored in PVC bags. All samples

were sent to the laboratory in Vienna, Austria, in cooling boxes with ice cartridges, where they were stored at 5°C until analysis.

Fig. 7 – Plots from the study area. Control, burned and roofed plots and the grid sampling scheme for soil sampling. Photo: Póvoas

Fig. 8 – Steel corer used for soil sampling. Photo: Póvoas.

2.4 Laboratory analysis

Potential soil GHG fluxes:

For each site (control and burned) three intact soil cores were oven-dried at 105°C for 24 hours to determine the gravimetric water content. Soil moisture content is expressed as water-filled pore space (WFPS), which embraces bulk density, porosity and particle density (2.65 g cm^{-3}). The calculated WFPS was considered to be representative for the other soil cores. After completion of the experiment each soil core was oven-dried at 105°C in order to determine the actual WFPS.

The 24 soil cores of each treatment were divided in 6 groups à 4 cylinders and adjusted to previously defined WFPS (10%, 20%, 40%, 60%, 80% and 100% WFPS) by drying or adding water at least 3 days before analysis started. Soil samples were incubated under 6 different temperatures (5, 10, 15, 22.5, 30, and 37.5°C). NO and CO_2 was measured with an automatic system (Schaufler et al., 2010). NO was detected with a chemoluminescence detector (HORIBA APNA 360) and CO_2 with a IR analyzer (EGM4, PP-systems). For NO and CO_2 flux determination 12 samples were incubated starting at 5°C for 8 hours (Fig. 11), followed by the manual gas sampling for N_2O and CH_4 (see below). Afterwards the temperature was increased in 5-7.5°C steps until 37.5°C and N_2O and CH_4 was sampled in between.

Leaf litter and ash was adjusted to 10%, 30% and 40% water content. The litter and ash samples were placed in stainless-steel cylinders (diameter: 7.2 cm, height: 5 cm). Filling height was 2 cm which is the mean litter depth in the field. NO, CO_2, N_2O and CH_4 from litter and ash samples were determined in the same way as the mineral soil samples.

Fig. 9 – Resin bags handmade by Brigitte Schraufstädter. Photo: Póvoas

N_2O and CH_4 measurements:

At the end of the determination of NO and CO_2 the samples were incubated in static chambers (adapted Kilner jars (Vol: 685 cm^3) for a period of ½ hour. The chambers are fitted with rubber septa in the lid for gas collection with a syringe. 25 ml of air was injected into each chamber at the beginning of the measurements cycle. 12 ml of gas sample was extracted from each chamber at intervals of 0, 15 and 30 min, and injected into sealed (rubber lid and aluminum cap) and evacuated sampling vials. The vials were sealed with silicon grease and stored at 5°C not exceeding 3 days of storage.

The gas samples were analyzed with a chromatography (AGILENT 6890N connected to an automated system sample-injection (AGILENT TECH G1888, Network HEADSPACE-SAMPLER) (Fig. 12). The oven temperature was set at 35°C. Nitrous oxide was detected with a 63Ni-electron-capture detector (ECD) (detector: 350°C) and CH_4 by a methanizer and a flame ionization detector (FID) (detector: 250°C).

Fig. 10 – Construction of the tunnels for the resin bags. Photo: Póvoas

Nitrogen was used as carrier gas for ECD with a flow rate of 25 ml min^{-1}, while Helium served as the carrier gas for the FID (flow rate: 3.5 ml min^{-1}). Calibration was performed using 1, 2.5 and 5 µl l^{-1} N_2O (Inc. Linde) and 2.5, 3.5 and 5 µl l^{-1} CH_4 (Inc. Linde) (in N_2). Detection limits are 0.021 ppm N_2O and 0.382 ppm CH_4. Fluxes of N_2O (µg N_2O-N m^{-2} h^{-1}) and of CH_4 (µg CH_4-C m^{-2} h^{-1}) were determined by headspace concentration changes over time in the test chambers. Mean values are shown with standard error (S.E.).

Water Content (WC%):

For the determination of the WC% we put 5g of each sieved and/or ground sample at 105°C for 24 hours in the drying oven (Co: Hereaus). After this period we weighed the samples and calculated the amount of water in the samples.

pH measurements:

For the determination of pH we mixed 2g of each sieved and/or ground sample with 25 ml 0.01 M $CaCl_2$-solution. The pH of samples was measured with a pH-Meter (Co: Franz Morat KG GmbH & Co).

Fig. 11 – Incubated samples in the measuring system. Photo: Póvoas

Chloroform fumigation extraction (CFE) - microbial biomass N and C:

Microbial biomass C and N were determined using a modified version of the CFE method (Schinner et al., 1996). Eleven grams of sieved soil and 5g of ground litter/ash samples were placed in 100 ml Erlenmeyer flask that were placed in an Exsiccator for fumigation. With a pump the Exsiccator was subjected to vacuum for 5 minutes. Then the Exsiccator was decompressed for 2 minutes. After this period the Exsiccator was again subjected to vacuum for 7 minutes, and then the Exsiccator was depressurized. This procedure was repeated five times. The soil samples in the Erlenmeyer flasks were kept inside a desiccator with sodium lime and wet filter papers within a chloroform atmosphere for 24 h at 25 °C. After fumigation the samples were split into two 5g soil samples/2g litter or ash and the remaining 1g of soil was withdrawn. Twenty-five ml of 2 M KCl solution were added to the samples that were then shaken for 30 min and afterward filtered through N-free filters. Control samples were processed using the same procedure.

We added 25 ml 2M KCl solution to the samples, which where then shaken for 30 minutes in the shaker (Co: GLF Gesellschaft für Labortechnik mbH). The solutions were filtered through N-free filter paper and frozen immediately.

Fig. 12 – AGILENT 6890N connected to an automated system sample-injection (AGILENT TECH G1888, Network HEADSPACE-SAMPLER. Photo: Póvoas.

Solutions were diluted with distilled water in 1.5 ml Eppis reaching a total of 200 µl. 200 µl of reagent and 50 µl of sodium citrate were mixed into the diluted samples. The samples were placed for 30 minutes in a water bath at a temperature of 100°C, mixed with 500 µl v/v Ethanol, pipetted into microtiter plates (250 µl of each sample) and measured immediately at 570 nm wavelength in a photometer (Co: SZABO-SCANDIC HandelsgmbH & Co KG).

Microbial biomass C and N contents in µg g^{-1} dry matter were calculated by subtracting C and N contents of the control sample from mean C and N contents of the two fumigated samples.

Microbial biomass C was estimated from the difference of organic carbon measured by a TOC/TN analyzer in KCl extract of fumigated and unfumigated soils.

Calculation of µg ninhydrin-reactive-N g^{-1} dw: VP * V * 100 / EW * ml * %dw = µg Ninhydrin-reactive-N * g^{-1} dw. (VP: total value the photometer (µg N); V: total volume of extract (ml); EW: weighing (g); ml: filtrate aliquot; 100 * $\%^{-1}$ dw: dry weight).

NO_3^- and NH_4^+ in leachate:

In the laboratory resin bags were carefully washed with distilled water to remove any soil and placed in plastic bottles which were filled with 100 ml 1 M NaCl solution. Samples were shaken with 690-700 rotation min-1 for 30 minutes. Solutions were then filtered in 200 ml bottles, for nitrate and ammonium extraction. This procedure was performed twice. The extracted samples were frozen immediately.

The resin bags were immediately regenerated after the extraction. They were placed in a solution of 1.6 M NaCl in plastic bottles of 200 ml and shaken of a period of 30 min. After the 30 minutes in the Shaker resin bags were rinsed four times with distilled water and dried. For the NH_4^+ and NO_3^- analysis, extracted samples were analyzed as mentioned below.

NO_3^-, NH_4^+ from soil/litter/ash samples:

NH_4^+ and NO_3^- concentrations were determined from the 3 sieved soil samples per plot according to Kandeler (1996). 2.5g of soil, litter/ash sample were weighted into plastic bottles of 200 ml and extracted with 25 ml or 50 ml 0.1M KCL solution for soil samples and litter and ash samples, respectively. The solution was shaken for 30 minutes at a rotation of 690-700/min. The solutions were then filtered and frozen until analysis.

Nitrate analysis:

For nitrate analysis, we used the chemical components hydrochloric acid 32%, Vanadium (III) Chloric; N-(1-Naphtyl) ethylenediamine- dihydrochloride; Sulfanilic acid and Potassium nitrate.

Samples were diluted directly in microtiter plates, with the final volume of 100 µl. Calibration curve was also pipetted in microtiter plates. Then up to 100 µl vanadium (III) chloric solution and 100 µl mixed solution was pipette into the samples. To make the mixed solution we mixed the same volume of solution N-(1-Naphtyl) ethylenediamine and Sulfanilic acid. We subsequently incubated the microtiter plates with prepared samples at 37°C in the dark for 30 minutes. After incubation, the samples were immediately measured at 540 nm wavelength on a photometer (Co: SZABO-SCANDIC HandelsgmbH & Co KG)

Ammonium analysis:

For ammonium analysis we used the following chemicals: Sodium nitroprusside, Sodium salicylate, NaOH pellets, Dichloroisocyanuric sodium salt and Ammonium chloride.

The samples were diluted in Eppis to achieve the desired concentration. Then we mixed the samples with 100 µl Dichloroisocyanuric solution and 250 µl mixed solution, which was designed by mixing the same volume of Sodium hydroxide solution, Nitroprusside salicylate and distilled water. We left the samples to stand for 30 minutes, after this period we pipetted 250 µl of the calibration curve and samples in a microtiter, which was immediately measured at 660 nm wavelength in a colorimetric method in a photometer.

The following formula was used for calculation of results: (VP * V * 100) / (ws * %dw) = µg NO_3-N g^{-1} dw. (VP: total sample the photometer; V: total volume of extract (ml); ws: wet soil (g); 100 * $\%^{-1}$ dw: factor of dry matter).

N-mineralization:

Three times 5g of each soil sample and three times 2.5g of each litter and ash sample. For the blank values, we repeated this step. The blank samples were mixed in 100 ml bottles with 15 ml distilled water and 15 ml of 2M KCl solution. The samples for incubation were mixed without 2M KCl solution. Samples were shaken for

30 minutes at a rotation 690-700/min. Blank sample solutions were filtered and frozen until the NH_4^+ measurements. The samples for incubation were placed in an oven with 40°C for a 7 days period. After this time the solutions were mixed with 15 ml of 2M KCl solution in a shaker for 30 minutes at 690-700/min rotation. The solutions were filtered and frozen until NH_4^+ analysis.

Measurement of NH_4^+ was done as previously mentioned. The difference of blank sample and incubated sample gives the mineralized N.

Microbial community composition: Phospholipid Fatty Acid Analysis (PLFA):

For PLFA analysis we used a modified method after Frostegård et al. (1991) and Hackl et al. (2005). Water content of the soil samples was measured prior to the procedure for adjusting the method and further calculations, then 1.5g fresh (leaf litter, ash and) soil sample was extracted with a chloroform:methanol:citrate buffer mixture (1:2:0.8, v/v/v). The lipids were separated into neutral lipids, glycolipids and phospholipids on a silica acid column. The phospholipids were subjected to a mild alkaline methanolysis. The extraction with fatty acid methyl esters were analyzed with a HP 6890 Series gas chromatograph instrument equipped with a 7683 Series injector and auto sampler on a HP-5 capillary column (50.0 m, 0.20 mm, 0.33 µm) and detected with a FID (flame ionization detector) using Helium as carrier gas (Hewlett Packard, Wilmington, Delaware, USA). Temperatures of the injector and detector were 280°C and 350°C, respectively. The injected sample volume was 1 µl (spitless mode injection). The initial oven temperature of 70°C was maintained for 1.5 min, and then subsequently raised by 30°C min^{-1} to 160°C, by 4°C min^{-1} to 270°C, and by 30°C min^{-1} to the final temperature of 300°C, which was held for 39 minutes. All GC measurements included a blank sample with the internal standard (peak 19:0, nonadeconoate fatty acid), one sample with a standard qualitative bacterial acid methyl esters mix (BAC mix) and one sample with a standard qualitative fatty acid methyl esters mix (FAME mix; both Sigma Aldrich Co., St. Louis, MO) for easier identification of the fatty acid peaks.

In total 32 peaks were detected per sample. The areas measured by GC-FID were used for calculating the abundance of PLFA markers in nmol g^{-1} dw. For

characterizing the community structure we used the terminal-branched saturated PLFA peaks i15:0, a15:0, i16:0, a16:0, i17:0, a17:0 as marker for Gram positive bacteria (Federle, 1986; Zelles, 1997), the mono-unsaturated and cyclopropyl saturated peaks 16:1ω5, 16:1ω9, 17:1ω9, cy17:0, 18:1ω11, cy19:0 were used as indicators for Gram negative bacteria and the PLFA peaks 14:0, 15:0, 17:0 for unspecific bacteria (Frostegård et al. 1993;). 18:2ω6,9 was used as fungal PLFA marker (Kaiser et al., 2010). The methylic, mid-chain-branched saturated PLFA peaks 10Me16:0, 10Me17:0, 10Me18:0 were used as indicators for actinomycetes (Frostegård et al., 1993). We used 16:1ω11 (Olsson et al., 1999) as arbuscular mycorrhiza peak and the peaks 20:4ω6 and 20:0 were used as indicators for protozoa (White et al., 1996). Total PLFA bacteria were calculated of the sum of Gram negative, Gram positive and unspecific bacteria.

3 Results

3.1 Methane fluxes

The variability of CH_4 fluxes was high in our samples (Fig. 13 and 14). Methane fluxes from litter and soil (burned and unburned) showed no general trend and no significant differences could be observed between the two treatments, the incubation temperature or the water content. However, we could find a tendency of higher uptake rates in dry samples.

3.2 Carbon dioxide fluxes

Litter: At 20-40% WFPS, CO_2 emissions proved to be quite predictable, both for leaf litter and ash samples emissions increased with the rise of temperature, with maximum emission occurring between 22.5°C and 30°C, and lower emissions at 37.5°C. The lowest emission rates were observed at 20% WFPS at 37.5°C (2.92 ± 1.74 µg CO_2-C m^{-2} h^{-1}), and the highest rates were observed in 40% WFPS at 30°C (1415.48 ± 932.89 µg CO_2-C m^{-2} h^{-1}). At 20% WFPS, ash samples showed higher emissions than leaf litter. But litter samples with 30% and 40% WFPS had higher emission rates for leaf litter (Fig. 15).

Soil: Like leaf litter and ash, mineral soil samples followed the expected patterns. In all incubations, samples showed increasing emissions with a rise of temperature, reaching maximum emission at 30°C and dropping again at 37.5°C (Fig. 16). For all incubations, control plots had higher emissions than burned plots. The lowest emission rates were observed in 10% WFPS in control plots (9.60 ± 8.12 µg CO_2-C m^{-2} h^{-1}), and in burned plots (8.01 ± 9.64 µg CO_2-C m^{-2} h^{-1}) at 5°C and 10°C respectively. Emissions also increased as WFPS increased, reaching a maximum for control plots (597.34 ± 556.04 µg CO_2-C m^{-2} h^{-1}) and burned plots (556.27 ± 278.43 µg CO_2-C m^{-2} h^{-1}) at 40% WFPS, and in control plots (606.10 ± 468.96 µg

CO_2-C m^{-2} h^{-1}) and burned plots (492.88 ± 152.30 µg CO_2-C m^{-2} h^{-1}) at 60% WFPS at 30°C; at 80% and 100% WFPS emissions decreased again.

In our study, we observed that the ideal moisture for CO_2 emissions was between 40% and 60% in both burned soil samples and control samples. The CO_2 emissions rose exponentially with temperature and humidity, but at 37.5°C the emissions dropped. The same occurred when samples were above 80% WFPS. All samples had a CO_2 emission peak at 30°C, except at 30% humidity—where the peak of control soil samples was 22.5°C. Leaf litter samples also had higher emissions than ash samples, except at 20% humidity, where emissions were higher in ash samples.

3.3 Nitrous oxide

Litter: In leaf litter, for both control and burned plots (ash samples) we observed an increase in nitrous oxide emissions as WFPS increased. In 10% WFPS, N_2O was mainly taken up. In 40% WFPS, nitrous oxide emissions of both burned and control plots decreased with the temperature rise, with a small uptake at 37.5°C in control (-0.95 ± 5.25 µg N_2O-N m^{-2} h^{-1}) and burned plots (-0.45 ± 5.05 µg N_2O-N m^{-2} h^{-1}) (Fig. 15). The peak emission rate was at 40% WFPS at 10°C for control plots (12.73 ± 14.09 µg N_2O-N m^{-2} h^{-1}) and at 5°C for burned plots (17.58 ± 2.25 µg N_2O-N m^{-2} h^{-1}).

Soil: In soil samples, nitrous oxide emissions were higher at higher temperatures and increased with soil moisture, which increased significantly from 15°C to 22.5°C in all incubations—both in control and burned plots (Fig. 16). Control plots had an emission peak at 30°C, with a drop in emissions at 37.5°C at all WFPS—except in 10% WFPS. The maximum emission rate for control plots (253.03 ± 331.01 µg N_2O-N m^{-2} h^{-1}) occurred at 80% WFPS. However burned plots had peak emissions at 37.5°C with 100% moisture (642.86 ± 890.66 µg N_2O-N m^{-2} h^{-1}). Variability within the soil cores was high and therefore no significant differences could be found between control and burned soil.

3.4 Nitric oxide

Litter: In litter samples we observed NO emissions at 20% WFPS, which increased as the temperature rose, reaching a maximum emission at 30°C (8.8 ± 2.37 µg NO-N m^{-2} h^{-1}). There was a small uptake at 37.5°C. In ash samples NO was taken up at all temperatures and all WFPS, except at 30% WFPS. From litter samples we observed higher NO emissions with the rise of temperature, but emissions were reduced at 37.5°C. Ash samples showed the highest uptake in 20% WFPS, with a peak at 37.5°C (-13.95 ± 1.48 µg NO-N m^{-2} h^{-1}), but a reduced uptake as WFPS increased. On the other hand, leaf litter samples had an optimum uptake in 40% WFPS, with a peak at 15°C (-8.24 ± 2.58 µg NO-N m^{-2} h^{-1}) (Fig 15).

Soil: At 10% WFPS there was nitric oxide uptake between 5°C and 15°C in control plots with maximum uptake at 10°C (-14.0 ± 9.72 µg NO-N m^{-2} h^{-1}). For burned plots in 10% WFPS, there was uptake between 5°C and 10°C, with a peak at 10°C (-2.84 ± 2.62 µg NO-N m^{-2} h^{-1}). All soil samples showed the same pattern: increased emissions at higher temperatures; emissions of burned soil samples were relatively higher than control soil samples, but variability was high in burned soil. The highest emission rate for burned soil samples was found in 60% WFPS, with a peak at 37.5°C (291.40 ± 339.64 µg NO-N m^{-2} h^{-1}). Reduced rates were found in 80% and 100% WFPS. However, emissions had an optimum WFPS in control soil samples at 40%, with a peak at 37.5°C (82.49 ± 72.84 µg NO-N m^{-2} h^{-1}), reduced emissions with increasing WFPS (80% and 100%). Lowest emission rates were observed at 100% WFPS (Fig 16).

3.5 Water content (WC%)

The higher WC% was found on the first sampling day in unburned soil samples (29.8 ± 1.75%), and on 26 September 2009 in burned soil samples (18.8 ± 3.18%). In litter samples we observed the highest WC (19.9 ± 8.51%) on the first day and for ash samples (8.7 ± 6.37%) on 26 September 2009. For soil samples—burned and unburned (12.14 ± 1.10% and 12.9 ± 2.00%)—as well as for litter (10.06 ± 3.5%)

and ash samples (3.7 ± 1.1%), we observed a gradual decrease until 6 October 2009. In both soil (18.0 ± 1.86% for unburned plots and 16.7 ± 1.69% for burned ones) and ash samples (11.0 ± 2.39%), there was a slight WC increase on 9 November 2009, because of a light rain that occurred during the last two sampling days. Leaf litter WC increased considerably (22.1 ± 2.85%) in the last sampling day due to the high moisture absorption capacity of leaf litter in relation to the soil and ash. For burned soil samples, as well as for ash samples, WC was lower on almost all days in relation to leaf litter and unburned soil samples—except for 6 October 2009, when burned plots had higher WC (12.9 ± 2.0%) than unburned plots (12.4 ± 1.1%) (Fig. 17).

3.6 pH

In the period between September and November 2009, there was no significant change in soil pH, and the average pH was around 5.45 ± 0.13. After burning the soil pH rose by 0.3 (Fig. 18).

The average pH of the litter was between 4.42 ± 0.47 and 4.65 ± 0.04, and the pH of the ashes was between 7.67 ± 0.76 and 7.87 ± 0.74 (Fig. 18).

3.7 Microbial community composition (PLFA´s)

Gram+ bacteria: All burned samples had a lower concentration than control samples (Fig. 19). Microbial community didn't show large variations with regard to gram+ bacteria over time for litter and soil in both control plots and burned plots. The mean concentration of control soil samples was between 19.54 ± 3.32 and 26.92 ± 7.23 nmol g^{-1} dw, and for burned soil samples, 14.95 ± 1.12 and 21.96 ± 1.63 nmol g^{-1} dw. Ash samples had lower rates than litter samples—the mean was between 5.81 ± 2.36 and 9.79 ± 0.73 nmol g^{-1} dw. The mean of the litter samples was between 34.62 ± 7.98 and 37.22 ± 7.79 nmol g^{-1} dw.

In mineral soil samples we found positive correlations only for burned treatments. These were gram+ bacteria with WC%, NH_4^+ concentration, N-mineralization, TN, TC (Table 2-3).

Gram- bacteria: The concentration of gram-bacteria didn't vary in control soil samples over time (Fig. 19), (47.27 ± 12.66 to 56.51 ± 16.55 nmol g^{-1} dw). Burned soil samples had lower rates than control samples, but these also didn't differ over time, (29.77 ± 3.62 to 46.49 ± 4.51 nmol g^{-1} dw).

In leaf litter samples we observed significantly higher rates than in ash samples. Concentrations reached their peak (138.64 ± 26.51 nmol g^{-1} dw) in October, and a minimum in September (107.37 ± 23.71 nmol g^{-1} dw). We observed a small increase until October; in November gram- bacteria experienced a slight decrease.

In ash samples, we observed the opposite of leaf litter samples: samples experienced a decrease from September until November. Highest concentrations were observed shortly after burning (31.21 ± 3.59 nmol g^{-1} dw) and lowest concentrations (16.92 ± 5.42 nmol g^{-1} dw) were measured in November.

Unspecific bacteria: The unspecific bacteria suffered a gradual decrease in its concentration in control soil samples (Fig. 19) from September (39.10 ± 8.19 nmol g^{-1} dw) to October (32.13 ± 9.99 nmol g^{-1} dw). Rates increased (37.48 ± 11.60 nmol g^{-1} dw) on November. Burned plots experienced a gradual decrease over time, reaching a peak (27.30 ± 2.18 nmol g^{-1} dw) in September, and a minimum (20.18 ± 1.92 nmol g^{-1}dw) in November. In ash samples we found negative correlations between gram+ bacteria, sum of bacteria and fungi and NO_3^- and positive correlations with NH_4^+.

On 22 September 2009 rates of leaf litter samples were 154.64 ± 50.23 nmol g^{-1} dw; on 26 September 2009 they were 217.54 ± 60.24 nmol g^{-1} dw and in October they were 219.60 ± 50.46 nmol g^{-1} dw. During these days no differences were observed. In November, however, we observed a decrease in the rate (166.87 ± 46.96 nmol g^{-1} dw). In ash samples the peak was observed in November (29.43 ± 9.08 nmol g^{-1} dw), the minimum (17.96 ± 7.0 nmol g^{-1} dw) was observed in October. In September after burning (26.25 ± 5.75 nmol g^{-1} dw) and in November no differences in concentration were observed.

Leaf litter samples had significantly higher concentrations than ash and mineral soil samples (control and burned).

Sum of bacteria: In control soil samples we could find a slight increase of bacteria (Fig. 19) until November (117.87 ± 33.57 nmol g^{-1} dw). In burned plots, we observed the opposite, with an increase over time—reaching its peak in September (82.83 ± 6.28 nmol g^{-1} dw) and its minimum (65.37 ± 7.91 nmol g^{-1} dw) in November.

Leaf litter samples had higher concentrations than burned, control and ash samples. From September until October there were no significant changes, but from September until November there was a decrease in concentrations.

Fungi: After burning fungal biomass was reduced (Fig. 20). Concentrations of fungal biomass increased from September onwards reaching a peak (17.85 ± 11.03 nmol g^{-1} dw) in November. Burned plots had significantly lower concentration than control plots, the lowest value (4.14 ± 0.30 nmol g^{-1} dw) was observed in November, and the peak (9.86 ± 1.98 nmol g^{-1} dw) in October.

Leaf litter samples had higher values than ash, control soil and burned soil samples. From September (176.11 ± 80.34 nmol g^{-1} dw) until October (579.17 ± 111.2 nmol g^{-1} dw), samples experienced a gradual increasing, but in November concentration decreased (224.18 ± 31.96 nmol g^{-1} dw). Ash samples didn't show significant differences (28.18 ± 9.65 to 37.0 ± 13.53 nmol g^{-1} dw). Fungal biomass was negatively correlated to pH (Table 4).

Actinomycetes: Actinomycetes showed no significant differences in control samples (Fig. 20) over time (15.77 ± 3.87 to 17.03 ± 2.98 nmol g^{-1} dw). Burned plots had a mean concentration between 10.05 ± 1.11 and 12.87 ± 1.17 nmol g^{-1} dw, and in November less actinomycetes were detected.

Leaf litter samples showed no significant differences between September and October and their mean was between 14.23 ± 4.3 and 15.22 ± 2.71 nmol g^{-1} dw. In November rates reached their peak (18.26 ± 4.75 nmol g^{-1} dw). In ash samples we

observed an increase of concentration over time, with highest values (6.33± 0.63 nmol g^{-1} dw) recorded in September, and lowest (3.74 ± 1.05 nmol g^{-1} dw) in November.

Protozoa: Over time we didn't observe significant differences in protozoa in control plots (Fig. 20), and the mean was between 4.53 ± 1.14 and 5.30 ± 0.96 nmol g^{-1} dw. Burning didn't not change the amount of protozoa from September until October, but concentrations decreased slightly in November 2009 (2.58 ± 0.44 nmol g^{-1} dw).

In relation to control, burned and ash samples, leaf litter samples had higher rates, suffering a gradual increase from 22 September 2009 (20.97 ± 5.48 nmol g^{-1} dw) to November (33.87 ± 4.03 nmol g^{-1} dw). Ash samples had a peak (7.11 ± 1.94 nmol g^{-1} dw) on 26 September 2009, and between October (4.24 ± 1.57 nmol g^{-1} dw) and November (3.79 ± 1.53 nmol g^{-1} dw) we didn't observe significant differences.

In leaf litter samples there were only negative correlations between protozoa and NO_3^-, NH_4^+ and N-mineralization (Table 5)

Arbuscular mycorrhiza (AM): We observed that burned plots didn't suffer significant changes in *AM* (Fig. 20), and that its average was between 3.19 ± 0.38 and 3.62 ± 0.47 nmol g^{-1} dw. Also we did not observe changes in control plots from September and October. In November, on the other hand, we observed a slight increase (6.24 ± 1.75 nmol g^{-1} dw).

AM concentrations were relatively lower in leaf litter samples in relation to control soil samples. The minimum value observed was in September (3.11 ± 1.08 nmol g^{-1} dw), but its peak was in October (4.66 ± 1.67 nmol g^{-1} dw). In the last two sampling days we observed a small increase in relation to the September samples, but not a significant increase. In ash samples we didn't observe significant changes, the mean being between 0.99 ± 0.18 nmol g^{-1} dw and 1.63 ± 0.37 nmol g^{-1} dw.

3.8 Nitrogen in the microbial biomass (Nmic)

In control plots the Nmic peak was observed (47.01 ± 15.05 µg N g^{-1} dw) in soil samples in September. From September to October we observed a significant decrease of Nmic (5.26 ± 3.93 µg N g^{-1} dw), which rose significantly (28.56 ± 4.14 µg N g^{-1} dw) on 9 November 2009.

The highest rate (29.91 ± 7.06 µg N g^{-1} dw) in burned soil samples was observed in September. From September to October rates declined significantly (1.12 ± 1.94 µg N g^{-1} dw). From October to November we observed an increase in Nmic (6.94 ± 2.65 µg N g^{-1} dw).

In leaf litter samples, the highest Nmic concentration (48.14 ± 3.55 µg N g^{-1} dw) occurred in November. There was also a drop in Nmic with time, reaching its minimum (12.62 ± 11.05 µg N g^{-1} dw) in October. In ash samples, there was no difference in Nmic for September and October.

3.9 NO_3^- and NH_4^+

Variability within the plots was high. The highest nitrate concentration (19.55 ± 10.69 µg NO_3-N g^{-1} dw) occurred in September in control soil samples (Fig. 21). The lowest rate (2.63 ± 2.28 µg NO_3-N g^{-1} dw) was measured in October. In November we observed a considerable increase (8.60 ± 4.68 µg NO_3-N g^{-1} dw) in NO_3^- concentration, in relation the September samples. Burned plots the highest rate (11.62 ± 6.48 µg NO_3-N g^{-1} dw) was measured in November, and the lowest rate was observed (3.09 ± 3.25 µg NO_3-N g^{-1} dw) in October. In soil samples, as well as in burned and control plots, the lowest value observed matched with the lowest WC%, in addiction we found a significant correlation (Table 2-3).

In leaf litter and ash samples there were no significant differences, the highest concentration (6.79 ± 2.23 µg NO_3-N g^{-1} dw) was found in October, and the lowest (3.24 ± 2.12 µg NO_3-N g^{-1} dw) in November. For leaf litter samples we observed a drop in concentration over time, the highest value was 3.60 ± 0.56 µg NO_3-N g^{-1} dw, in September, and the lowest (1.37 ± 0.63 µg NO_3-N g^{-1} dw) in November 2009 (Fig. 21).

We observed significantly higher NH_4^+ concentration in burned plots compared to control plots. In soil samples NH_4^+ concentration was positively correlated to water content in soil samples (Table 3-4). The highest concentration for burned and control plots was measured in September (53.16 ± 15.41 and 49.27 ± 3.25 µg NH_4-N g^{-1} dw). The NH_4^+ concentration in mineral soil samples exhibited the lowest values in the drier period, the lowest rate were measured in October in burned (29.84 ± 7.92 µg NH_4-N g^{-1} dw), and control samples (13.04 ± 3.71 µg NH_4-N g^{-1} dw). In November concentrations increased again in control plots (control: 21.77 ± 2.45 µg NH_4-N g^{-1} dw and burned: 47.42 ± 12.69 µg NH_4-N g^{-1} dw) (Fig. 22).

Similar to soil samples, we observed a higher NH_4^+ concentration in ash samples in relation to leaf litter samples. Furthermore, ash samples had their lowest value on drier days, with the peak (40.06 ± 13.76 µg NH_4-N g^{-1} dw) in September, and the lowest rate (24.88 ± 15.67 µg NH_4-N g^{-1} dw) in October. Leaf litter samples reached a peak (23.16 ± 4.91 µg NH_4-N g^{-1} dw) in September, and the lowest concentration was measured (2.75 ± 0.79 µg NH_4-N g^{-1} dw) in November (Fig. 22).

3.10 Nitrogen mineralization (Nmin)

In soil samples, Nmin was highest in unburned samples (183.45 ± 6.90 µg N g^{-1} dw $week^{-1}$) on 22 September 2009, and the lowest rate (86.5 ± 17.51 µg N g^{-1} dw $week^{-1}$) occurred in October (Fig. 23). In burned plots the highest mineralization rate (145.43 ± 26.75 µg N g^{-1} dw $week^{-1}$) occurred on 26 September 2009; the lowest rate (83.13 ± 12.03 µg N g^{-1} dw $week^{-1}$) was observed in October. We observed a decrease in nitrogen mineralization in burned and control plots over time, reaching a minimum in October and rising again in November.

In leaf litter samples, Nmin was low compared to soil samples. The highest Nmin rate (16.06 ± 13.99 µg N g^{-1} dw $week^{-1}$) was recorded in September, and the lowest rate in November.

We observed that on drier days less N was mineralized than on wetter days for soil samples. However, variability within the plots was high and no significant differences were observed.

We also observed a dependency of NH_4^+, NO_3^- and N-mineralization rates with water content for soil samples (Table 2-3). We didn't observe this pattern in leaf litter and ash samples.

3.11 Nitrogen leaching

Variability within the plots was high and therefore no significant differences could be observed between control and burned plots. We observed a higher NO_3^- and NH_4^+ leaching in burned plots on rainy days, but during dry days leaching was insignificant. NO_3^- leaching was much higher than NH_4^+ leaching, as expected.

The analysis of NH_4^+ leaching showed that despite the fire, slightly more NH_4^+ was leached from burned plots (Fig. 24). The highest leaching rate (20.21 ± 9.14 µg NH_4-N m^{-2} d^{-1}) occurred in January 2010 in burned plots, where also the highest rate (14.40 ± 4.94 µg NH_4-N m^{-2} d^{-1}) were measured from control plots. The NH_4^+ leaching rate in control plots for the first three sampled months remained almost unchanged. The lowest rate in control and burned plots (7.44 ± 3.67 and 13.00 ± 5.98 µg NH_4-N m^{-2} d^{-1}) was measured in February 2010.

Burned plots showed the highest NO_3^- leaching, except in November 2009. In control plots (5.00 ± 3.51 µg NO_3-N m^{-2} d^{-1}) as well as in burned plots (6.53 ± 5.18 µg NO_3-N m^{-2} d^{-1}) the lowest NO_3^- leaching rate occurred in October. In the first two month after burning NO_3^- leaching was low as precipitation was low. In the last three months, the time when precipitation is high in this area, we observed a significant increase in burned plots, reaching a maximum (245.08 ± 125.15 µg NO_3-N m^{-2} d^{-1}) in January 2010. In the unburned plots there was a significant increase in the last two months reaching a maximum leaching (89.07 ± 150.30 µg NO_3-N m^{-2} d^{-1}) in February 2010.

4 Discussion

4.1 Methane

We found a tendency toward CH_4 uptake in the lower WFPS (Fig. 13-14). Castro et al. (1994) and Borkenet et al. (2000) both found a negative relationship between humidity and CH_4 uptake, and this corroborates our results. We found a high variability in the soil cores. CH_4 uptake and CH_4 production occurs simultaneously (Schlaoufer et al., 2010).

4.2 Carbon dioxide

In our study we observed that the optimum WFPS for CO_2 emissions was between 40% and 60% for both the burned samples and the control soil samples (Fig. 16). For leaf litter and ash samples the optimum WFPS was 40%, however we did not incubated them at higher WC% as the maximum Water Holding Capacities was already reached (Fig. 15). The CO_2 emissions saw an exponential increase relative to the temperature and WFPS increase, but suffered a drop at 37.5°C. For the soil samples with values higher than 80% WFPS, CO_2 emissions started to decrease, the maximum emission rates occurring between 40% and 60% WFPS. The emission peak for all the analysed WFPS factors was at 30°C—except in 30% WFPS to the litter samples, the emission peak of which was at 22.5°C. The optimum temperature for CO_2 emissions is, therefore, 30°C.

Bowden et al. (1998) observed a drop in the respiration rates in very dry or very humid conditions, which corroborates our results. Schaufler et al. (2010) observed that the majority of CO_2 emissions occurred at 40% humidity. Pinto et al. (2002) observed a positive correlation between water content and fluxes of CO_2. Schaufler et al. (2010) also found an exponentially significant increase in CO_2 emissions with the rise of soil temperature. This can be explained by the fact that the microorganisms need humidity for their biochemical functions; consequently, in samples with low humidity the respiration rates were low, and with an increase in

humidity the respiration rates rose, reaching their peak between 40% and 60%. Emissions dropped with the increase of humidity above 60%. This can be explained by the substrate porosity, which due to the high humidity index creates an anaerobic environment, since water fills practically all the substrate pores, thus avoiding oxygenation and ending the microorganism's aerobic respiration.

In general, CO_2 emissions were lower in the burned samples than in the control samples in all analysed WFPS and temperatures. This does not corroborate the results of Rutigliano et al. (2007), which suggest an increase of CO_2 not only during combustion but also during the period after burning. Hamman et al. (2007), on the other hand, found a lower level of respiration in the burned samples than in their controls, just as our results indicate. A possible reason could be the death of the microbiota, plants and roots through fire, added to the fact that after burning, carbon became unavailable to microorganisms (Albrecht et al., 1995).

Fierro et al. (2007) and Pinto et al. (2002) observed in the field that humidity is the determinant factor for CO_2 emissions in the burned samples, but that temperature did not play a significant role. Tejedor (2010) found in the field a decrease in the emitted CO_2 flux of the soil samples during the dry period. Also, a dropin the CO_2 flux in the field after fire was observed, which corresponds with our laboratory observations.

4.3 Nitrous oxide

We observed a small uptake of N_2O in 10% and 20% WFPS in the burned soil samples as well as in their controls (Fig. 16). The emissions in 10%, 20%, and 40% WFPS were very low. Similar results were found by Levine et al. (1996), where it was observed that after burning, N_2O fluxes in dry savannah soils were below the detection limits. Other studies showed similar results (Pinto et al., 2002), where N_2O fluxes were also under the detection limits. This can possibly be explained by looking at the local ecosystem characteristics: savannah soils are thoroughly dry during most of the year. As in our study area, N_2O emissions are very low to almost non-existent, and a small uptake of N_2O existed in the very dry conditions in our laboratory analysis, as well as in the field (Tejedor, 2010).

We also observed an increase of emissions with an increase in WFPS and temperature, reaching a peak in the wettest soil condition.

Schaufler et al. (2010) found a nonlinear increase of N_2O with temperature increase and a positive correlation with soil humidity when analysing different European ecosystems, which corroborates our results. Another study performed by Weitz et al. (1998) in a rainforest in Costa Rica corroborates our results, where immediately after burning there was a short-term N_2O emission peak, and this increase could be linked with the decomposition of the existent NO_2^- in ashes, the same explanation Neff et al. (1995) gave for the increase of NO rates.

Davidson (1991) and Davidson et al. (2000) observed a maximum emission of N_2O between 50% and 70% WFPS, corroborating therefore our results, where the N_2O emission peak was found to be between 60% and 80% WFPS for the control samples. For burned samples the peak occurred in 100% WFPS.

Inclán et al. (2010) studied different forests in central Spain in burned and control areas and detected differences between the N_2O fluxes. The N_2O emissions correlated to the rainfall of the previous day and the soil humidity. In addition, in the most humid months the N_2O fluxes were higher, whereas in the dry months the N_2O fluxes decreased, concurring again with our results. The N_2O uptake was observed in higher temperatures and in lower moisture contents, corroborating part of our data; in one instance we observed uptake at the lowest temperatures—not at the highest. This could have occurred because of various factors, such as the microbial quality of the soil, its biochemical, physical and other qualities, substrate quality, nutrient availability, ecological matters among the microbial communities, relations, interrelations, competition and other factors.

Tejedor (2010) measured N_2O in the field, in the same location as our study. It was observed that the N_2O fluxes in this ecosystem in burned and control samples were very low or non-existent, despite the high level of NH_4^+ and NO_3^-. In the field, there were no ideal conditions for the occurrence of significant emissions of N_2O for samples below 60% WFPS. In the laboratory, the emissions started to increase at a

WC% higher than 60%, which never occurred in the field. High rates of NH_4^+ after burning could indicate a high nitrification potential in this soil.

4.4 Nitric oxide

NO was taken up at low WC% but was emitted in wetter soil (Fig. 15-16).

An emission peak of NO between 50% and 60% WFPS was observed by Davidson (1991) as well as in Davidson et al. (2000), corroborating our results. Nevertheless, another author observed an emission peak of NO far below 60% WFPS (Del Prado et al., 2006). Concerning temperature, Schaufler et al. (2010) observed a nonlinear increase in the NO emissions relative to the temperature, corroborating the findings of our study.

Other authors (Davidson et al., 1993; Cardenas et al., 1993) show biogenic emissions of NO being severely regulated by humidity and nitrogen concentration in the soil. Levine et al. (1996) also detected that through burning and humidity the NO fluxes increased in savannah soils. Neff et al. (1995) detected a significant flux of NO from the soil to the atmosphere after deforestation and biomass burning. All these results show an increase of NO emissions after burning and corroborate our study.

We also observed that NO values were very high—far beyond expected. One explanation can be found in Neff et al. (1995) or Weitz et al. (1998). The burning produces an ash layer rich in NO_2^- resulting in a brief dissociation of the NO_2^- to NO through the biotic action (denitrification) in the soil, increasing therefore the production of NO. This sudden increase in the production of NO ends one to three days after burning, according to Neff et al. (1995). In our study we observed that this period can last up to three weeks after burning, which was the period between gathering samples and laboratory analyses.

Normally after fire, a great amount of NH_4^+ is deposited with the ashes, which was also found in our study. This high amount of ammonium in the soil may also indicate higher emissions of NO, through the activity of nitrifying and denitrifying bacteria, if soil conditions (i.e. soil moisture) are favourable. As in our study, the NH_4^+ rates are high to explain this higher NO fluxes and we suggest that nitrification was a

main process for NO production that these high levels of NO can only be explained as Neff et al. (1995) and Weitz et al. (1998) suggest.

When correlating PLFA groups and the amounts of NH_4^+ and NO_3^- in the ash samples, we observed a positive correlation between NH_4^+ and the PLFA groups: gram+ bacteria, fungi and the sum of bacteria. These groups showed a negative correlation with NO_3^-. One explanation is that these microorganisms would be involved in N mineralization, increasing therefore the amount of NH_4^+ content in the substrate (consequently positively correlated with its PLFA group) and at the same time becoming a factor in the denitrification process, using NO_3^- as an energy source. Nitric oxides (NO_x) are eliminated from the environment, thus increasing the populations of the before mentioned PFLA groups, whereas the NO_3^- concentration would drop (negatively correlating with these populations). Actually, it was expected that some positive correlation between NO_3^- and a PLFA group would be found. It was expected that, with the increase of the amount of NH_4^+ in the substrate, inorganic N offered to the nitrifying microorganisms would be higher, and consequently the amount of NO_3^- in the substrate would be higher, which would end in a positive correlation between the NO_3^- and the nitrifying PLFA group; this, however, we did not find to be the case.

4.5 Microbial community composition

Concerning microbial community, there are very distinct and even conflicting results among previous studies. In our study, all the burned samples analysed had lower PLFA concentrations than their controls—soil and leaf litter. Therefore, in soil samples there were no great differences between burned and control samples, whereas between leaf litter and ash samples, the data showed significant differences.

Esquilín et al. (2007) observed after burning, a reduction of fungi at 5 cm soil depth in a stone-mountain forest in Colorado. Hamman et al. (2007) had similar results in mid-elevation forests in central Colorado. A reduction of the fungi biomarkers in samples with high as well as low burning intensity was. After burning, a reduction of fungi mycelia was also found by Rutigliano et al. (2007) in a shrubland in

southern Italy. Ponder et al. (2009) also observed a drop in fungi markers after burning in a Missouri savannah in the United States. These results corroborate our observations.

On the other hand, Gordon et al. (2007) in his study found higher amounts of fungi and bacteria after heating. These results are similar to those of Díaz-Raviña et al. (2006), who observed a slight increase in the fungi biomarker rates after the heating of soils, by treatment with a fire retarder in humid cambisols in Friol, Galicia, Spain. Mubyana-John et al. (2007) also found an increase of fungi after burning in Okavango Delta in Botswana. All these results are in disagreement with our findings.

In a forest in southwest Spain, Bárcenas-Moreno et al. (2011) found a large increase in the PLFA biomarker for fungi when compared to the control, having a maximum value in acid soils after induced temperature increase. In basic soils, both heated and controlled, the values were low. If the increase of the fungal growth after heating is not taken into consideration, these results correlate with our findings. After burning, the soil pH indicated a small increase, becoming more alcaline, which probably affected the growth of the fungi mycelia negatively after burning, beside of the death of the fungal biomass. In the ash samples, pH increased considerably, directly affecting the mycelia growth.

The pH of leaf litter samples was lower than the pH of the control soil samples. Fungal mycelia were probably positively influenced by the low pH of leaf litter samples because the fungi PLFA biomarker was considerably higher in leaf litter compared to soil samples. Here we also have to consider the substrate structure, the leaf litter probably being more adequate for mycelia fixation (and microorganisms in general) relative to the soil (water retention, nutrient availability, organic C, etc.), therefore positively affecting fungal growth.

We could find a significant negative correlation between fungi and ash pH. Thus, it is clear that pH influences fungi growth since ash pH was higher and the PLFA biomarker for fungi was very low. We must consider the negative effect of fires on the microorganism populations, since fire probably destroyed most of microbiota. Corroborating this observation, Bárcenas-Moreno et al. (2011) suggests that the pH of

their analysed samples after burning was the main factor regulating population growth of microorganisms in their samples, where low pH stimulated the growth of fungi and high pH the growth of bacteria.

Esquilín et al. (2007) found a reduction in the bacterial population in soil samples. Our observations suggest a drop in the rate of bacterial populations as well as in the population of all analysed microorganisms up to a depth of 5 cm, probably in combination with the negative effect of the burning on microorganisms and the effect of low humidity.

Bárcenas-Moreno et al. (2011) did not observe differences in the bacterial PLFA groups with the heating of soil samples, either in acid nor basic soils. Rutigliano et al. (2007) observed an increase of microbial populations after burning. Ponder et al. (2009) also observed higher rates of the bacterial gram+ bacteria and gram- bacteria biomarkers after burning. On the other hand, Díaz-Raviña et al. (2006) observed a drop in the gram+ bacteria PLFAs, but an increase in the gram- bacteria. Mubyana-John, et al. (2007) also observed an increase on the rates of actinomycetes after burning, this result conflicting with ours.

Rutigliano et al. (2007) suggests a greater ability of bacteria to acquire the resources offered after burning—resources like organic C and other nutrients relative to fungi—to explain the low amount of fungi observed in their study. According to our results, fungal selection by pH is valid, but the relation of pH to the bacterial groups was not observed. We should also consider the suggestions of Rutigliano et al. (2007). We also suggest a negative influence of burning and the death of microbial soil communities.

After fire the microbial population was low compared to the control samples, showing a negative effect of fire on microorganism in the Mediterranean shrubland. Another important parameter observed was the dependence of fungi on pH. This PLFA group prefer a low pH, resulting in diminishing levels with the increase of pH. Preferred pH values are between 4.42 and 4.65 for the investigated area.

The gram+ bacteria probably had a significant role in the N mineralization in burned soil samples (Table 2). In the burned soil samples we observed positive correlations only between the gram+ bacteria and WC%, NH_4^+, N-mineralization, TN (Table 2). The only tested factor which showed no correlation with the gram+ bacterial group was the NO_3^- content. This shows the importance of this bacterial group in N transformation.

In leaf litter samples, however, there were only negative correlations. Protozoa correlated to NO_3^-, NH_4^+ and N-mineralization (Table 1.3), probably through competition; the group protozoa is controlled by groups responsible for the mineralization and/or denitrification. In ash samples there were negative correlations with NO_3^- and positive correlations with NH_4^+ between gram+ bacteria, all bacteria and fungi.

Gram+ bacteria, gram- bacteria, fungi and total bacteria are essential to nitrogen mineralization, and gram+ bacteria, fungi, unspecific bacteria and bacteria are essential to nitrogen uptake.

4.6 NH_4^+ and NO_3^-

We observed very high rates of NH_4^+ in the burned soil samples as well as in the ash samples relative to their respective control samples (Fig. 22). These results were already expected, which indicates a positive influence of fire on NH_4^+ rates in this type of ecosystem. Immediately after the fire, NO_3^- rates were very low in the burned soil samples relative to the control samples. Ten days after the fire, the nitrate concentration in the burned samples were slightly higher than the concentration of the controls, and after 44 days the NO_3^- rates in the burned samples were higher than in the controls (Fig. 21), showing a long term nitrification trend after burning, possibly due to the amount of NH_4^+ deposited together with the ashes. For both parameters we found a correlation with WC%, showing a probable dependence of both parameters on WC%. The ash samples presented higher rates of NO_3^- relative to the leaf litter samples, thus showing an immediate nitrification of NH_4^+ into NH_3 by the nitrifying bacteria. Conversely, we didn't find a correlation with WC%. Plants have played an important role regarding the high inorganic N levels, it will take several month until a

newly established vegetation compete with microorganisms for inorganic N, thus reducing again the high levels of NO_3^- and NH_4^+.

Rodríguez et al. (2009), studying a *Pinus canariensis* forest in La Palma (Canary Islands, Spain), observed significantly higher rates of NH_4^+ and NO_3^- in the burned samples relative to the controls, just as we observed in our study. Also Matson et al. (1987) reported a higher concentration of NH_4^+ and NO_3^- in the surface soil after clearing and burning, and this increase was sustained for six months. Another author (Andersson et al., 2004) observed in a woodland savannah in Ethiopia higher rates of NO_3^- from 1 to 90 days after burning, whereas the NH_4^+ rates were not affected within the same time period. The authors of this study suggest as a cause the immediate oxidation of NH_4^+ to NO_3^- through the stimulation of the nitrifying bacteria of the soil after burning, which also support our results.

Sardans et al. (2008) observed in a calcareous shrubland higher amounts of NO_3^- and a reduction in NH_4^+ after heating, probably with immediate nitrification of ammonium, which again partially supports our results. Choromanskaand DeLuca (2002) observed low concentrations of NH_4^+ in a *Pinus ponderosa* forest west of Montana after the heating of samples. The concentration of NO_3^- was high in relation to the control samples, but the soil already had low concentrations of NH_4^+ and high concentrations of NO_3^- before the heating—unlike our results. In the same study, it was observed that the concentrations of NH_4^+ and NO_3^- were lower with the heating of the samples associated with low water potential—corresponding with the results of our study of the soil samples.

We concluded that there is a probable dependence of the rates of NH_4^+, NO_3^- and N-min on the water content for the soil samples. If we observe the NH_4^+ and NO_3^- graphs, as well as the nitrogen mineralization, we observe a dependence of the NH_4^+, NO_3^- and the N-min on the WC% in the soil samples (Table 2-3); this pattern however did not occur in the litter and ash samples.

In a Mediterranean shrubland disturbed by fire in southern Italy, Fierro and Castaldi (2011) observed a significant increase of soil NH_4^+ after burning, and they reported that this pattern continued five months after the fire; this was identical to our

findings. In the same study they also observed an increase of NO_3^- in the burned plots. Nevertheless, this pattern was observed only on the first sampling date—unlike our study, where we observed a tendency for the nitrification process to continue over a longer period for soil samples. The authors, however, mentioned that the inorganic N was extremely low in all treatments after eight months. Since the duration of our study was less than two months, we do not know the amount of inorganic N that would have remained in the soil over a longer period of time.

In a Mediterranean shrubland in the Salento Peninsula in southern Italy, Dannenmann et al. (2010) observed an extractable soil content of NH_4^+ and NO_3^- higher in the burned plots than in the control plots.

4.7 Nitrogen leaching

In our study we observed a higher leaching in the burned plots relative to the control plots of NH_4^+ as well as NO_3^- (plots with environmental precipitation). NO_3^- had very high leaching potential in relation to NH_4^+ (Fig. 24). This result was expected, since nitrate is more soluble than ammonium, therefore it is more easily washed out from the soil. The comparative rates of NH_4^+ were similar, the highest rate occurring in January 2010, which coincides with a period of strong rainfall (data not shown). NO_3^- had higher rates between December 2009 and February 2010, reaching a peak in January, also coinciding with a strong period of rainfall.

A study made by Gordon et al. (2007) demonstrated higher nutrient leaching in enriched soil samples of grassland which were dried and then rewetted. We can assume that in our samples the burned soils went through at least a short-term enrichment process because of the nutrients deposited with the ash. With the rainfall these soils were re-wetted, increasing therefore the leaching rates of the deposited NH_4^+ as well as the NO_3^-; this corresponds with the conclusions of Gordon et al. (2007) and with what Sardans et al. (2008) proposed: over a long period in dried soils of a Mediterranean shrubland in Catalonia, a higher availability of NO_3^- was found. The authors suggest that this increase of NO_3^- in the soil could result in a loss through leaching through the torrential rains typical of this region. Our results support this

hypothesis. Once after burning and rainfall we observed a higher leaching of NO_3^-, the same as NH_4^+ relative to the controls. Generally, however, fire increases the N loss through leaching, mainly in the form of NO_3^-.

5 Conclusions

For the analyzed area, we concluded that fire reduced the emissions of CO_2. We also concluded that the WFPS for maximum CO_2 emissions is between 40% and 60% in the analyzed soil samples, whereas for CO_2 emissions from the leaf litter and ash samples, the maximum emissions were measured at a WC of 40% dry weight. Another conclusion is that the ideal temperature for CO_2 emissions is 30°C. We found this to be the case for the soil samples, for leaf litter samples as well as for the ash samples.

We found a tendency toward higher CH_4 uptake rates in burned soil in the lower WFPS range.

We also concluded that fire contributes positively to the increase of NH_4^+ as the concentration increased significantly immediately after a fire and maintained this pattern 46 days after the fire. Nevertheless, the influence of fire on the NO_3^- rates was not clear in this study, but we observed a slight increase with time, probably due to an increase in the nitrification of the abundant amount of NH_4^+ deposited after fire.

Furthermore, with fire there is a loss of NH_4^+ and NO_3^- via leaching over time.

The fire drastically increased NO and N_2O emission rates from the soil with the increase of temperature and WFPS. However this didn't happen in the field. The ideal pattern for emissions of NO was in 60% WFPS at 30°C and 37.5°C. This increase in the rates occurs due to nitrification and denitrification. This pattern continued for up to three weeks. Conversely, the ideal pattern for N_2O emissions at water logged conditions is 100% at 37.5°C. In the field, however, differences in N_2O emissions were not significant as soil moisture was too dry for denitrification.

Fire negatively affected microorganisms in the Mediterranean shrubland. Fungi were controlled mainly through the influence of pH, preferring lower pH.

6 Tables

Table 2 – R^2 and p values from analyzed parameters for burned soil samples. (bold = positiv, kursiv underlined = negative).

	B		TN [µg N g^{-1} dw]	pH	WC [%]	NO_3^-	NH_4^+	N_mineralization	gram+ bacteria	gram- bacteria	fungi	actinomyceten	unspecific bacteria	protozoa	bacteria all	arbuscular mycorrhizal
					%	µg N g^{-1} dw			nmol g^{-1} dw							
r^2	TN [µg N g^{-1} dw]			0,27	**0,45**	**0,46**	0,38	**0,59**	**0,64**	0,11	0,04	0,08	0,23	0,08	0,11	0,07
	pH				0,01	0,00	0,00	0,02	0,01	0,29	0,00	0,27	0,00	0,19	0,04	0,31
	WC [%]					**0,56**	**0,95**	**0,86**	**0,54**	0,01	0,05	0,00	0,12	0,02	0,13	0,03
	NO_3^- [µg N g^{-1} dw]						**0,55**	**0,65**	0,30	0,00	0,04	0,03	0,06	0,10	0,08	0,05
	NH_4^+ [µg N g^{-1} dw]							**0,83**	**0,50**	0,02	0,09	0,01	0,07	0,02	0,09	0,02
	N_min [µg N g^{-1} dw]								**0,69**	0,00	0,01	0,01	0,26	0,00	0,23	0,02
	gram+ bacteria	nmol g^{-1} dw								0,01	0,00	0,04	**0,64**	0,07	**0,55**	0,01
	gram- bacteria										**0,57**	**0,89**	0,33	**0,62**	**0,55**	**0,80**
	fungi											**0,41**	0,32	0,37	0,34	0,34
	actinomyceten												0,35	**0,63**	**0,57**	**0,67**
	unspecific bacteria													**0,44**	**0,91**	0,17
	protozoa														**0,53**	0,28
	bacteria all															0,39
	arbuscular mycorrhizal															
p-value	TN [µg N g^{-1} dw]			0,15	**0,05**	0,05	0,08	**0,02**	**0,01**	0,39	0,60	0,47	0,19	0,46	0,38	0,51
	pH				0,82	0,97	0,95	0,72	0,77	0,14	0,91	0,15	0,88	0,24	0,62	0,12
	WC [%]					**0,02**	**0,00**	**0,00**	**0,02**	0,80	0,57	0,87	0,37	0,75	0,33	0,63
	NO_3^- [µg N g^{-1} dw]						**0,02**	**0,01**	0,12	0,90	0,63	0,68	0,53	0,41	0,46	0,55
	NH_4^+ [µg N g^{-1} dw]							**0,00**	**0,03**	0,72	0,44	0,78	0,50	0,73	0,43	0,73
	N_min [µg N g^{-1} dw]								**0,01**	0,88	0,81	0,80	0,16	0,97	0,19	0,74
	gram+ bacteria	nmol g^{-1} dw								0,75	0,93	0,59	**0,01**	0,48	**0,02**	0,77
	gram- bacteria										**0,02**	**0,00**	0,10	**0,01**	**0,02**	**0,00**
	fungi											0,06	0,11	0,08	0,10	0,10
	actinomyceten												0,09	**0,01**	**0,02**	**0,01**
	unspecific bacteria													**0,05**	**0,00**	0,27
	protozoa														**0,03**	0,15
	bacteria all															0,07
	arbuscular mycorrhizal															

Table 3 – R^2 and p values from analyzed parameters for unburned soil samples. (bold = positiv, kursiv underlined = negative).

	c			TN [µg N g^{-1} dw]	pH	WC [%]	NO_3^-	NH_4^+	N_mineralization	gram+ bacteria	gram- bacteria	fungi	actinomyceten	unspecific bacteria	protozoa	bacteria all	arbuscular mycorrhizal
						%	µg N g^{-1} dw			nmol g^{-1} dw							
r^2	TN [µg N g^{-1} dw]				0	**0,58**	0	0,01	0,12	0,34	0,1	0,01	0,04	0,23	0,01	0,16	0,19
	pH					0,15	0,05	0,14	0,12	**0,44**	**0,52**	0,36	**0,5**	**0,67**	**0,48**	**0,45**	**0,48**
	WC [%]						0,03	**0,85**	**0,85**	0,32	0,04	0,02	0,02	0,16	0	0,09	0,04
	NO_3^- [µg N g^{-1} dw]							0	0,11	0	0,14	0,12	0,17	0,05	0,1	0,08	0,11
	NH_4^+ [µg N g^{-1} dw]								**0,66**	0,15	0,02	0,04	0	0,08	0,01	0,05	0,01
	N_min [µg N g^{-1} dw]									0,29	0,04	0	0	0,15	0	0,1	0,06
	gram+ bacteria	nmol g^{-1} dw									**0,61**	0,18	**0,6**	**0,82**	**0,43**	**0,45**	**0,65**
	gram- bacteria											**0,64**	**0,94**	**0,86**	**0,86**	**0,87**	**0,93**
	fungi												**0,56**	**0,55**	**0,63**	**0,6**	**0,59**
	actinomyceten													**0,82**	**0,83**	**0,72**	**0,85**
	unspecific bacteria														**0,66**	**0,7**	**0,84**
	protozoa															**0,65**	**0,81**
	bacteria all																**0,78**
	arbuscular mycorrhizal																
p-value	TN [µg N g^{-1} dw]				0,13	**0,03**	0,49	0,24	0,26	**0,02**	**0,01**	**0,04**	**0,01**	**0**	**0,01**	**0,02**	**0,01**
	pH					0,21	0,33	0,42	**0,05**	0,07	0,49	0,78	0,58	0,16	0,77	0,46	0,21
	WC [%]						0,61	**0,00**	**0,00**	**0,06**	0,51	0,64	0,7	0,2	0,92	0,34	0,55
	NO_3^- [µg N g^{-1} dw]							0,87	0,3	0,96	0,23	0,27	0,19	0,49	0,31	0,37	0,3
	NH_4^+ [µg N g^{-1} dw]								**0,00**	0,22	0,69	0,51	0,91	0,39	0,79	0,49	0,78
	N_min [µg N g^{-1} dw]									0,07	0,55	0,86	0,88	0,21	0,96	0,33	0,45
	gram+ bacteria	nmol g^{-1} dw									**0,00**	0,17	**0,00**	**0,00**	**0,02**	**0,02**	**0,00**
	gram- bacteria											**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**
	fungi												**0,01**	**0,01**	**0,00**	**0,00**	**0,00**
	actinomyceten													**0,00**	**0,00**	**0,00**	**0,00**
	unspecific bacteria														**0,00**	**0,00**	**0,00**
	protozoa															**0,00**	**0,00**
	bacteria all																**0,00**
	arbuscular mycorrhizal																

Table 4 – R^2 and p values from analyzed parameters for ash samples. (bold= positiv, kursiv and underlined = negative).

	Ash		pH	WC [%]	NO_3^-	NH_4^+	N_mineralization	gram+ bacteria	gram- bacteria	fungi	actinomyceten	unspecific bacteria	protozoa	bacteria all	arbuscular mycorrhizal
				%	µg N g^{-1} dw			nmol g^{-1} dw							
r^2	pH			0,06	**0,58**	*0,65*	0,01	0,29	0,27	*0,63*	0,13	0,38	0,03	*0,42*	0,04
	WC [%]				0,11	0,01	0,37	0,32	0,02	0,05	0,06	0,22	0,12	0,15	0,14
	NO_3^- [µg N g^{-1} dw]					*0,61*	0,24	*0,55*	0,18	*0,67*	0,08	*0,81*	0,06	*0,62*	0,01
	NH_4^+ [µg N g^{-1} dw]						0,00	**0,58**	**0,67**	**0,66**	**0,46**	**0,41**	**0,44**	**0,71**	0,18
	N_min [µg N g^{-1} dw]							0,04	0,13	0,01	0,12	0,15	0,07	0,00	0,14
	gram+ bacteria	nmol g^{-1} dw							**0,66**	**0,70**	**0,65**	**0,68**	**0,48**	**0,94**	**0,53**
	gram- bacteria									**0,52**	**0,93**	0,22	**0,70**	**0,75**	**0,71**
	fungi										0,37	**0,64**	0,23	**0,78**	0,21
	actinomyceten											0,16	**0,70**	**0,67**	**0,83**
	unspecific bacteria												0,11	**0,72**	0,13
	protozoa													**0,49**	**0,57**
	bacteria all														**0,51**
	arbuscular mycorrhizal														
p-value	pH			0,52	**0,03**	**0,02**	0,84	0,14	0,15	*0,01*	0,33	0,08	0,68	0,06	0,59
	WC [%]				0,41	0,81	0,08	0,11	0,70	0,58	0,54	0,20	0,36	0,30	0,32
	NO_3^- [µg N g^{-1} dw]					*0,02*	0,22	*0,03*	0,29	*0,01*	0,50	*0,00*	0,54	*0,02*	0,81
	NH_4^+ [µg N g^{-1} dw]						0,96	**0,03**	**0,01**	**0,01**	0,06	0,09	0,07	**0,01**	0,30
	N_min [µg N g^{-1} dw]							0,63	0,33	0,79	0,37	0,29	0,48	0,94	0,32
	gram+ bacteria	nmol g^{-1} dw							**0,01**	**0,00**	**0,01**	**0,01**	**0,04**	**0,00**	**0,03**
	gram- bacteria									**0,03**	**0,00**	**0,21**	**0,00**	**0,00**	**0,00**
	fungi										0,08	**0,01**	0,19	**0,00**	0,21
	actinomyceten											0,28	**0,00**	**0,01**	**0,00**
	unspecific bacteria												0,38	**0,00**	0,35
	protozoa													**0,04**	**0,02**
	bacteria all														**0,03**
	arbuscular mycorrhizal														

Table 5 – R^2 and p values from analyzed parameters for leaf litter samples. (bold = positiv, Kursiv and underlined = negative).

	Leaf Litter		pH	WC [%]	NO_3^-	NH_4^+	N_mineralization	gram+ bacteria	gram- bacteria	fungi	actinomyceten	unspecific bacteria	protozoa	bacteria all	arbuscular mycorrhizal
				%	µg N g^{-1} dw			nmol g^{-1} dw							
r^2	pH			0,01	0,00	0,07	0,08	0,22	0,02	0,13	0,30	0,24	0,14	0,06	0,15
	WC [%]				0,09	0,01	0,04	0,01	0,05	**0,37**	0,10	0,03	0,01	0,02	0,03
	NO_3^- [µg N g^{-1} dw]					0,34	**0,44**	0,00	0,01	0,01	0,09	0,01	*0,54*	0,00	0,03
	NH_4^+ [µg N g^{-1} dw]						0,23	0,14	0,22	0,07	0,06	0,12	*0,50*	0,24	0,07
	N_min [µg N g^{-1} dw]							0,07	0,01	0,10	0,02	0,07	*0,60*	0,01	0,11
	gram+ bacteria	nmol g^{-1} dw							**0,46**	0,07	**0,77**	0,01	0,03	0,26	**0,70**
	gram- bacteria									0,00	0,27	0,30	0,02	**0,70**	**0,60**
	fungi										0,08	0,03	**0,60**	0,00	0,07
	actinomyceten											0,02	0,02	0,07	**0,62**
	unspecific bacteria												0,04	**0,73**	0,00
	protozoa													0,04	0,00
	bacteria all														0,17
	arbuscular mycorrhizal														
p-value	pH			0,73	0,92	0,40	0,37	0,12	0,68	0,21	0,06	0,10	0,26	0,46	0,23
	WC [%]				0,34	0,75	0,54	0,82	0,50	**0,01**	0,32	0,61	0,77	0,66	0,64
	NO_3^- [µg N g^{-1} dw]					**0,05**	**0,02**	0,84	0,80	0,88	0,35	0,71	*0,01*	0,94	0,47
	NH_4^+ [µg N g^{-1} dw]						0,12	0,22	0,12	0,17	0,46	0,27	*0,01*	0,11	0,44
	N_min [µg N g^{-1} dw]							0,39	0,73	0,48	0,65	0,42	*0,01*	0,81	0,32
	gram+ bacteria	nmol g^{-1} dw							**0,02**	0,76	**0,00**	0,75	0,63	0,09	**0,00**
	gram- bacteria									0,68	0,09	0,06	0,10	**0,00**	**0,01**
	fungi										0,34	0,08	0,31	0,06	0,99
	actinomyceten											0,70	0,66	0,40	**0,00**
	unspecific bacteria												0,54	**0,00**	0,87
	protozoa													0,54	0,69
	bacteria all														0,20
	arbuscular mycorrhizal														

7 Figures

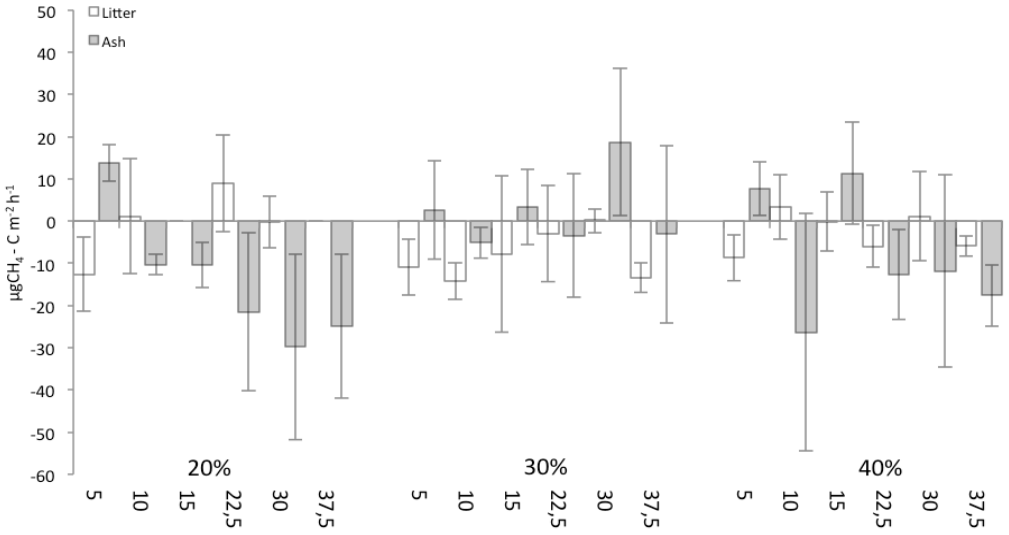

Fig. 13 – CH_4 fluxes from litter samples (white) and ash samples (grey) at different levels of moistures (10-40%) and temperatures (5-37.5°C).

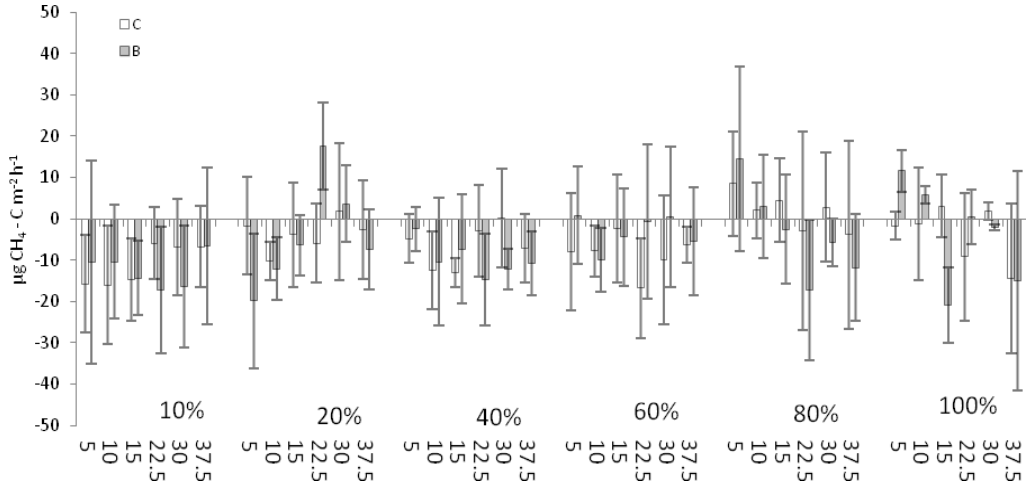

Fig. 14 – CH_4 fluxes from soil samples control (white) and burned samples (grey) at different levels of soil moisture (10-100%) and soil temperature (5-37.5°C).

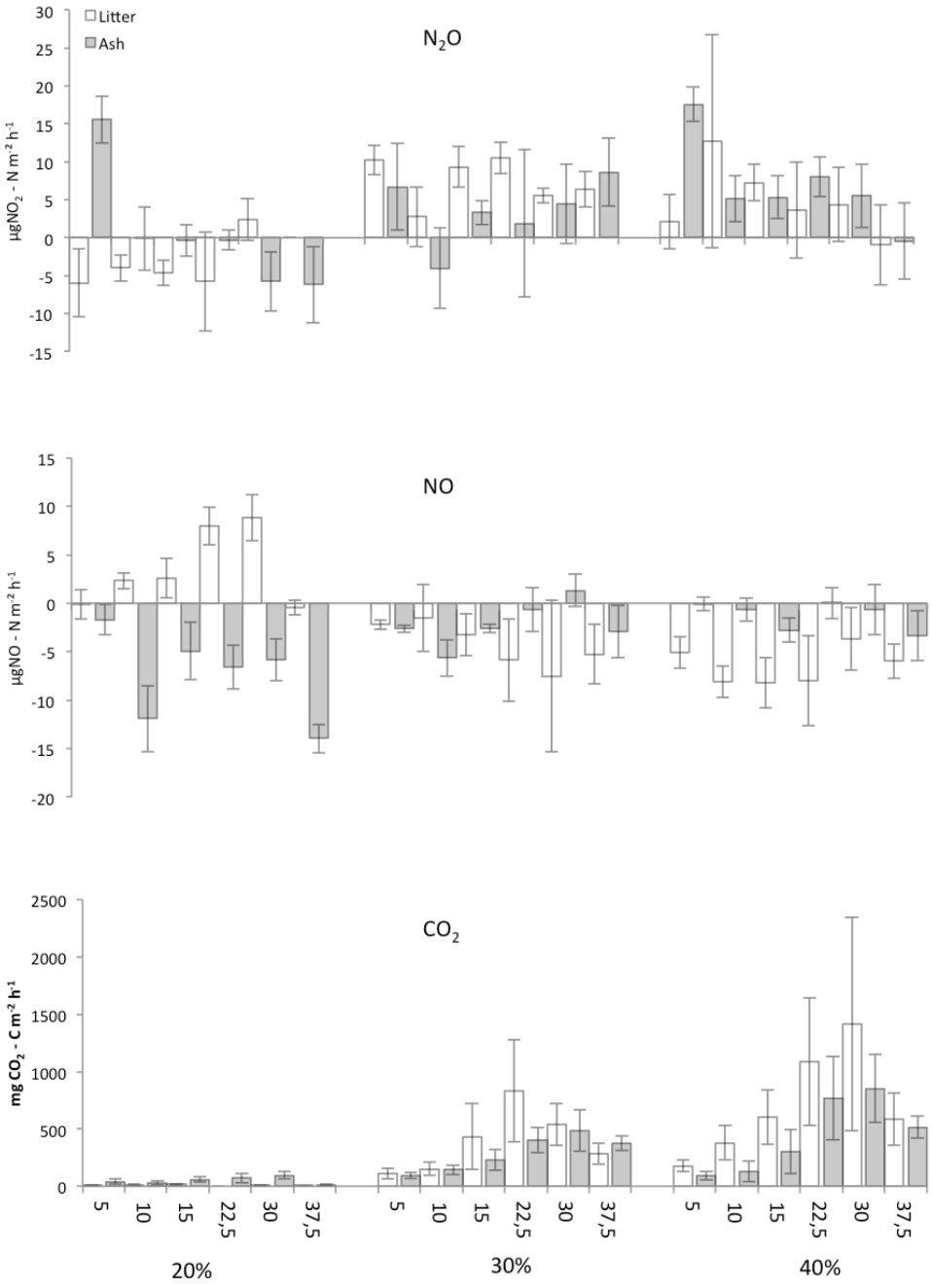

Fig. 15 – N_2O (a), NO (b) and CO_2 (c) fluxes from leaf litter (white) and ash samples (grey) at different moisture (20-40%) and temperatures (5-37.5°C)

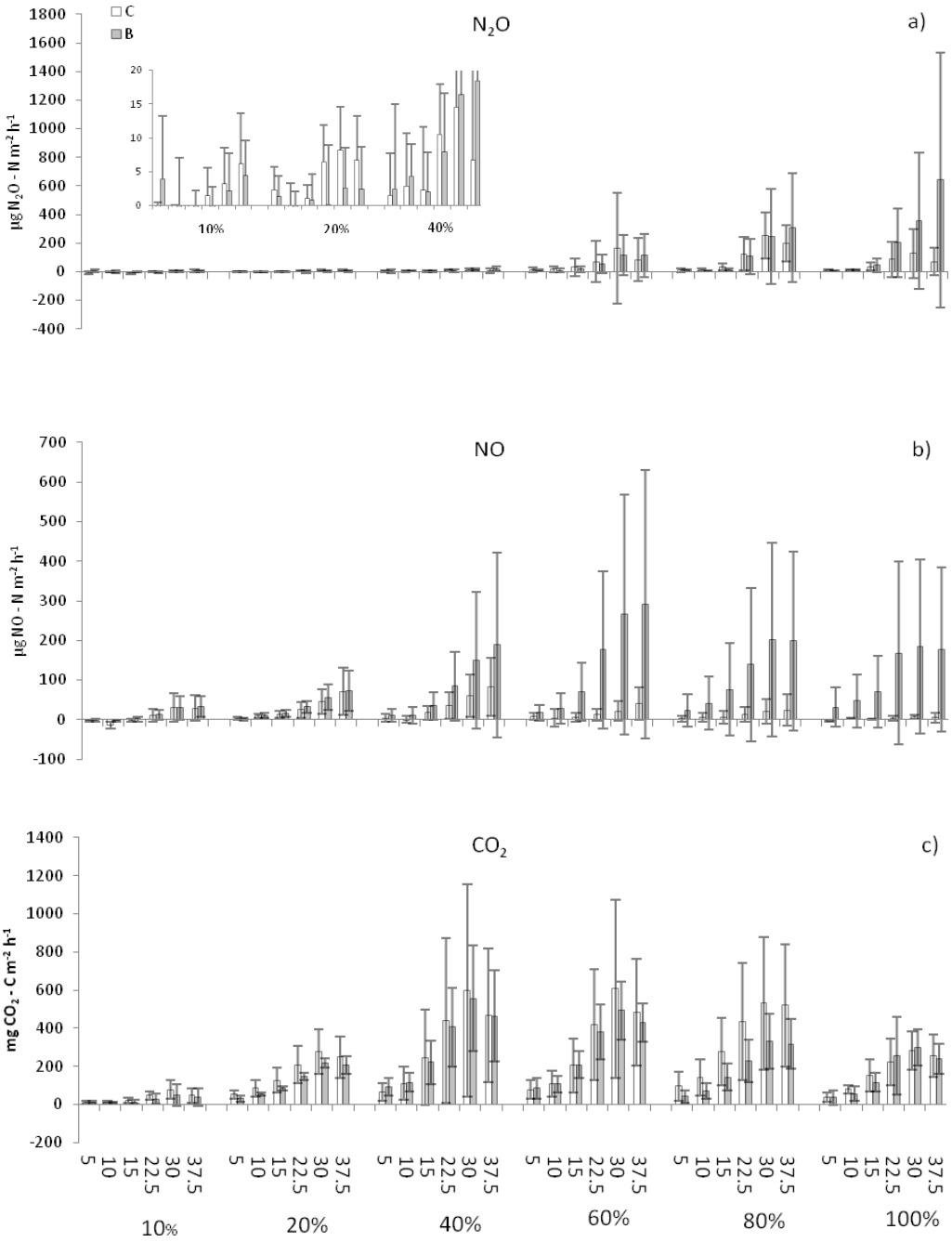

Fig. 16 – N_2O (a), NO (b) and CO_2 (c) fluxes at control (white) and burned (grey) plots at different soil moistures (10-100%) and soil temperatures (5-37.5°C)

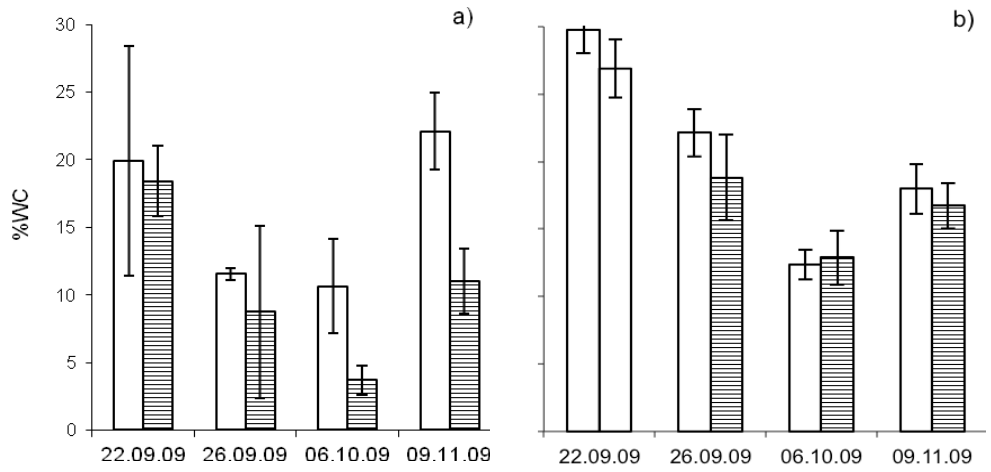

Fig. 17 – Water content (%) at unburned (blank bars) and burned (slashed bars) plots from leaf litter and ash samples (a) and mineral soil samples (b) over time

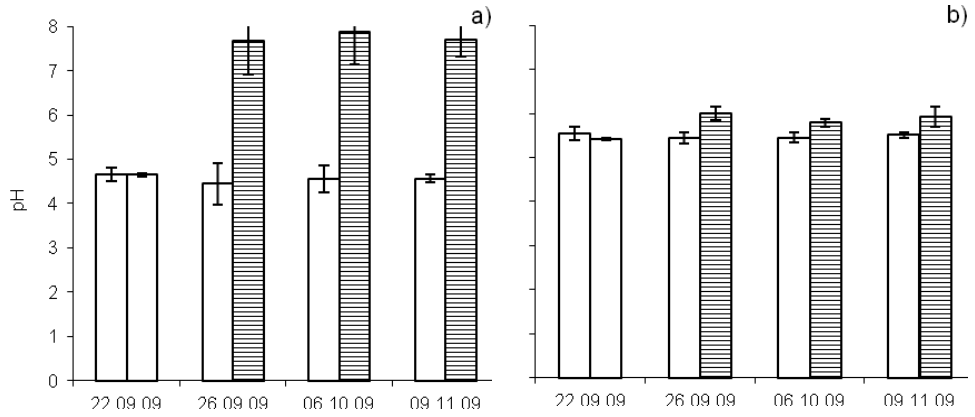

Fig. 18 – pH at unburned (blank bars) and burned (slashed bars) plots from leaf litter and ash samples (a) and mineral soil samples (b) over time

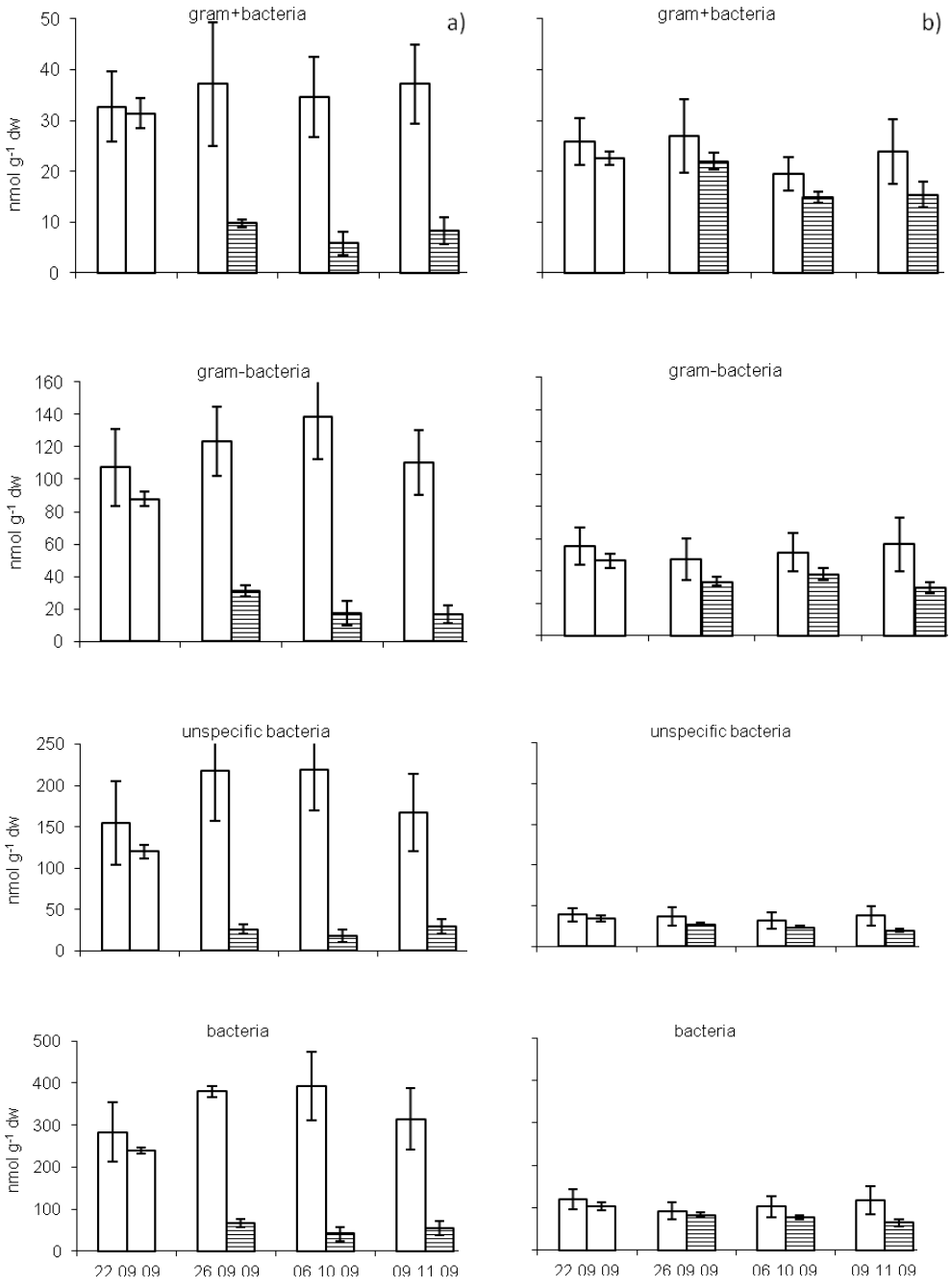

Fig. 19 – Gram+ bacteria, gram- bacteria, unspecific bacteria and bacteria (= sum of gram+ ; gram- ; unspecific bacteria) of leaf litter and ash samples (a) and burned soil and control soil samples (b).

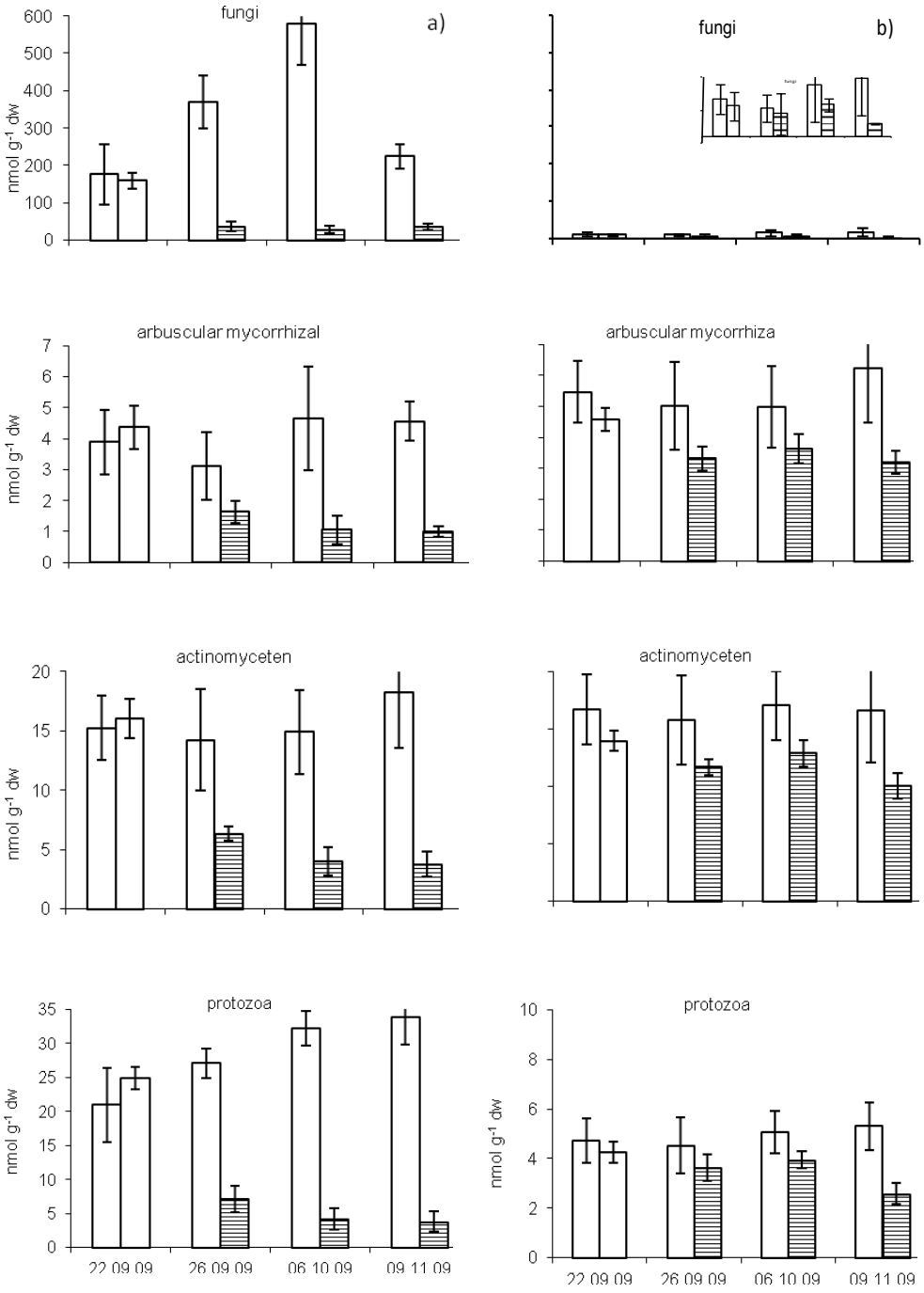

Fig. 20 – Fungi, arbuscular mycorrhiza, actinomyceten and protozoa of leaf litter and ash samples (a) and burned soil and unburned mineral soil samples (b).

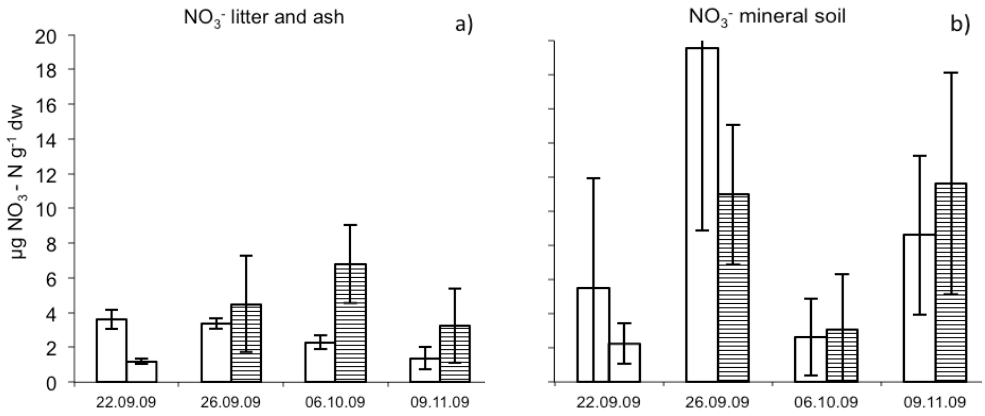

Fig. 21 – Nitrate concentration from unburned (blank bars) and burned (slashed bars) plots over time. (a) leaf litter and ash samples, (b) mineral soil samples.

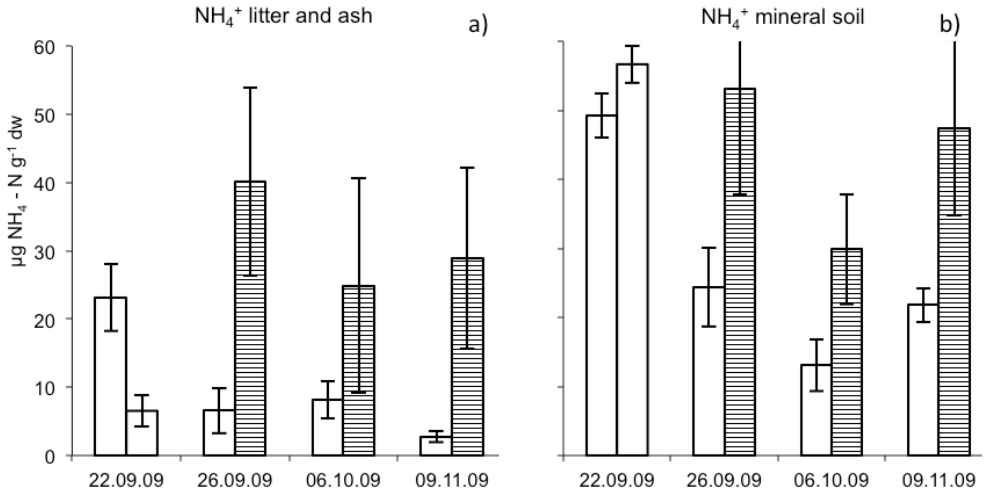

Fig. 22 – Ammonium concentration from unburned (blank bars) and burned (slashed bars) plots over time. (a) leaf litter and ash samples; (b) mineral soil samples.

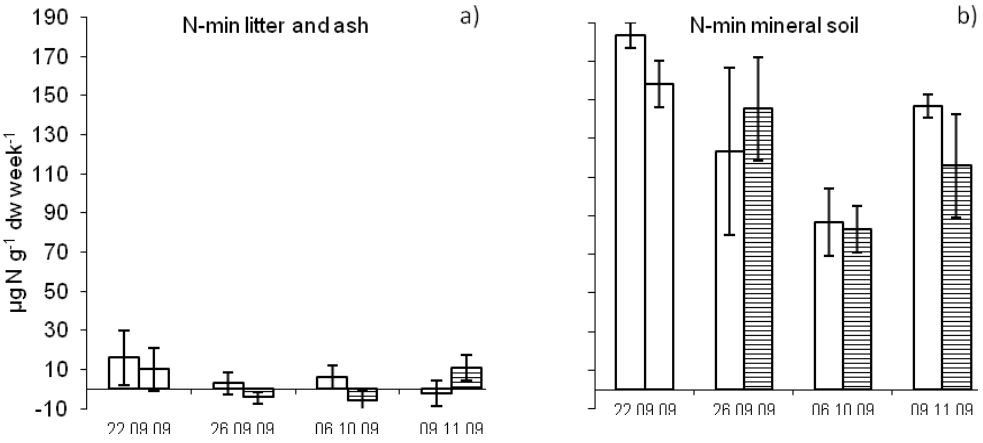

Fig. 23 – Nitrogen mineralization from unburned (blank bars) and burned (slashed bar) plots over time. (a) leaf litter and ash samples and (b) mineral soil samples.

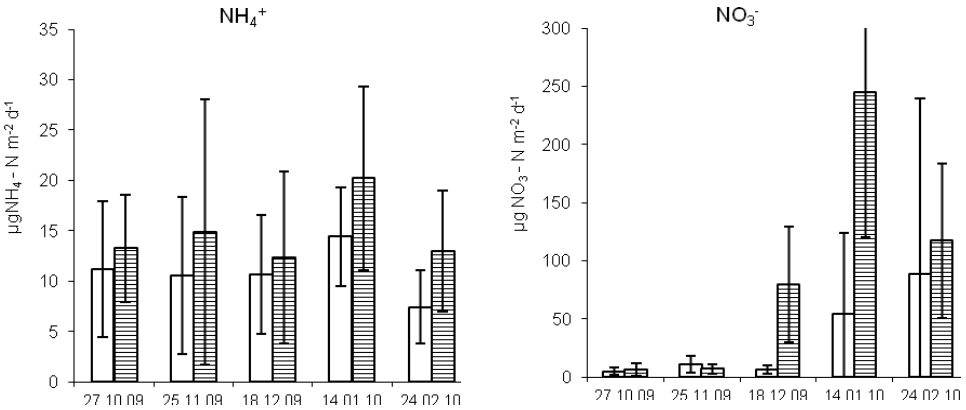

Fig. 24 – Ammonium and nitrate measured from resin bags from unburned (blank bars) and burned (slashed bars) plots over time

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Ich habe mich bemüht, sämtliche Inhaber der Bildrechte ausfindig zu machen und ihre Zustimmung zur Verwendung der Bilder in dieser Arbeit eingeholt. Sollte dennoch einer Urheberrechtsverletzung bekannt werden, ersuche ich um Meldung bei mir.

9 Curriculum Vitae

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Education:

2009-2012	Diploma thesis, Institute of Forest Ecology at the Federal Research and Training Centre for Forests, Natural Hazards and Landscape Department in Ecology in cooperation with the Department of Chemical Ecology and Ecosystem Research of the University of Vienna
	Field trip to Finca Quintos de Mora in Los Cortijos, Spain
06-07/2004	Specialization course about the ecology of the floodplain woods in the region Vale do Itajaí (South Brazil)
1998-2005	Bachelor and Licenciate Degree in Biology of the FURB Regional University Blumenau, Brazil
	Thesis: Impact of the physical state of the culture medium on the reproductive behaviour *in vitro* of the *Vriesea gegantea* and *Neoregelia cruenta*
05-06/1998	Symposium about the biology of mammals in Brazil

Relevant work experience:	
2005	Intern at the botanic lab at the University FURB – Blumenau/SC, Brazil
2003-2004	Collaborator in projects such as the restoration of riparian forests; creating green aeras in schools FAEMA (Fundação Municipal do Meio Ambiente / Umweltamt), Blumenau – Brazil
2001-2003	Biology teacher at the secondary schools Hermann Hering (CEDUP) and Pedro II; Blumenau - Brazil
07-10/1999	Chemistry and biology substitute teacher at the secondary schools Padre José Maurício and Pedro II; Blumenau - Brazil
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