FLUJOS DE GASES DE EFECTO INVERNADERO EN SUELOS FORESTALES Y AGROFORESTALES DEL CENTRO DE LA PENÍNSULA IBÉRICA

Carla Yolanda Uribe Vallejos
Madrid, 2013

TESIS DOCTORAL
FLUJOS DE GASES DE EFECTO INVERNADERO EN SUELOS FORESTALES Y AGROFORESTALES DEL CENTRO DE LA PENÍNSULA IBÉRICA

GREENHOUSE GAS FLOWS IN FOREST AND AGROFORESTRY SOILS IN THE CENTER OF THE IBERIAN PENINSULA

Autora:
Carla Yolanda Uribe Vallejos. Licenciada en Ciencias Ambientales

Directoras:
Rosa María Inclán Cuartas. Doctora en Ciencias Biológicas
Helga Van Miegroet. Doctor in Forest Soils and Mineral Cycling

Madrid, 2013
Tribunal nombrado por el Mg/co. y Excmo. Sr. Rector de la Universidad Politécnica de Madrid, el día........de........... de 2013

Presidente D. ........................................................................................................
Vocal D. ..............................................................................................................
Vocal D. ..............................................................................................................
Vocal D. ..............................................................................................................
Secretario D. ........................................................................................................
Suplente 1 D. ......................................................................................................
Suplente 2 D. ......................................................................................................

Realizado el acto de defensa y lectura de la Tesis Doctoral
el día ........de.............de 2013 en.................................................................

Calificación .......................................................................................................

EL PRESIDENTE LOS VOCALES

EL SECRETARIO
Durante el tiempo de realización de esta Tesis Doctoral he disfrutado de una Beca del Programa Nacional de Formación de Personal Investigador del Centro de Investigaciones Energéticas, Medioambientales y Tecnológicas (CIEMAT).

Este trabajo estuvo financiado por los proyectos FLUSULCA (CGL2006-02922) del Ministerio de Ciencia e Innovación, EMAPGEI (CGL2009/07031) del Ministerio de Ciencia e Innovación y el proyecto (SUM2006-00034-CO2) del Ministerio de Educación y Ciencia.

La investigación presentada en esta Tesis Doctoral se ha realizado en el Departamento de Medioambiente del CIEMAT, Madrid.
Abstract

Due to the complexity of the processes that control the exchange of carbon (C) and nitrogen (N) gasses between soils and the atmosphere in forest and agroforestry ecosystems, understandable uncertainties exist as regards the estimation of greenhouse gas (GHG) fluxes and the soil sink capacity at regional and global scale under different forms of land use and disturbance regimes. These uncertainties justify the need to characterize the exchange dynamics of GHG between the atmosphere and soils in Mediterranean terrestrial ecosystems, particularly in the current context of climate change and the associated increase in temperature, drought periods, heavy rainfall events, and increased risk of wildfires, which affect not only the C and N pools but also the soil C sink capacity of these ecosystems. Within this context, the aims of the present thesis were, firstly, to quantify and characterize the fluxes of carbon dioxide (CO$_2$), nitrous oxide (N$_2$O) and methane (CH$_4$) as well as the C and N stocks in Quercus ilex, Quercus pyrenaica and Pinus sylvestris stands affected by wildfires, and secondly, to study the effects of Quercus ilex canopy and management on both soil respiration and C and N pools in dehesa systems in the center of Iberian Peninsula.

Soil CO$_2$, N$_2$O and CH$_4$ fluxes, and soil physical-chemical and biological parameters were studied under the different treatments and ecosystems considered in this study. The results showed seasonal and spatial variations in soil respiration within small geographic areas, mainly controlled by soil temperature and moisture in addition to soil carbon and nitrogen stocks in mixed pine–oak forest ecosystems on the north facing slopes of the Sierra de Guadarrama in Spain.

The analysis of long term effects of wildfires (6–8 years) revealed that annual carbon losses through soil respiration from burned sites in Quercus ilex, Quercus pyrenaica and Pinus sylvestris stands were 450 gCm$^{-2}$yr$^{-1}$, 790 gCm$^{-2}$yr$^{-1}$ and 1220 gCm$^{-2}$yr$^{-1}$, respectively; with burned sites emitting 43%, 22% and 11% less in burned as opposed to non-burned sites due the loss of trees. Fire may alter both N$_2$O and CH$_4$ fluxes although the magnitude of such variation depends on the site, soil characteristics and seasonal climatic conditions. The burned sites showed higher CH$_4$ oxidation in Q. ilex stands, and lower oxidation rates in P. sylvestris stands. A reduction in N$_2$O fluxes in Q. pyrenaica stands was detected at burned sites along with changes in soil microclimate; higher soil temperature and lower soil moisture content. Exchangeable
ations, the C/N ratio, pH, fine root and microbial biomass were also found to decrease at burned sites. Although the soil organic carbon was not significantly altered, the quality of the organic matter changed, displaying a decrease in labile carbon and a relative increase in refractory forms, leading to lower sensitivity of soil respiration to temperature (Q_{10} values) at burned sites.

The results from the dehesa study show that light grazing and superficial tilling practices used in the studied dehesa system in Spain had a slight but non-consistent impact on soil respiration and soil microclimate over the study period. The reduction in soil respiration in the dehesa system due to the effects of grazing was around 12%. However, increments of 3Mg/ha in C stocks and 0.3 Mg/ha in N stocks in grazed soils were observed. Although no clear effect of tilling on soil respiration was found, a decrease of 3.5 Mg/ha in C stocks and 0.3 Mg/ha in N stocks was detected for tilled soils. The presence of a tree canopy induced increases in soil respiration, soil C and N stocks, while soil moisture was found to play an important role in soil respiration temperature response.

Our results suggest that the use of standard models to estimate soil respiration in small geographical areas may not be adequate unless other factors are considered in addition to soil temperature. Furthermore, the different responses of GHG flux to climatic shifts, many years after the occurrence of wildfire, highlight the need to include these shifts in C dynamics in future research undertaken in Mediterranean ecosystems. Furthermore, divergent responses in soil respiration and soil C and N stocks to grazing or tilling practices in Dehesa systems, and the influence of tree canopy on soil respiration and soil nutrient content, illustrate the importance of maintaining beneficial management practices. Moreover, the carbon sequestration capacity of the Dehesa system studied may be enhanced through improvements in the management applied.

It is hoped that the information obtained through this research will contribute towards improving our understanding of the dynamics and balance of C in Mediterranean systems, and help predict the impact of climate change on the exchange of C between forest and agroforestry ecosystems and the atmosphere.
Resumen

Debido a la complejidad de los procesos que controlan el intercambio de gases de carbono (C) y nitrógeno (N) entre el suelo y la atmósfera, en los sistemas forestales y agroforestales, son comprensibles las incógnitas existentes respecto a la estimación de los flujos de los gases de efecto invernadero (GEI) y la capacidad como reservorios de carbono de los suelos, bajo diferentes formas de uso y regímenes de alteración a escala regional y global. Esta escasez de información justifica la necesidad de caracterizar la dinámica de intercambio de GEI en los ecosistemas Mediterráneos, en especial en el contexto actual de cambio climático, y el incremento asociado de temperatura y períodos de sequía, alteración de los patrones de precipitación, y el riesgo de incendios forestales; cuyas consecuencias afectarán tanto a los compartimentos de C y de N del suelo como a la capacidad de secuestro de C de estos ecosistemas. Dentro de este contexto se enmarca la presente tesis doctoral cuyo objetivo ha sido cuantificar y caracterizar los flujos de dióxido de carbono (CO₂), de óxido nitroso (N₂O) y de metano (CH₄), junto con los stocks de C y N, en suelos forestales de Quercus ilex, Quercus pyrenaica y Pinus sylvestris afectados por incendios forestales; así como el estudiar el efecto de la gestión y la cubierta arbórea en la respiración del suelo y los stocks de C y N en una dehesa situada en el centro de la Península Ibérica.

De manera que los flujos de CO₂, N₂O y CH₄, y los parámetros físico-químicos y biológicos del suelo fueron estudiados en los diferentes tratamientos y ecosistemas a lo largo del trabajo que se presenta. Los resultados obtenidos muestran la existencia de variaciones temporales y espaciales de la respiración del suelo dentro de una escala geográfica pequeña, controladas principalmente por la temperatura y la humedad del suelo; y por los contenidos de C y N del suelo en un bosque de Pinus sylvestris en la vertiente norte de la Sierra de Guadarrama , en España.

El análisis de los efectos de los incendios forestales a largo plazo (6-8 años) revela que las pérdidas anuales de C a través de la respiración del suelo en las zonas quemadas de Quercus ilex, Quercus pyrenaica y Pinus sylvestris fueron 450 gCm⁻²yr⁻¹, 790 gCm⁻²yr⁻¹ y 1220 gCm⁻²yr⁻¹, respectivamente; lo que representa una reducción del 43%, 22% y 11% en comparación con las zonas no quemadas de dichas especies, debido a la destrucción de la masa arbórea. El efecto del fuego también alteró los flujos N₂O y CH₄ del suelo, de una forma diferente en los distintos ecosistemas y estacionalidades.
Los incendios también afectaron los parámetros microclimáticos de los suelos forestales, observándose un incremento de la temperatura del suelo y una disminución de la humedad en los emplazamientos quemados que en los no quemados. Los cationes intercambiables, el pH, el cociente C/N, el contenido en raicillas y la biomasa microbiana también disminuyeron en las zonas quemadas. Aunque el C orgánico del suelo no se alteró de manera significativa, si lo hizo la calidad de la materia orgánica, disminuyendo el carbono lábil y aumentando las formas recalcitrantes lo que se tradujo en menor sensibilidad de la respiración del suelo a la temperatura (valores de Q10) en las zonas quemadas.

Los resultados del estudio realizado en la Dehesa muestran que las actividades silvopastorales estudiadas afectaron levemente y de forma no constante a la respiración del suelo y las condiciones microclimáticas del suelo. Se observó una reducción 12% de la respiración del suelo por efecto del pastoreo no intensivo. Sin embargo, se observaron incrementos de 3Mg/ha en los stocks de C y de 0.3 Mg/ha en los stocks de N en los suelos pastoreados en comparación con los no pastoreados. Aunque, no se observó un claro efecto de la labranza sobre la respiración del suelo en nuestro experimento, sin embargo si se observó una disminución de 3.5 Mg/ha en las reservas de C y de 0.3 Mg/ha en las de N en los suelos labrados comparados con los no labrados. La copa del arbolado influyó de forma positiva tanto en la respiración del suelo, como en los stocks de C y N de los suelos. La humedad del suelo jugó un papel relevante en la sensibilidad de la respiración a la temperatura del suelo.

Nuestros resultados ponen de manifiesto la sensibilidad de la respiración del suelo a cambios en la humedad y los parámetros edáficos, y sugieren que la aplicación de modelos estándar para estimar la respiración del suelo en áreas geográficas pequeñas puede no ser adecuada a menos que otros factores sean considerados en combinación con la temperatura del suelo. Además, las diferentes respuestas de los flujos de gases de efecto invernadero a los cambios, años después de la ocurrencia de incendios forestales, destaca la necesidad de incluir estos cambios en las futuras investigaciones de la dinámica del carbono en los ecosistemas mediterráneos. Por otra parte, las respuestas...
divergentes en los valores de respiración del suelo y en los contenidos de C y N del suelo observados en la dehesa, además de la contribución de la copa de los árboles en los nutrientes del suelo ilustran la importancia de mantener la gestión tradicional aplicada en beneficio de la capacidad de almacenar C en la dehesa estudiada.

La información obtenida en este trabajo pretende contribuir a la mejora del conocimiento de la dinámica y el balance de C en los sistemas mediterráneos, además de ayudar a predecir el impacto del cambio climático en el intercambio de C entre los ecosistemas forestales y agroforestales y la atmósfera.
CONTENT

Chapter 1

Introduction
  1. Background
  2. Objectives and structure
  3. General methodology and study area
  4. Bibliography

Chapter 2

Carbon dioxide fluxes across the Sierra de Guadarrama, Spain

Chapter 3

Effect of wildfires on soil respiration in three typical mediterranean forest ecosystems in Madrid, Spain

Chapter 4

N₂O and CH₄ fluxes in undisturbed and burned Holm oak, Scots pine and Pyrenean oak forests in central Spain

Chapter 5

Pasture, tillage and canopy effects on carbon dioxide fluxes in a Spanish dehesa

General discussion

General conclusions

Agradecimientos

Annexes
Abbreviations

Rs: Soil respiration
Ts: Soil temperature
Ms: Soil moisture
Qi: Quercus ilex
Qp: Quercus pyrenaica
Ps: Pinus sylvestris
NB: Non burned
B: Burned
NGNT: Non grazed-non tilled
NGT: Non grazed-tilled
GNT: Grazed- non tilled
GT: Grazed tilled
WFPS: Water filled pores space
CMBT: Soil microbial biomass carbon
TOC: Total organic carbon
DOC: Water extractable organic carbon
SOC: Soil organic carbon
SOM: Soil organic matter
HS: Humic substances
HA: Humics acids
FA: Fulvic acids
Chapter 1

INTRODUCTION
# CONTENT

1.1 BACKGROUND ............................................................................................................. 3

1.1.1 Climate Change: causes and effects.......................................................................... 3

1.1.2 Climate change and terrestrial ecosystem............................................................... 10

1.1.2.1 Terrestrial carbon cycle........................................................................................ 13

1.1.2.2 The soil respiration ............................................................................................. 14

1.1.2.3 Factors controlling soil respiration....................................................................... 15

1.1.2.4 Soil temperature and moisture .............................................................................. 15

1.1.2.5 Substrate and ecosystem productivity.................................................................. 16

1.1.2.6 Temporal and spatial variation in soil respiration.................................................. 17

1.1.2.7 Methane and Nitrous oxide fluxes in forest soils................................................... 19

1.1.2.8 Methane ............................................................................................................... 20

1.1.2.9 Methane production ............................................................................................. 20

1.1.2.10 Methane oxidation .............................................................................................. 21

1.1.2.11 Nitrous oxide ...................................................................................................... 21

1.1.2.12 Nitrogen cycle ..................................................................................................... 22

1.1.2.13 Nitrification ......................................................................................................... 24

1.1.2.14 Denitrification ..................................................................................................... 25

1.1.3 Other N\textsubscript{2}O forming processes in soils......................................................... 25

1.1.3.1 Chemodenitrification ............................................................................................. 25

1.1.4 The influence on wildfires in greenhouse gases efflux............................................. 26

1.2 OBJECTIVES AND STRUCTURE .............................................................................. 27

1.2.1 The general objective............................................................................................... 27

1.3 GENERAL METHODOLOGY AND STUDY AREA ................................................. 29

1.3.1 Field studies:......................................................................................................... 29

1.3.2 Field work methodology: ....................................................................................... 30

1.3.3 Analytical laboratory techniques ............................................................................ 31

1.3.4 Statistical Analysis .................................................................................................. 31

1.4 REFERENCES ............................................................................................................ 41
1.1 BACKGROUND

1.1.1 Climate Change: causes and effects

According to the United Nations Framework Convention on Climate Change (UNFCCC), climate change is a change of climate which is attributed directly or indirectly to human activity that alters the composition of the global atmosphere and which is in addition to natural climate variability observed over comparable time periods. Changes in the state of the climate can be identified by changes in the mean and/or the variability of its properties, persisting over an extended period, typically decades or longer. The Intergovernmental Panel on Climate Change (IPCC) defined climate change as any change in climate over time, whether due to natural variability or as a result of human activity (IPCC 2007).

Changes in the atmospheric concentrations of greenhouse gases GHG (water vapor, carbon dioxide (CO₂), methane (CH₄), nitrous oxide (N₂O), halocarbons (gases containing fluorine, chlorine), tropospheric ozone, and aerosols) as well as land cover and solar radiation alter the energy balance of the climate system (Figure 1.1) and are drivers of climate change.

The global atmospheric concentrations of GHG reached a new high (since pre-industrial times) in 2010 and the rate of increase has accelerated, according to the World Meteorological Organization’s (WMO 2011). The anthropogenic GHG emissions relating to energy use (63%), land use change (16%) and agricultural activities (13%) (Kroon et al. 2010), have intensified the natural GHG effect causing global warming. The greenhouse effect is caused by long wave radiation being reflected by the Earth back into the atmosphere and then reflected back by trace gases in the cooler upper atmosphere (Figure 1.1), thus causing additional warming of the Earth’s surface (Smith 2001). Consequently, the global surface temperature increased by 0.74 ± 0.18 °C during last 100 years (1906-2005) (IPCC 2007).
Figure 1.1 Global energy flows (W m\(^{-2}\)). The broad arrows indicate the schematic flow of energy in proportion to their importance. Source: (Trenberth 2009).

Warming of the climate system is evidenced by observations of increases in global average air and ocean temperatures (Figure 1.2), with the associated risks of widespread melting of snow and ice leading to a rise in global average sea level. Furthermore, numerous long-term changes in other aspects of climate have also been observed. Between 1900 and 2005, precipitation increased significantly in eastern parts of North and South America, northern Europe and northern and central Asia. By contrast, the area extending from the Mediterranean through central Europe into European Russia and Ukraine has experienced decreases in precipitation of up to 20% in some areas (Dore 2005). Globally, the area affected by drought has probably increased since 1970 (Huntington 2006). In addition, certain extreme weather events have changed in frequency and/or intensity over the last 50 years; hot days and nights as well as heat waves have become more frequent (IPCC 2007). The resulting changes in the energy balance due to these factors are expressed as radiative forcing\(^1\), which is used to compare warming or cooling influences on global climate (IPCC 2007). Between 1990 and 2010, there was a 29% increase in radiative forcing from greenhouse gases. CO\(_2\) accounted for 80% of this increase (WMO 2011).
Radiative forcing is a measure of the influence a factor has in altering the balance of incoming and outgoing energy in the Earth-atmosphere system and is an index of the importance of the factor as a potential climate change mechanism. Radiative forcing values are expressed in watts per square metre (W/m\(^2\)).
CO₂ remained almost constant at around 280 parts per million over a period of approximately 10,000 years prior to the start of the industrial era in the mid-18th century (WMO 2011). Today, the atmospheric abundance of CO₂ has increased to 389 parts per million (number of molecules of the gas per million molecules of dry air) (Table 1.1). This is primarily due to emissions from combustion of fossil fuels, deforestation and changes in land-use (Figure 1.3). Between 2009 and 2010, the atmospheric abundance of CO₂ increased by 2.3 parts per million. The rate of increase in CO₂ concentration during the past century is at least an order of magnitude greater than the world has seen for the last 20 kyr (Prentice et al. 2001).

**Figure 1.3** Global anthropogenic greenhouse gas emissions expressed as the percentage of total CO₂ equivalent. Source: (IPCC, 2007).

CH₄ contributes about 14% to the overall global increase in radiative forcing and is the second most important greenhouse gas after CO₂. Before the start of the industrial era, atmospheric methane was about 700 parts per billion (number of molecules of the gas per billion molecules of dry air) (Table 1.1). Since 1750, it has increased 158%, mostly because of activities such as cattle-rearing, rice planting, fossil fuel exploitation and landfills. Human activities now account for 60% of CH₄ emissions, with the remaining
40% being from natural sources such as wetlands. After a period of temporary relative stabilization from 1999 to 2006, atmospheric CH$_4$ has again risen.

N$_2$O contributes about 8% to the overall global increase in radiative forcing. It is emitted into the atmosphere from natural and man-made sources, including the oceans, biomass burning, fertilizer use and various industrial processes. It is now the third most important greenhouse gas. The atmospheric burden of nitrous oxide in 2010 was 323.2 parts per billion (Table 1.1), 20% higher than in the pre-industrial era. It has grown at an average rate of about 0.75 parts per billion over the past ten years, mainly as a result of the use of nitrogen containing fertilizers, including manure, which has profoundly affected the global nitrogen cycle. Its impact on climate, over a 100 year period, is 298 times greater than equal emissions of carbon dioxide. It also plays an important role in the destruction of the stratospheric ozone layer which protects us from the harmful ultraviolet rays of the sun.

**Table 1.1** Main greenhouse gases. Source: (IPCC, 2007; WMO, 2011).

<table>
<thead>
<tr>
<th>Greenhouse gases</th>
<th>Chemical formula</th>
<th>Pre-industrial concentration (ppbv)</th>
<th>Concentration in 2010 (ppbv)</th>
<th>Atmospheric lifetime (years)</th>
<th>Global warming potential (GWP)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon-dioxide</td>
<td>CO$_2$</td>
<td>280000</td>
<td>389000</td>
<td>Variable</td>
<td>1</td>
</tr>
<tr>
<td>Methane</td>
<td>CH$_4$</td>
<td>700</td>
<td>1808</td>
<td>12</td>
<td>21</td>
</tr>
<tr>
<td>Nitrous oxide</td>
<td>N$_2$O</td>
<td>275</td>
<td>323</td>
<td>114</td>
<td>310</td>
</tr>
</tbody>
</table>

GWP*: The Intergovernmental Panel on Climate Change (IPCC) has used the Global Warming Potential as a method for comparing the potential climatic impact of emissions of different greenhouse gases. The GWP is the time-integrated radiative forcing due to a pulse emission of a given gas, over a given time period or horizon (100 year) relative to a pulse emission of carbon dioxide.
Although some halocarbons such as chlorofluorocarbons (CFCs) previously used as refrigerants, propellants in spray cans and solvents, are decreasing slowly as a result of international action to preserve the Earth’s protective ozone layer, the concentrations of other gases such as hydrochlorofluorocarbon (HCFCs) and hydrofluorocarbons (HFCs), which have replaced CFCs and are less harmful to the ozone layer, are increasing rapidly. These two classes of compounds are potent greenhouse gases and last much longer in the atmosphere than CO₂. The combined radiative forcing attributable to halocarbons is 12%. Anthropogenic contributions to aerosols (primarily sulphate, organic carbon, black carbon, nitrate and dust) together produce a cooling effect and also influence precipitation patterns.

The consequences of changes atmospheric composition are not known, but according to the IPCC (2001a) it is possible that the consequences for the environment and sustainable development could be severe. Hence, strategies aimed at mitigating and adapting the increase in atmospheric GHGs are being developed by national and international administrations. The Intergovernmental Panel on Climate Change (IPCC) was jointly established by the World Meteorological Organization (WMO) and the United Nations Environment Programme (UNEP) in 1988. Its terms of reference include (i) to assess available scientific and socio-economic information on climate change and its impacts and on the options for mitigating climate change and adapting to it and (ii) to provide, on request, scientific/technical/socio-economic advice to the Conference of the Parties (COP) to the United Nations Framework Convention on Climate Change (UNFCCC).

Since 1990, the IPCC has produced a series of Assessment Reports, Special Reports, Technical Papers, methodologies and other products that have become standard works of reference, widely used by policymakers, scientists and other experts (IPCC 2001a; IPCC 2007). As part of an effort to reduce global emissions of greenhouse gases (GHGs), which are expected to contribute to a significant warming of the earth’s climate, the Kyoto Protocol (KP) to the United Nations Framework Convention on Climate Change, signed in Kyoto in December 1997, includes binding GHG emissions targets for the world’s industrial economies for the period 2008-2012 (McKibbin and Wilcoxen 2002).
According to the KP, carbon (C) sequestration in terrestrial sinks can be used to offset GHG emissions. The KP states in Article 3.3 that “net changes in greenhouse gas emissions by sources and removals by sinks resulting from direct human-induced land-use change and forestry activities, limited to afforestation, reforestation and deforestation since 1990, measured as verifiable changes in C stocks in each commitment period, shall be used to meet the commitments”. Likewise, the special report on land use, land-use change and forestry (LULUCF) prepared in response to a request from the UNFCCC, revealed the important impact of land use and forestry changes on C stocks in the C pools of the terrestrial ecosystem and between the terrestrial ecosystem and the atmosphere (Watson 2000). Management and/or conversion of land uses (e.g. forests, croplands and grazing lands) affect sources and sinks of CO$_2$, CH$_4$ and N$_2$O (IPCC 2007).

In 2012, the United Nations Climate Change Conference was held at the Qatar National Convention Centre in Doha from 26 November to 8 December 2012. The conference reached an agreement to extend the life of the KP, which had been due to expire at the end of 2012, until 2020, and to reify the 2011 Durban Platform. The wording adopted by the conference incorporated for the first time the concept of "loss and damage", an agreement in principle that richer nations could be financially responsible to other nations for their failure to reduce C emissions.

The GHG emissions by industrialized countries currently exceed 220 billion tonnes of CO$_2$ equivalent, which is far more than the maximum of 150 billion agreed by the KP for the period 2008-2012. The Spanish ratification of the KP in 2002 implied a commitment that emissions would be no more than 15% above the 1990 level. The general character of the mitigation measures and the long-term approach entails the need to develop certain instruments as part of a strategic plan. If the policies to mitigate climate change and measures related to sustainable development remain as at present, global GHG emissions will continue to grow over the coming decades (IPCC 2007) which would have a substantial effect on the equilibrium and dynamic of the planet's ecosystems.
KP recognized the importance of land ecosystems in the global C cycle and recommends the protection, enhancement and quantification of terrestrial biospheric sinks for anthropogenic CO\textsubscript{2} emissions (Cramer et al. 2001).

Forests are the main reserve of terrestrial C stocks (Lal 2005). Forest ecosystems cover approximately 4.1 billion hectares globally (Dixon and Wisniewski 1995), corresponding to 31% of total land area (FAO 2010). The world’s forests have been estimated to contain up to 80% of all aboveground C (Dixon et al. 1994) although their contribution to the total varies with latitude and land use (Malhi et al. 2002). In the absence of natural or human disturbance, all forest systems would be potential C sinks, as indeed they were during Paleozoic and Mesozoic times, when much of the current fossil fuels were sequestered (Conard and Solomon 2008). On a global scale, the land cover ecosystems sequester approximately one-third of the anthropogenic emission in plant and soil pools (Schimel et al. 2001). European forests absorb 7-12 % of European anthropogenic CO\textsubscript{2} emissions (Janssens et al. 2003b). Even mature forests continue to store C in vegetation, litter and soils although the rate of sequestration may decrease greatly in older forests (Dixon and Krankina 1993; Dixon et al. 1994; Kasischke et al. 1995; Kurz and Apps 1999).

In recent decades, agroforestry ecosystems have gained prominence as an option for sequestering C on agricultural land due to their capacity to sequester significant amounts of C while the bulk of the land remains in agricultural production (Schoeneberger 2009). An extensive literature points to a general trend of increasing soil C sequestration in agroforestry in comparison to other land-use practices, with the exception of forest ecosystems (Ramachandran Nair et al. 2010). Agroforestry has been defined by the World Agroforestry Centre (www.icraf.cgiar.org) as a dynamic, ecologically based, natural resource management system which, through the integration of trees on farms and in the agricultural landscape, diversifies and sustains production for increased social, economic and environmental benefits for land users at all levels. In the first IPCC Technical Paper entitled: Technologies, policies and measures for mitigating climate change, agroforestry was included in both the forestry and agricultural sections (Schoeneberger 2009). Furthermore, in the IPCC Technical Paper-V, agroforestry was
identified as an activity that “can sequester C and have beneficial effects on biodiversity because it creates more biologically diverse systems than conventional agricultural lands” (Gitay et al. 2002).

In the Mediterranean basin, a traditional agroforestry system termed ‘the Dehesa’ (Spain) or ‘Montado’ (Portugal) has been an important and influential part of the landscape for many centuries (Eichhorn et al. 2006), covering around 5-6 million hectares of the Iberian Peninsula (Sundseth 2009). Although Dehesas are widespread in the Iberian Peninsula, they have not generally been included in large scale studies to assess soil C sequestration capacity (Romanya et al. 2007). Today, Dehesas generally consist of a mosaic of scattered, widely-spaced oak trees combined with crops, pasture and shrubs. This Dehesa landscape is the result of the different management practices carried out over the years, namely agriculture, livestock husbandry, forestry and hunting activities (Moreno and Obrador 2007). In these systems, shaped by human activity, there is a need to quantify the effects of climate change and management practices on their C capacity (Nair et al. 2006).

The amount of C sequestered at a given site reflects the long-term balance between C uptake and release mechanisms. Because the flux rates are large, changes such as shifts in land cover and/or land use practices that affect pools and fluxes of C, hold important implications for the C cycle and the earth’s climate system (Ramachandran Nair et al. 2009). Forest ecosystems are currently disappearing at a rate of 13 million ha/year (FAO 2012) and there are serious doubts as to whether the terrestrial sink capacity will be operative in the long term (IPCC 2007). Furthermore, changes in land use and land management, especially deforestation, cause significant changes in terrestrial C storage. Deforestation at low latitudes produce 1.6 ± 0.4 Pg of C per year (Dixon et al. 1994) and the emissions associated with land use changes account for 3 Pg y⁻¹ (Ciais et al. 2010).

Over two-thirds of the terrestrial C in forest ecosystems is contained in soils (Dixon et al. 1994). Soils play a particularly important role in the C cycle (Schulze and Freibauer 2005). The soil C stock may comprise as much as 85% of the terrestrial C stock in boreal forests, 60% in temperate forests and 50% in tropical forests (Dixon et al. 1994; Lal 2005). Soils contain between 1200 and 1500 Pg of C in the first 1m of soil profile (Lal 2004), although the amount reported by different authors varies (Dixon et al. 1994; Guo and Gifford 2002; Malhi et al. 2002; Schimel et al. 1994). This quantity increases to 2300
Pg of C when the entire profile is taken into account (Jobbágy and Jackson 2000). The soil C pool is three times greater than the atmospheric pool (770 Pg and 3.8 times larger than the vegetation pool 610 Pg). A reduction of 1 Pg in the soil C pool is equivalent to an atmospheric CO$_2$ enrichment of 0.47 ppmv (Lal 2001).

Considerable uncertainty exists regarding the sink capacity of soils under different forms of land-use, especially under future climate conditions (Cramer et al. 2001; IPCC 2001a) or under regimes of ecosystem disturbance, which are typical in certain regions (Jandl and Olsson 2011). Climate change forecasts for the Mediterranean area (IPCC 2007) indicate that the Mediterranean basin is one of the more susceptible zones as regards climate change, and predictions indicate increases in temperature, drought periods and heavy rainfall events, possibly contributing to greater levels of disturbance in the different ecosystems. These events predicted for the Mediterranean area would cause a further reduction in soil organic C (SOC) and would lead to land degradation, particularly in places which are prone to land erosion (e.g. mountain slopes and semiarid areas) (Durán Zuazo et al. 2006). However, the biology underlying the C storage capacity of terrestrial systems is rather complex, due the great heterogeneity of vegetation and soils, and the effects of human land use and management.

Likewise, although the seasonally-dry ecosystems are potentially able to act as efficient sinks for atmospheric CH$_4$ and significant sources of N$_2$O (Castaldi et al. 2004; Castaldi et al. 2006; Potter et al. 1996) less information is available for these type of ecosystems. Trace gas emissions from Spanish forest soils have rarely been studied (Merino et al. 2004), and therefore there is a little understanding of the spatial and temporal variability of N$_2$O and CH$_4$ exchange in Spanish forest soils or the soil parameters that control this exchange. The importance of forest type or tree species and the effects of land disturbances are also poorly understood. A high level of uncertainty exists as to the contribution (source or sink) of Spanish forests to the regional, continental, and global N$_2$O and CH$_4$ balance.
1.1.2.1 Terrestrial carbon cycle

Plants acquire CO$_2$ from the atmosphere by diffusion through stomata into leaves, the amount of CO$_2$ fixed from the atmosphere (converted to carbohydrate during photosynthesis), is known as gross primary production (GPP). Terrestrial GPP has been estimated at about 123 ± 8 PgC/yr (Beer et al. 2010). About half of the organic C compounds are incorporated into new plant tissues (leaves, stems and roots), and the other half is released back into the atmosphere through autotrophic respiration (Rp) (Figure 1.4). Rp can be separated into aboveground plant respiration (Ra) and belowground plant respiration (Rb). Annual plant growth is the difference between photosynthesis and autotrophic respiration, and is referred to as net primary production (NPP). The C fixed in NPP is returned to the atmospheric CO$_2$ pool through two processes: heterotrophic respiration (Rh) by decomposers (bacteria and fungi feeding on dead tissue and exudates) and herbivores; and combustion in natural or human-set fires (Figure 1.4).

![Figure 1.4. Schematic C Cycle in terrestrial ecosystems.](image)
Soils receive dead organic material mainly from the plant cover. This material is decomposed by the biota and partly mineralized. It is released into the atmosphere as CO$_2$ and CH$_4$ or by leaching into groundwater (Schulze and Freibauer 2005). The mechanism of soil C sequestration involves the decomposition of plant residues and other organic materials that serve as a source of C and nutrients for microbial communities and vegetation. Part of this C is released back into the atmosphere as CO$_2$ during respiration or is incorporated into the living biomass. The live microbial biomass is mixed with organic residuals of dead plants and dead microbes to form soil organic matter (SOM) (Luo and Zhou 2006); which is a continuum of compounds with different degrees of decomposition and stabilization and, therefore, turnover rates (Trumbore 1997; von Lützow et al. 2007). Leaves and fine roots usually live from several months up to a few years before death, whereas woody tissue may grow for hundreds of years in forests, constituting stable SOM (Figure 1.4). About a third of SOM breaks down much more slowly and could be present for a long time in soils. SOM represents a significant C store and is intimately associated with soil organic carbon (SOC) (Martens 2000). SOC comprises a variety of fractions that differ in terms of decomposability and possess very heterogeneous structures (Wander 2004). Changes in the ability of soils to store SOC can have positive or negative feedbacks on atmospheric CO$_2$ levels (Davidson and Janssens 2006; Rustad et al. 2000)

1.1.2.2 The soil respiration

Soil respiration, which is an important flux in the terrestrial C cycle (Dixon et al. 1994), is the sum of autotrophic respiration from plant metabolic activity and heterotrophic respiration from the decomposition of organic material by microbes (Hanson et al. 2000; Luo and Zhou 2006). Soil respiration may account for between 56% and 67% of total ecosystem respiration (Janssens et al. 2001; Lavigne et al. 1997; Longdoz et al. 2000), although this contribution may differ from one ecosystem to another, depending fundamentally on site biomass (Longdoz et al. 2000), vegetation type and age of stands (Buchmann 2000). Photosynthesis has also been reported to affect soil respiration (Hogberg et al. 2001; Janssens et al. 2001). The availability of photosynthetic C is the primary and fundamental limitation to root respiration (Janssens et al. 2001). Moreover, organic matter deposition and exudates from vegetation play an important role
in microbial and root respiration. Estimates of the magnitude of the soil respiration ranged from 68 Pg C yr\(^{-1}\) (Raich and Schlesinger 1992) to 100 Pg C yr\(^{-1}\) (Musselman and Fox 1991). Raich and Potter (1995), using a global model, updated the estimate of global soil respiration to 77 Pg C yr\(^{-1}\). Similar values of 78 \(\pm\)12 Pg were found by Lal (2007). At global scale, soil respiration releases C at a rate which is more than one order of magnitude greater than the anthropogenic emission and is only exceeded by gross primary productivity (Bahn et al. 2008; Janssens et al. 2001; Longdoz et al. 2000), which is estimated to range from 100 to 120 Pg C yr\(^{-1}\) (Rustad et al. 2000). Small changes in soil respiration can critically alter the balance of atmospheric CO\(_2\) concentration. As a result of growing concern about climate change and the increasingly recognized importance of the role of soils as potential C sinks, much effort is being devoted to improving our understanding of the interaction between environmental variables and soil respiration at local, regional and global scales.

### 1.1.2.3 Factors controlling soil respiration

Soil respiration depends on numerous biotic and abiotic factors related to the ecosystem and environment. Critical factors reported to influence rates of soil respiration are: soil temperature and moisture (Boone et al. 1998; Buchmann 2000; Davidson et al. 1998; Inclán et al. 2007; Longdoz et al. 2000; Lloyd and Taylor 1994); vegetation and substrate quality (Olsen and Van Miegroet 2010; Raich and Schlesinger 1992); quantity and quality of SOM (Trumbore 2000); net ecosystem productivity (Raich and Potter 1995; Schlesinger 1977); relative allocation of NPP above- and belowground (Boone et al. 1998); population and community dynamics of the aboveground vegetation and belowground flora and fauna (Raich and Schlesinger 1992) and land use and or disturbance regimes such as wildfires (Conard and Solomon 2008; Johnson et al. 2007; Weber 1990).

### 1.1.2.4 Soil temperature and moisture

Temperature influences root respiration via its effects on root growth; respiration in young roots being more susceptible to temperature than old roots. Soil temperature also has a direct influence on the microbial decomposition process. Soil respiration is
typically modeled through a $Q_{10}$ function or as a step relationship based on temperature response curves (Rustad et al. 2000).

The $Q_{10}$ exponential model based on the Arrhenius equation is that which is most frequently used to predict respiration rates from temperature (Boone et al. 1998; Buchmann 2000; Raich and Potter 1995); where the parameter $Q_{10}$ describes the sensitivity of respiratory processes to temperature (Janssens and Pilegaard 2003). Based on recently compiled data, the estimated values of $Q_{10}$ for soil respiration vary widely from little more than 1 to more than 10, depending on the geographic locations and ecosystem types (Davidson et al. 1998; Janssens et al. 2003b; Kirschbaum 1995; Lloyd and Taylor 1994; Raich and Schlesinger 1992). However, rather than temperature sensitivity, $Q_{10}$ is now considered to reflect a combined response to fluctuations in temperature, root biomass, moisture conditions, and perhaps other variables such as substrate (Boone et al. 1998; Davidson et al. 1998; Janssens and Pilegaard 2003).

Soil moisture influences soil respiration directly though physiological processes of roots and microorganisms (Davidson and Janssens 2006) and indirectly via diffusion of substrates and $O_2$ (Linn and Doran 1984). Soil $CO_2$ efflux reaches the maximal rate in intermediate soil moisture levels; and decreases under dry conditions or at high soil moisture content (under anaerobic conditions) (Bowden et al. 1998; Xu and Baldocchi 2004). The effect of moisture on soil respiration assumes particular importance in ecosystems where the water content is limited (Almagro et al. 2009; Asensio et al. 2007; Curiel Yuste et al. 2003; Davidson et al. 2000; Inclán et al. 2007; Janssens and Pilegaard 2003; Marañón-Jiménez et al. 2011; Rey et al. 2002; Xu and Qi 2001) such as in Mediterranean ecosystems.

1.1.2.5 Substrate and ecosystem productivity

Substrate supply directly from canopy photosynthesis exerts a strong influence on soil respiration (Hogberg et al. 2001). However, the heterogeneity in substrate quality and the multiple sources of supply make it extremely difficult to derive simple relationships between substrate supply and respiratory $CO_2$ production (Raich and Tufekciogul 2000). Although respiratory $CO_2$ release is linearly proportional to substrate availability, the rate at which the substrates are converted to $CO_2$ varies with substrate types (Raich and
Tufekciogul 2000). Plant phenology has an important influence on soil respiration, mainly through different timing of root growth, root turnover, and litterfall (Curiel Yuste et al. 2007). C is the main component of SOM, which is a continuum of compounds with different degrees of decomposition and stabilization and therefore, different turnover rates (Trumbore 1997; von Lützow et al. 2007).

Litter provides substantial amounts of C substrate to microbial respiration (Raich and Tufekciogul 2000). As a consequence, soil respiration usually increases with the amount of litter (Boone et al. 1998; Maier and Kress 2000; Sulzman et al. 2005). Although on global scales, soil respiration is correlated with ecosystem productivity (Janssens et al. 2001; Raich and Schlesinger 1992; Reichstein et al. 2003); the interaction between the demand for carbohydrates (as regulated by the soil environment) and the aboveground capacity to supply carbohydrates (as determined by photosynthesis), together govern the belowground carbon flux and soil respiration (Luo and Zhou 2006) in the different ecosystems.

1.1.2.6 Temporal and spatial variation in soil respiration

The spatial and temporal variations in soil respiration result from variations in environmental variables (soil temperature and precipitation mainly), biochemical processes of respiration, vegetation and transport processes of CO₂ gas (Raich et al. 2002). Diurnal variation in soil respiration can be explained by soil temperature (Rayment and Jarvis 2000) and photosynthesis (Tang et al. 2005a) fundamentally. However, fluctuations in atmospheric pressure and humidity may also affect the diurnal patterns of CO₂ emission from soils (Law et al. 2001). Greater relative humidity at night in arid ecosystems leads to higher soil respiration rates than during the day due to increased microorganism activity (Edwards 1975). On a weekly time-scale, variations in soil CO₂ efflux may be induced by synoptic weather events and changes in photosynthetic assimilation (Ekblad and Högberg 2001; Tang et al. 2005a).

Seasonal variation in soil CO₂ efflux has been observed in almost all ecosystems, driven largely by changes in moisture, temperature, photosynthate production or a combination of these. In Mediterranean ecosystems in summer, moisture becomes a limiting factor, whereas in winter, the limiting factor is temperature (Almagro et al. 2009;
Curiel Yuste et al. 2003; Inclán et al. 2007). A number of studies have reported maximum soil respiration values in spring and autumn (Almagro et al. 2009; Asensio et al. 2007; Inclán et al. 2007; Joffre et al. 2003), when soil temperature and soil moisture conditions are optimal for stimulating both plant and microbial activity (Dickmann et al. 1996; Xu and Baldocchi 2004).

Spatial variability in soil respiration occurs on various scales, from a few square centimeters to several hectares up to global scale (Rayment and Jarvis 2000). At landscape scale, the spatial variability in soil respiration is caused largely by variations in climate, topography, soil characteristics, vegetation types, patchy areas and edges of patches, and disturbance history (Maestre and Cortina 2003; Olsen and Van Miegroet 2010). It is necessary to understand the spatial variability of soil CO$_2$ efflux in order to make a representative estimate of the regional C budget. Due to the high heterogeneity of natural soil, spatial differences in soil respiration have been observed in various ecosystems (Law et al. 2001; Xu and Qi 2001).

Regional patterns of soil respiration have been examined through synthesis of data from eddy-covariance flux networks (Janssens et al. 2001; Serrano-Ortiz et al. 2009; Valentini et al. 2000) and through transect studies in different ecosystems. In general, wetter and warmer regions exhibit higher rates of soil respiration and decomposition of organic matter than colder and drier regions when all other variables are similar.

Altitudinal gradients are often used to examine environmental regulation of soil respiration. Numerous natural gradient studies (e.g., latitudes, altitudes, topography, and successional ages), have been carried out to examine variations in soil respiration (Conant et al. 1998; Rodeghiero and Cescatti 2005; Schindlbacher et al. 2010; Simmons et al. 1996). Understanding the spatial and temporal variation in soil respiration within small geographic areas is essential to accurately assess the C budget on a global scale.

Studies undertaken along elevation gradients indicate that soil C concentrations or stocks increase with altitude in mountainous terrains (Rodeghiero and Cescatti 2005; Schindlbacher et al. 2010). Microbial respiration increases with elevation from 1900 to 2300 m, possibly due to differences in soil carbon pool sizes (Conant et al. 1998). Field studies carried out in the mountains of more arid areas indicate that soil moisture is an important factor controlling soil C stocks and fluxes along elevation gradients.
Greenhouse gas flows in forest and agroforestry soils in the center of the Iberian Peninsula

(Buchmann 2000; Rodeghiero and Cescatti 2005). Furthermore, topography induced microclimates can affect soil respiration rates by constraining microclimatic factors, such as soil temperature and moisture (Davidson et al. 1998). Other studies suggest that certain chemical and biological factors such as nitrogen (N) and C dynamics co-varying with soil moisture regulate the spatial distribution of soil C losses through decomposition (Garten Jr and Hanson 2006). The findings of some studies suggest a correlation between soil respiration rates and tissue N concentration (Janssens et al. 2010), so differences in N availability among sites and changes in N availability through N deposition or global change may alter soil respiration rates. The ways in which N content affect litter decomposition and therefore microbial respiration is somewhat complex (Magill and Aber 1998). Early stages of litter decomposition may respond positively to elevated N deposition, since microorganisms on high C/N ratio litter material need to immobilize N for the decomposition (Berg 2000), whereas during the later stages, when the easily decomposable organic matter has been processed, decomposition may actually decrease with N availability (Janssens et al. 2010). However, the mechanism whereby N addition is accompanied by a decrease in decomposition and increase in soil C stocks is still unclear (Janssens et al. 2010).

1.1.2.7 Methane and Nitrous oxide fluxes in forest soils

Forest soils are the main reservoirs of CH₄ and N₂O, both biologically produced and consumed by soil microorganisms (Conrad et al. 1989; Chapuis-Lardy et al. 2007; Firestone and Davidson 1989). The net N₂O and CH₄ exchange fluxes between soils and the atmosphere are controlled by the balance of concurrent production and consumption mechanisms in the forest soils.
1.1.2.8 Methane

Forest soils can act as potential sources or sinks for atmospheric CH₄; positive flux (emission) and negative flux (deposition). A special group of soil microorganisms, known as methanogenic bacteria, are responsible for CH₄ production. Recent estimates put global CH₄ emissions from soil at between 150 and 250 Tg CH₄ yr⁻¹ (IPCC 2001a), with a quarter to a third of the total emitted from the wet soils of high latitudes (Walter et al. 2001). Another group, called methanotrophs, is responsible for CH₄ consumption. CH₄ consumption by soil microbes is estimated at around 10⁻⁵¹ Tg CH₄ yr⁻¹ (Dutaur and Verchot 2007; IPCC 2001a; Potter et al. 1996). Most of the CH₄ consumption occurs in the well-drained soils of temperate and tropical areas (Le Mer and Roger 2001).

Plants affect methane because most of the plant biomass is situated below-ground, where methane production also takes place (Saarinen 1996). It is therefore not surprising that methane fluxes were first found to correlate with vegetation biomass (Schimel 1995). Vegetation can promote methanogenesis by providing root exudates and easily decomposable litter, which contains carbohydrates, organic and amino acids and phenolic compounds (Rovira 1969). Experimental evidence also suggests that methane flux increases with the photosynthetic activity of plants (Whiting and Chanton 1993). The significantly lower methane fluxes from unvegetated surfaces compared to vegetated surfaces highlights the importance of plants in the substrate supply (Smith et al. 2000). Temperature and moisture also play an important role in the magnitude and direction of the CH₄ flux.

1.1.2.9 Methane production

Methane is formed as a terminal stage of a complex anaerobic degradation chain by methanogenic bacteria. Strictly anaerobic conditions and a suitable C source are required for methanogenesis to occur. Methanogenesis is mainly achieved via three pathways: carbonate reduction, fermentation of acetate and other organic acids, and fermentation of various methylated substrates (Figure 1.5).
Figure 1.5 Diagram of methane production. The upper part shows the carbon flows, the lower part concerns the electron-acceptor cycling. Rectangular boxes represent material, rounded boxes represent processes. The thicknesses of the lines qualitatively represent typical sizes of the flows. Source: (Segers and Kengen 1998).

1.1.2.10 Methane oxidation

Opposite reaction for methane production, converts CH\(_4\) to CO\(_2\) (Wang and Ineson 2003). Given the importance of soils as methane sinks, a relatively large number of studies have been conducted to measure CH\(_4\) oxidation rates in various types of soils (Born et al. 1990; Kravchenko et al. 2002). Methane oxidation rates are known to be higher in forest soils than in grasslands or agricultural lands (Bender and Conrad 1995). The main variables controlling CH\(_4\) oxidation rates are soil water content and inorganic nitrogen in the soils (Castro et al. 1995; Jang et al. 2006). Inorganic nitrogen, ammonium in particular, is known to inhibit enzymes involved in methane oxidation (Wang and Ineson 2003).

1.1.2.11 Nitrous oxide

Terrestrial systems are major sources of atmospheric N\(_2\)O, accounting for about 8% of the current GHG effect (IPCC 2007). On a global scale, soils are a major source of
N$_2$O (approximately 60% of all sources) (Firestone and Davidson 1989). Due to the high rates of deposition of nitrogen compounds, European forests are a significant source of N oxides (Kesik et al. 2005). In the soil, N$_2$O can be produced through several coexisting processes that are regulated by the number of different forms of N and the O$_2$ concentration. The nitrification and denitrification processes dominate N$_2$O production in soils, although N$_2$O can also be formed in the soil chemically via chemodenitrification (Johnson 1992). In order to comprehend the complexity of N$_2$O formation, it is necessary to understand the major N$_2$O production and consumption processes described below.

1.1.2.12 Nitrogen cycle

In terrestrial ecosystems, the main factors affecting N cycling are the climatic, edaphic and landscape conditions, the sum of N inputs via N deposition and biological N fixation. N cycling in terrestrial ecosystems is characterized by a variety of N transformations involving both organic and inorganic (ammonium and nitrate) N species and the uptake/immobilization of N by microbes and plants, as shown in (Figure 1.6).

Dinitrogen (N$_2$) is the main component of the earth’s atmosphere and although N is essential for all forms of life, N$_2$ cannot be used directly by biological systems to synthesize the chemicals required for growth, maintenance and reproduction. Many bacteria and algae, especially the symbiotic bacteria of the genus *Rhizobium*, are able to fix molecular nitrogen (N$_2$) from the atmosphere. Besides N fixation, decomposition of SOM is a major source of mineral N to plants and microorganisms (Figure 1.6). The loss of N from the soil occurs via leaching of nitrate (NO$_3^-$) to waterways, or through gaseous losses as nitrogen oxides (N$_2$O; NO) or molecular nitrogen (N$_2$) into the atmosphere. Although gaseous losses of N to the atmosphere form only a minor part of N cycling within the biosphere, losses in the form of N$_2$O and NO play a crucial role in determining the scope of climate change.
Figure 1.6 Scheme of major ecosystem processes of N cycling, including internal N retention pathways. Dashed lines indicate plant processes; solid lines show microbial processes; red dashed and solid lines indicate competitive processes between plants and microorganisms. The blue lines are hydrological transport pathways. Source: (Rennenberg et al. 2009).

During the decomposition process, SOM is cleaved from large polymers to largely bio-available monomers which are accessible to both plants and microbes. Depolymerization of organic matter is carried out by extracellular enzymes of fungi and bacteria (Jackson et al. 2008). Microbes can further degrade these organic monomers to form ammonium (NH$_4^+$) through ammonification or N mineralization mechanics. NH$_4^+$ as well as organic N can also be oxidized to nitrate (NO$_3^-$) through nitrification mechanics (Figure 1.7). Both NH$_4^+$ and NO$_3^-$ can either be taken up by plants or immobilized by microorganisms and converted to N$_2$ through denitrification mechanics (Figure 1.7).
Figure 1.7 Transformation of mineral nitrogen in soil. Source: (Wrage et al. 2001).

1.1.2.13 Nitrification

Nitrification is the biological oxidation of NH$_4^+$ or ammonia (NH$_3$) via hydroxylamine to nitrite (NO$_2^-$) or nitrate (NO$_3^-$) (Figure 1.7). Nitrification of NH$_4^+$ increases the probability that the converted N$_2$ is lost from the ecosystems, since the end products NO$_2^-$ / NO$_3^-$ are susceptible to losses by leaching along hydrological pathways or further reduction to gaseous NO, N$_2$O and N$_2$ via denitrification (Figure 1.7).

Nitrification can be performed both along heterotrophic and autotrophic pathways. Autotrophic nitrification is performed in two steps by different groups of microorganisms (Costa et al. 2006; Wrage et al. 2001). In a first step, an ammonia oxidizer oxidizes NH$_4^+$ or ammonia to hydroxylamine (NH$_2$OH) and further to NO$_2^-$. *Nitrosomonas europaea* is the best recognized autotrophic ammonia oxidizer. In a second step a nitrite-oxidizer (e.g. *Nitrobacter*) converts NO$_2^-$ to NO$_3^-$.

While autotrophic nitrifiers use the oxidation of NH$_4^+$ or NO$_2^-$ as an energy source for CO$_2$ fixation, heterotrophic nitrifiers use organic N. Heterotrophic nitrifiers may be able to oxidize both NH$_4^+$ and organic N compounds. Nitrification is in general an aerobic process. The oxygen availability in the soil exerts a high influence. The optimum soil water content for nitrification is, depending on the soil texture, in the range of approximately 30%–60% water-filled pore space (Davidson 1993). However, autotrophic nitrification is affected by soil pH and the optimum pH for nitrification is thought to be pH 5.5–6.5 (Weslien et al. 2009). Another potential
environmental control on nitrification is soil temperature (Castaldi 2000). Nitrogen is crucial for the growth and activity of heterotrophic soil microorganisms. Heterotrophic microorganisms in the soil prefer NH$_4^+$ over NO$_3^-$ (Rice and Tiedje 1989) due to energy costs. Nevertheless, microbial NO$_3^-$ immobilization is a significant process in a wide range of terrestrial ecosystems (Booth et al. 2005), especially in relatively undisturbed soils of seminatural ecosystems (Rennenberg et al. 2009).

1.1.2.14 Denitrification

Denitrification is defined as the respiratory bacterial reduction of NO$_3^-$ or NO$_2^-$ to nitrogen oxides or molecular nitrogen (Einsle and Kroneck 2005) (Figure 1.7). N$_2$O is an obligatory intermediate of the reduction process catalyzed by the enzymes nitrate reductase, nitrite reductase, nitric oxide reductase and nitrous oxide reductase (Einsle and Kroneck 2005). Denitrifying microorganisms are often facultative aerobic bacteria that are able to reduce nitrogen oxides when O$_2$ becomes limiting. Denitrifying microorganisms derive energy mostly from organic substrates; hence denitrification is mostly limited by the amount of readily decomposable organic substrates in the soil.

N additions stimulate plant primary production (LeBauer and Treseder 2008). In N rich environments, N fertilization could exacerbate the conditions of N saturation, resulting in N leaching and runoff and causing little change in soil respiration. There is growing evidence of reduced soil respiration from chronic N addition experiments (Burton et al. 2004; Janssens et al. 2010) in parallel with a decline in soil microbial biomass (Treseder 2008).

1.1.3 Other N$_2$O forming processes in soils

1.1.3.1 Chemodenitrification

Small amounts of N$_2$O can be formed in the chemical decomposition of NO$_2^-$ or other intermediates from the oxidation of NH$_4^+$ to NO$_2^-$. This process usually takes place at low pH, and the major product is NO, although some N$_2$O may also be produced (Bremner 1997).
Production and emission of N$_2$O from soil are controlled by several factors, including aeration, water content (Davidson 1993), soil temperature (Castaldi 2000), organic N and C content, pH (Weslien et al. 2009) and soil characteristics.

1.1.4 The influence on wildfires in greenhouse gases efflux

Climatic warming, which is generally more pronounced in boreal and temperate zones of the Northern Hemisphere, is expected to continue to accelerate over the next century, leading to more severe droughts and the likelihood of more frequent and severe fires in many parts of the world (IPCC 2007). Forest fires are highly sensitive to climate change since fire behavior responds immediately to fuel moisture, which is affected by precipitation, relative humidity, air temperature and wind speed (Flannigan et al. 2000).

Ecosystem models using satellite data estimate an average annual global emission from biomass burning of around 1.3 to 3.4Gt C per year (Hoelzemann et al. 2004). Moreover, apart from CO$_2$, other components of this emission such as CH$_4$, black C and other aerosols, though emitted in smaller amounts, can have much stronger climate forcing coefficients per unit mass (IPCC 2007).

Wildfires may lead to soil degradation, including deterioration of structure as well as losses of soil organic matter and nutrients (Shakesby 2011). The role of wildfire in the storage and release of C is largely a function of the fire regime (frequency, size, seasonality, and severity of fires etc) and the type of ecosystem; as a result of severe fires with loss of vegetation and soils, a forest system may lose the ability to capture C, acting only as a source of C (Litton et al. 2003). Hence, the importance of accurately quantifying the regional and global effects of wildfires on C stocks (Conard and Solomon 2008).

By far the greatest impact of fire on the global C cycle is the pulse release of large amounts of previously sequestered C in above ground biomass in the form of CO$_2$, along with both short and long term changes in SOC dynamics and net ecosystem exchange (NEE) (Amiro et al. 2003; Dore et al. 2008; Van Miegroet and Olsson 2011). The fire affects SOC in two ways: in the first instance through the loss and chemical modification of SOC during the burn event and then during the post fire period through changes in soil properties (Van Miegroet and Olsson 2011).
Approximately 85% of the total burnt area corresponds to the EU Mediterranean region (San-Miguel-Ayanz et al. 2012); about 65,000 fires take place every year in the European region, burning on average around half a million ha of forested areas (San-Miguel-Ayanz et al. 2013). In the Mediterranean area, wildfires represent the most important natural disturbance due to the climatic characteristics, the influence of human activity (Lloret et al. 2002; Naveh 1990; Trabaud 1994) and climate change scenarios (IPCC 2007; Sabaté et al. 2002).

Wildfires can lead to significant C pool losses as well as changes in the soil parameters that control the CO₂, N₂O and CH₄ exchanges. Therefore, determining the impact of fire on soil ecosystems and the emission/deposition of greenhouse gases is crucial in order to assess the potential consequences of the predicted increase in wildfires in Mediterranean ecosystems.

1.2 OBJECTIVES AND STRUCTURE

1.2.1 The general objective

Due to the complexity of the processes that control the exchange of C and N gasses between soils and the atmosphere in forest and agroforestry ecosystems, understandable uncertainties exist as regards the estimation of greenhouse gas (GHG) fluxes and the soil sink capacity at regional and global scale under different forms of land use and disturbance regimes.

These uncertainties justify the need to characterize the exchange dynamics of GHG between the atmosphere and soils in Mediterranean ecosystems, particularly in the current context of climate change and the associated increase in temperature, drought periods, heavy rainfall events, and increased risk of wildfires, which affect not only the C and N pools but also the soil C sink capacity of these ecosystems.

Within this framework, the general objective of the present thesis is to quantify and characterize the GHG fluxes (CO₂, N₂O and CH₄) along with C and N stocks in Quercus ilex, Quercus pyrenaica and Pinus sylvestris forests as well as a ‘dehesa’ ecosystem in the center of Spain.
The research was divided into five specific objectives:

1. To study the temporal and spatial effect on the soil CO$_2$ efflux in a small Mediterranean forest catchment.

2. To contribute to a better understanding of soil CO$_2$, N$_2$O and CH$_4$ exchange in Mediterranean forest ecosystems under different disturbance regimes caused by forest fires.

3. To determine the post-wildfire changes in the biotic and abiotic soil parameters and their relationship with changes in greenhouse gas exchange from forest soils.

4. To establish the effects of fire on C pools, stocks and fluxes in three typically Mediterranean forests in the center of Spain.

5. To study the soil CO$_2$ efflux and soil C and N stocks in agroforestry soils under different management activities.

In order to meet these objectives, the present document includes the following Chapters:

- **Chapter 2**: This Chapter deals with the effect of spatial variation within small geographical areas on soil CO$_2$ fluxes. This line of research is essential to improving the accuracy of carbon budget assessments on a global scale. It involved establishing transects at different altitudinal gradients in a southern Mediterranean mixed pine-oak forest ecosystem on the north face of the Sierra de Guadarrama in Spain where CO$_2$ fluxes, physical-chemical and microbiological properties of the soil were measured over a period of one year.

- **Chapter 3**: In this Chapter we evaluate the long term effects of wildfires on C efflux in three typical Mediterranean forest ecosystems by comparing soil C fluxes in non-burned and burned areas. During the experiment, the effect of forest fires on the physical-chemical and biological soil parameters (bulk density; pH, C and N content; microbial biomass, soil organic components etc) as well as their impact on soil C efflux were examined. The investigation was conducted in *Quercus ilex*, *Quercus pyrenaica* and *Pinus sylvestris* forest stands located in the area surrounding Madrid (Spain) over two successive years. Soil temperature and soil moisture measurements
taken during the study were integrated into two different models in order to predict the response of soil C efflux to future changes in soil microclimate conditions.

- **Chapter 4:** The aim of this Chapter is to examine the long-term effect of wildfires on N\textsubscript{2}O and CH\textsubscript{4} soil-atmosphere exchange in Holm oak, Pyrenean Oak and Scots pine forest stands located in the Sierra de Guadarrama, Madrid (Spain). These greenhouse gases were measured over a period of eighteen months in mature stands and post-fire stands. Along with gas fluxes, physical-chemical and biological soil parameters were measured during the experiment.

- **Chapter 5:** To determine the influence of tree canopy and traditional silvopastoral management practices (grazing and tilling) on soil CO\textsubscript{2} fluxes and soil C and N stocks in a dehesa system. This work was undertaken in a typical dehesa ecosystem, Dehesón del Encinar in the center of Spain (Oropesa, Toledo).

1.3 GENERAL METHODOLOGY AND STUDY AREA

In the development of the present thesis, different methodologies have been employed, including field studies, field work methodology, laboratory analytical techniques and the use of statistical tools in the data analysis:

1.3.1 Field studies:

The research work described in the different Chapters of the present thesis is based on field studies. In order to achieve the specific objectives of each Chapter, different experimental approaches were established which included the selection of study areas, the environmental parameters to be studied throughout the investigation, the most appropriate techniques for the field work, the sampling/measurement frequency and the duration of the studies.
1.3.2 Field work methodology:

The study areas used in the thesis are situated at different locations around the center of the Iberian Peninsula (Figure 1.8). The studies concerning forest ecosystems were conducted in the Sierra de Guadarrama (Madrid). Chapter 2 was conducted on the northern face of the Sierra de Guadarrama (Valsaín, Segovia), while Chapter 3 and Chapter 4 were conducted on the southern face of the Guadarrama mountain range, in three of the most representative Mediterranean forest types present in the region around Madrid (*Quercus ilex* in Torrelodones, *Quercus pyrenaica* in El Escorial and *Pinus sylvestris* in Los Molinos). The agroforestry ecosystem study described in Chapter 5, was undertaken in a typical dehesa ecosystem, *Dehesón del Encinar* in the center of Spain (Oropesa, Toledo).

The main characteristics of the selected study areas were determined in different plots. These include the geographical coordinates, elevation, slope, and aspect. Furthermore, in each of the study plots, a forest inventory was carried out in which each tree was mapped and total height along with diameter at breast height were measured.

Additionally, the forest understory consisting of shrub-type vegetation and grasses was identified. The historical meteorological data from each area was obtained from the Spanish National Meteorological Service and a small meteorological station was installed at the dehesa.

In the studies conducted in the forest situated on the southern face of the Sierra de Guadarrama (Chapter 3 and 4), the chemical and physical properties of the whole soil profile (0–100 cm) were characterized at the beginning of the experiment at each study site. The soils were classified according to the FAO (1998) classification.

The management practices employed in the different studies areas (coppice, silvopastoral practices etc) along with the characteristics of the wildfires was provided by local environmental agencies.

In each study area, the necessary devices were installed for collecting and measuring the environmental parameters studied in each of the Chapters. The different
device designs were those most commonly used in this kind of studies; the maps sketch of devices are show in the Annex of this thesis.

1.3.3 Analytical laboratory techniques

Most of the environmental parameters (soil carbon and nitrogen content; total dissolved organic carbon, soil microbial biomass carbon, soil pH and electrical conductivity, water-filled pore space, soil moisture content, soil particle density, the concentrations of the exchangeable cations, the alkali-extracted humic substances, soil mineral N, and soil mineralogy, etc) were analyzed in the laboratories of the CIEMAT (Research Center for Energy, Environment and Technology).

Soil classification was performed by the soil group at the Department of Silvopascicultura of the Polytechnic University of Madrid. Likewise, the N₂O and CH₄ gases were analyzed by the Department of Agricultural Chemistry at the Polytechnic University of Madrid.

1.3.4 Statistical Analysis

Different statistical tools were used (parametric and non-parametric analysis) according to the type of data set and the objectives of each Chapter. These analyses include correlations, analysis of variance, t-test, linear models (one-dimensional variables), multiple linear regression models (multidimensional variables), exponential models etc. All statistical analysis was performed using the Statistica 6.0 (StatSoft. Inc., Tulsa. USA) and Sigma Plot 6.0 software packages (SystatSoft. Inc., San Jose, USA).
Figure 1.8 Map of the Iberian Peninsula showing the locations of the study areas investigated in the present thesis. The locations of Valsaín, Los Molinos, El Escorial and Torrelodones belong to forestry areas in the Sierra de Guadarrama (Madrid) and Oropesa belongs to the dehesa area located in Toledo. Source: ‘Google Earth’.
Figure 1.9 Experimental plot located in mixed forest of *Pinus sylvestris* and *Quercus pyrenaica* in the Valsaín forest (Segovia).
Figure 1.10 Non-burned area located in a Holm oak wood (Quercus ilex subsp. ballota) in Torrelodones municipality (Madrid).
Figure 1.11 Burned area located in a Holm oak wood (*Quercus ilex* subsp. *ballota*) in Torrelodones municipality (Madrid).
Figure 1.12 Non-burned area located in forest of *Quercus pyrenaica* Willd. in Escorial municipality (Madrid).
Figure 1.13 Burned area (above), and one of the burned plots (below) located in the Quercus pyrenaica forest stand located in Escorial municipality (Madrid).
Figure 1.14 Non-burned area located in a pine forest of *Pinus sylvestris* L located in Los Molinos municipality (Madrid).
Figure 1.15 *Adenocarpus hispanicus* vegetation in burned area located in former pine forest in Los Molinos municipality (Madrid).
Figure 1.16 Experimental areas located in the agroforestry system “Dehesón del Encinar” in Oropesa (Toledo).
1.4 REFERENCES


Firestone M K and Davidson E A 1989 Microbiological basis of NO and N₂O production and consumption in soil.


Chapter 1
Introduction


forest during a summer drought. Agricultural and Forest Meteorology 110, 27-43.


Luo Y and Zhou X 2006 Soil Respiration and the Environment. San Diego, CA.


Nair P K R, Bannister M E, Nari V D, Alavalapati J R R and Ellis E 2006 Silvopasture in south-eastern United States: more than just a new name for an old practice. CABI.


Greenhouse gas flows in forest and agroforestry soils in the center of the Iberian Peninsula


Watson R T 2000 Land use, land-use change, and forestry: a special report of the intergovernmental panel on climate change. Cambridge University press, Cambridge, UK.


Chapter 2

CARBON DIOXIDE FLUXES ACROSS
THE SIERRA DE GUADARRAMA, SPAIN
Chapter 2

CARBON DIOXIDE FLUXES ACROSS
THE SIERRA DE GUADARRAMA, SPAIN


European Journal of Forest Research (2010) 129, 93-100
ABSTRACT

Understanding the spatial and temporal variation in soil respiration within small geographic areas is essential to accurately assessment carbon budget on the global scale. In this study we investigated the factors controlling soil respiration in an altitudinal gradient in a southern Mediterranean mixed pine-oak forest ecosystem in the north face of the Sierra de Guadarrama in Spain. Soil respiration was measured in five Pinus sylvestris L. plots over a period of one year by means of a closed dynamic system (LI-COR 6400). Soil temperature and water content were measured at the same time as soil respiration. Other soil physico-chemical and microbiological properties were measured during the study.

Measured soil respiration ranged from 6.8 μmol·m⁻²·s⁻¹ to 1.4 μmol·m⁻²·s⁻¹, showing highest values at plots situated at higher elevation. Q₁₀ values ranged between 1.30 and 2.04, while R₁₀ values ranged between 2.0 and 3.6. The results indicate that soil respiration seasonal variation was mainly controlled by soil temperature and moisture. Among sites, soil carbon and nitrogen stocks, regulate soil respiration in addition to soil temperature and moisture.

Our results suggest that application of standard models to estimate soil respiration for small geographic areas may not be adequate unless other factors are considered in addition to soil temperature.

**Keywords:** soil respiration, microbial biomass carbon, carbon and nitrogen stocks, soil water content, soil temperature, Pinus sylvestris, altitudinal transects.
# CONTENT

2.1  INTRODUCTION .............................................................................................................. 57

2.2  MATERIAL AND METHODS ......................................................................................... 58

2.2.1 Study site ...................................................................................................................... 58

2.2.2 Experimental design ................................................................................................. 58

2.2.3 Soil respiration, temperature and moisture measurements ...................................... 60

2.2.4 Soil properties ............................................................................................................. 61

2.2.4.1 Soil sampling ........................................................................................................... 61

2.2.4.2 Analysis of soils ....................................................................................................... 61

2.2.4.3 Calculation of soil carbon and nitrogen stocks ...................................................... 63

2.2.5 Statistical analysis and modelling ................................................................................ 63

2.3  RESULTS ....................................................................................................................... 64

2.3.1 Soil respiration ........................................................................................................... 64

2.3.2 Soil temperature and moisture .................................................................................. 66

2.3.3 Influence of temperature and moisture on soil respiration ...................................... 66

2.3.4 Influence of soil parameters on soil respiration ......................................................... 69

2.4  DISCUSSION AND CONCLUSIONS .............................................................................. 69

2.5  ACKNOWLEDGEMENTS ............................................................................................... 72

2.6  REFERENCES ............................................................................................................... 72
2.1 INTRODUCTION

The characterization of soil respiration ($R_s$) response through time and space is increasingly important to identify dominant sources and sinks of carbon (C) and parameterize carbon cycling to represent forest-atmosphere interactions in global modelling studios (Cox et al. 2000; Goh 2004). In the last decade, major efforts have been made to understand the environmental drivers of soil respiration. Soil temperature ($T_s$) and soil moisture ($M_s$) explain most of the variation of this parameter (Davidson et al. 1998; Rey et al. 2002), but additional factors such as forest type, soil fertility, soil texture, stand age, plant photosynthetic activity and topography also affect soil respiration in forested ecosystems (Rodeghiero and Cescatti 2005; Dilustro et al. 2005; Tang et al. 2005; Kang et al. 2003).

However, the identification of the environmental factors controlling the variability of $R_s$ still remains a difficult task surrounded by large uncertainties, especially in forest soil in mountainous regions, and when it is necessary to consider the effects of disturbances associated with forest fires. This is of major relevance in the Mediterranean forest ecosystems that are highly vulnerable to climate change. Numerous studies along elevation gradients indicate that soil C concentrations or stocks increase with altitude in mountainous terrain (Rodeghiero and Cescatti 2005; Garten et al. 2006). Field studies in the mountains of more arid areas indicate that soil moisture is an important factor controlling soil C stocks and fluxes in elevation gradients (Wang et al. 2000; Li et al. 2007). Topography induced microclimates can affect $R_s$ rates by constraining microclimatic factors, such as soil temperature and moisture (Kang et al. 2003; Li et al. 2007). Other studies suggest that several chemical and biological factors such as nitrogen (N) and C dynamics co-varying with soil moisture regulate the spatial distribution of soil C losses through decomposition (Garten et al. 2006).

The purpose of this study was investigated the main factors controlling seasonal and spatial variation of soil respiration along an elevation gradient in the northern face of the Sierra de Guadarrama mountains (Central Spain).
2.2 MATERIAL AND METHODS

2.2.1 Study site

The study took place in Central Spain (Valsaín, Segovia), located in the northern face of the Sierra de Guadarrama (40° 51’N, 4°3’W). The total area of Valsaín Forest is 10.672 ha, covered mainly by Scots pine (*Pinus sylvestris* L.). Other species present include oak (*Quercus pyrenaica* Willd.) and montane broom (*Cytisus purgans* (L.) Boiss.), with small areas of Holm oak (*Quercus ilex* subsp. *ballota* (Desf.) Samp.) and riparian forest. The herbaceous layer is largely made up of grasses which develop in spring, dry off in summer and regrow to some extent after the first autumn rains.

The climate is nemoro-mediterranean. The rainfall distribution is irregular, with a drought period in summer of approximately 2 months and an annual average rainfall of 1.600-1.400 mm. Mean temperatures ranged between 1.5-2.7°C during winter and 19.7-20.3°C during summer. Geologically, granites are predominant. Soils are classified as Humic Cambisols or Typic Haplumbrepts.

2.2.2 Experimental design

A transect was established in a 64 ha *P. sylvestris* L. watershed spread along an elevation gradient ranging from 1.320 to 1.592 m asl. Four sampling plots with dimensions 10 m x 15 m were designated within the experimental site. An additional site (plot 100) was selected at a higher elevation (1.700 m asl.) an area burned 2 years before (Table 2.1). The tree density was 220 tree/ha, with a mean height 30 m and a mean diameter at breast height of 41 cm. The mean age of the stands was 120 years. The general aspect of the site was NE with a mean slope of 30%. The leaf area index (LAI) was 6.42 m²·m⁻². The historical meteorological data collected in the area found that temperature decreased around 0.65°C for every 100 m and annual precipitation increased by about 100 mm every 100 m (Lopez Arias M, personal communication). A summary of the site characteristics is given in Table 2.1.
Table 2.1 Summary of site characteristics of six study plots spread along an altitudinal gradient in a *Pinus sylvestris* L. forest in the northern Sierra de Guadarrama Mountains.

<table>
<thead>
<tr>
<th></th>
<th>Plot 14</th>
<th>Plot 22</th>
<th>Plot 33</th>
<th>Plot 42</th>
<th>Plot 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latitude (N, deg.)</td>
<td>40º 50’ 58”</td>
<td>40º 50’ 39”</td>
<td>40º 50’ 59”</td>
<td>40º 51’ 15”</td>
<td>40º 49’ 15”</td>
</tr>
<tr>
<td>Longitude (W, deg.)</td>
<td>4º 02’ 52”</td>
<td>4º 02’ 52”</td>
<td>4º 02’ 52”</td>
<td>4º 02’ 34”</td>
<td>4º 04’ 2”</td>
</tr>
<tr>
<td>Elevation (masl)</td>
<td>1592</td>
<td>1579</td>
<td>1380</td>
<td>1320</td>
<td>1700</td>
</tr>
<tr>
<td>Slope (%)</td>
<td>22</td>
<td>50</td>
<td>20</td>
<td>67</td>
<td>49</td>
</tr>
<tr>
<td>Aspect (deg)</td>
<td>30º</td>
<td>100º</td>
<td>60º</td>
<td>110º</td>
<td>-</td>
</tr>
<tr>
<td>Total Porosity (%)</td>
<td>62</td>
<td>61</td>
<td>55</td>
<td>64</td>
<td>67</td>
</tr>
<tr>
<td>Particle Density (g/cm³)</td>
<td>2.57</td>
<td>2.64</td>
<td>2.63</td>
<td>2.64</td>
<td>2.54</td>
</tr>
<tr>
<td>WFPS (%)</td>
<td>58.4</td>
<td>50.5</td>
<td>51.9</td>
<td>45.0</td>
<td>28.8</td>
</tr>
<tr>
<td>pH (1:2)</td>
<td>5.6</td>
<td>5.8</td>
<td>5.8</td>
<td>5.7</td>
<td>5.4</td>
</tr>
<tr>
<td>EC (µS/cm)</td>
<td>171</td>
<td>156</td>
<td>119</td>
<td>164</td>
<td>216</td>
</tr>
<tr>
<td>C stocks (kg C·m⁻²)</td>
<td>5.57 ± 0.16</td>
<td>7.76 ± 0.20</td>
<td>7.37 ± 0.30</td>
<td>5.34 ± 0.27</td>
<td>7.06 ± 0.30</td>
</tr>
<tr>
<td>N stocks (kg N·m⁻²)</td>
<td>0.30 ± 0.01</td>
<td>0.34 ± 0.003</td>
<td>0.38 ± 0.01</td>
<td>0.27 ± 0.006</td>
<td>0.42 ± 0.02</td>
</tr>
<tr>
<td>C/N</td>
<td>18.7 ± 0.4</td>
<td>23.1 ± 0.7</td>
<td>19.3 ± 1.4</td>
<td>19.4 ± 1.1</td>
<td>16.9 ± 0.2</td>
</tr>
<tr>
<td>TOC (g/kg)</td>
<td>56.8 ± 1.6</td>
<td>75.3 ± 2.0</td>
<td>62.8 ± 2.6</td>
<td>56.8 ± 2.9</td>
<td>86.1 ± 3.6</td>
</tr>
<tr>
<td>C-MBT (g/kg)</td>
<td>1.30 ± 0.23</td>
<td>1.14 ± 0.02</td>
<td>0.71 ± 0.05</td>
<td>0.60 ± 0.05</td>
<td>0.18 ± 0.008</td>
</tr>
</tbody>
</table>

Means with different letters within site are significantly different using Tukey’s HSD at the 0.05 level.

Air temperature and precipitation during the experimental period were obtained from the weather station in Puerto de Navacerrada (1.860 m asl: Figure 2.1).
Figure 2.1 Precipitation and mean temperature recorded during the experimental period. Data were obtained from the weather station in Puerto de Navacerrada located approximately 2 km from the site of the study.

2.2.3 Soil respiration, temperature and moisture measurements

Measurements of $R_s$, $T_s$ and $M_s$ were conducted between spring 2005 and summer 2006 randomly in each forest plot on a monthly basis, using a closed dynamic system LI-6400 coupled to a LI-6400-9 soil chamber (LI-COR Inc., Lincoln, NE, USA). Measurements were made between 10:00 a.m. and 16:00 p.m. to minimize the diurnal variation in soil respiration using PVC collars (10 cm diameter and 4.5 cm length) which were inserted into the soil 2.5 cm depth (to avoid root severing) at least one week prior to investigation and left in place throughout the course of the experiment. The measurement of $R_s$ consisted of placing the chamber on the collar, scrubbing the CO$_2$ to sub-ambient levels, and measuring the flux rate as it rose from 15 ppm below to 15 ppm above the atmospheric value. Soil respiration sampling was not performed on days following a rain event to avoid an overestimation of the efflux due to CO$_2$ displacement from soil pores (Rey et al. 2002). A total of 12 collars were placed in plots 14, 22 and 42; whereas 9 collars were employed in plot 33 and 6 in plot 100.
Soil moisture content and soil temperature in the top 10 cm of soil were measured next to each soil respiration measurements with a time-domain reflectometry system (TRIMEGM, IMKO GmbH, Ettlingen, Germany) and a thermocouple sensor (Omega Engineering, Stamford, CT). Soil water content was measured at three points around each collar.

2.2.4 Soil properties

2.2.4.1 Soil sampling

In March 2006, soil samples were taken at three different sites in each plot. Five soil cores were extracted (10 cm deep x 8 cm diameter) beneath the organic layer and composited. Soils were kept separately in plastic bags and rapidly transported on ice in a dark cooler to the laboratory and stored in a refrigerator (4°C) prior to sampling processing.

Composite mineral samples were separated in two subsamples for determination of physico-chemical and biological soil properties. The subsample for biological properties were sieved through a 2 mm mesh and stored at 4°C until they were processed. The remaining sample was air dried at room temperature for 2-3 days, and sieved through a 2 mm sieve to remove stones, gravel, and coarse debris. Soil passing the 2 mm sieve was grounded and homogenized using a mortar and stored in an airtight bottle prior to determine physico-chemical properties.

2.2.4.2 Analysis of soils

Soil pH and electrical conductivity (EC) was measured in a 1:2 aqueous extract. The pH was measured by means of an ORION 720A pH-meter. Electrical conductivity measurements were performed by means of an ORION 115 conductimeter. The total C and N analyses were carried out by using a LECO TruSpec analyser. The organic carbon was analyzed with a TOC-VCSH analyser (SHIMADZU, Shimadzu Scientific Instruments, Kyoto, Japan).

Soil moisture content (Mₜ) and dry density values were determined on separate and unaltered core samples taken 50 mm depth using standard core steel samplers (28.5 mm
internal diameter and 34.3 mm high). Soil bulk dry density was calculated from the dimensions of the samplers, taking into account the oven-dried weight as a known volume of soil. The soil moisture content or gravimetric water content is defined as the ratio between the weight of water and the weight of dry solid expressed as a percentage. The weight of water was determined as the difference between the weight of the sample and its weight after oven drying at 110 °C for 24 hours (UNE Standard 103-300-93). Water filled-pore space (WFPS) was calculated as:

$$WFPS = \frac{(w.c. \times \gamma_s)}{(1 - (\rho_d/\gamma_s))},$$

Where w.c. is the gravimetric water content, $\gamma_s$ is the soil particle density and $\rho_d$ is the bulk dry density. The particle density or grain density was measured on a powdered oven-dried specimen using the pycnometer’s method with water (UNE Standard 103-302).

Soil microbial biomass carbon was determined by microwave irradiation-extraction method (Islam and Weil 1998). The organic carbon in the extracts of the control and microwaved soil were analyzed with TOC-V CSH analyzer (SHIMADZU, Shimadzu Scientific Instruments, Kyoto, Japan). The soil microbial biomass C (CMBT) was calculated as:

$$C_{MBT} = \frac{C_{EXTMW}}{0.213}$$

Where, $C_{EXTMW}$ is the net flush of C, obtained from the difference between the extracted C in microwaved soil samples minus the extracted C in control soil samples, and 0.213 is a constant to compensate the fraction of extracted carbon by 0.5 M K$_2$SO$_4$. 


2.2.4.3 Calculation of soil carbon and nitrogen stocks

Carbon and N stocks in the top 10 cm of mineral soil (kg·m$^{-2}$) were estimated by multiplying values of percent C and N in the mineral soil by soil-bulk density measurements from each plot.

2.2.5 Statistical analysis and modelling

Plot-level seasonal temperature response of Rs was calculated by means of a $Q_{10}$ function as mentioned by Janssens and Pilegaard (2003), which is called temperature sensitivity of Rs:

$$Rs = R_{10} \times Q_{10}^{((T-10)/10)}$$  \hspace{1cm} (E2.1)

Where, $Rs$ (µmolCO$_2$·m$^{-2}$·s$^{-1}$) is the soil respiration flux, $R_{10}$ is the simulated Rs at a soil temperature of 10°C which is often used to compare the Rs characteristics of ecosystems, $Q_{10}$ is the temperature sensitivity of Rs (the respiratory flux at one temperature over the flux at a temperature 10°C lower), and $T$ is the measured soil temperature (°C). The functions were fitted to the measured data by means of a nonlinear least square fitter (Sigma Plot 6.0).

All statistical analyses were performed using the Statistica 6.0 software package (StatSoft. Inc., Tulsa. USA). Site (Plot) and time effects for soil respiration, soil temperature and soil moisture were tested with a repeated measures analysis of variance (ANOVA). One-way analysis of variance was used in order to test for effects of stand on soil respiration in the sites where there is no a complete set of data and to analyse the differences in soil properties between plots. The normal distribution and homogeneous variance were tested by the Kolmogorov-Smirnov and Levene’s tests, respectively. Post-hoc comparisons were tested using Tukey’s HSD test, calculated at the 5% level. Whenever necessary, data were transformed as $(x+1)^{0.5}$ or logs x. Correlation analyses from data of the entire data set were used to examine relationship between soil respiration rates and soil properties.
2.3 RESULTS

2.3.1 Soil respiration

Soil respiration values during the experimental period (June 2005-July 2006) (Figure 2.2) varied from $6.8 \pm 0.2 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in June 2005 to $1.4 \pm 0.1 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in February 2006. Soil respiration across the six study sites showed a typical seasonal pattern with maximums in spring and summer and minimums in winter. Seasonal pattern were similar in the different plots, although temporal fluctuations were more limited in plots 42 and 100.

Repeated measures analysis of variance indicated that Rs was different among sites ($F_{3,131} = 31.871; p = 0.0$) and time of the year ($F_{5,655} = 205.29; p = 0.0$). Mean values in plot 22 were intermediate ($p<0.05$) between the highest values of plots 14 and 33 and the lowest values of plot 42 during July 2005, June 2006 and July 2006. Plot 100 showed low values ($p<0.05$), similar to those found in plot 42, and significantly different from the rest of the plots.
Figure 2.2 Seasonal variations of observed soil respiration ($R_s$, µmol·m$^{-2}$·s$^{-1}$) (a), volumetric water content ($M_s$, %)(b), soil temperature at 10-cm soil depth ($T_s$, ºC) (c) for study plots in Valsaín during the period 2005-2006. Asterisk indicates significant differences at the 0.05 level.
2.3.2 Soil temperature and moisture

Both soil temperature and moisture content varied according to the season. Maximum soil temperature coincided with minimum soil moisture during the summer, and minimum soil temperatures were recorded in winter when soil moisture was highest (Figure 2.2).

Soil temperature varied with site \((p=0.0)\), time \((p=0.0)\) and site over time \((p=0.0)\). There was a significant site effect \((p<0.05)\), with plot 42 exhibiting the highest soil temperature followed by plot 22, 33 and finally by plot 14. Soil temperature was highest in July 2006, declined in June 2005, followed by May 2006 and March 2006 and dropped to a minimum in February 2006. Plot 100 showed highest values during May 2006 and June 2006.

Soil moisture differed over time \((p=0.0)\), site \((p=0.0)\) and site over time \((p=0.0)\). Soil moisture was highest \((p<0.05)\) in plots 14 and 33 and lowest in plots 22 and 42. Soil moisture during May 2006 was higher \((p<0.05)\) in plot 14, while no significant difference was observed among the other plots. During June 2006, soil moisture was higher in plot 14 than in plot 42. For all treatments, soil moisture rose to a maximum in winter and spring months and declined steadily through June to a minimum in July.

2.3.3 Influence of temperature and moisture on soil respiration

Not surprisingly, both soil temperature and moisture were highly significant in a regression analysis explaining a large percentage of the seasonal variability in soil respiration. Soil moisture showed significant negative correlations to seasonal soil respiration for plot 14 \((r^2=0.28, r=-0.53, p=0.0)\), plot 22 \((r^2=0.39, r=-0.63, p=0.0)\), plot 33 \((r^2=0.36, r=-0.60, p=0.0)\) and plot 42 \((r^2=0.06, r=-0.25, p=0.0)\); while positive correlations were associated with plot 100 \((r^2=0.10, r=0.32, p=0.00)\).

Soil respiration was satisfactorily represented as a function of temperature, using the \(Q_{10}\)-type model fitted to the entire dataset (Figure 2.3, a, b). In plot 100 soil respiration and soil temperature were not correlated. The temperature-based \(Q_{10}\) models had similar \(Q_{10}\) values ranging between 1.30 and 2.04. The \(R_{10}\) values ranged between 2.0 and 3.61.
Figure 2.3 (a) Soil CO$_2$ efflux versus soil temperature as measured in Spanish Scots pine forest. The solid line represents the best fitting Q$_{10}$ function. Functions were fitted through the entire dataset. R$^2$ represents the proportion of variability in soil respiration explained by soil temperature.
Figure 2.3 (b) Soil CO$_2$ efflux versus soil temperature as measured in Spanish Scots pine forest. The solid line represents the best fitting $Q_{10}$ function. Functions were fitted through the entire dataset. $R^2$ represents the proportion of variability in soil respiration explained by soil temperature.
In plots 33 and 42, an interaction between soil temperature and soil moisture explained a 40% and 36% of the variability, respectively (Table 2.2).

**Table 2.2** Soil respiration as a function of temperature and moisture content, using a fitted linear curve through the entire dataset.

<table>
<thead>
<tr>
<th>Model</th>
<th>$r^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plot 33 SR = 3.02 + 0.13 x ST - 0.04 x SM</td>
<td>0.40</td>
<td>0.0</td>
</tr>
<tr>
<td>Plot 42 SR = -0.19 + 0.13 x ST + 0.04 x SM</td>
<td>0.35</td>
<td>0.0</td>
</tr>
</tbody>
</table>

2.3.4 **Influence of soil parameters on soil respiration**

Soil carbon stocks varied between $5.57 \pm 0.2$ kg·m$^{-2}$ (plot 14) and $7.76 \pm 0.2$ kg·m$^{-2}$ (plot 22), while soil nitrogen content varied from $0.27 \pm 0.01$ kg·m$^{-2}$ (plot 42) to $0.42 \pm 0.02$ kg·m$^{-2}$ (in plot 100) (Table 2.1). Regarding microbial biomass carbon (C-MBT), plot 14 showed the highest content, while burned plot (plot 100) had the lowest. Soil respiration (Table 2.3) was positively correlated with C and N stocks and WFPS, and negatively correlated to total porosity. Although not significantly, soil respiration tends to increase with C-MBT, TOC, pH and C/N.

2.4 **DISCUSSION AND CONCLUSIONS**

The values of soil respiration in this area are in a range comparable with those measured in other Mediterranean forest (Rey et al. 2002; Rodeghiero and Cescatti 2005; Tedeschi et al. 2006). The annual $Q_{10}$ derived from the dataset were lower than the global median of 2.4 (Raich and Schlesinger 1992) and the range data (2.0-6.3) reported for forest ecosystems (Janssens et al 2003). Our estimates of $R_{10}$ (2.0-3.6) are in agreement with the range (0.7-4.9 μmol·m$^{-2}$·s$^{-1}$) observed in European forests (Janssens et al. 2003). We found similar $Q_{10}$ and $R_{10}$ values as were observed by Tang et al. 2005b and Rodeghiero and Cescatti 2005 in Mediterranean ecosystems. The reasons for reduced temperature sensitivity in Valsaín forest are unclear, depending on the environmental constraints (generally low soil moisture content) and the substrate.
The results indicate that $R_s$ seasonal variation was mainly dominated by $T_s$ (Figure 2.3) and that soil moisture also had an important influence on $R_s$. However, the influence of $M_s$ on the $R_s$ depends on the site. In plot 100 the temporal variation of $R_s$ was mainly controlled by soil moisture, and in plots 33 and 42 the interaction between $T_s$ and $M_s$ explained most of the variability observed. Although it is difficult to separate completely the influence of $T_s$ and $M_s$ imposed on $R_s$, our short-term observation showed that $R_s$ responded mainly to $T_s$ in winter and that the interaction of soil temperature and moisture on $R_s$ was especially apparent during summer months when low soil moisture limited soil microbial activity and root growth despite warm soil temperature (Figure 2.2).

In the Valsaín mountains, in situ measurements of soil respiration decline with declining altitude in contrast with other field studies that indicate correlations between $R_s$ and elevation (Wang et al. 2000; Rodeghiero and Cescatti 2005; Garten et al. 2006). Whereas the warmest temperatures were associated with the lowest elevation site (plot 42), the highest rates of $R_s$ were observed at the highest elevation plots (14, 22, and 33). The elevation factor alone may not explain the $R_s$ sensitivity by itself. Topography produces spatially heterogeneous $M_s$ and thus soil respiration. In our study, more mesic north-facing slopes (plot 14 and 33) showed higher $R_s$ relative to less mesic south-facing slopes (plot 22 and 42) despite higher $T_s$ at south-facing slopes.

The role of temperature and soil moisture in $R_s$ under field conditions is not always straightforward and might be influenced by vegetation covers, litter chemistry and thickness, soil organic matter, variables rates of belowground root growth and activity, N availability and soil characteristics. All these factors potentially interact in complex ways through effects on decomposition. In our experiment we obtained $R_s$, $Q_{10}$ and $R_{10}$ values higher in the cooler, wetter site relative to the warmer, drier site. This is in agreement with Xu and Qi (2001). If we compare this trend with C-MBT (decrease in the same way) we could explain this behaviour by a faster response of the microbial community to warming when easily degradable substrate ability is also high.

In our experiment, the positive correlations between $R_s$, $Q_{10}$ and $R_{10}$ values and C and N stocks, TOC, C-MBT and WFPS (Table 2.3), indicated that microbial substrate quantity and nitrogen-rich conditions promote higher $Q_{10}$ and $R_s$ values. Atmospheric deposition and litterfall inputs could influence $R_s$ along the altitudinal transect.
**Table 2.3** Correlation coefficients between various soil variables and soil respiration ($R_s$, $\mu$mol m$^{-2}$ s$^{-1}$) at March 2006 in *Pinus sylvestris* L. forest in the Sierra de Guadarrama.

<table>
<thead>
<tr>
<th>Variable</th>
<th>$R_s$</th>
<th>$Q_{10}$</th>
<th>$R_{10}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.stocks (kg·C·m$^{-2}$)</td>
<td>0.65</td>
<td>0.32</td>
<td>0.42</td>
</tr>
<tr>
<td>N.stocks (kg·N·m$^{-2}$)</td>
<td>0.65</td>
<td>0.35</td>
<td>0.53</td>
</tr>
<tr>
<td>C/N</td>
<td>0.20</td>
<td>0.06</td>
<td>0.04</td>
</tr>
<tr>
<td>C-MBT (g·kg$^{-1}$)</td>
<td>0.28</td>
<td>0.86</td>
<td>0.52</td>
</tr>
<tr>
<td>TOC (g·kg$^{-1}$)</td>
<td>0.39</td>
<td>0.26</td>
<td>0.21</td>
</tr>
<tr>
<td>Particle Density (g·cm$^{-3}$)</td>
<td>0.06</td>
<td>-0.64</td>
<td>-0.53</td>
</tr>
<tr>
<td>Total Porosity (%)</td>
<td>-0.69</td>
<td>-0.18</td>
<td>-0.49</td>
</tr>
<tr>
<td>WFPS (1:2)</td>
<td>0.68</td>
<td>0.80</td>
<td>0.72</td>
</tr>
<tr>
<td>pH</td>
<td>0.48</td>
<td>-0.30</td>
<td>-0.14</td>
</tr>
<tr>
<td>EC (µS·cm$^{-1}$)</td>
<td>-0.50</td>
<td>0.19</td>
<td>-0.15</td>
</tr>
</tbody>
</table>

Bold numbers indicate a significant correlation at $p < 0.05$.

Prior studies along the elevation gradient in Valsaín indicated a decline in litterfall inputs and N deposition with decreasing altitude (Gonzalez et al. 1994; Garcia del Barrio et al. 1997). At low elevations, both higher mean annual temperatures and lower site N availability potentially contribute to lower soil C stocks and shorter turnover time for labile soil C. The highest values of $R_s$ in plot 14 also could be related to higher pine density and regeneration in this plot, and from differences in root respiration. Results from studies of other forests indicate that fine root biomass is directly associated with tree basal area (Chen et al. 2004).

Our results reveal that fire can alter the level of C, N, pH, and C-MBT in the soil. The increases in C concentration during the first years after fire could be attributed to unburnt remains from dead roots which slowly incorporate into the soil. C/N ratios were lower in the burst soils than in the unburned soils. An alteration of nitrogen by fire is a complex process. Fire alters the amount of nitrogen in the soil by volatilisation of some into the atmosphere and by converting the nitrogen into different nitrogen compounds. The pH decreased after fire. The burn plot exhibited reduced microbial biomass C compared to mature plots. This data revealed that fire altered the microbial diversity of the soil ecosystem. After the fire, C-MBT was very low, which indicates that microbial diversity had decreased significantly even 2 years after the occurrence of the disturbance. Decreases in microbial biomass have been widely reported after fire. $R_s$, $Q_{10}$ and $R_{10}$ were lower than in unburned plots.

From the results obtained, it can be concluded that under similar climatic conditions and soil types within a small geographical area variation in soil temperature, soil
moisture, carbon and nitrogen stocks and C-MBT can influence $R_s$ in southern *Pinus sylvestris* L. forest. Thus, changes in soil parameters over small scales need to be considered when modelling landscape-level $R_s$. Furthermore, a better understanding of how natural disturbances affects C and N cycling is crucial for predicting how changes in climate and disturbance regimens will affect the exchange of C between forest ecosystems and the atmosphere.

2.5 ACKNOWLEDGEMENTS

This research was conducted in the framework of Spanish HU2005-0023, AGL2004-01941 and CGL 2006-02922/CLI Projects, and the European COST 639 (BurnOut) Project. The authors want to express their gratitude to Mr. Javier Dones, Mr.Manuel Lopez Arias and Mr. Jose M. Grau for their valuable assistance with field aspects and the data provided.

2.6 REFERENCES


Chapter 3

EFFECT OF WILDFIRES ON SOIL RESPIRATION IN THREE TYPICAL MEDITERRANEAN FOREST ECOSYSTEMS IN MADRID, SPAIN
Chapter 3

EFFECT OF WILDFIRES ON SOIL RESPIRATION IN THREE TYPICAL MEDITERRANEAN FOREST ECOSYSTEMS IN MADRID, SPAIN


Plant and Soil journal, published on line (2013). DOI 10.1007/s11104-012-1576-x
ABSTRACT

Mediterranean forests are vulnerable to numerous threats including wildfires due to a combination of climatic factors and increased urbanization. In addition, increased temperatures and summer drought lead to increased risk of forest fires as a result of climate change. This may have important consequences for C dynamics and balance in these ecosystems. Soil respiration was measured over 2 successive years in Holm oak (*Quercus ilex* subsp. *ballota*; Qi); Pyrenean oak (*Quercus pyrenaica* Willd; Qp); and Scots pine (*Pinus sylvestris* L.; Ps) forest stands located in the area surrounding Madrid (Spain), to assess the long term effects of wildfires on C efflux from the soil, soil properties, and the role of soil temperature and soil moisture in the variation of soil respiration.

Soil respiration, soil temperature, soil moisture, fine root mass, microbial biomass, biological and chemical soil parameters were compared between non burned (NB) and burned sites (B).

The annual C losses through soil respiration from NB sites in Qi, Qp and Ps were 790, 1010, 1380 gCm$^{-2}$yr$^{-1}$, respectively, with the B sites emitting 43%, 22% and 11% less in Qi, Qp and Ps respectively. Soil microclimate changed with higher soil temperature and lower soil moisture in B sites after fire. Exchangeable cations and the pH also decreased. The total SOC stocks were not significantly altered, but 6-8 years after wildfires, there was still measurably lower fine root and microbial biomass, while SOC quality changed, indicated by lower the C/N ratio and the labile carbon and a relative increase in refractory SOC forms, which resulted in lower Q$_{10}$ values.

We found long term effects of wildfires on the physical, chemical and biological soil characteristics, which in turn affected soil respiration. The response of soil respiration to temperature was controlled by moisture and changed with ecosystem type, season, and between B and NB sites. Lower post-burn Q$_{10}$ integrated the loss of roots and microbial biomass, change in SOC quality and a decrease in soil moisture.

**Keywords:** soil respiration; wildfires; climate change; soil parameters; *Quercus ilex*; *Quercus pyrenaica*; *Pinus sylvestris*.
Chapter 3

**Effect of wildfires on soil respiration in three typical Mediterranean forest ecosystems in Madrid, Spain**

**CONTENT**

3.1 INTRODUCTION ........................................................................................................... 79
3.2 MATERIALS AND METHODS ...................................................................................... 80
  3.2.1 Study sites and experimental design ................................................................. 80
  3.2.2 Soil respiration (Rs), soil temperature (Ts) and soil moisture (Ms) measurements .............................................................. 82
  3.2.3 Soil sampling and analysis .................................................................................. 83
  3.2.4 Fine root sampling and analysis ....................................................................... 84
  3.2.5 Statistical analysis and modelling ..................................................................... 85
3.3 RESULTS ...................................................................................................................... 86
  3.3.1 Soil respiration, soil temperature and soil moisture .......................................... 86
  3.3.2 Soil parameters and fine roots .......................................................................... 90
  3.3.3 Correlations and modelling ............................................................................. 94
  3.3.3.1 Soil parameters and Rs .............................................................................. 94
  3.3.3.2 Rs and soil temperature and soil moisture .................................................. 94
3.4 DISCUSSION .............................................................................................................. 100
  3.4.1 Rs, soil temperature and soil moisture ............................................................. 100
  3.4.2 Soil parameters and soil respiration ................................................................. 102
  3.4.3 Correlation between Rs and soil parameters .................................................... 104
3.5 CONCLUSION ........................................................................................................... 105
3.6 ACKNOWLEDGMENTS ............................................................................................ 106
3.7 REFERENCES ............................................................................................................ 106
3.8 SUPPLEMENTARY MATERIAL .................................................................................. 113
3.1 INTRODUCTION

Forests constitute the most important carbon (C) pool in terrestrial ecosystems (Dixon et al. 1994). An average of 69-75% of this C is found below-ground in living biomass and soil organic matter pools (Dixon et al. 1994; Keith et al. 1997). Soils contain the largest terrestrial organic C stock, representing about two-thirds of terrestrial C (Schimel et al. 2000). Second only to gross photosynthesis, soil respiration (R_s), estimated at around 75 Pg C yr\(^{-1}\) (Schlesinger and Andrews 2000), exceeds all other terrestrial-atmospheric carbon fluxes. Given the importance of R_s in the global C cycle, it is important to obtain better estimates of R_s and to improve our understanding of the interactions between environmental variables and R_s. The R_s is the sum of autotrophic respiration (R_a) from plant metabolic activity and heterotrophic respiration (R_h) from the decomposition of organic material by microbes (Hanson et al. 2000; Wang et al. 2010). Both components are affected by temperature and moisture as well as by the physical, chemical and biological properties of the soil (Boone et al. 1998; Borken et al. 2006; Inclán et al. 2010).

Fire is a natural ecological factor in Mediterranean-basin and the main agent of disturbance (Naveh 1990). The vulnerability of Mediterranean forests to frequent wildfires is due not only to climatic factors (Lloret et al. 2002) but also to human activity (land conversion, inadequate land use planning and management, etc). More than 4 million ha in the Mediterranean region of Spain have been affected by wildfires in the last 20 years (Moreno et al. 1998). In the future, warmer and drier conditions associated with global climate change are expected to increase the area affected by wildfires (Flannigan et al. 2000; IPCC 2007; Peñuelas et al. 2005). The effects of wildfires and the alterations on fire patterns could lead to important changes in terrestrial C storage and atmospheric chemistry. More commonly the short term effects of fires on forest soils and R_s have been evaluated (Almagro et al. 2009; Irvine et al. 2007; Neary et al. 1999; Rodríguez et al. 2009). However, less information is available on the long term effects of wildfires (Dore et al. 2008; Durán et al. 2010; Sullivan et al. 2011; Wang et al. 2001). The magnitude of the soil CO_2 efflux after a fire depends on climatic factors, the post-fire forest management (Marañón-Jiménez et al. 2011) loss and chemical transformation of the soil organic carbon (SOC) pools, often leading to
increased resistance to chemical and biological breakdown (Certini 2005; Knicker 2007), and rate of recovery process of the vegetation (González-Perez et al. 2004; Irvine et al. 2007; Litton et al. 2003; Van Miegroet and Olsson 2011), the change in the balance between autotrophic and heterotrophic respiration (Irvine et al. 2007), the alteration in fine root dynamics (Hart et al. 2005) and soil parameters (Certini 2005). The objectives of the present study, conducted in the three most representative Mediterranean forest types of the region around Madrid were to (1) characterize the long term effects of wildfire on \( R_s \), (2) study the fire effects on the physicochemical and biological parameters of forest soils and (3) compare the sensitivity of \( R_s \) to soil temperature and moisture in non-burned and burned sites.

### 3.2 MATERIALS AND METHODS

#### 3.2.1 Study sites and experimental design

The research was conducted in three forest stands (Table 3.1) close to Madrid (Spain): A Holm oak stand (*Quercus ilex* subsp. *ballota*; hereafter Qi); a Pyrenean Oak forest stand (*Quercus pyrenaica* Wild; hereafter Qp); and a Scots pine forest stand (*Pinus sylvestris* L.; hereafter Ps). All the stands are located within protected natural areas. The forest understory in Qi consists of shrub-type vegetation and grasses, including *Cistus ladanifer*, *Lavandula stoechas*, *Rosmarinus officinalis*, *Crataegus monogyna*. In Qp, other species present include scattered *Fraxinus angustifolia*, *Acer pseudoplatanus*, *Juniperus oxycedrus*, *Cytisus scoparius*, *Genista florida*, *Rubus ulmifolius*, *Crataegus monogyna*, *Prunus spinosa*, *Lonicera peryclimenum* and *Tamus communis*. In Ps, the understory is dominated by shrubs (*Genista cinerea*, *Adenocarpus hispanicus*, *Rosa canina*, *Santolina rosmarinifolia*) and grasses (*Pteridium aquilinum*, *Rumex scutatus*, *Stipa gigantea*). The forests are located on granitic bedrock and soils are Haplic Umbrisols (FAO 1998). The climate is Mediterranean-type characterized by extreme temperatures; very hot in summer and cold in winter.
Table 3.1 Site characteristics of the three forest types.

<table>
<thead>
<tr>
<th></th>
<th>Quercus ilex</th>
<th>Quercus pyrenaica</th>
<th>Pinus sylvestris</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Location</td>
<td>N 40° 35' 39.3'' W 3° 55' 30.5''</td>
<td>N 40° 35' 39.7'' W 3° 55' 31.7''</td>
<td>N 40° 34' 07.8'' W 4° 08' 54.8''</td>
</tr>
<tr>
<td>Stand age (yr)</td>
<td>40</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Mean precipitation (mm)</td>
<td>655</td>
<td>50</td>
<td>–</td>
</tr>
<tr>
<td>Mean air temperature (°C)</td>
<td>14</td>
<td>13</td>
<td>8</td>
</tr>
<tr>
<td>Elevation (m.a.s.l)</td>
<td>985</td>
<td>1020</td>
<td>1409</td>
</tr>
<tr>
<td>Slope (%)</td>
<td>0</td>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td>Aspect (deg)</td>
<td>a.w.</td>
<td>a.w.</td>
<td>135°</td>
</tr>
<tr>
<td>Tree height (m)</td>
<td>3.1</td>
<td>7.5</td>
<td>16</td>
</tr>
<tr>
<td>DBH (cm)</td>
<td>14</td>
<td>18</td>
<td>27</td>
</tr>
<tr>
<td>Fine earth fraction (%)</td>
<td>71</td>
<td>70</td>
<td>65</td>
</tr>
<tr>
<td>Coarse earth fraction (%)</td>
<td>29</td>
<td>30</td>
<td>74</td>
</tr>
<tr>
<td>Clays (%;&lt;2µm)</td>
<td>20</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td>Silt +Sand (%;&gt;2µm)</td>
<td>80</td>
<td>85</td>
<td>81</td>
</tr>
</tbody>
</table>
Rainfall is scarce (below 900 mm/year [see Table 3.1]), especially in summer, and is concentrated in spring and autumn. Qi, falls in mesomediterranean and Qp and Ps in supramediterranean bioclimates according to Rivas-Martínez (2004).

Wildfires have occurred in part of these forests at different times in the past. Qi suffered a fire in 2004, affecting approximately 12 ha; in Qp the fire occurred in 2002, affecting around 10 ha; and around 71 ha of forest were burned in Ps in 2001. Although we do not know the temperature reached (i.e. the fire intensity), the fires resulted in the death of trees and forest floor vegetation and the fires were classified as crown fires (Scott and Reinhardt 2001). According to Knicker (2007) fuel load must be large enough for the flames to reach the canopy to develop crown fires. Consequently, it is likely that the sites were subject to high-intensity fires.

In each forest stand we selected two 50 m x 50 m areas: one unaffected by fire (non burned-control, NB) and the other burned (B) with the distance between the B and NB areas from 150 m to 2.5 km. The NB and B areas at each forest site are similar in terms of slope and aspect. The B area in each stand is devoid of trees and the vegetation mainly comprises the previously described understory shrub species associated with each ecosystem. In the B areas in Qi and Qp, the understory is sparse, whereas in Ps the vegetation cover is dominated by the leguminous *Adenocarpus hispanicus*, which is endemic to the Sistema Central (Spain).

### 3.2.2 Soil respiration (R_s), soil temperature (T_s) and soil moisture (M_s) measurements

Measurements of R_s, T_s and M_s were conducted from June 2007 to May 2009 on a monthly basis using a closed dynamic system LI-6400 coupled to an LI-6400-9 soil chamber (LI-COR inc., Lincoln, NE, USA). Measurements were taken between 10 am and 4 pm to minimize the diurnal variation. We randomly placed 12 PVC collars in each of the 6 areas per area (diameter 10 cm x height 4.5 cm), inserted in the soil at a depth of 2.5 cm to limit root severing, for a total of 72 collars. These were positioned at least 1 week prior to the start of the experiment and left in place throughout the course of the investigation. Green plant material growing within the ring was clipped as close to the soil as possible and removed along with coarse materials prior to each sampling.
date. The measurements of $R_s$ were performed by placing the chamber on the collar, scrubbing the CO$_2$ to sub-ambient levels and measuring the flux rate as it rose from 15 ppm below to 15 ppm above the atmospheric value according to LI-6400 owner manual. The $R_s$ measurement was not performed on days following a rain event to avoid an overestimation of the efflux due to CO$_2$ displacement from soil pores (Rey et al. 2002). $T_s$ and $M_s$ were measured at three points close to each collar at the same time as the $R_s$ using a thermocouple sensor (Omega Engineering, Stamford, CT) and a time-domain reflectometry system (TRIMEGM, IMKO GmbH, Ettlingen, Germany).

3.2.3 Soil sampling and analysis

Soil samples were taken in June and October in 2007, January, June, August and October in 2008 and March in 2009. Three 2 x 2 m$^2$ plots were delineated in each area. After removal of plant debris on the surface, three undisturbed soil cores (diameter 6 cm x height 5 cm) were taken to determine bulk density and three composite soil samples (core diameter 8 cm x height 10 cm) were taken to determine microbial biomass and physicochemical parameters in each area. These samples were kept separately in plastic bags and rapidly transported on ice in a dark cooler to the laboratory and stored in a refrigerator (4ºC) until analysis. Bulk density was calculated from the dimensions of the samplers and the dry weight (110ºC for 48 h) of the undisturbed soil samples. Composite samples were separated into two subsamples. One sub-sample was sieved through a 2 mm mesh and soil microbial biomass carbon (C-MBT) was determined using the microwave irradiation extraction method with K$_2$SO$_4$ (Islam and Weil 1998). The organic carbon in the microwave-treated and untreated extracts was analysed using a Shimadzu TOC-VCSH analyzer (Shimadzu Scientific Instruments, Kyoto, Japan). The C-MBT was estimated as the difference between the extracted C in microwave soil minus the extracted C in unmicrowaved soil (Islam and Weil 1998). The remaining sample was air dried at room temperature for 2–3 days, sieved (2 mm mesh), ground and homogenized using a pestle, and stored in an airtight bottle until further analysis. The pH of the air dried soil was potentiometrically measured using a glass electrode in a 1:5 (volume fraction) suspension of soil in 0.01 mol/l calcium chloride solution (pH in CaCl$_2$) in accordance with the ISO 10390. The concentrations of the exchangeable cations were determined in the BaCl$_2$ solution 0.1 mol/l extract of the soil (2.5 g), using
an inductively coupled plasma spectrometer (ICP) Varian 735-ES (Varian, Inc., Mulgrave, Australia). A LECO TruSpec CHN elemental analyzer (LECO Corp., St. Joseph, USA) was used for total C and N determination in soil samples. The C and N stocks were calculated based on soil organic C and N concentration of the fine fraction (g/kg), bulk density (g/cm$^3$) and content of fines to a depth of 10 cm. The water extractable organic carbon (DOC) was analysed by following the method proposed by Ghani et al. (2003). The supernatants were filtered through a 0.45 μm filter and organic C was measured using a Shimadzu TOC-VCSH analyzer.

In November in 2008 a fractionation of soil organic carbon (SOC) was performed. The alkali-extracted (<2.0 mm soil) humic substances (HS) were fractionated by an acid-extraction into humic acids (HA) and fulvic acids (FA) (Khan and Schnitzer 1972; Kononova 1962). The SOC contents of the different fractions were measured in a Shimadzu TOC-VCSH analyzer. The level of humification of HA was determined by $E_4/E_6$ method using spectroscopy (Chen et al. 1977). Soil mineralogy was determined by X-ray diffraction (Philips X PertMPD, PANalytical B.V., The Netherlands) and infrared spectroscopy (Nicolet 6700, ThermoScientific., USA).

### 3.2.4 Fine root sampling and analysis

In September in 2009, soils cores (diameter 8 cm x height 10 cm) consisting of organic-mineral soil were taken from each 2 x 2 m$^2$ plots in each area to estimate root biomass and N content in roots, for a total of 18 samples. The samples were transported to the laboratory, where they were washed and the fine roots between 2 and 5 mm were separated manually (Gaitán and Penón 2003). The root fractions per soil sample were placed in separated paper bags, dried at 65 ºC for 48 h, and weighed. A fraction of roots was crushed and the total N content was measured using a LECO TRUSPEC analyzer (LECO Corp., St. Joseph, USA).
3.2.5 Statistical analysis and modelling

Data were checked for homogeneity of variances (Levene test) and normality (Kolmogorov-Smirnov test), and if necessary, data were transformed as logx or log(x + n). Differences in Rs, Ts and Ms were evaluated using a three-way “repeated measures” analysis of variance (ANOVA), considering forest type (ecosystem), time (month) and disturbance (burn/no burn). Post-hoc comparisons were performed using Tukey’s HSD test. A one-way ANOVA was used to test differences in soil parameters and root characteristics between NB and B sites. A Spearman-rank correlation test was used to examine the relationship between Rs and soil parameters across the entire dataset and for each season using periodic measurements (Dytham 2011).

To test the influence of soil microclimate as a driver of Rs two different approaches were used. First, a model for Rs was calculated using soil temperature as an independent variable in the apparent Q₁₀ function, as in Janssens and Pilegaard (2003). This model was applied in NB and B sites in each forest type separately.

\[ Rs = R_{10} \times Q_{10}^{(T_s-10)/10} \]  \hspace{1cm} (E3.1)

Where Rs (µmol m⁻² s⁻¹) is the soil respiration, R₁₀ is the simulated Rs at a soil temperature of 10°C, Q₁₀ is the temperature sensitivity of Rs (the respiratory flux at a given temperature over the flux at a temperature 10 ºC lower), and Tₛ (ºC) is the measured soil temperature at 10 cm.

Second, the model proposed by Tang et al. (2005b), which uses soil temperature and soil moisture as independent variables, along with one categorical variable, representing the fire effect in this study, was also employed. This model was applied in each forest type:

\[ \ln (Rs) = \beta_0 + \beta_1 T_s + \beta_2 M_s + \beta_3 M_s^2 + \beta_4 K \] \hspace{1cm} (E3.2)

Where Rs (µmol m⁻² s⁻¹) is the soil respiration, Tₛ (ºC) is the measured soil temperature at 10 cm, Mₛ (%) the volumetric soil water content at 10 cm, and \( \beta \) represents the coefficient estimates. A categorical variable (K) represents the fire effect, where K = 0 for the non-burned sites, and K = 1 for the burned sites. As in Tang et al.
(2005) we used a backward elimination process to eliminate any variables that failed to pass the statistical t-test and F-test.

All statistical analyses were performed using the Statistica 6.0 software package (StatSoft. Inc., Tulsa, USA) with 5% probability level for significance. The $Q_{10}$ functions were fitted using Sigma Plot 6.0. The annual C emission was calculated from average $R_s$ values in each of the plots ($\mu$mol·m$^{-2}$·s$^{-1}$), extrapolated to a year and expressed as g·C·m$^{-2}$·yr$^{-1}$.

3.3 RESULTS

3.3.1 Soil respiration, soil temperature and soil moisture

Mean $R_s$ rates ($\mu$mol·m$^{-2}$·s$^{-1}$) during the experiment were 2.09 ± 0.21 in QiNB and 1.20 ± 0.11 in QiB; 2.68 ± 0.24 in QpNB and 2.08 ± 0.27 in QpB; 3.64 ± 0.19 in PsNB and 3.23 ± 0.22 in PsB. Our estimates of annual C losses through $R_s$ from NB soils were 790 gCm$^{-2}$yr$^{-1}$ for Qi, 1010 gCm$^{-2}$yr$^{-1}$ for Qp and 1380 gCm$^{-2}$yr$^{-1}$ for Ps, respectively. For B soils the estimates were 450 gCm$^{-2}$yr$^{-1}$ for Qi, 790 gCm$^{-2}$yr$^{-1}$ for Qp and 1220 gCm$^{-2}$yr$^{-1}$ for Ps, respectively. In the B soils $R_s$ was lower relative to NB soils ($p < 0.05$), $T_s$ (º C) increased and $M_s$ (%) decreased in most cases in Qi (Figure 3.1 a, b, c) and Qp (Figure 3.2, a, b, c). In Ps the differences in $R_s$ values between NB and B plots were less pronounced (Figure 3.3, a, b, c). Overall post-burn $R_s$ decreased by 43 % in Qi, 22 % in Qp and 11 % in Ps. There were significant differences in $R_s$ between forest types ($p < 0.0001$) and seasons ($p < 0.0001$) at the three study sites. Ps showed the highest $R_s$ values followed by Qp and finally by Qi. $R_s$ values were lowest during the coldest winter months as well as in the hottest and driest months (Figure 3.2.1, a; Figure 3.2, a; Figure 3.3, a); they were highest in autumn and spring.
Figure 3.1 Temporal variation in soil respiration ($R_s$: µmol·m$^{-2}$·s$^{-1}$) (a), soil temperature ($T_s$: ºC) (b) and soil moisture ($M_s$: %) (c) below Quercus ilex from June 2007 to May 2009. Data are mean values and standard error at each sampling (n=12).
Chapter 3

Effect of wildfires on soil respiration in three typical Mediterranean forest ecosystems in Madrid, Spain

Figure 3.2 Temporal variation in soil respiration ($R_s$; $\mu$mol·m$^{-2}$·s$^{-1}$) (a), soil temperature ($T_s$; ºC) (b) and soil moisture ($M_s$; %) (c) below Quercus pyrenaica from June 2007 to May 2009. Data are mean values and standard error at each sampling (n=12).
Figure 3.3 Temporal variation in soil respiration ($R_s$: µmol·m$^{-2}$·s$^{-1}$) (a), soil temperature ($T_s$: ºC) (b) and soil moisture ($M_s$: %) (c) below *Pinus sylvestris* from June 2007 to May 2009. Data are mean values and standard error at each sampling (n=12).
3.3.2 Soil parameters and fine roots

No significant differences were observed between B and NB SOC stocks (Table 3.2). The soil N stock was higher in B plots than NB plots only in Ps stand. The C/N ratio was lower in B plots of Qi and Ps than in NB plots. Soil pH and exchangeable Ca, K and Mg were lower in B plots of Qp and Ps compared to NB controls. Only in Ps was exchangeable Al detectable, with higher values in B than NB plots.

However, differences in the labile pool of SOC such as C-MBT and DOC were found. The size of the soil microbial community was lower at the B sites than NB sites in all three forest types, although the effect was most pronounced in Qp. When expressing the C-MBT as a percentage of the total organic C (C-MBT x 100/ SOC), the only significant differences were found in Qp, where lower ratios were lower in QpB (0.49% ± 0.05) than in QpNB (0.81% ± 0.06). The DOC was also lower in B plots than in NB plots in Qp and Ps (Table 3.2).

Using humus fractions, in absolute values HA values were higher in the B plots, while the contribution of the FA fraction was lower and the HA/FA ratio increased as a result of the fire in the three studied plots (Table 3.3). Total concentrations of humic substances (HS) were lower in Qi and Ps B plots than in NB control plots. The values observed for E₄/E₆ were low (3.8 to 4.7) indicating a high humification degree of the HA (Table 3.3).

In Qi and Ps, fine root biomass (kg·m⁻²) was lower in B plots (Table 3.4), but not in Qp. Root N content (g·N·m⁻²) followed biomass and was higher in NB than B plots in Qi and Ps.
Table 3.2 Mean values and standard errors (in brackets) of the main soil properties (top 10 cm) for the three studied forest.

<table>
<thead>
<tr>
<th>Soil parameters</th>
<th>Quercus ilex</th>
<th>Quercus pyrenaica</th>
<th>Pinus sylvestris</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-2004-</td>
<td>-2002-</td>
<td>-2001-</td>
</tr>
<tr>
<td>SOC Stock (Mg ha⁻¹)</td>
<td>18.5 (1.4)</td>
<td>16.7 (0.9)</td>
<td>27.2 (1.0)</td>
</tr>
<tr>
<td></td>
<td>27.1 (1.2)</td>
<td>56.0 (5.7)</td>
<td>55.8 (3.2)</td>
</tr>
<tr>
<td>N Stock (Mg ha⁻¹)</td>
<td>1.14 (0.08)</td>
<td>1.16 (0.06)</td>
<td>1.92 (0.10)</td>
</tr>
<tr>
<td></td>
<td>1.93 (0.07)</td>
<td>3.12 (0.23)</td>
<td>4.26 (0.25)</td>
</tr>
<tr>
<td>C/N</td>
<td>16.1 (0.4)</td>
<td>14.5 (0.4)</td>
<td>14.4 (0.3)</td>
</tr>
<tr>
<td></td>
<td>14.0 (0.2)</td>
<td>17.5 (0.6)</td>
<td>13.1 (0.1)</td>
</tr>
<tr>
<td>pH (CaCl₂)</td>
<td>5.9 (0.1)</td>
<td>5.8 (0.0)</td>
<td>5.8 (0.0)</td>
</tr>
<tr>
<td></td>
<td>5.4 (0.0)</td>
<td>4.1 (0.0)</td>
<td>3.7 (0.0)</td>
</tr>
<tr>
<td>C-MBT (mg/kg)</td>
<td>153 (17)</td>
<td>138 (13)</td>
<td>280 (26)</td>
</tr>
<tr>
<td></td>
<td>186 (23)</td>
<td>275 (51)</td>
<td>198 (38)</td>
</tr>
<tr>
<td>DOC (mg/kg)</td>
<td>213 (16)</td>
<td>211 (10)</td>
<td>366 (22)</td>
</tr>
<tr>
<td></td>
<td>244 (14)</td>
<td>421 (36)</td>
<td>246 (24)</td>
</tr>
<tr>
<td>CEC (cmol/Kg)</td>
<td>Ca²⁺</td>
<td>1.89 (0.3)</td>
<td>2.53 (0.1)</td>
</tr>
<tr>
<td></td>
<td>1.45 (0.0)</td>
<td>1.75 (0.1)</td>
<td>0.70 (0.0)</td>
</tr>
<tr>
<td></td>
<td>Mg²⁺</td>
<td>0.16 (0.0)</td>
<td>0.44 (0.0)</td>
</tr>
<tr>
<td></td>
<td>0.15 (0.0)</td>
<td>0.19 (0.0)</td>
<td>0.10 (0.0)</td>
</tr>
<tr>
<td></td>
<td>0.10 (0.0)</td>
<td>0.03 (0.0)</td>
<td>0.03 (0.0)</td>
</tr>
<tr>
<td></td>
<td>K</td>
<td>0.30 (0.0)</td>
<td>0.42 (0.0)</td>
</tr>
<tr>
<td></td>
<td>0.25 (0.0)</td>
<td>0.28 (0.0)</td>
<td>0.19 (0.0)</td>
</tr>
<tr>
<td></td>
<td>0.19 (0.0)</td>
<td>0.16 (0.0)</td>
<td>0.16 (0.0)</td>
</tr>
<tr>
<td></td>
<td>Na</td>
<td>0.04 (0.0)</td>
<td>0.05 (0.0)</td>
</tr>
<tr>
<td></td>
<td>0.04 (0.0)</td>
<td>0.04 (0.0)</td>
<td>0.05 (0.0)</td>
</tr>
<tr>
<td></td>
<td>0.04 (0.0)</td>
<td>0.04 (0.0)</td>
<td>0.04 (0.0)</td>
</tr>
<tr>
<td></td>
<td>Al³⁺</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>0.32 (0.0)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>0.38 (0.0)</td>
</tr>
</tbody>
</table>

Numbers in bold indicate significant statistical differences between non burned and burned plots.
Data represent average across sampling dates (n = 21). (c) Concentrations less than 3x10⁻³ (cmol/Kg)
Chapter 3

Effect of wildfires on soil respiration in three typical Mediterranean forest ecosystems in Madrid, Spain

Table 3.3 Distribution of SOC fractions (g C Kg⁻¹) in NB and B soils. In parentheses, percentages are relative to total SOC.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Qi</th>
<th>Qp</th>
<th>Ps</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NB</td>
<td>B</td>
<td>NB</td>
</tr>
<tr>
<td>SOC</td>
<td>17.0</td>
<td>15.5</td>
<td>40.3</td>
</tr>
<tr>
<td>DOC</td>
<td>0.23 (1.59)</td>
<td>0.24 (1.59)</td>
<td>0.41 (1.01)</td>
</tr>
<tr>
<td>Fulvics acid (FA)</td>
<td>2.4 (15.8)</td>
<td>2.4 (15.8)</td>
<td>4.9 (12.5)</td>
</tr>
<tr>
<td>Humics acid (HA)</td>
<td>3.8 (21.8)</td>
<td>3.8 (21.8)</td>
<td>6.6 (17.6)</td>
</tr>
<tr>
<td>FA+HA (HS)</td>
<td>7.8 (57)</td>
<td>7.6 (49)</td>
<td>13.3 (34)</td>
</tr>
<tr>
<td>HA/FA</td>
<td>1.6</td>
<td>2.1</td>
<td>2.1</td>
</tr>
<tr>
<td>E₄/E₆</td>
<td>3.8</td>
<td>4.46</td>
<td>4.09</td>
</tr>
</tbody>
</table>

Data derived from the samples taken in November 2008 (n = 3).

Numbers in bold indicate significant statistical differences between non burned (NB) and burned (B) plots. Anova (p < 0.05).

E₄/E₆ data derived from one composite soil sample.
Table 3.4 Root biomass and root N content in non burned and burned plots.

<table>
<thead>
<tr>
<th></th>
<th>Quercus ilex</th>
<th>Quercus pyrenaica</th>
<th>Pinus sylvestris</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non Burned</td>
<td>Burned</td>
<td>Non Burned</td>
</tr>
<tr>
<td>Biomass (kg m(^{-2}))</td>
<td>0.54 (0.19)</td>
<td><strong>0.19</strong> (0.04)</td>
<td>0.57 (0.13)</td>
</tr>
<tr>
<td>Nitrogen content (g N m(^{-2}))</td>
<td><strong>4.43</strong> (1.56)</td>
<td><strong>1.99</strong> (0.45)</td>
<td>5.33 (1.21)</td>
</tr>
</tbody>
</table>

Data is presented as means and values in parentheses are standard errors (n = 9). Bolds numbers indicates significant differences (p < 0.05). Asterisks indicates significant differences at p = 0.1.
3.3.3 Correlations and modelling

3.3.3.1 Soil parameters and Rs

The average Rs rates ($\mu$mol·m$^{-2}$·s$^{-1}$) obtained during the experiment were positively related to SOC and C-MBT contents; and negatively related to pH (Figure 3.4). However, the Spearman correlations analysis (Table 3.5) for each plot showed that in QpB Rs and pH were positively correlated, while Rs was negatively correlated to C-MBT in QiB during the dry months.

3.3.3.2 Rs and soil temperature and soil moisture

Using all data (a.d) obtained during the experiment, the relationship between the Rs and T$_s$ was positive (Table 3.6). In Qi and Qp plots the fit was better for NB than B plots. We found that the $Q_{10}$ values estimated for B plots were lower than NB plots in each ecosystem (Table 3.7). The correlation for each season showed different patterns (Table 3.6). The best correlations between Rs and T$_s$ were found in winter and autumn in most cases. The $Q_{10}$ values during these seasons indicated that T$_s$ could explain from 40% to 75% of the variability in Rs (Table 3.7). In spring and summer the relationship between Rs and T$_s$ was low in Qi and Qp sites, when the best fit was found between Rs and M$_s$ (Table 3.6). When linear equation (2) was applied, between 20 and 50% of the variation in Rs could be explained by soil temperature (T$_s$), soil moisture (M$_s$) and fire effects (K) in the three ecosystems across all data as well as summer only (Table 3.8). The $\beta_4$ coefficient values: -0.40, -0.19 and -0.13 for Qi, Qp and Ps, respectively indicated a Rs decrease of 40%, 19% and 13% due to fire effects (Table 3.8). It should be noted that in this model, the $\beta_1$ coefficients were low and in most cases smaller than the $\beta_2$ coefficients, with the latter generally higher in summer (Table 3.8), suggesting a greater effect of M$_s$ on Rs during summer months.
Figure 3.4 Relationship between average values of soil respiration ($R_s$: $\mu$mol·m$^{-2}$·s$^{-1}$) versus average values of soil parameters (SOC: soil organic carbon, C-MBT: soil microbial biomass carbon, pH). *Quercus ilex* (Qi); *Quercus pyrenaica* (Qp); *Pinus sylvestris* (Ps); Non burned (NB); Burned (B). 

$$y = 0.9581 + 0.037x$$

$R^2 = 0.72; p < 0.03$

$$y = 0.2080 + 0.011x$$

$R^2 = 0.65; p < 0.05$

$$y = 6.185 - 0.714x$$

$R^2 = 0.45; p < 0.1$
Table 3.5 Spearman correlation coefficients between soil respiration (Rs) and soil parameters, with all periodic data (a) (2007-2009) and the dry (b) and wet (c) months.

**a) With all periodic data obtained during the experiment**

<table>
<thead>
<tr>
<th>Soil parameters</th>
<th>Quercus ilex</th>
<th>Quercus pyrenaica</th>
<th>Pinus sylvestris</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non Burned</td>
<td>Burned</td>
<td>Non Burned</td>
</tr>
<tr>
<td>C Stock</td>
<td>0.15</td>
<td>0.05</td>
<td>-0.37</td>
</tr>
<tr>
<td>N Stock</td>
<td>0.28</td>
<td>0.20</td>
<td>-0.29</td>
</tr>
<tr>
<td>C/N</td>
<td>-0.24</td>
<td>-0.35</td>
<td>-0.02</td>
</tr>
<tr>
<td>pH</td>
<td>0.27</td>
<td>-0.31</td>
<td>-0.30</td>
</tr>
<tr>
<td>C-MBT</td>
<td>-0.16</td>
<td>-0.35</td>
<td>-0.42</td>
</tr>
</tbody>
</table>

**b) In dry months:**

<table>
<thead>
<tr>
<th>Soil parameters</th>
<th>Quercus ilex</th>
<th>Quercus pyrenaica</th>
<th>Pinus sylvestris</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non Burned</td>
<td>Burned</td>
<td>Non Burned</td>
</tr>
<tr>
<td>C Stock</td>
<td>0.08</td>
<td>-0.08</td>
<td>-0.60</td>
</tr>
<tr>
<td>N Stock</td>
<td>0.60</td>
<td>0.14</td>
<td>0.20</td>
</tr>
<tr>
<td>C/N</td>
<td>-0.60</td>
<td>-0.11</td>
<td>-0.60</td>
</tr>
<tr>
<td>pH</td>
<td>0.08</td>
<td>-0.54</td>
<td>-0.08</td>
</tr>
<tr>
<td>C-MBT</td>
<td>-0.14</td>
<td><strong>-0.77</strong></td>
<td>-0.42</td>
</tr>
</tbody>
</table>

**c) In wet months:**

<table>
<thead>
<tr>
<th>Soil parameters</th>
<th>Quercus ilex</th>
<th>Quercus pyrenaica</th>
<th>Pinus sylvestris</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non Burned</td>
<td>Burned</td>
<td>Non Burned</td>
</tr>
<tr>
<td>C Stock</td>
<td>0.54</td>
<td>0.65</td>
<td>-0.60</td>
</tr>
<tr>
<td>N Stock</td>
<td>0.54</td>
<td>0.25</td>
<td>-0.60</td>
</tr>
<tr>
<td>C/N</td>
<td>-0.25</td>
<td>0.31</td>
<td>0.42</td>
</tr>
<tr>
<td>pH</td>
<td>0.48</td>
<td>0.49</td>
<td>0.55</td>
</tr>
<tr>
<td>C-MBT</td>
<td>0.14</td>
<td>0.48</td>
<td>-0.48</td>
</tr>
</tbody>
</table>

Bold number indicate significant correlation ($p < 0.05$)
### Table 3.6 Spearman correlation coefficients (r) between periodic soil respiration (Rs), soil temperature (Ts) and soil moisture (Ms) during the period 2007-2009.

<table>
<thead>
<tr>
<th></th>
<th>Rs</th>
<th>All data</th>
<th>Winter</th>
<th>Spring</th>
<th>Summer</th>
<th>Autumn</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non Burned</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quercus ilex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tₚ</td>
<td>0.40</td>
<td>0.66</td>
<td>0.31</td>
<td>0.13</td>
<td>0.78</td>
<td></td>
</tr>
<tr>
<td>Mₛ</td>
<td>0.20</td>
<td>-0.55</td>
<td>0.15</td>
<td>0.37</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>Burned</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tₛ</td>
<td>0.23</td>
<td>0.81</td>
<td>0.27</td>
<td>0.22</td>
<td>0.78</td>
<td></td>
</tr>
<tr>
<td>Mₛ</td>
<td>0.04</td>
<td>-0.79</td>
<td>0.08</td>
<td>0.48</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td><strong>Burned</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non Burned</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tₚ</td>
<td>0.49</td>
<td>0.64</td>
<td>0.45</td>
<td>-0.15</td>
<td>0.88</td>
<td></td>
</tr>
<tr>
<td>Mₛ</td>
<td>-0.02</td>
<td>0.32</td>
<td>0.31</td>
<td>0.39</td>
<td>-0.58</td>
<td></td>
</tr>
<tr>
<td>Quercus pyrenaica</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tₛ</td>
<td>0.28</td>
<td>0.84</td>
<td>0.23</td>
<td>-0.24</td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td>Mₛ</td>
<td>0.14</td>
<td>-0.42</td>
<td>0.63</td>
<td>0.60</td>
<td>-0.45</td>
<td></td>
</tr>
<tr>
<td><strong>Non Burned</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tₚ</td>
<td>0.41</td>
<td>0.54</td>
<td>0.66</td>
<td>0.22</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>Mₛ</td>
<td>-0.15</td>
<td>0.27</td>
<td>-0.08</td>
<td>0.04</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>Pinus sylvestris</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tₛ</td>
<td>0.42</td>
<td>0.68</td>
<td>0.21</td>
<td>0.36</td>
<td>0.64</td>
<td></td>
</tr>
<tr>
<td>Mₛ</td>
<td>-0.08</td>
<td>0.07</td>
<td>-0.13</td>
<td>-0.07</td>
<td>0.51</td>
<td></td>
</tr>
</tbody>
</table>

Numbers in bold indicate significant statistical correlation (p<0.05).
*Asterisks indicates significant correlation at p = 0.1
Table 3.7 Exponential relationship between $R_s$ and $T_s$, $Q_{10}$, $R_{10}$ parameters and squared coefficients of regression ($R^2$) calculated with all data and for each season.

Equation 1: $Rs = R_{10} \times Q_{10}^{((T_s-10)/10)}$

<table>
<thead>
<tr>
<th>Species</th>
<th>Plots</th>
<th>Measures</th>
<th>$Q_{10}$</th>
<th>$R_{10}$</th>
<th>$R^2$</th>
<th>P</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Qi</em></td>
<td>NB</td>
<td>a.d</td>
<td>1.38</td>
<td>1.88</td>
<td>0.13</td>
<td>&lt;0.0001</td>
<td>323</td>
</tr>
<tr>
<td></td>
<td></td>
<td>w</td>
<td>1.90</td>
<td>1.84</td>
<td>0.29</td>
<td>&lt;0.0001</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td></td>
<td>sp</td>
<td>1.10</td>
<td>2.40</td>
<td>0.01</td>
<td>n.s</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td></td>
<td>s</td>
<td>1.38</td>
<td>1.82</td>
<td>0.01</td>
<td>n.s</td>
<td>124</td>
</tr>
<tr>
<td></td>
<td></td>
<td>au</td>
<td>2.30</td>
<td>1.76</td>
<td>0.54</td>
<td>&lt;0.0001</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>a.d</td>
<td>1.12</td>
<td>1.15</td>
<td>0.04</td>
<td>&lt;0.0001</td>
<td>348</td>
</tr>
<tr>
<td></td>
<td></td>
<td>w</td>
<td>1.53</td>
<td>1.28</td>
<td>0.40</td>
<td>&lt;0.0001</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>sp</td>
<td>1.41</td>
<td>1.17</td>
<td>0.12</td>
<td>n.s</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td></td>
<td>s</td>
<td>1.66</td>
<td>0.58</td>
<td>0.07</td>
<td>n.s</td>
<td>119</td>
</tr>
<tr>
<td></td>
<td></td>
<td>au</td>
<td>1.89</td>
<td>1.12</td>
<td>0.62</td>
<td>&lt;0.0001</td>
<td>87</td>
</tr>
<tr>
<td><em>Qp</em></td>
<td>NB</td>
<td>a.d</td>
<td>1.67</td>
<td>2.30</td>
<td>0.22</td>
<td>&lt;0.0001</td>
<td>426</td>
</tr>
<tr>
<td></td>
<td></td>
<td>w</td>
<td>2.21</td>
<td>2.18</td>
<td>0.40</td>
<td>&lt;0.0001</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td></td>
<td>sp</td>
<td>1.74</td>
<td>2.64</td>
<td>0.13</td>
<td>n.s</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>s</td>
<td>0.48</td>
<td>6.05</td>
<td>0.03</td>
<td>n.s</td>
<td>169</td>
</tr>
<tr>
<td></td>
<td></td>
<td>au</td>
<td>2.21</td>
<td>1.97</td>
<td>0.75</td>
<td>&lt;0.0001</td>
<td>130</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>a.d</td>
<td>1.31</td>
<td>1.71</td>
<td>0.08</td>
<td>&lt;0.0001</td>
<td>440</td>
</tr>
<tr>
<td></td>
<td></td>
<td>w</td>
<td>2.73</td>
<td>2.02</td>
<td>0.67</td>
<td>&lt;0.0001</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td></td>
<td>sp</td>
<td>1.49</td>
<td>2.42</td>
<td>0.07</td>
<td>n.s</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td></td>
<td>s</td>
<td>0.50</td>
<td>4.41</td>
<td>0.05</td>
<td>n.s</td>
<td>176</td>
</tr>
<tr>
<td></td>
<td></td>
<td>au</td>
<td>1.78</td>
<td>1.35</td>
<td>0.68</td>
<td>&lt;0.0001</td>
<td>134</td>
</tr>
<tr>
<td><em>Ps</em></td>
<td>NB</td>
<td>a.d</td>
<td>1.68</td>
<td>3.18</td>
<td>0.13</td>
<td>&lt;0.0001</td>
<td>420</td>
</tr>
<tr>
<td></td>
<td></td>
<td>w</td>
<td>12.2</td>
<td>4.60</td>
<td>0.35</td>
<td>n.s</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td></td>
<td>sp</td>
<td>4.65</td>
<td>3.31</td>
<td>0.31</td>
<td>n.s</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td></td>
<td>s</td>
<td>2.21</td>
<td>2.57</td>
<td>0.05</td>
<td>n.s</td>
<td>177</td>
</tr>
<tr>
<td></td>
<td></td>
<td>au</td>
<td>2.34</td>
<td>3.17</td>
<td>0.31</td>
<td>&lt;0.0001</td>
<td>115</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>a.d</td>
<td>1.38</td>
<td>2.86</td>
<td>0.13</td>
<td>&lt;0.0001</td>
<td>424</td>
</tr>
<tr>
<td></td>
<td></td>
<td>w</td>
<td>5.86</td>
<td>4.12</td>
<td>0.42</td>
<td>n.s</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td></td>
<td>sp</td>
<td>1.39</td>
<td>3.70</td>
<td>0.03</td>
<td>n.s</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td></td>
<td>s</td>
<td>2.21</td>
<td>1.72</td>
<td>0.10</td>
<td>&lt;0.0001</td>
<td>162</td>
</tr>
<tr>
<td></td>
<td></td>
<td>au</td>
<td>2.20</td>
<td>2.67</td>
<td>0.35</td>
<td>&lt;0.0001</td>
<td>121</td>
</tr>
</tbody>
</table>

*Quercus ilex (Qi)*; *Quercus pyrenaica (Qp)*; *Pinus sylvestris (Ps)*; Non burned (NB); Burned (B); All data (a.d); Winter (w); Spring (sp); Summer (s); Autumn (au)
Table 3.8 Linear relationship between soil respiration ($R_s$), soil temperature ($T_s$), soil moisture ($M_s$) and fire effects ($K$). Coefficients, standard error and the squared coefficients of regression ($R^2$) are reported for all data and for the summer season.

<table>
<thead>
<tr>
<th></th>
<th>$\beta_0$</th>
<th>$\beta_1$</th>
<th>$\beta_2$</th>
<th>$\beta_3$</th>
<th>$\beta_4$</th>
<th>$R^2$</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qi a.d</td>
<td>1.37 ± 0.17</td>
<td>0.04 ± 0.00</td>
<td>0.18 ± 0.03</td>
<td>-0.008 ± 0.00</td>
<td>-0.40 ± 0.05</td>
<td>0.30</td>
<td>287</td>
</tr>
<tr>
<td>s</td>
<td>-0.89 ± 0.48</td>
<td>0.12 ± 0.02</td>
<td>0.38 ± 0.10</td>
<td>-0.026 ± 0.01</td>
<td>-0.54 ± 0.12</td>
<td>0.50</td>
<td>75</td>
</tr>
<tr>
<td>Qp a.d</td>
<td>1.27 ± 0.16</td>
<td>0.05 ± 0.00</td>
<td>0.19 ± 0.01</td>
<td>-0.006 ± 0.00</td>
<td>-0.19 ± 0.05</td>
<td>0.40</td>
<td>320</td>
</tr>
<tr>
<td>s</td>
<td>2.95 ± 0.47</td>
<td>-0.05 ± 0.02</td>
<td>0.32 ± 0.05</td>
<td>-0.018 ± 0.00</td>
<td>-0.15 ± 0.08</td>
<td>0.50</td>
<td>123</td>
</tr>
<tr>
<td>Ps a.d</td>
<td>2.43 ± 0.17</td>
<td>0.05 ± 0.00</td>
<td>0.07 ± 0.02</td>
<td>-0.002 ± 0.00</td>
<td>-0.13 ± 0.05</td>
<td>0.20</td>
<td>274</td>
</tr>
<tr>
<td>s</td>
<td>2.02 ± 0.51</td>
<td>0.12 ± 0.02</td>
<td>-0.19 ± 0.08</td>
<td>0.017 ± 0.00</td>
<td>-0.38 ± 0.11</td>
<td>0.25</td>
<td>81</td>
</tr>
</tbody>
</table>

Equation 2: $\ln (R_s) = \beta_0 + \beta_1 T_s + \beta_2 M_s + \beta_3 M_s^2 + \beta_4 K$; ($p < 0.001$)

$Quercus$ ilex ($Qi$); $Quercus$ pyrenaica ($Qp$); $Pinus$ sylvestris ($Ps$); All data (a.d); Summer (s)
3.4 DISCUSSION

Our results show that 6-8 years after a severe wildfire SOC stocks were unaffected. However the quality of the SOC showed a consistent shift towards lower lability and greater resistance-complexity, resulting in a decline of sensitivity of soil respiration to soil temperature ($Q_{10}$). Contrary to most (short-term) post-fire studies, we observed a general acidification of the B soil, indicated by a decline in exchangeable cations, a decline in pH and some observations of greater exchangeable Al$^{3+}$. The fire effects on physical-chemical characteristics of soils were more pronounced in Ps than in the other two vegetation types. The rate of post-fire plant recovery in B areas caused a decline in root biomass (and C inputs) which subsequently lowered microbial populations and overall soil respiration.

3.4.1 Rs, soil temperature and soil moisture

The $R_s$ values obtained were similar to those previously found in other stands in the Sierra de Guadarrama (Inclán et al. 2010). The highest $R_s$ values in spring and in autumn, as in other Mediterranean ecosystems (Almagro et al. 2009; Asensio et al. 2007; Inclán et al. 2007; Inclán et al. 2010; Joffre et al. 2003), could be due to the optimal soil temperature and soil moisture conditions that stimulate both plant and microbial activity during these seasons (Dickmann et al. 1996; Xu and Baldocchi 2004). Annual C losses via $R_s$ from the NB sites are similar to the reported on different Mediterranean forests ranging from 700 to 1184 gCm$^{-2}$yr$^{-1}$ (Almagro et al. 2009; Janssens et al. 2001; Raich and Schlesinger 1992; Tang et al. 2005b).

Even several years after severe wildfires the $R_s$ values in B sites remained lower during much of the time when compared to the NB sites, and annual C losses were 10-43% lower in B compared to NB sites. These results agree with findings of studies conducted in a variety of forest ecosystems in which a reduction in $R_s$ following wildfires has also been reported (Amiro et al. 2003; Litton et al. 2003; Marañón-Jiménez et al. 2011; Sullivan et al. 2011). The long term effects of wildfire on $R_s$ depend on several factors including forest characteristics, forest management and fire characteristics, such as fire intensity (Certini 2005; González-Perez et al. 2004). These
Greenhouse gas flows in forest and agroforestry soils in the center of the Iberian Peninsula

Factors can directly affect post-fire vegetation recovery, survival of microbial populations, and changes in the amount and quality of SOC (Van Miegroet and Olsson 2011).

The B site could continue to be a C source 5-8 years after the fire. It is expected that the B forest will not shift from C source to C sink until the forest has fully regenerated. Many authors suggest that the rate of forest recovery and the time required for the ecosystem to shift from C source to C sink following fire will depend on ecosystem characteristics and the intensity and frequency of the fire events (Amiro et al. 2001; Kowalski et al. 2004). In our case, the drier climatic characteristics of the Mediterranean area appear to slow post-fire recovery, as reported by Savage and Mast (2005) and Dore et al. (2008).

In general, the higher $T_s$ measured in B plots was due to the reduced tree cover after the fire, increasing exposure of the soil surface to solar radiation (Iverson and Hutchinson 2002) and a decrease in transpiration, limiting the cooling effect of latent energy at B sites. Only in the winter months was $T_s$ higher in NB plots than in B plots in the three ecosystems, possibly because the vegetation cover reduced heat loss from the soil. In addition, the lower $M_s$ in B plots could be due greater soil surface evaporation associated with the higher soil surface temperatures in these plots (Kobziar and Stephens 2006; O'Neill and Oppenheimer 2002; Sullivan et al. 2011). Hence, even a considerable time after a wildfire event, B plots exhibited a different soil climate, associated with changes in soil moisture dynamics and increased solar heating (Shakesby and Doerr 2006).

In the long term the microbial biomass progressively recovers depending on the post fire vegetation dynamics and its effect on soil microclimate and C inputs (Hart et al. 2005; Litton et al. 2003). The size of the soil microbial community was lower at the B sites presenting C-MBT values (138-280 mg/kg) similar to those previously reported (Dilustro et al. 2005; Inclán et al. 2010). The decrease in microbial populations immediately following fire events has been widely reported (Inclán et al. 2010; Prieto-Fernández et al. 1998; White et al. 2004). Lower C-MBT values long-term after fire have been also found by Dumontet et al.(1996) and Litton et al.(2003). This lower C-MBT in the B compared to the NB sites suggests a reduced heterotrophic contribution to total soil respiration, explaining part of the $R_s$ diminution in the QpB plot (Mabuhay
et al. 2006). The lower fine root biomass content in B plots might have contributed to the observed declines in $R_s$ in B plots, associated with the removal of trees by fire and lack of post-fire re-establishment of trees in B plots (Inclán et al. 2007; Irvine et al. 2007; Litton et al. 2003; Ryu et al. 2009; Sullivan et al. 2011). Our results agree with those of previous studies which also reported reduction in fine root biomass several years after burning and canopy removal (Litton et al. 2003; Ryu et al. 2009; Sullivan et al. 2011). The tendency of B sites to emit less CO$_2$ probably reflects the loss of root respiration and/or the lack of production of labile root exudates due to plant death, as well as the changes in the SOC quality.

### 3.4.2 Soil parameters and soil respiration

The decreased post-fire C/N ratio is a phenomenon frequently cited for several types of post-fire soils (Fernández et al. 1999; González-Perez et al. 2004; Ryu et al. 2009). The SOC and N stocks in the mineral soil tend to recover over time eventually reaching the pre-disturbing levels (Johnson et al. 2004; Norris et al. 2009). Although no statistically significant differences in SOC were observed between B and NB sites, the differences in the C/N could be due to a difference in the C and N consumption by fire, where more C is lost and relatively more N becomes stabilized. Soil NH$_4^+$ levels often increase following fire, because of heat induced decomposition of organic N in soil and also by inorganic N inputs from ash (Certini 2005; Neary et al. 1999) causing disproportionately loss of N compared to C pools. Furthermore, the differences in the C/N could be due to a difference in recovery C vs. N pools after fire, the lost of N is often replaced and even exceeded by inputs from N fixers after fire (Johnson et al. 2008). Especially in the case of Ps, the increase in N stocks of the soil could be due to N fixation by the leguminous *Adenocarpus hispanicus*. Johnson and co-workers (Johnson et al. 2007; Johnson et al. 2004) had also noted that site occupancy with N-fixers plays a crucial role in ecosystem C recovery in the dry montane forest western USA where wildfires significantly reduce N availability.

The reduction in pH in B plots of Qp and Ps, accompanied by a depletion in exchangeable bases could be related to greater erosion of the ash layer or leaching by rainwater (Ulery et al. 1993) especially since the level of precipitation in these
ecosystems was higher than in Qi. A decrease in soil pH after fire has also been reported by Fernandez et al. (2001) and Ryu et al. (2009). Loss of exchangeable bases has further been related to a fire-induced decline in cation exchange capacity associated with the loss of organic matter (Oswald et al. 1998). Soil pH regulates chemical reactions and a number of enzymes in microorganisms, many of which are pH-dependent (Luo and Zhou 2006). The decrease in soil pH could have influenced the growth and proliferation of soil microbes as well as soil respiration and Q_{10} values in B plots of Qp and Ps. Also the decrease in soil pH in PsB could lead to greater mobilization of the Al^{3+} from organic matter. These findings agree with those of previous studies carried out by Fernandez et al. (2001).

Despite lack of significant differences in total SOC stocks between B and NB site, the different parameters used this study to characterize SOC quality indicate a preferential loss of more labile SOC and a greater preponderance of recalcitrant SOC forms, including a decline in DOC and C-MBT and a shift in the HA/FA composition (Almedros et al. 1990; Certini 2005; González-Perez et al. 2004; Shibata et al. 2003). The increase in SOC recalcitrance is indicated by the HA (formed by condensed structures) accumulation in the superficial B soils layer that are considered an index of humification intensity (Certini 2005; Van Miegroet and Olsson 2011). Moreover the increment in the HA/FA ratio after fire could be due to the decrease of FA fractions that were transformed into an acid-insoluble macromolecule (HA) by aromatization and condensation phenomena (Vergnoux et al. 2011). The E_{d}/E_{o} ratios obtained in this study (3.8 to 4.8) are all similar and characteristic of humic acids (Khan and Schnitzer 1972; Kononova 1962). However it is difficult to interpret the differential post-burn responses in terms of fire-induced changes in humification. The influence of fire on the labile fractions of SOC depends on fire types and severity (Choromanska and DeLuca 2002; Walstad et al. 1990) and the time after fire (Boerner et al. 2004; Döckersmith et al. 1999; Gundale et al. 2005; Rutigliano et al. 2007).

The shift in SOC quality from more easily decomposable forms towards more recalcitrant SOC compounds which are more resistant to chemical and biological breakdown could contribute to the formation of stable SOC pools, and could explain the lower R_{s} values in B plots. The Q_{10} values and the correlation between R_{s} and T_{s} obtained for NB plots were higher than for B plots in Qi and Qp when all the data was
considered, revealing that $R_s$ displays less sensitivity to $T_s$ following fire. This lower sensitivity to $T_s$ might be related to the loss of labile fraction of SOC (Hernández et al. 1997; Marschner and Kalbitz 2003; Van Miegroet and Olsson 2011) combined with lower $M_s$ conditions (Conant et al. 2004; Liski et al. 1999) in the B plots.

### 3.4.3 Correlation between $R_s$ and soil parameters

The strongest correlation between $R_s$ and $T_s$ were obtained in winter and autumn in all the forest areas and the weakest correlation were found in summer. $T_s$ and $M_s$ explained most of the variation in $R_s$, but their relative role changed with seasons, as also observed in numerous other studies conducted in seasonally dry areas (Almagro et al. 2009; Asensio et al. 2007; Inclán et al. 2010; Marañón-Jiménez et al. 2011; Olsen and Van Miegroet 2010; Rey et al. 2002; Xu and Qi 2001). We observed that $M_s$ had two opposite effects on $R_s$. When $M_s$ was greater than 5% in Qi, and greater than 10% in Qp and Ps during the wet seasons, the $R_s$ responded positively to $T_s$ and negatively to $M_s$. When $M_s$ was below this threshold during dry season, the $R_s$ responded negatively to $T_s$ (or with weaker correlation). High water content can slow down diffusion of O$_2$, which impedes decomposition and CO$_2$ production, while low soil water content can inhibit soil microbial activity and root respiration (Davidson et al. 1998).

The $Q_{10}$ values for the different areas and seasons were within the range previously reported for Mediterranean ecosystems (Almagro et al. 2009; Inclán et al. 2010; Reichstein et al. 2002; Xu and Baldocchi 2004; Xu and Qi 2001). Our results reveal that $Q_{10}$ exhibits a seasonal pattern regardless of site; with $Q_{10}$ values consistently greater in winter and autumn. A number of authors also point to $T_s$ and $M_s$ as important explanatory factors for the seasonal variation in $Q_{10}$ (Borken et al. 1999; Cernusak et al. 2006; Conant et al. 1998; Davidson et al. 2000). Janssens and Pilegaard (2003) further stated that $Q_{10}$ is not simply a reflection of temperature sensitivity, but rather expresses the combined response to fluctuations in temperature, root biomass, moisture conditions, and perhaps other variables that directly or indirectly have been affected by fire (Boone et al. 1998). The application of a linear model helped to better understand the effects of $T_s$, $M_s$ and wildfires on $R_s$. 

104
Linear regression revealed that $R_s$ was also positively correlated with SOC stocks and C-MBT. The results agree with Priess and Fölster (2001) which related the decomposition process and hence microbial respiration with SOC increases. Different authors suggest that soil C could be one of main determinants of $R_s$, particularly at large spatial and temporal scales (Davidson and Janssens 2006; Giardina and Ryan 2002; Ryan and Law 2005). However, $R_s$ was negatively correlated with C-MBT in QiB during dry months, probably, as a response mechanism of the $R_s$ to water scarcity during this time. This drought stress may cause the lysis of microorganisms in the soil, enhancing the availability of C with a rapid mineralization of tissues of dead microbial biomass to surviving microorganisms (Borken and Matzner 2009).

The Spearman rank correlation data showed poor correlation between $R_s$ and soil parameters. In QpB, $R_s$ was positively correlated with pH, several studies described a similar positive correlation of pH and $R_s$ (Ellis et al. 1998; Reth et al. 2005).

3.5 CONCLUSION

In the *Quercus ilex*, *Quercus pyrenaica* and *Pinus sylvestris* forests around Madrid, we found long term effects of wildfires on the physical, chemical and biological characteristics of the soil, which in turn affect soil respiration, with burn effects most pronounced in *Pinus sylvestris*. Wildfires alter C flux from soils due to their impact on tree vegetation and physical and chemical soil parameters. In burned plots, soil temperatures and refractory SOC were higher, while soil moisture, fine root, labile carbon and microbial biomass were lower. Collectively these changes in soil microclimate and SOC quality explain the observed decreases in $R_s$. Soil respiration was largely controlled by soil temperature only if sufficient soil moisture was available, hence the critical importance of soil moisture to C dynamics and C source-sink strength in Mediterranean ecosystems. The $Q_{10}$ model explained part of the variation in soil respiration in each ecosystem, but the application of a linear model that includes soil moisture improved the explanation of the variation in soil respiration observed during the experiment. The lower $Q_{10}$ values in burned probably reflect the combined effect of loss of root respiration and lack of production of labile root exudates, lower soil microbial biomass, fire-induced changes in the SOC quality, and decreases in soil
moisture. The results from this study indicate that various chemical and biological factors (such as fine root biomass, soil microbial biomass, C/N and SOC quality) co-vary with soil temperature and soil moisture to control the soil respiration in these ecosystems.

The different responses of C emission to climatic shifts many years after the occurrence of wildfire highlights the need include these shifts in C dynamics in future research in order to more accurately assess the C balance in Mediterranean forests and better predict the impact of climatic change on the exchange of C between forest ecosystems and the atmosphere.

3.6 ACKNOWLEDGEMENTS

This research was conducted in the framework of the Spanish CGL2006-02922/CLI and CGL2009-07031. The authors would like to express their gratitude to Stoyan Holding LTD., Patrimonio Nacional and Community of Madrid for the use of their forest sites. We thank Dr. Robert Jandl for his constructive suggestion to this work. We also thank two anonymous referees for constructive comments on the manuscript.

3.7 REFERENCES


Chapter 3

Effect of wildfires on soil respiration in three typical Mediterranean forest ecosystems in Madrid, Spain


108


Luo Y and Zhou X 2006 Soil Respiration and the Environment. San Diego, CA.


Moreno J M, Vázquez A and Vélez R 1998 Recent history of forest fires in Spain. Backhuys


3.8 SUPPLEMENTARY MATERIAL

S.1. Carbon content in litterfall and litter

S.1.1 Litterfall

S.1.1.1 Methodology

C inputs through litterfall were measured simultaneously with the experimental measurements described in this Chapter (Table S.1.1.2). The samples of litterfall were taken in four collector (0.29 m$^2$/per collector) in each non burned areas from the forest ecosystems investigated. The samples were collected once a month from 2007 to 2009. Samples were oven-dried at 65°C/48h; the leaves were separate from the rest of the samples (lichen, small branch fruits, etc), weighed and ground before the analysis of carbon content (using a LECO TRUSPEC analyzer) in the laboratory. We observed that carbon content in leaves biomass and total biomass had similar values, around 50% of C.

S.1.1.2 Table with mean values of Carbon content in total biomass of litterfall in non burned areas of *Quercus ilex*; *Quercus pyrenaica* and *Pinus sylvestris*.

<table>
<thead>
<tr>
<th>Litterfall (gCm$^{-2}$yr$^{-1}$)</th>
<th><em>Quercus ilex</em></th>
<th><em>Quercus pyrenaica</em></th>
<th><em>Pinus sylvestris</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Non burned Burned</td>
<td>152</td>
<td>142</td>
<td>336</td>
</tr>
</tbody>
</table>

(—) Burned areas were avoided of trees.

S.1.2 Organic layer

S.1.2.1 Methodology

To study the differences in carbon content in the forest soil organic layer the organic layer at the soil surface were sampled with a frame of 25 by 25 cm in three different points by area in non burned and burned sites in June 2007; June 2008 and September 2009 during the experiment of Chapters 3 and 4. The organic and mineral
soil should were sampled at exactly the same locations (sample the mineral soil underneath the organic layer that has already been removed for sampling). Organic layer were dried at 65º/ 48h, weighed and ground and homogenized using a pestle to measurement C content in the laboratory.

S.1.2.2 Table with mean values of carbon content in organic layer in non burned and burned areas of *Quercus ilex*, *Quercus pyrenaica* and *Pinus sylvestris*

<table>
<thead>
<tr>
<th></th>
<th>Organic layer (g C m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Quercus ilex</em></td>
</tr>
<tr>
<td>Non burned</td>
<td>490</td>
</tr>
<tr>
<td>Burned</td>
<td>360</td>
</tr>
</tbody>
</table>
Chapter 4

$\text{N}_2\text{O}$ AND $\text{CH}_4$ FLUXES IN UNDISTURBED AND BURNED HOLM OAK, SCOTS PINE AND PYRENEAN OAK FORESTS IN CENTRAL SPAIN
Chapter 4

N₂O and CH₄ fluxes in undisturbed and burned Holm oak, Scots pine and Pyrenean oak forests in central Spain

Biogeochemistry journal (2012) 107, 19-41
ABSTRACT

We investigated N$_2$O and CH$_4$ fluxes from soils of Quercus ilex, Quercus pyrenaica and Pinus sylvestris stands located in the surrounding area of Madrid (Spain). The fluxes were measured for 18 months from both mature stands and post fire stands using the static chamber technique. Simultaneously with gas fluxes, soil temperature, soil water content, soil C and soil N were measured in the stands. Nitrous oxide fluxes ranged from -11.43 to 8.34 µg N$_2$O-N·m$^{-2}$·h$^{-1}$·in Q. ilex, -7.74 to 13.52 µg N$_2$O-N·m$^{-2}$·h$^{-1}$·in Q. pyrenaica and -28.17 to 21.89 µg N$_2$O-N·m$^{-2}$·h$^{-1}$·in P. sylvestris. Fluxes of CH$_4$ ranged from -8.12 to 4.11 µg CH$_4$-C·m$^{-2}$·h$^{-1}$·in Q. ilex, -7.74 to 3.0 µg CH$_4$-C·m$^{-2}$·h$^{-1}$·in Q. pyrenaica and -24.46 to 6.07 µg CH$_4$-C·m$^{-2}$·h$^{-1}$·in P. sylvestris. Seasonal differences were detected; N$_2$O fluxes being higher in wet months whereas N$_2$O fluxes declined in dry months. Net consumption of N$_2$O was related to low N availability, high soil C contents, high soil temperatures and low moisture content. Fire decreased N$_2$O fluxes in spring. N$_2$O emissions were closely correlated with previous day’s rainfall and soil moisture. Our ecosystems generally were a sink for methane in the dry season and a source of CH$_4$ during wet months. The available water in the soil influenced the observed seasonal trend. The burned sites showed higher CH$_4$ oxidation rates in Q. ilex, and lower rates in P. sylvestris. Overall, the data suggest that fire alters both N$_2$O and CH$_4$ fluxes. However, the magnitude of such variation depends on the site, soil characteristics and seasonal climatic conditions.

**Keywords**: Greenhouse flux, fire, soil water content, soil temperature, Quercus pyrenaica, Pinus sylvestris, Quercus ilex.
# CONTENT

4.1 INTRODUCTION ........................................................................................................ 119
4.2 MATERIALS AND METHODS .................................................................................. 122
4.2.1 Study sites and experimental design .................................................................. 122
4.2.2 N₂O and CH₄ flux measurements ...................................................................... 125
4.2.3 Meteorological data ......................................................................................... 126
4.2.4 Soil samples ..................................................................................................... 127
4.2.5 Statistical analysis ......................................................................................... 128
4.3 RESULTS ................................................................................................................. 128
4.3.1 Environmental conditions ................................................................................ 128
4.3.2 Soil parameters ............................................................................................... 133
4.3.3 N₂O fluxes ....................................................................................................... 135
4.3.4 CH₄ fluxes ....................................................................................................... 142
4.4 DISCUSSION ............................................................................................................ 146
4.4.1 Nitrous oxide fluxes ......................................................................................... 146
4.4.1.1 Seasonal variation ...................................................................................... 146
4.4.1.2 Effects of forest type .................................................................................. 150
4.4.1.3 Effects of fire .............................................................................................. 152
4.4.2 Methane fluxes ............................................................................................... 154
4.4.2.1 Seasonal variation ...................................................................................... 154
4.4.2.2 Effects of forest type .................................................................................. 155
4.4.2.3 Effects of fire .............................................................................................. 156
4.5 CONCLUSIONS ..................................................................................................... 157
4.6 ACKNOWLEDGEMENTS ....................................................................................... 158
4.7 REFERENCES .......................................................................................................... 158
4.1 INTRODUCTION

Nitrous oxide (N$_2$O) and methane (CH$_4$) are greenhouse gases (GHG) that interact with long-wave (infrared) solar radiation and, as a result, contribute to ‘greenhouse warming’. Global atmospheric concentrations of N$_2$O and CH$_4$ have increased markedly as a result of human activities since 1750 and now far exceed pre-industrial values (from 270 to 320 ppb N$_2$O and from 700 to 1782 ppb CH$_4$ in 2006). The observed increase in N$_2$O is largely attributed to agricultural activity. Increased livestock production and fossil fuel use are the main reasons for the atmospheric increase in CH$_4$ (IPCC 2007).

Although less abundant than CO$_2$, the Global Warming Potential of N$_2$O and CH$_4$ are about 310 and 21 times respectively, greater than CO$_2$ (IPCC 2007); and together they are responsible for more than 21% of the radiative forcing of Earth’s energy balance (IPCC 1992). N$_2$O is also involved in the destruction of the stratospheric ozone layer (Cicerone 1979).

Soils play a key role in the global budget of N$_2$O and CH$_4$, as both gases are biologically produced and consumed by soil microorganisms (Conrad 1989; Firestone and Davidson 1989; Chapuis-Lardy et al. 2007). Soils are usually considered as net sources of atmospheric N$_2$O. Total annual emissions from natural soils have been estimated to be 6.0 Tg N-N$_2$O yr$^{-1}$ and 3.5 Tg N-N$_2$O yr$^{-1}$ from agricultural soils (IPCC 2007), but with large uncertainties. The principal sources of N$_2$O in soils are microbial nitrification, nitrifier denitrification and denitrification, with the latter ones also being able to consume N$_2$O (Granli and Bøckman 1994; Schmidt et al. 2004). Nitrifying microbes convert soil ammonium ($\text{NH}_4^+$) to nitrate ($\text{NO}_3^-$) under aerobic conditions. Anaerobic denitrifiers reduce nitrogen oxides to nitric oxide (NO), N$_2$O and finally N$_2$, when there is sufficient carbon (C) and available NO$_3^-$; incomplete conversion results in the emission of N$_2$O. These microbial processes can occur simultaneously in adjacent anaerobic and aerobic micro-sites within the soil profile (Firestone and Davidson 1989; Potter et al. 1996b). There are some indications that soils may also function as significant sinks for atmospheric N$_2$O and that the soil N$_2$O reduction has decreased over recent decades (Chapuis-Lardy et al. 2007; Goldberg and Gebauer 2009). The main
environmental factors controlling $N_2O$ production in forest soils are soil nitrogen (N) status, soil moisture, precipitation, aeration, pH, atmospheric nitrogen deposition, temperature and tree species composition (Butterbach-Bahl et al. 2002; Schindlbacher et al. 2004; Kitzler et al. 2006; Skiba et al. 2004; Skiba et al. 2009; Weslien et al. 2009; Pilegaard et al. 2006).

$CH_4$ is produced by groups of anaerobic Archea, which require a low redox potential (Philippot et al. 2009). Therefore, high $CH_4$ fluxes are typical of wetlands, rice paddies or environments where anoxic fermentation is favoured. The impact of these sources is only partly balanced by removal processes in upland soils (Houghton et al. 1996). The extent of $CH_4$ removal by terrestrial oxidation is uncertain, with best estimates between 20 and 51 Tg $CH_4$ yr$^{-1}$ (Potter et al. 1996 a; Smith et al. 2000). Soils have been identified as a significant biological sink for atmospheric $CH_4$ due to the oxidation of $CH_4$ to methanol ($CH_3OH$) for energy by methanotrophic bacteria, generally in well-drained, aerobic soils (Mosier et al. 1991; Papen et al. 2001). Forests are generally recognized as the largest sinks for $CH_4$, while $CH_4$ uptake by disturbed soils tends to be small (Smith et al. 2000). The diffusivity of $CH_4$ through the soil profile is the primary limiting factor upon $CH_4$ oxidation and this is influenced by soil moisture and bulk density. Furthermore, soil N status can limit $CH_4$ oxidation directly by inhibiting/competing with the monooxygenase enzyme of methanotrophs (Castro et al. 1995).

An important scientific priority of terrestrial trace gas studies is to create data bases of trace gas fluxes for different ecosystems, which could be used to parameterize and validate biogeochemical models (Potter et al. 1996 a,b; Parton et al. 2001). However, much of the experimental data currently available has been collected in temperate regions (Smith et al. 2000), boreal forests, prairies, agricultural areas and tropical forest. Less information is available on seasonally-dry ecosystems, which are potentially able to act as efficient sinks for atmospheric $CH_4$ and significant sources of $N_2O$ (Potter et al. 1996 a,b; Castaldi et al. 2004, 2006; Wolf et al. 2010). This knowledge is particularly evident for Mediterranean type ecosystems. Trace gas emissions from Spanish forest soils have rarely been studied (Merino et al. 2004), and therefore there is a little understanding of the spatial and temporal variability of $N_2O$ and $CH_4$ exchange in Spanish forest soils or the soil parameters that control this exchange. The importance of
forest type or tree species and the effects of land disturbances are also poorly understood. A high level of uncertainty exists as to the contribution (source or sink) of Spanish forests to the regional, continental, and global N₂O and CH₄ balance.

Fire is among the most relevant form of ecosystem disturbance affecting nutrient cycling in Mediterranean forest ecosystems (Johnson et al. 2005), significantly affecting the soil-plant system (Wienhold and Klemmedson 1992). Over the last few decades, the number of wildfires has increased in this region destroying thousands of hectares every year. Interest in the effects of fire on forest soil fertility and soil-atmosphere exchange of N₂O and CH₄ has recently heightened due to the concern that climate change may increase fire frequency and intensity (Westerling et al. 2006).

The short term effects of fires on forest soils have been widely studied (Rodriguez et al. 2009; Poth et al. 1995; Castaldi and Fierro 2005, Certini 2005; Neary et al. 1999; Castaldi and Aragosa 2002; Inclán et al. 2010). However, less information is available on the long-term effects of wildfires (Wan et al. 2001; Johnson et al. 2009; Durán et al. 2009). Wildfires may lead to soil degradation, including deterioration of structure as well as losses of soil organic matter and nutrients (Giovannini et al. 1988; Gillon et al. 1995; Prieto-Fernández et al. 1998; Inclán et al. 2010). The abovementioned effects of fire along with its impact on soil microbial populations can significantly alter soil C and N transformation rates and ultimately the C and N cycles of forest ecosystems (Johnson et al. 2009). Fire can have substantial long-term effects on ecosystem C and N by causing changes in vegetation, often through the facilitation of occupancy of the burned site by N-fixing vegetation, which in turn can indirectly cause long-term increases in ecosystem C capital in N-limited ecosystems (Johnson et al. 2005).

Immediately after burning, nitrification and denitrification can be significantly reduced, probably as a direct effect of fire on soil microorganisms (Castaldi and Aragosa 2002). The capacity of the soil to retain water can decrease after fire, as both water holding capacity and the ability to resist evaporation may be reduced in burned soil, slowing down nitrification and denitrification processes. Increases in N mineralization and microbial activity have been reported after initial post-fire stages as an effect of transient increases in temperature, water content and labile sources of C and N for microbes (Rutigliano et al. 2007). These initial increases are short-lived and 10 years after the last fire, significant decreases in net mineralization rates were observed.
indicating slow recovery of N turnover (Durán et al. 2009). It was expected that increasing NH$_4^+$ content in the soil would reduce methanotrophic activity; since it has been hypothesized that NH$_3$ can act as a competitive substrate for methane monooxygenase (Bédard and Knowles 1989). Nitrite, formed during NH$_4^+$ oxidation by methanotrophs, may also have a role in the inhibiting effect of NH$_4^+$ addition on soil CH$_4$ oxidation capacity. Soil NH$_4^+$ levels often increase following fire because of heat-induced decomposition of organic N in soil and possibly by inorganic N inputs from ash (Certini 2005; Neary et al. 1999). Post-fire concentrations of NH$_4^+$ were found to decrease in the long-term (Durán et al. 2009). Hence, fluxes of N$_2$O and CH$_4$ may increase or decrease in forest soils after fire depending on the time elapsed since the disturbance. As a consequence of the strong seasonality of rainfall, opposite situations might occur such as the inability of microbes to exploit the increased mineral N content, due to summer drought, or significant N losses by denitrification and leaching during winter months, in which more significant precipitation events are concentrated. Greater differences between burned and unburned plots might be expected on spring sampling dates in comparison to winter sampling dates due to higher soil temperatures (Gallardo and Schlesinger 1994).

The aim of this work was to study the long-term effect of wildfires on N$_2$O and CH$_4$ soil-atmosphere exchange in three typical Mediterranean type forest ecosystems in the surrounding area of Madrid (Spain). We hypothesized that the effects of fire-induced type conversion from forest to grass-shrub mosaic on N$_2$O and soil CH$_4$ flux depends on forest site, soil characteristics and seasonal climatic conditions.

4.2 MATERIALS AND METHODS

4.2.1 Study sites and experimental design

The research was conducted in three forest stands (Table 4.1) near Madrid (Spain): A Holm oak stand (Quercus ilex subsp. ballota; hereafter QI); a Pyrenean oak forest stand (Quercus pyrenaica Willd.; hereafter QP); and a Scots pine forest stand (Pinus sylvestris L.; hereafter PS). All the stands are located within protected areas that have been managed in the past. Oaks have been coppiced for centuries to provide firewood, charcoal and grazing for domestic livestock. In the last 50 years, the use of fossil fuels
has led to a drop in the value of firewood and the near abandonment of coppice management systems. Consequently, many of these forests currently exhibit very high densities and are more susceptible to fire. PS stands have been managed in a relatively consistent, systematic manner; harvesting trees through successive uniform thinning interventions, with a cycle of around 120 years and a regeneration period of 20 years. Today, forests are managed using ecologically sustainable methods aimed at reducing the fuel potential for forest fires and increasing the resilience of forests to possible future fires through selective clearing, pruning, cutting and removal.

The forest understory in QI consists of shrub-type vegetation and grasses, including *Cistus ladanifer, Lavandula stoechas, Rosmarinus officinalis, Crataegus monogyna*. In QP, other species present include scattered *Fraxinus angustifolia, Cytisus scoparius, Genista florida, Rubus ulmifolius, Crataegus monogyna, Prunus spinosa, Lonicera peryclimenum* and *Tamus communis*. In PS, the understory is dominated by shrubs (*Genista cinerea, Adenocarpus hispanicus, Rosa canina, Santolina rosmarinifolia*) and grasses (*Pteridium aquilinum, Rumex scutatus, Stipa gigantea*).

The forests are located on granitic bedrock. Soils are Haplic Umbrisols (FAO, 1998). The main properties of these soils are shown in Table 4.1. The climate is continental Mediterranean in QP and QI and mountain-Mediterranean in PS, which is situated at higher altitude. The continental Mediterranean climate is characterized by extreme temperatures: very hot in the summer and very cold in winter. Rainfall is scarce, especially in summer and is concentrated in spring and autumn. During the winter, this precipitation is usually in the form of snow. In general, rainfall remains below 900 mm per year (Table 4.1). The mountain-Mediterranean climate tends to be cold and damp. The mean annual minimum and maximum temperatures at the different locations vary between 2.4 °C and 24.1 °C in QI, 2.6 °C and 23.1 °C in QP and -2.9 °C and 7.5° C in PS.
Table 4.1 Site characteristics of the different studied forest in Madrid, Spain.

<table>
<thead>
<tr>
<th>Vegetation</th>
<th>Non burned</th>
<th>Burned</th>
<th>Non burned</th>
<th>Burned</th>
<th>Non burned</th>
<th>Burned</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quercus illex</td>
<td>Quercus illex</td>
<td>Quercus pyrenaica</td>
<td>Quercus pyrenaica</td>
<td>Pinus sylvestris</td>
<td>Pinus sylvestris</td>
<td></td>
</tr>
<tr>
<td>Location</td>
<td>N 40° 35' 39.3'' W 3° 55' 30.5''</td>
<td>N 40° 35' 39.7'' W 3° 55' 31.7''</td>
<td>N 40° 34' 07.8'' W 4° 08' 54.8''</td>
<td>N 40° 34' 06.3'' W 4° 08' 55.2''</td>
<td>N 40° 43' 21.2'' W 4° 07' 20.1''</td>
<td>N 40° 44' 08.5'' W 4° 06' 21.9''</td>
</tr>
<tr>
<td>Stand age (yr)</td>
<td>40</td>
<td>–</td>
<td>50</td>
<td>–</td>
<td>120</td>
<td>–</td>
</tr>
<tr>
<td>Mean precipitation (mm)</td>
<td>655</td>
<td>655</td>
<td>788</td>
<td>788</td>
<td>895</td>
<td>895</td>
</tr>
<tr>
<td>Mean air temperature (°C)</td>
<td>14</td>
<td>14</td>
<td>13</td>
<td>13</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Elevation (m.a.s.l)</td>
<td>985</td>
<td>986</td>
<td>1020</td>
<td>1017</td>
<td>1409</td>
<td>1397</td>
</tr>
<tr>
<td>Slope (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>40</td>
<td>25</td>
</tr>
<tr>
<td>Aspect (deg)</td>
<td>a.w.</td>
<td>a.w.</td>
<td>a.w.</td>
<td>a.w.</td>
<td>135º</td>
<td>90º</td>
</tr>
<tr>
<td>Tree height (m)</td>
<td>3.1</td>
<td>–</td>
<td>7.5</td>
<td>–</td>
<td>16</td>
<td>–</td>
</tr>
<tr>
<td>DBH (cm)</td>
<td>14</td>
<td>–</td>
<td>18</td>
<td>–</td>
<td>27</td>
<td>–</td>
</tr>
<tr>
<td>pH (CaCl₂)</td>
<td>5.9 a</td>
<td>5.8 ab</td>
<td>5.8 ab</td>
<td>5.4 b</td>
<td>4.1 c</td>
<td>3.7 c</td>
</tr>
<tr>
<td>C (g/kg)</td>
<td>20.9 c</td>
<td>18.6 c</td>
<td>34.3 bc</td>
<td>37.0 b</td>
<td>73.4 a</td>
<td>64.1 a</td>
</tr>
<tr>
<td>N (g/kg)</td>
<td>1.3 c</td>
<td>1.3 c</td>
<td>2.4 bc</td>
<td>2.6 b</td>
<td>4.0 a</td>
<td>4.8 a</td>
</tr>
<tr>
<td>C/N</td>
<td>16.1 b</td>
<td>14.1 c</td>
<td>14.1 c</td>
<td>14.0 cd</td>
<td>17.5 a</td>
<td>13.2 d</td>
</tr>
<tr>
<td>Soil type</td>
<td>Haplic Umbrisol</td>
<td>Haplic Umbrisol</td>
<td>Haplic Umbrisol</td>
<td>Haplic Umbrisol</td>
<td>Haplic Umbrisol</td>
<td>Haplic Umbrisol</td>
</tr>
<tr>
<td>Clays (%;&lt;2µm)</td>
<td>20 ab</td>
<td>15 ab</td>
<td>28 a</td>
<td>12 b</td>
<td>11 b</td>
<td>19 a</td>
</tr>
<tr>
<td>Silt +Sand (%;&gt;2µm)</td>
<td>80 a</td>
<td>85 a</td>
<td>72 b</td>
<td>88 a</td>
<td>89 a</td>
<td>81 a</td>
</tr>
</tbody>
</table>

Different letters in each line indicate significant statistical differences (p < 0.05).

Wildfires have occurred in these forests at different times in the past. QI suffered a fire in August 2004, affecting approximately 12 ha; in QP the fire occurred in August 2002, affecting around 10 ha; and around 71 ha of forest were burned in PS in August 2001. The intensity and severity of the fire was assessed according to the amount and height of bark charring on tree stems. Based on this data as well as on information provided by local environmental agencies, all the fires at the plots were classified as crown fires.

In each forest stand we selected two (50 m x 50 m) plots: one affected by fire (burned, B) and the other unaffected (unburned-control, NB). The B and NB plots at each forest site are similar in terms of slope/aspect. The burned area in each stand is devoid of trees and the vegetation mainly comprises the previously described understory shrub species associated with each ecosystem. In the burned plots located in QI and QP, the understory is sparse whereas in PS, the vegetation cover is dominated by the leguminous *Adenocarpus hispanicus*, which is endemic of the Sistema Central (Spain) and is capable of fixing atmospheric N due to a symbiotic relationship with Rizobium bacteria.

### 4.2.2 \( \text{N}_2\text{O} \) and CH\(_4\) Flux Measurements

Fluxes of \( \text{N}_2\text{O} \) and CH\(_4\) between the soil and the atmosphere were measured using the closed chamber technique. Measurements were taken approximately on a monthly basis from December 2007 until September 2009. Gas measurements were carried out as described in Kitzler et al. (2006). Gas emissions were measured using four manual chambers installed randomly in each plot.

A manual chamber consists of an aluminium frame (1 x 1 x 0.05 m). A single-wall rigid polyethylene light-dome (volume: 70 L) with a compressible seal at the bottom was fixed onto the frame by means of 2 elastics bands fixed to the soil. Large chambers were chosen to account for small scale variability. Air samples from the chambers were taken using 100 ml gas-tight syringes at intervals of 0, 0.5 and 1 h after the closure of the chamber in order to measure the increase or the decrease in concentration inside the chamber. These samples, which were taken between 9.00 and 13.00 GMT, were then injected into gas tight head-space vials (20 ml). The linearity of the emission was tested
and additional measurements every 0.5 h showed that the increase in CH₄ and N₂O concentrations remained linear for up to 2 hours.

The vials with the gas samples were transported to the laboratory and analysed using a gas chromatograph (HP-6890) equipped with a headspace autoanalyzer (HT3). HP-Plot Q capillary columns transported gas samples from the injector to a 63Ni electron-capture detector to measure N₂O and to a flame ionization detector (FID) fitted with a methanizer to measure CH₄. N₂ served as carried gas with a flow rate of 30 ml/min⁻¹. The gas-chromatograph was cross-calibrated with standard gases. The temperatures of the injector, oven and detector were 50 ºC, 50 ºC, and 300 ºC respectively.

Emissions of N₂O (µg N₂O-N m⁻² h⁻¹) and CH₄ (µg CH₄-C m⁻² h⁻¹) were determined from the rate of change in the concentration in the air inside the chambers during the 1-h sampling period. This was estimated as the slope of the linear regression between concentration and time (after corrections for temperature) and from the ratio between chamber volume and soil surface area. Annual accumulate emissions for each plot (kg N₂O-N ha⁻¹, kg CH₄-C ha⁻¹) were calculated using the time-weighted average.

4.2.3 Meteorological data

Soil moisture or water content (%) was measured continuously by capacitance (ECH₂O, Decagon Devices, Inc., USA); and soil temperature (ºC) by thermocouples (TMCx-HD, Onset Computer Corporation) at soil depth of 10 cm in each plot. Data were stored at intervals of 0.5 h in the data logger (HOBO® H8, Onset Computer Corporation).

During each gas flux sampling, soil moisture content and soil temperature in the top 10 cm of soil were measured next to each gas measurement chamber using a time-domain reflectometry system (TRIMEGM, IMKO GmbH, Ettlingen, Germany) and a thermocouple sensor (Omega Engineering, Stamford, CT).

Air temperature (ºC) and daily precipitation (l/m²) in each forest stand was taken from the nearest meteorological stations belonging to the Spanish National Meteorological Service.
4.2.4 Soil samples

Three square 2 x 2 m² plots were marked out in close proximity to the chambers. In every season (winter, January 2008, March 2009; spring, June 2008; summer, August 2008; autumn, October 2008) 3 samples of the mineral soil were taken from each square plot, pooled and sieved through a 2-mm sieve (using a metal cylinder 10 cm high and 8 cm in diameter). Soil samples from each of the square plots (n=3) were analysed for soil mineral N (total N, NO₃⁻ and NH₄⁺), total dissolved organic carbon (DOC), total carbon (C) and water-filled pore space (WFPS).

The chemical and physical properties over the whole soil profile (0-100 cm) were characterized at the beginning of the experiment at each study site. The soil characteristics from the fist layer of the profile are shown in Table 4.1.

Nitrate, NH₄⁺ and DOC were extracted from the soil samples. To measure soluble nitrate an aliquot of the homogenized moist soil (2 g) was shaken for one hour with 20 ml of Milli-Q water at room temperature. The supernatant was filtered through 0.45 μm Millipore filter and the extraction solution was analyzed. To measure the ammonium, an aliquot of the homogenized moist soil (10 g) was shaken for one hour with 20 ml of 1M KCl solution at room temperature. The supernatant was filtered through 0.45 μm Millipore filter and the extraction solution was analyzed. For DOC analysis 30 ml of MILLI-Q water was added to 3 g moist soil. The tubes were then extracted at room temperature (25°C) for 30 minutes in a shaker at 30 rpm, centrifuged (20 minutes, 3500 rpm) and the supernatant was filtered through 0.45 μm pore size Millipore filter. The water sample was first acidified to a pH value of 2. In this way, the carbonates and hydrogen carbonates are transformed into carbon dioxide. The CO₂ is then removed by sparging with carrier gas. What remains is a solution of non-volatile organic carbon.

The total amounts of C and N in the soils were determined using a LECO TRUSPEC analyzer (LECO Corp., St. Joseph, USA). DOC was analyzed using Shimadzu’s TOC-V (SHIMADZU, Shimadzu Scientific Instruments, Kyoto, Japan). Nitrate was determined using a Dionex ICS-200 Ion Chromatography System (Dionex Corp., Sunnyvale, USA). Ammonium was determined using an Orion Research 901 MicroIonalyzer. The pH of the soils was potentiometrically measured in the supernatant suspension of a 1:5 soil: CaCl₂ liquid (v/v) mixture by means of an ORION 720A pH-
Chapter 4

N$_2$O and CH$_4$ fluxes in undisturbed and burned Holm oak, Scots pine and Pyrenean oak forests in central Spain

meter. Soil mineralogy (0-10 cm) was determined by X-ray diffraction (Philips X’Pert – MPD) and Infrared spectroscopy (Nicolet 6700).

WFPS was calculated by dividing volumetric water content by total porosity. Volumetric water contents were calculated by multiplying gravimetric water content by bulk dry density. Total porosity was calculated as (1-(bulk dry density/particle density)) x 100 %, using a particle density of 2.65 g. cm$^{-3}$.

4.2.5 Statistical analysis

Prior to analysis, the data were checked for homogeneity of variances and for normal distribution. Whenever necessary, data were transformed as logx or log(x+n). Differences in soil emissions, soil temperature, soil moisture, and soil chemistry data were evaluated using a three-way “repeated measures” analysis of variance (ANOVA) considering site type (ecosystem), time (month) and states (fire). Post-hoc comparisons were tested using Tukey’s HSD test.

Differences between the cumulative fluxes of the different states (B and NB) in each forest stand were carried out using the t-test.

The relationships between fluxes and soil and meteorological data were investigated using Pearson correlation. All tests were performed at the 5% probability level. All statistical evaluation of the data was performed using the Statistica 6.0 software package (StatSoft. Inc., Tulsa. USA).

4.3 RESULTS

4.3.1 Environmental conditions

The amount of precipitation recorded at each site during the study period (December 2007-September 2009) was: 923 mm in the QI stand, 1049 mm in the QP stand and 940 mm in the PS stand. Historical mean precipitations at each site, averaged over a period of 10 years (1999-2009) are shown in Table 4.1. Rainfall was concentrated in the winter, spring and autumn months, with the PS stand receiving the highest amount of rainfall (Figure 4.1,c). Both soil temperature and moisture content
varied according to the season. Maximum soil temperatures coincided with minimum soil moisture during the summer, and minimum soil temperatures were recorded in winter when soil moisture was highest. Average daily surface soil temperatures ranged from 4.0 to 43.6 °C in the QI stand, from 5.6 to 36.6 °C in the QP stand, and from 4.7 to 36.3 °C in the PS stand (Figure 4.1,c). Soil temperature was often greater ($p < 0.05$) at the burned sites than at the undisturbed site (Figure 4.1,a,b,c). Soil temperature in summer was highest in QI stands.

Average daily soil moisture ranged from 0.1 to 14 % in the QI stand, from 2 to 23 % in the QP stand, and from 2 to 43 % in the PS stand (Figure 4.1,b). Soil moisture in the surface soil was generally lower ($p < 0.05$) at the burned sites. Soil moisture presented the highest values in the QP plots followed by the PS and finally by the QI plots.

Surface soil WFPS varied seasonally in response to rainfall (data not shown). Soil WFPS ranged from 1.1 to 43.5 % in the QI stand, from 3.6 to 48 % in the QP stand and from 4.2 to 50.1 % in the PS. The highest WFPS values were recorded in October 2008, June 2008 and March 2009. The QI stand had the lowest WFPS values ($p = 0.01$).
Figure 4.1 (a) Monthly mean precipitation, soil temperature and volumetric soil water content (0-10 cm mineral soil depth) at the Quercus ilex stand in Madrid, Spain (December 2007-September 2009). SMB= Soil moisture at burned site; SMNB= Soil moisture at unburned site; STB= Soil temperature at burned site; STNB= Soil temperature at unburned site.
Figure 4.1 (b) Monthly mean precipitation, soil temperature and volumetric soil water content (0-10 cm mineral soil depth) at the Quercus pyrenaica stand in Madrid, Spain (December 2007-September 2009). SMB = Soil moisture at burned site; SMNB = Soil moisture at unburned site; STB = Soil temperature at burned site; STNB = Soil temperature at unburned site.
Figure 4.1 (c) Monthly mean precipitation, soil temperature and volumetric soil water content (0-10 cm mineral soil depth) at the *Pinus sylvestris* stand in Madrid, Spain (December 2007-September 2009). SMB= Soil moisture at burned site; SMNB= Soil moisture at unburned site; STB= Soil temperature at burned site; STNB= Soil temperature at unburned site.
4.3.2 Soil parameters

The mean total soil C values in the PS stands were higher than in the QP and QI stands (Table 4.1). Nitrogen values among the forest stands follow the same pattern as C, with PS and QP exhibiting the highest values. The C/N ratio was lower in burned plots located in QI and PS stands. The soil pH values were lower in the PS stands. The clay content of the soil was higher in PSB and QPNB. The Silt + Sand content of the soil were lower in QPNB.

The concentration of DOC in soil water varied seasonally ($p = 0.006$), being high in June 2008 and declining in August 2008 (Figure 4.2). Soil DOC was greater in PS and QP stands as well as in undisturbed stands from these forest species. The site x states (fire) interaction in the ANOVA analysis showed that in the case of soils in the QI stands ($p = 0.04$) there were no differences in the DOC values between burned and unburned plots. In the deciduous oak QP stands, DOC concentrations were lower in January 2008 and March 2009 when there were no leaves on the trees.

Soil $\text{NH}_4^+$ and $\text{NO}_3^-$ concentrations in the surface soil varied seasonally ($p < 0.05$) (Figure 4.2). Soil $\text{NO}_3^-$ concentrations were highest in June 2008 and lowest in October 2008 in PS and QP stands. In the QI stands, the highest $\text{NO}_3^-$ values were found in August 2008. Soil $\text{NH}_4^+$ concentrations were greatest in March 2009 with the exception of PS stands, which peaked in August 2008. Burned stands exhibited higher $\text{NO}_3^-$ values than the unburned stands in June 2008 and lower values in August 2008, with the exception of the QP stands in August 2008 where burned soils presented higher $\text{NO}_3^-$ values than unburned soils. Soil $\text{NH}_4^+$ concentrations ($p = 0.09$) tended to be lower in October 2008 and March 2009 in burned stands compared to control stands. The highest $\text{NO}_3^-$ and $\text{NH}_4^+$ values were found in PS and QP stands.

Soil pH was negatively correlated with total soil C ($r = -0.93$, $p = 0.0$) and total soil N ($r = -0.95$, $p = 0.0$). Soil $\text{NH}_4^+$ was positively correlated with soil moisture ($r = 0.79$, $p = 0.05$), WFPS ($r = 0.84$, $p = 0.03$), DOC ($r = 0.81$, $p = 0.04$), total soil C ($r = 0.77$, $p = 0.05$), total soil N ($r = 0.71$, $p = 0.05$), and previous day’s rainfall ($r = 0.85$, $p = 0.02$). Soil DOC was negatively correlated with soil temperature ($r = -0.89$, $p = 0.01$), and positively correlated with soil moisture ($r = 0.95$, $p = 0.003$).
Figure 4.2 Soil nitrate (NO$_3^-$)(a), ammonium (NH$_4^+$)(b), dissolved organic C (DOC) (c), over time at the three forest stands at Madrid, Spain (2008-2009). Values represent means of three replicates (µg/g). QIB=Quercus ilex burned; QINB= Quercus ilex unburned; PSB= Pinus sylvestris burned; PSNB= Pinus sylvestris unburned; QPB= Quercus pyrenaica burned; QPNB= Quercus pyrenaica unburned.
4.3.3 N\textsubscript{2}O fluxes

Nitrous oxide fluxes ranged from -11.43 to 8.34 µg N\textsubscript{2}O-N m\textsuperscript{-2}·h\textsuperscript{-1}·in QI stands, -7.74 to 13.52 µg N\textsubscript{2}O-N m\textsuperscript{-2}·h\textsuperscript{-1}·in QP stands and -28.17 to 21.89 µg N\textsubscript{2}O-N m\textsuperscript{-2}·h\textsuperscript{-1}·in PS stands (Figure 4.3). A significant time effect on N\textsubscript{2}O fluxes was observed, whereas the effects of site and fire were not statistically significant (Table 4.2). The emissions of N\textsubscript{2}O from the soils of QI stands were the lowest. In unburned stands, minimum values were recorded in July 2008, September 2008, October 2008, April 2009 and July 2009. The maximum values were found in February 2008, April 2008, June 2008 and June 2009. In burned stands, the minimum values were recorded in June 2008, July 2008 and August 2008.

In QP stands, the time x site x fire interaction (\(p = 0.01\)) (Figure 4.3 b, Table 4.2) revealed that fire reduced N\textsubscript{2}O emissions in February 2008, June 2008, May 2009 and June 2009.

In PS stands (Figure 4.3 c), fire reduced N\textsubscript{2}O emissions in February 2008, April 2008 and June 2009. However we observed an increase in N\textsubscript{2}O emissions from burned soils in May 2008, July 2008 and October 2008.

Table 4.2 Results of three-way repeated measures analysis of variance on N\textsubscript{2}O flux from the three studied sites.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Sum of squares</th>
<th>Degr. of Freedom</th>
<th>Mean Ssquare</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1894.11</td>
<td>1</td>
<td>1894.11</td>
<td>3.239252</td>
<td>0.00</td>
</tr>
<tr>
<td>Forest species (S)</td>
<td>24.69</td>
<td>2</td>
<td>12.34</td>
<td>0.21108</td>
<td>0.81</td>
</tr>
<tr>
<td>Fire (F)</td>
<td>1.98</td>
<td>1</td>
<td>1.98</td>
<td>0.03389</td>
<td>0.85</td>
</tr>
<tr>
<td>S x F</td>
<td>277.83</td>
<td>2</td>
<td>138.91</td>
<td>237.565</td>
<td>0.13</td>
</tr>
<tr>
<td>Error</td>
<td>760.16</td>
<td>13</td>
<td>58.47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time (Month. M)</td>
<td>2957.30</td>
<td>17</td>
<td>173.95</td>
<td>270.881</td>
<td>0.00</td>
</tr>
<tr>
<td>M x S</td>
<td>1438.22</td>
<td>34</td>
<td>42.30</td>
<td>0.65869</td>
<td>0.92</td>
</tr>
<tr>
<td>M x F</td>
<td>3044.42</td>
<td>17</td>
<td>179.08</td>
<td>278.861</td>
<td>0.00</td>
</tr>
<tr>
<td>M x F x S</td>
<td>3761.83</td>
<td>34</td>
<td>110.64</td>
<td>172.287</td>
<td>0.01</td>
</tr>
<tr>
<td>Error</td>
<td>14192.55</td>
<td>221</td>
<td>64.22</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Bold numbers indicate significant differences at \(p < 0.05\) level.
During early summer (June 2008), elevated N$_2$O emissions coincided with elevated soil moisture content, surface soil temperatures (Figure 4.1), previous day’s rainfall, and soil mineral N concentrations (Figure 4.2). In October 2008, lower NO$_3^-$ coincided with lower NH$_4^+$ and lower N$_2$O fluxes (Figure 4.2 and 3). N$_2$O sinks were observed during dry months.

In the QI burned plots (Table 4.3), mean N$_2$O flux was best correlated with soil temperature ($r = -0.38$). In non-burned plots, N$_2$O flux was correlated with DOC ($r = -0.71$) and soil moisture ($r = 0.46$). In burned QP soils, the N$_2$O flux was correlated with DOC ($r = -0.89$) and previous day’s rainfall ($r = -0.62$). N$_2$O fluxes from unburned soils were correlated with previous day’s rainfall ($r = 0.56$ and $r = 0.44$). In PS burned soils, mean N$_2$O flux was best correlated with the previous day’s rainfall ($r = 0.53$). In non-burned PS plots, the best fitting was found with DOC ($r = -0.73$).

The N$_2$O emissions from all ecosystems (Table 4.4), were positively correlated with previous day’s rainfall ($r = 0.23$, $r = 0.32$) and soil moisture ($r = 0.19$), and negatively correlated with soil temperature ($r = -0.20$).

Mean annual N$_2$O emissions were related with mean annual values of soil parameters (Figure 4.4). Mean annual N$_2$O emissions were negatively related with soil pH ($r = -0.30$; ns), and positively correlated with soil DOC ($r = 0.79$, $p = 0.06$), soil NH$_4^+$ ($r = 0.90$, $p = 0.01$), WFPS ($r = 0.67$, $p = 0.1$). Log-transformed N$_2$O fluxes were negatively correlated with the C/N-ratio ($r = -0.84$; $p = 0.03$) and positively correlated with soil clay content ($r = 0.83$; $p = 0.03$).

Mean N$_2$O emissions averaged over the entire experimental period were identical in all the plots (Table 4.5). The N$_2$O cumulative fluxes (kg N$_2$O-N ha$^{-1}$·year$^{-1}$) were significantly ($p <0.05$) higher in unburned plots (0.54 kg N$_2$O-N ha$^{-1}$) than in burned plots (0.34 kg N$_2$O-N ha$^{-1}$) in the QP stands. In PS and QI stand no significant differences were found between burned plots compared to non-burned plots (Table 4.5).
Figure 4.3 Nitrous oxide flux ($\text{N}_2\text{O}-\text{N} \ \mu\text{g m}^{-2}\text{h}^{-1}$) over time at a *Quercus ilex* stand (a), a *Quercus pyrenaica* (b) and a *Pinus sylvestris* stand (c) in Madrid, Spain (December 2007-September 2009). Gas flux values represent means (± SE) of four replicates. B=Burned site; NB=Unburned site.
Table 4.3 Pearson correlation coefficients between real-time fluxes and soil parameters for the period 2007-2009 in each of the forest stands.

<table>
<thead>
<tr>
<th></th>
<th>Quercus ilex</th>
<th>Quercus pyrenaica</th>
<th>Pinus sylvestris</th>
<th>Quercus ilex</th>
<th>Quercus pyrenaica</th>
<th>Pinus sylvestris</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non burned</td>
<td>Burned</td>
<td>Non burned</td>
<td>Burned</td>
<td>Non burned</td>
<td>Burned</td>
</tr>
<tr>
<td>Soil temperature</td>
<td>-0.20</td>
<td>-0.38*</td>
<td>-0.25</td>
<td>0.00</td>
<td>0.00</td>
<td>-0.19</td>
</tr>
<tr>
<td>Soil moisture</td>
<td>0.46*</td>
<td>0.06</td>
<td>0.37*</td>
<td>-0.10</td>
<td>0.13</td>
<td>0.08</td>
</tr>
<tr>
<td>WFPS</td>
<td>0.26</td>
<td>0.01</td>
<td>0.10</td>
<td>-0.20</td>
<td>-0.12</td>
<td>0.40</td>
</tr>
<tr>
<td>P1DB</td>
<td>0.04</td>
<td>0.22</td>
<td><strong>0.56</strong></td>
<td><strong>-0.62</strong></td>
<td>0.23</td>
<td>0.42*</td>
</tr>
<tr>
<td>DOC</td>
<td><strong>-0.71</strong></td>
<td><strong>-0.07</strong></td>
<td><strong>0.66</strong></td>
<td><strong>-0.89</strong></td>
<td><strong>-0.73</strong></td>
<td>0.44</td>
</tr>
<tr>
<td>NO3-</td>
<td>-0.22</td>
<td>0.13</td>
<td>0.84*</td>
<td>-0.93*</td>
<td>0.57</td>
<td>0.81*</td>
</tr>
<tr>
<td>NH4+</td>
<td>-0.12</td>
<td>-0.18</td>
<td>0.02</td>
<td>-0.98*</td>
<td>0.80</td>
<td>-0.86</td>
</tr>
</tbody>
</table>

1) At 10 cm.
2) WFPS= Water-filled pore space.
3) P1DB= Precipitation one day before gas sample measurements.
4) MP4DB= Mean precipitation of four days before gas sample measurements.

*Asterisk indicates tendencies (*p = 0.05-0.1)
Bolded values refer to significant differences (p<0.05)
Figure 4.4 Relationship between average annual N₂O fluxes (N₂O-N µg m⁻² h⁻¹) versus average mean soil parameters. QIB=Quercus ilex burned; QINB= Quercus ilex unburned; PSB=Pinus sylvestris burned; PSNB= Pinus sylvestris unburned; QPB= Quercus pyrenaica burned; QPNB= Quercus pyrenaica unburned.
Table 4.4 Pearson correlation coefficients between real-time fluxes and soil parameters for the period 2007-2009 in all forest stands.

<table>
<thead>
<tr>
<th></th>
<th>N$_2$O</th>
<th>logN$_2$O</th>
<th>CH$_4$</th>
<th>$^1$Soil temperature</th>
<th>$^1$Soil moisture</th>
<th>$^2$WFPS</th>
<th>$^3$P1DB</th>
<th>$^4$MP4DB</th>
<th>NO$_3^-$</th>
<th>NH$_4^+$</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>N$_2$O</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>logN$_2$O</td>
<td>-0.02</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH$_4$</td>
<td>-0.16*</td>
<td>-0.07</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^1$Soil temperature</td>
<td>-0.20</td>
<td>-0.18*</td>
<td>-0.09</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^1$Soil moisture</td>
<td>0.19*</td>
<td>0.21</td>
<td>-0.05</td>
<td>-0.66</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^2$WFPS</td>
<td>0.05</td>
<td>0.19*</td>
<td>-0.04</td>
<td>-0.63</td>
<td>0.85</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^3$P1DB</td>
<td>0.23</td>
<td>0.00</td>
<td>-0.17*</td>
<td>-0.05</td>
<td>0.19*</td>
<td>0.26</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^4$MP4DB</td>
<td>0.32</td>
<td>0.02</td>
<td>-0.10</td>
<td>-0.11</td>
<td>0.22</td>
<td>0.26</td>
<td>0.72</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>-0.04</td>
<td>-0.27</td>
<td>0.01</td>
<td>0.58</td>
<td>-0.55</td>
<td>-0.59</td>
<td>0.00</td>
<td>-0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NH$_4^+$</td>
<td>-0.01</td>
<td>-0.31</td>
<td>-0.07</td>
<td>-0.03</td>
<td>-0.01</td>
<td>-0.07</td>
<td>--</td>
<td>0.63</td>
<td>-0.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>-0.17</td>
<td>0.11</td>
<td>0.25*</td>
<td>0.22</td>
<td>-0.18</td>
<td>0.11</td>
<td>-0.33*</td>
<td>-0.24*</td>
<td>0.22</td>
<td>-0.49</td>
<td></td>
</tr>
</tbody>
</table>

1) At 10 cm.
2) WFPS = Water-filled pore space.
3) P1DB = Precipitation one day before gas sample measurements.
4) MP4DB = Mean precipitation of four days before gas sample measurements.

*Asterisk indicates tendencies (*p = 0.05-0.1)
Bolded values refer to significant differences (p <0.05)
Table 4.5 Estimated mean and cumulative emission of N$_2$O and CH$_4$ across forest sites in Madrid during the experiment.

<table>
<thead>
<tr>
<th>Species</th>
<th>N$_2$O N mean (µg m$^{-2}$ h$^{-1}$)</th>
<th>N$_2$O N (Kg ha$^{-1}$) cumulative</th>
<th>CH$_4$ C mean (µg m$^{-2}$ h$^{-1}$)</th>
<th>CH$_4$ C cumulative (Kg h$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non burned</td>
<td>Burned</td>
<td>Non burned</td>
<td>Burned</td>
</tr>
<tr>
<td><em>Quercus ilex</em></td>
<td>2.3</td>
<td>2.3</td>
<td>0.37</td>
<td>0.35</td>
</tr>
<tr>
<td><em>Quercus pyrenaica</em></td>
<td>4.1</td>
<td>2.2</td>
<td>0.54</td>
<td>0.34</td>
</tr>
<tr>
<td><em>Pinus sylvestris</em></td>
<td>3.2</td>
<td>3.3</td>
<td>0.43</td>
<td>0.36</td>
</tr>
</tbody>
</table>
4.3.4 CH$_4$ fluxes

Fluxes of CH$_4$ ranged from -8.12 to 4.11 µg CH$_4$-C m$^{-2}$·h$^{-1}$·in QI stands, -7.74 to 3.0 µg CH$_4$-C m$^{-2}$·h$^{-1}$·in QP stands, and -24.46 to 6.07 µg CH$_4$-C m$^{-2}$·h$^{-1}$·in PS stands (Figure 4.5). Differences in CH$_4$ fluxes among sites were significant ($p <0.05$) and PS and QP stands were found to absorb more CH$_4$ than QI stands.

Soil CH$_4$ flux varied over the measurement period (Table 4.6). CH$_4$ uptake was highest in dry months (June, July) and February 2008. A net source of CH$_4$ was found during wet months. Differences between burned and unburned stands in relation to CH$_4$ fluxes were identified in soils under QI. Fire increased CH$_4$ uptake in June 2008, May 2009 and July 2009 (Figure 4.5a). In burned QP stands (Figure 4.5b), CH$_4$ uptake was higher than at the unburned stand during June 2008 and July 2008 (not significant). However, in June 2009, July 2009 and September 2009, the unburned stand showed more CH$_4$ consumption.

In PS stands, CH$_4$ uptake was higher in undisturbed soil than in burned soil in February 2008 and June 2009 (Figure 4.5c). Mean CH$_4$ fluxes (Table 4.3) were correlated with WFPS ($r = 0.45$) in unburned QI soils. In burned QP soils, mean CH$_4$ flux was correlated with previous day’s rainfall ($r = -0.61$, $r = -0.46$) and NO$^3$- ($r = -0.96$). CH$_4$ fluxes were correlated with NH$_4^+$ ($r = -0.99$) in unburned soils. No significant correlation was found between mean CH$_4$ flux and the studied parameters in PS stands. No significant correlations were found between mean CH$_4$ flux from all ecosystems (Table 4.4) and the studied soil parameters.

Mean annual CH$_4$ fluxes were related with mean annual values of soil parameters (Figure 4.6). Mean annual CH$_4$ flux were positively related with soil clay content ($r = 0.62$, $p = 0.1$), and negatively correlated with soil DOC ($r = -0.61$, $p = 0.1$), soil NH$_4^+$ ($r = -0.30$, ns) and soil moisture ($r = -0.61$, $p = 0.1$).

The mean fluxes (µg CH$_4$-C m$^{-2}$·h$^{-1}$) averaged over the experimental period were not significantly different (Table 4.5). Fire increased CH$_4$ uptake cumulative fluxes (kg CH$_4$-C ha$^{-1}$) for QI ($B = -0.31$ kg CH$_4$-C ha$^{-1}$; NB = -0.09 kg CH$_4$-C ha$^{-1}$). In PS plots, fire reduced CH$_4$ uptake ($B = -0.20$ kg CH$_4$-C ha$^{-1}$; NB = -0.52 kg CH$_4$-C ha$^{-1}$). In QP no
clear differences were found between burned and un-burned plots (B= 0.31 kg CH$_4$-C ha$^{-1}$; NB = -0.35kg CH$_4$-C ha$^{-1}$).

**Table 4.6** Results of three-way repeated measures analysis of variance on CH$_4$ flux from the three studied sites.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Sum of squares</th>
<th>Degr. of Freedom</th>
<th>Mean square</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>4144.63</td>
<td>1</td>
<td>4144.62</td>
<td>1.048556</td>
<td>0.00</td>
</tr>
<tr>
<td>Forest species (S)</td>
<td>2985.13</td>
<td>2</td>
<td>1492.56</td>
<td>377.606</td>
<td>0.05</td>
</tr>
<tr>
<td>Fire (F)</td>
<td>832.98</td>
<td>1</td>
<td>832.98</td>
<td>210.737</td>
<td>0.17</td>
</tr>
<tr>
<td>S x F</td>
<td>2102.32</td>
<td>2</td>
<td>1051.16</td>
<td>265.935</td>
<td>0.11</td>
</tr>
<tr>
<td>Error</td>
<td>4743.24</td>
<td>12</td>
<td>395.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time (Month. M)</td>
<td>20179.60</td>
<td>17</td>
<td>1187.03</td>
<td>289.059</td>
<td>0.00</td>
</tr>
<tr>
<td>M x S</td>
<td>34538.99</td>
<td>34</td>
<td>1015.85</td>
<td>247.374</td>
<td>0.00</td>
</tr>
<tr>
<td>M x F</td>
<td>19202.66</td>
<td>17</td>
<td>1129.56</td>
<td>275.065</td>
<td>0.00</td>
</tr>
<tr>
<td>M x F x S</td>
<td>33643.98</td>
<td>34</td>
<td>989.52</td>
<td>240.964</td>
<td>0.00</td>
</tr>
<tr>
<td>Error</td>
<td>83773.59</td>
<td>204</td>
<td>410.65</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Bold numbers indicate significant differences at $p < 0.05$ level.
Figure 4.5 Methane flux (CH$_4$-C µg m$^{-2}$h$^{-1}$) over time at a *Quercus ilex* stand (a), a *Quercus pyrenaica* (b) and a *Pinus sylvestris* stand (c) in Madrid, Spain (December 2007-September 2009). Gas flux values represent means (± SE) of four replicates. B=Burned site; NB=Unburned site.
Figure 4.6 Relationship between average annual CH$_4$ fluxes (CH$_4$-C µg m$^{-2}$h$^{-1}$) versus average annual soil parameters. QIB= *Quercus ilex* burned; QINB= *Quercus ilex* unburned; PSB= *Pinus sylvestris* burned; PSNB= *Pinus sylvestris* unburned; QPB= *Quercus pyrenaica* burned; QPNB= *Quercus pyrenaica* unburned.
4.4 DISCUSSION

4.4.1 Nitrous oxide fluxes

4.4.1.1 Seasonal variation

Our soils are weak N$_2$O emitters and temporary sinks for atmospheric N$_2$O fluxes. The low N$_2$O fluxes presented in this work were also reported in previous studies concerning Mediterranean forest soils (Castaldi et al. 2006; Rosenkranz et al. 2006; Pilegaard et al. 2006). N$_2$O fluxes were temporally variable. Seasonal differences indicated that in wet months N$_2$O fluxes were high and in dry months N$_2$O fluxes declined. The parameters that best explain the seasonal variation in N$_2$O flux in all the forest ecosystems are soil temperature, soil moisture and previous day’s rainfall. Production of N$_2$O in soils is primarily driven by microbial processes such nitrification and denitrification (Firestone and Davidson 1989), therefore soil temperature is a key variable affecting the emission rates of both gases. Emissions of N$_2$O increase with increasing soil temperatures (Skiba et al. 1998) due to the fact that rates of enzymatic processes generally increase with temperature as long as other factors (e.g. substrate or moisture) are not limiting. Soil water acts as a transport medium for NO$_3^-$ and NH$_4^+$ and influences the rate of O$_2$ supply and thereby controls whether aerobic processes such nitrification or anaerobic processes such as denitrification dominate within the soil. In our case the relationship between hourly N$_2$O fluxes in all ecosystems are negatively correlated with soil temperature and positively correlated with soil moisture. This pattern is typical in seasonally dry ecosystems, revealing the fact that soil water content is a limiting factor in N$_2$O fluxes. Most of the temporal variation observed within a given site was explained by precipitation.

In general, dry and warm summer soils can be affected by drought, which favours soil aeration and is thus thought to make conditions unfavourable for N$_2$O production by denitrification (Castaldi 2000) or nitrifier denitrification. Furthermore, drought limits the overall activity of soil microorganisms, reducing the amount of N cycled in the ecosystem.
Therefore, lack of available N together with the low water content could contribute
to a limitation of the N\(_2\)O production in the dry season. In Mediterranean ecosystems,
total N tends to be low (Rovira and Vallejo 1997); and mineralization rates are generally
low as a consequence of allelopathic compounds leached from plants and the quality of
the sclerophyllous leaf, typical of such ecosystems (Gallardo and Merino 1992). This
might result in low availability of mineral N in the soil, which could limit the rate of
both nitrification and denitrification.

During the dry period many microorganisms and plants die, thereby increasing the
concentrations of labile N and C available in the soil. The first rainfall on dry soil
produces pulses of N\(_2\)O (Davidson 1993). The initial fluxes are thought to be caused by
the accumulation of inorganic N in dry soils and the reactivation of water-stressed
bacteria upon wetting, which then metabolize the available inorganic N (Davidson
1993). The significant increase in N\(_2\)O fluxes in wet months observed in our study may
be explained at least in part by the positive correlation found between N\(_2\)O fluxes and
the previous day’s rainfall.

The significant increase in N\(_2\)O emissions in the wet months could be due to a
combined effect of increased soil water content and mineralization. N\(_2\)O production
improves as soil water content increases and aeration becomes restricted, with optimum
values around 60 %WFPS (Davidson 1991; Castaldi 2000). Maximum N\(_2\)O emissions
were measured between 80 % and 95 % WFPS in a laboratory experiment with soil
cores from different forests in Europe (Schindlbacher et al. 2004). The correlation
between N\(_2\)O and WFPS is a well-known relationship associated with both nitrification
and denitrification (Davidson 1992). In the case of this field data, WFPS was not a
significant parameter affecting N\(_2\)O emission; a logarithmic transformation of N\(_2\)O
emission improved the significance of the correlation, and N\(_2\)O fluxes tend to increase
with monthly mean values of WFPS although the relationship stated is not significant.
However log-transformed data correlated with soil water content. Smith et al. (1998)
also found that emission increases with increasing water content. Castaldi et al. (2004)
found no significant correlation between N\(_2\)O fluxes and WFPS in savanna ecosystems.
The coarse texture and the low water retention capacity of these soils limit the
likelihood of anaerobic microsites developing in which N\(_2\)O production could take place
(Firestone and Davidson 1989; Smith 1990). In our study sites, WFPS was always
below 60%; the value at which O\textsubscript{2} diffusion is sufficiently reduced to allow for a sharp increase in N\textsubscript{2}O production (Davidson 1991).

Soil emissions of N\textsubscript{2}O are often positively correlated with inorganic N availability (Skiba et al. 2009). However, no clear relationship could be established between N\textsubscript{2}O fluxes and soil NO\textsubscript{3}\textsuperscript{−} in the present study. The possible existence of nitrification processes in these well-drained soils may explain the lack of correlation between N\textsubscript{2}O fluxes and NO\textsubscript{3}\textsuperscript{−} content since the nitrification process (NO\textsubscript{3}\textsuperscript{−} production) is opposed to denitrification process.

In seasonally dry ecosystems, the nitrification rate is controlled by low water contents during part of the year (Bate, 1981), and by allelopathic compounds (Gallardo and Merino 1992). In this type of ecosystems, soil C and N cycling may be also affected by the timing of plant biomass turnover relative to seasonal patterns of temperature and soil moisture. As the summer dry-season develops, perennial plants reduce growth rates and increase litterfall. The annual grasses were senescence with the onset of the summer. In these soils, soil availability would be expected to increase during the early-summer due to the pulse of high C/N leaf and root litter inputs. The highest DOC values in our study were found in June 2008 (early summer) as was expected. In warm and dry areas, the highest concentrations of inorganic N were found in winter and spring or early-summer, when moisture favours mineralization, and the lowest concentrations were found during the summer, after the period of maximum plant growth and uptake. In our case, the highest soil NO\textsubscript{3}\textsuperscript{−} content was observed in June 2008 (late spring-early summer) in all forest types, and also in March 2009 (winter) in the case of PS stands. This increment in NO\textsubscript{3}\textsuperscript{−} might explain the increases observed in N\textsubscript{2}O fluxes during these months.

We also found a peak in NO\textsubscript{3}\textsuperscript{−} and NH\textsubscript{4}\textsuperscript{+} in PS and QINB plots, in August 2008. Surface soils of semiarid ecosystems tend to accumulate NO\textsubscript{3}\textsuperscript{−} during the summer (Davidson 1990). This NO\textsubscript{3}\textsuperscript{−} accumulation suggests that microbial N immobilization is limited by C availability, which is inconsistent with the expectation that plant detrital inputs during the summer stimulate soil C availability. One explanation for this inconsistency is that by the time annual grasses senesce in late spring (May-June), the soil may already be too dry for microbes to colonize the newly released detritus.
The highest N₂O emissions were observed in February 2008, June 2008 and June 2009, in QPNB and PSNB plots. There are various parameters that might have contributed to the N₂O peak. The increase in soil moisture after rainfall, may have caused an increase in microbial activity. On the other hand, the level of N-deposition and the NO₃⁻ in the soil might have played an important role in the sudden release of N₂O (Davidson et al. 2000). The highest amounts of NO₃⁻ and NH₄⁺ via throughfall reached the forest floor during these months (data not shown). A correlation was identified between N₂O flux and throughfall NO₃⁻ deposition. This N₂O peak coincided with higher concentrations of available inorganic N in the soil. Clear relationships between input of nitrogen to the forest and N₂O emissions have been reported previously (Butterbach-Bahl et al. 2002; Kitzler et al. 2006).

Net consumption of N₂O has been reported in forests (Fenn et al. 1996; Rosenkranz et al. 2006; Kitzler et al. 2006; Goldberg and Gebauer 2009). Net negative N₂O fluxes reported in the literature vary widely, from −0.0014 to −484 µg N₂O-N m⁻²·h⁻¹ (Chapuis-Lardy et al. 2007). The net negative N₂O fluxes measured in our study fall within this range. Most N₂O uptake has been reported to occur under conditions of low mineral nitrogen availability and high soil moisture (Chapuis-Lardy et al. 2007), during denitrifying processes, which mainly occur under prevailing anoxic conditions. These conditions favour microbial N₂O consumption, because NO₃⁻ is preferred as an electron acceptor over N₂O, and high soil moisture may support the reduction of N₂O to N₂ due to anaerobic conditions. One difference between the two periods in which a sink effect was observed in our study was the WFPS, which was lower in summer (July 2008, August 2008) than in spring and fall (June 2008, October 2008). The negative fluxes observed in October 2008 in QPNB, QIB, QINB and PSNB plots may be explained by the low soil NO₃⁻ content observed and the high soil moisture. In summer, the sink effect observed may have been produced by a different pathway.

There are few reports in the literature that point to a soil N₂O sink function under dry conditions (Rosenkranz et al. 2006, Goldberg and Gebauer 2009) but underlying reasons have not yet been identified. Rosenkranz et al. (2006) linked negative fluxes in Mediterranean forest soils to very low N availability and high soil C content. They considered aerobic denitrification by heterotrophic nitrifiers as a possible pathway. Yamulki et al. (1995) linked low emission rates, as well as net negative fluxes, to high
temperatures and low soil moisture content. An increase in soil temperature positively influences microbiological activity and gas diffusion, while it negatively affects the solubility of \( \text{N}_2\text{O} \). This parameter combination coincides with our site properties and may explain our observation of net atmospheric \( \text{N}_2\text{O} \) uptake. In July 2008 and August 2008, the \( \text{N}_2\text{O} \) sink may be related to high DOC content and high soil temperature, combined with low levels of soil moisture and nitrogen.

### 4.4.1.2 Effects of forest type

The differences identified between the \( \text{N}_2\text{O} \) emissions from the different forest soils studied can most likely be explained by differences in soil properties and soil moisture. Differences among species were also identified as regards the quantity of C and N on the forest floor, which can be explained by interspecific differences in litter production and quality (Finzi et al. 1998, Pilegaard et al. 2006).

Soil nitrogen and C concentrations, as well as soil moisture were higher in PS and QP stands. DOC, total C and \( \text{NH}_4^+ \) were positively correlated with soil moisture and negatively correlated with soil temperature. Total mean \( \text{N}_2\text{O} \) emissions were positively correlated with soil moisture, WFPS, DOC and \( \text{NH}_4^+ \), and negatively correlated with soil temperature.

Among the different sites, the QI stands showed the lowest \( \text{N}_2\text{O} \) fluxes. Low soil \( \text{N}_2\text{O} \) fluxes have previously been documented in similar type ecosystems (Castaldi et al. 2006). This type of ecosystem is characterized by acidic, porous soils with rapid drainage and poor water retention capacity. Furthermore, due to the limited amount of substrate (C and N) that can be used by microbes, \( \text{N}_2\text{O} \) emissions decrease (Firestone and Davidson 1989). The precipitation and soil moisture levels in the stands of this forest were lower than in the other studied stands, while soil temperatures were higher. Castaldi et al. (2006) reviewed published data regarding \( \text{N}_2\text{O} \) fluxes from soils of savannas and seasonally-dry ecosystems and concluded that \( \text{N}_2\text{O} \) fluxes were strongly limited by soil characteristics.

Under QP and PS, the higher organic matter input on the forest floor increases the nutrient content and improves soil structure. Moreover, the shading of soil surfaces, due to the higher vegetation cover, reduces the tendency of the surface to dry. This could
explain the differences in N$_2$O fluxes from QI stands. Another reason for the difference in N-oxide emission between QI stands and the others forest stands, is the differences in N-deposition. In PS stands, N deposition in throughfall was 12 kg ha$^{-1}$ yr$^{-1}$ while in QI stands it was 2 kg ha$^{-1}$ yr$^{-1}$.

Shifts in the dominant vegetation type may alter soil C availability through differences in the quantity and quality of plant detritus inputs (Carrera et al. 2003), particularly in semiarid ecosystems where soil organic C and N pools are small. Increased C inputs, through differences in foliage and root turnover among different vegetation types, may increase microbial N demand and reduce N available for plant uptake. By contrast, increased quality of plant detritus inputs (i.e. lower litter C/N ratios) may result in greater N availability due to decreased microbial demand for inorganic N per unit organic C consumed (Carrera et al. 2003). This study revealed a negative correlation between log-N$_2$O total mean emission and the C/N ratio. This is in accordance with the findings of Ambus et al. (2006) and Pilegaard et al. (2006). A similar negative relationship has been identified between net nitrification and C/N ratio in forests throughout Europe (Persson et al. 2000). Klemedtsson et al. (2005) found that where C/N ratios are low (below 15-20), other parameters such as climate and pH increase in importance as predictors for N$_2$O emission.

In PS stands where N$_2$O fluxes were higher, the pH values were low. There was a (non significant) negative correlation between N$_2$O flux and pH. The negative correlation between N$_2$O and soil pH is a well-known relationship (Weslien et al. 2009). Di-nitrogen oxide reductase is inhibited by acidic pH, thus enhancing N$_2$O emissions (Firestone and Davidson 1989; Skiba et al. 1993). Similar results have been found in Dutch forest soils (Martihainen and Boer 1993) where acidic conditions favour N$_2$O production from both autotrophic and heterotrophic nitrifiers. In contrast, it has been demonstrated that pH values below 5 decrease N$_2$O production (Granli and Bøeckman 1994).

There was a negative correlation (non significant) between pH and NH$_4^+$ in soil, which could be due to the acidifying effect of NH$_4^+$ in soil. The sites with high N$_2$O emission also consistently exhibited low pH (PS). Soil pH was negatively correlated with total soil C ($r = -0.93$, $p = 0.0$) and total soil N ($r = -0.95$, $p = 0.0$), which may be the result of differences in humic acid concentration and or types.
The observed variations in N₂O fluxes were positively related to variations in clay content. In QPNB plots, the N₂O emissions might be related to higher clay content and higher mean pH values, which provide favourable conditions for N₂O production by denitrification. N₂O production by denitrification and nitrification improves as soil water content increases and aeration becomes restricted, with optimum values reached at around 60 %WFPS (Davidson 1991; Castaldi 2000). In this case, with WFPS values at around 30 %, the higher proportion of fine soil particles improves the capacity of the soil to retain water, as well as the amount of soil micropores (as opposed to macropores) where O₂ limitation might occur more easily, thus favouring N₂O production.

4.4.1.3 Effects of fire

As stated in our first hypothesis the effect of fire on soil N₂O flux depends on forest site, soil characteristics and seasonal climatic conditions. The N₂O cumulative fluxes were higher in unburned plots (0.54 kg N₂O-N ha⁻¹) than in burned plots (0.34 kg N₂O-N ha⁻¹) in the QP stands. In the QI and PS stands no significant differences were found between burned compared to non-burned plots. When N₂O cumulative fluxes were compared between burned plots in the different forest stands, we found that PS had the highest values.

In QP, the decrease in cumulative fluxes in burned plots compared to non-burned may be explained by the different characteristics of each stand. In QPNB, vegetation cover in conjunction with the presence of higher clay content in the soil protects the soil from desiccation. The higher soil moisture content in this plot over the entire experimental period could explain the higher N₂O fluxes.

In QI, the absence of differences in N₂O cumulative fluxes might be explained by the time elapsed since the wildfire. In this forest, the wildfire was the most recent of those studied (August 2004); four years prior to the experimental period. The temporal transient increase in N content following the fire may explain the absence of differences in N₂O fluxes between burned and un-burned plots. Fire usually causes an immediate increase in available N pools. By way of explanation for the increase in mineral-N, most authors point to the rapid decomposition of organic-N compounds produced by the combustion of organic matter (Certini 2005).
However in the PS plots, where the fire occurred at an earlier date (2001), we expected a decrease in soil N content and N$_2$O fluxes since this effect is often observed in the long-term. The absence of differences in N$_2$O fluxes between burned and unburned plots in this case might be explained by the invasion of the burned plot by the N-fixing leguminous plant *Adenocarpus hispanicus*. This plant is a pioneer species which is particularly invasive following fire activity since it is especially adapted to fire. *Adenocarpus hispanicus* seeds which have lain dormant in forest litter for many years are activated by fire and winter weather, resulting in prolific germination following a wildfire. Nitrogen fixing plants can increase soil N content as well as cycling rates (Rothe et al. 2002). Consequently in PSB stands, lost N may be replaced by inputs from N fixers following a fire. In other forest and woodland soils, the presence of N fixing vegetation has been shown to increase soil N$_2$O production (Angoa-Perez et al. 2004, Benedikt et al. 2009). However, significant differences in total N were not detected between burned and unburned plots in our forest stands.

We observed different patterns in N$_2$O fluxes between burned and unburned stands over the course of the year. We expected that on the spring sampling dates (June 2008), the differences in inorganic N and N$_2$O levels between burned and unburned plots would be greater than on the winter sampling dates due to higher soil temperature and moisture levels, which would promote higher microbial activity (Gallardo and Schlesinger 1994). However, this pattern was only observed in QP stands.

In burned soils, N$_2$O fluxes were low in wet and hot months (spring). A possible explanation for this is that the water retention capacity of the soil as well as its ability to resist evaporation might be negatively affected when this ecosystem is disturbed by wildfire, further slowing the processes of nitrification and denitrification. A significant decrease in net mineralization rates in comparison to unburned plots was still observed in a Pinus forest in Spain 17 years after a fire (Durán et al. 2009), indicating slow recovery of N turnover in these forests. Another reason for the low N$_2$O fluxes could be the competition between microbes and plants for N during the vegetative growth period.

However this argument can not be used to explain the increase in N$_2$O flux in burned soils under PS (May 2008, June 2008, October 2008) and QI (May 2008). In these months, higher soil temperatures and moistures measured in these plots might increase the rate of decomposition during this period. Higher temperatures were
recorded in burned plots, probably as a consequence of the reduced plant canopy which leads to higher levels of irradiance reaching the soil surface. In PS stands, leguminous plants might have increased the inorganic nitrogen content in the soil during this period. Moreover, the PS burned stands exhibited higher NO$_3^-$ values than the unburned stands in June 2008.

In August 2008, the N$_2$O flux in PSB plots was lower than in unburned plots. Soils NO$_3^-$ values in PSB were also lower. This may be explained again by the presence of *Adenocarpus hispanicus*. Leguminous plants require a higher concentration of N than plants of other families, which is not dependent on the assimilation methods of individual plants. Thus, when atmospheric-N fixation is not possible, such as during decreased water availability in summer, leguminous plants must increase N uptake from the soil (Peoples and Craswell 1992). Water stress affects the N-fixing process more significantly than N assimilation and uptake. Consequently, the lower water content may have diminished soil mineralization and N-fixation rates more than N uptake, resulting in a decrease in soil N. In winter (March 2009), NO$_3^-$ is higher in PSB plots. A higher concentration of N-rich organic matter from litterfall beneath the legume canopy may explain the higher NO$_3^-$ in the soil (Jones et al. 2005). However, there were no differences in soil N$_2$O flux, probably due to the lower soil moisture content in the burned stand during this month.

### 4.4.2 Methane fluxes

#### 4.4.2.1 Seasonal variation

In general, our ecosystems appeared to act as methane sinks. However during wet months, they could also be a net source of CH$_4$. The magnitude of the observed net CH$_4$ uptake is in the range of that reported for Mediterranean and temperate nitrogen limited forest ecosystems (Merino et al. 2004; Rosenkranz et al. 2006; Castaldi and Fierro 2005; Castaldi et al. 2006). Well aerated soils are known to be significant sinks for atmospheric CH$_4$. Castaldi and Fierro (2005) also observed higher CH$_4$ oxidation rates during dry, warm periods in a *Q. ilex* stand in Italy.
The observed seasonal trend was influenced by soil water availability. However, no significant correlations were found between soil water content and CH$_4$. The observed seasonal trend is consistent with other studies (Butterbach-Bahl and Papen 2002; Castaldi and Fierro 2005, Castaldi et al. 2006; Sullivan et al. 2008) in which CH$_4$ oxidation rates increased during the drier season. During the wet season, when soils were wetter, net CH$_4$ emissions have been reported (Butterbach-Bahl and Papen 2002).

The shift from CH$_4$ sink to CH$_4$ source in well-aerated soils has been related to the formation of anaerobic microsites where CH$_4$ production could occur. Verchot et al. (2000) found that CH$_4$ flux variability was predicted by a linear combination of WFPS and soil respiration, so that net CH$_4$ emissions corresponded to the periods of higher soil respiration and water content. Thus, although CH$_4$ emissions are typically associated with heavy textured and water saturated soils, the development of anaerobic microsites in which CH$_4$ production might take place, might also be hypothesized to occur in medium and light textured soil (Verchot et al. 2000). The mechanism might be similar to that proposed by Smith (1990) to explain N$_2$O production and denitrification in aerobic soils. Zones of intense microbial activity might develop in association with easily decomposable fresh organic matter, and if the water content in the soil is sufficiently high to result in O$_2$ consumption by respiration at a faster rate than O$_2$ diffusion from the surrounding areas, then anaerobic conditions might be reached and CH$_4$ produced. This is consistent with the observed increase in net CH$_4$ production reported in this study, when the increase in soil water content also corresponds with the growing season, which implies higher root and microbial respiration rates (data not shown).

4.4.2.2 Effects of forest type

PS and QP stands absorb more CH$_4$ than QI stands. CH$_4$ uptake is supposed to be strongly affected by soil texture (Born et al. 1990) and soil moisture (Borken et al. 2000) due to their effect on the diffusion velocities of CH$_4$ and O$_2$ (Potter et al. 1996 a).

CH$_4$ absorption increases with soil DOC content and C/N ratio. There is a relationship between clay content and CH$_4$ consumption. The forest plot with the highest clay content exhibited lower CH$_4$ uptake. This may be due to a reduced
diffusion rate of atmospheric CH$_4$ into the soil. Boeckx and Van Cleemput (1996) found a lower CH$_4$ consumption rate in fine-textured soils than coarse-textured soils.

The optimum pH for CH$_4$ oxidation was 5.5. In our case we observed CH$_4$ oxidation at pH values below this threshold (3.5-5). Acidophilic methanotrophs and others can be active in soils at pH 4 (Dedysh et al. 2000; Radajewski et al. 2002). This may explain why CH$_4$ oxidation appeared to be independent of pH in forest soils, in accordance with reports review by Borken and Brumme (1997).

Increasing NH$_4^+$ and NO$_3^-$ concentrations were associated with decreasing CH$_4$ consumption rates. NH$_4^+$ production appears to have an inhibitory effect on CH$_4$ oxidation because ammonium mono-oxidase and methane mono-oxidase enzymes are linked in methanotrophs (Bedard and Knowles 1989). However, NO$_3^-$ inhibition on CH$_4$ oxidation is less likely.

4.4.2.3 Effects of fire

Fire increases CH$_4$ uptake cumulative fluxes in QI (B= -0.31 kg CH$_4$-C ha$^{-1}$; NB= -0.09 kg CH$_4$-C ha$^{-1}$). This suggests no direct effect of fire on soil capacity to oxidize CH$_4$ or that the microbial population recovers within four years after fire. The amount of soil heating would only be enough to destroy a small fraction of the total soil microorganism population within the greater soil profile. Poth et al. (1995) found that soil CH$_4$ uptake significantly increases after fire in savanna soils burned 30 days earlier. They hypotheses that burning might increase the diffusion of CH$_4$ into the soil, and hence CH$_4$ oxidation by opening roots channels and removing litter layers.

In QP stands, no clear differences were found between burned and unburned plots (B= -0.31 kg CH$_4$- ha$^{-1}$; NB=0.35 kg CH$_4$-C ha$^{-1}$). In PS stands, fire decreases CH$_4$ uptake (B= -0.20 kg CH$_4$-C ha$^{-1}$; NB= -0.51kg CH$_4$-C ha$^{-1}$). This may be due to the increasing inorganic N in the soil as a consequence of the N-fixing species present in this plot. Increasing NH$_4^+$ content in the soil should lead to a reduction in methanotrophic activity because NH$_3$ might be a competitive substrate for methane monooxygenase (Castaldi and Fierro 2005). Another explanation is the combination of higher temperature, less structured soil and smaller quantity of soil nutrients that could
be detrimental to the methanotrophic population of these soils and their CH$_4$ oxidation capacity.

Low CH$_4$ oxidation values in PSB may also be explained by the low pH values observed in this plot. Negative effects of pH on CH$_4$ oxidation were noted by Hütsch et al. (1994). The probable explanation in their case was that acid precipitation had caused a release of heavy metals, such as aluminium, iron, manganese and cadmium. This may also explain the results obtained in our study, in which we have detected an increase in exchangeable acidity (EA), the release of exchangeable Al$^{3+}$ from organic matter and the diminution of Ca$^{2+}$, Mg$^+$, K$^+$ and Na$^+$. 

4.5 CONCLUSIONS

The forest soils in the surrounding area of Madrid were mostly weak N$_2$O emitters and significant sinks for atmospheric CH$_4$. The nutrient status in forest soils had an effect on the fluxes of N$_2$O and CH$_4$. Mean fluxes of N$_2$O and CH$_4$ were highest in Scots Pine and Pyrenean oak stands. A weak N$_2$O uptake from the atmosphere into the soil was observed in fall and summer. The shift from CH$_4$ sink to CH$_4$ source was observed in wet months.

N$_2$O and CH$_4$ seasonal variation were mainly related to soil water availability. The impact of fire on the fluxes of N$_2$O and CH$_4$ differed from one ecosystem to another and from one season to another. The burned sites showed higher CH$_4$ oxidation in Q. ilex stands, and lower oxidation rates in P. sylvestris stands. Fire decreases N$_2$O fluxes in Q. pyrenaica stands.

Thus, the results confirm the hypothesis that was being tested: that the impact of fire on CH$_4$ and N$_2$O flux might strongly depend on the climatic seasonal patterns, ecosystem type and main soil characteristics.

Due to the lack of information on fluxes in Mediterranean ecosystems, it is difficult to compare their contribution to the local, regional and global flux of N$_2$O and CH$_4$. This emphasizes the necessity for better estimates of atmospheric CH$_4$ and N$_2$O fluxes, which can only be achieved through an improved understanding of the underlying processes and supplementary field data.
4.6 ACKNOWLEDGEMENTS

This research was conducted in the framework of the Spanish CGL2006-02922/CLI and CGL2009-07031 and the European COST 639 (BurnOut). The authors want to express their gratitude to Stoyan Holding LTD., Patrimonio Nacional and Community of Madrid for the employment of their forest sites.

4.7 REFERENCES


Borken W, Brumme R (1997) Liming practice in temperate forest ecosystems and the effects on CO₂, N₂O and CH₄ fluxes. Soil Use Manage 13: 251-257
Butterbach-Bahl K, Breuer L, Gasche R, Willibald G, Papen H (2002) Exchange of trace gases between soils and the atmosphere in Scots pine forest ecosystems of the northeastern German lowlands 1. Fluxes of N$_2$O, NO/NO$_2$ and CH$_4$ at forest sites with different N-deposition. For Ecol Manage 167: 123-134
Butterbach-Bahl K, Papen H (2002) Four years continuous record of CH$_4$ exchange between the atmosphere and untreated and limed soil of a N-saturated spruce and beech forest ecosystem in Germany. Plant Soil 240: 77-90


Goldberg SD, Gebauer G (2009) Drought turns a Central European Norway spruce forest soil from an N$_2$O source to a transient N$_2$O sink. Glob Chang Biol 15: 850-860


denitrification in European forest soil. In: Schulze ED (ed) Carbon and nitrogen cycling in European forest ecosystems. Springer-Verlag, Berlin, pp 297-340


Rodríguez A, Durán J, Fernández-Palacios JM, Gallardo A (2009) Spatial pattern and scale of soil N and P fractions under the influence of a leguminous shrub in a Pinus canariensis forest. Geoderma 51: 303-310


Chapter 4

$N_2O$ and $CH_4$ fluxes in undisturbed and burned Holm oak, Scots pine and Pyrenean oak forests in central Spain
Chapter 5

PASTURE, TILLAGE AND CANOPY EFFECTS ON CARBON DIOXIDE FLUXES IN A SPANISH DEHESA
Chapter 5

PASTURE, TILLAGE AND CANOPY EFFECTS
ON CARBON DIOXIDE FLUXES
IN A SPANISH DEHESA

C. Uribe, R. Inclán, L. Hernando, M. Román, MA. Clavero, S. Roig, H. Van Miegroet

Sent to Plant and Soil journal (2013)
ABSTRACT

There is increasing interest in carbon sequestering capacity of agroforestry systems especially in relation to climate change. Adequate use of silvopastoral practices in dehesa systems may contribute to their sustainability improving soil carbon (C) and nitrogen (N) capacity while reducing the CO$_2$ flux from the soil to the atmosphere. The response of soil respiration to grazing and tilling practices and trees canopy influence were studied in a dehesa ecosystem in the center of Spain from July 2008 to February 2010. Four different treatments were established: non grazed-non tilled (NGNT); non grazed-tilled (NGT); grazed-non tilled (GNT) and grazed and tilled (GT). Measurement of soil respiration ($R_s$), soil temperature ($T_s$), soil moisture ($M_s$), soil C and N stocks were taken in all the treatments.

Results showed $R_s$ reductions by grazing effect of 12 % across all experiment. However, increments of 3Mg/ha in C stocks and 0.3 Mg/ha in N stocks in grazing soils were observed. Although, not clear effect by tilling effect on soil respiration was found, a decreasing of 3.5 Mg/ha in C stocks and 0.3 Mg/ha in N stocks on soil C and N stocks was detected in tilling soils. Tree canopy induced increases in soil respiration, soil C and N stocks; and decreases in $T_s$ were observed; otherwise grazed effect decreased tree canopy influence on annual C losses by soil respiration. The soil moisture was relevant in constrains the soil respiration temperature response during the experiment, and apparent Q$_{10}$ values were only significant during the wettest time.

Grazing and tree canopy had a positive influence in the ability to storage soil C and N stocks while superficial tilling show a negative effect on soil C and N store capacity in this study. Indicating that maintain the beneficial practices and improve the management of tilled applied in this area may have important consequences in carbon sequestration capacity in this dehesa system.

**Keywords:** grazing practices; tilled practices; dehesa; soil respiration; soil microclimates; soil C stock; soil N stock; *Quercus ilex*; climate change.
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1</td>
<td>INTRODUCTION</td>
<td>171</td>
</tr>
<tr>
<td>5.2</td>
<td>MATERIAL AND METHODS</td>
<td>173</td>
</tr>
<tr>
<td>5.2.1</td>
<td>Study area</td>
<td>173</td>
</tr>
<tr>
<td>5.2.2</td>
<td>Experimental layout</td>
<td>175</td>
</tr>
<tr>
<td>5.2.3</td>
<td>Soil sampling and analysis</td>
<td>176</td>
</tr>
<tr>
<td>5.2.4</td>
<td>Statistical analysis</td>
<td>176</td>
</tr>
<tr>
<td>5.3</td>
<td>RESULTS</td>
<td>177</td>
</tr>
<tr>
<td>5.3.1</td>
<td>Soil respiration (Rs), soil temperature (Ts) and soil moisture (Ms)</td>
<td>177</td>
</tr>
<tr>
<td>5.3.1.1</td>
<td>Pasture and tillage treatments</td>
<td>177</td>
</tr>
<tr>
<td>5.3.1.2</td>
<td>Influence of tree canopy</td>
<td>181</td>
</tr>
<tr>
<td>5.3.2</td>
<td>Soil carbon and N stocks</td>
<td>184</td>
</tr>
<tr>
<td>5.4</td>
<td>DISCUSSION</td>
<td>184</td>
</tr>
<tr>
<td>5.4.1</td>
<td>Grazing influences</td>
<td>184</td>
</tr>
<tr>
<td>5.4.2</td>
<td>Tillage influences</td>
<td>188</td>
</tr>
<tr>
<td>5.4.3</td>
<td>Tree and grazing interactions</td>
<td>189</td>
</tr>
<tr>
<td>5.5</td>
<td>CONCLUSIONS</td>
<td>190</td>
</tr>
<tr>
<td>5.6</td>
<td>ACKNOWLEDGMENTS</td>
<td>191</td>
</tr>
<tr>
<td>5.7</td>
<td>REFERENCES</td>
<td>191</td>
</tr>
</tbody>
</table>
5.1 INTRODUCTION

Dehesas are multipurpose agrosilvopastoral systems of extensive utilisation, where native trees, mostly holm Quercus ilex and cork Quercus suber oaks, are spaced out or inserted in a continuum of grasslands or shrubland matrix (Díaz et al. 1997; Gómez-Limón and Fernández 1999; Montero et al. 2000; Ramachandran Nair et al. 2009). As a result, dehesa systems are distinguished by a systematic combination of agricultural, pastoral and forestry uses, resulting in different vegetation structures depending on land-use (Cubera and Moreno 2007; Joffre et al. 1999). Dehesas cover about 5-6 million hectares of the Iberian peninsula (Sundseth 2009) and have influenced the landscape for many centuries in the Mediterranean basin (Eichhorn et al. 2006).

Nevertheless in recent years, the sustainability of dehesas is in question due to the trend towards more intensive and simplified management (Cubera and Moreno 2007; Papanastasis 2004) and the insufficient regeneration of trees (Díaz et al. 1997; Plieninger et al. 2003; Plieninger and Wilbrand 2001; Pulido et al. 2001). Trees produce important environmental services: they enhance the nutrient availability, improve soil water holding capacity, ameliorate environmental stress below their canopy (Gallardo et al. 2000), determine the diversity and density of many animals groups (Olea and San Miguel-Ayanz 2006), and stimulate primary production in the herbaceous layer under canopies (Hussain et al. 2009). Furthermore, trees constitute an important means to capture and store carbon (C) atmospheric in biomass and soils (Malhi et al. 2008). More recently, there has been increasing interests in the C sequestering capacity of agroforestry systems, especially under the afforestation and reforestation mitigating strategies under the Kyoto Protocol (IPCC 2007; Ramachandran Nair et al. 2009). Agroforestry systems are believed to have a higher potential to sequester C than either pastures or field crops (Kirby and Potvin 2007; Ramachandran Nair et al. 2009; Sanchez 2000; Sharrow and Ismail 2004). However, arid and semiarid sites have lower C sequestration potential than fertile and humid sites (Jose 2009; Ramachandran Nair et al. 2009).

The use of appropriate silvopastoral practices in dehesa systems may contribute to their sustainability, improve soil quality and productivity, and reduce the soil CO₂ flux from the soil to the atmosphere. Tillage is commonly used in dehesas for various
periodical crops, to control shrub encroachment, favour the grass layer, and obtain complementary fodder for grazing animals (useful for dry and cold seasons) (Cubera and Moreno 2007; Moreno and Obrador 2007). In Spain it is common to apply a systematic periodical tillage of mouldboard ploughing in dehesas to prevent potential compaction problems and encourage seedling emergence and crop performance (López-Garrido et al. 2011).

Different types of livestock are usually employed in dehesas to make best use of the varied resources (Moreno and Obrador 2007); grazing of cattle, sheep, Iberian pigs, etc can influence plant community structure, soil properties, and the distribution and cycling of nutrients within the plant-soil system (Schuman et al. 1999). Previous studies in Mediterranean agroecosystems have shown that tillage increases soil respiration ($R_s$) in the short-term (Álvaro-Fuentes et al. 2007; López-Garrido et al. 2009; Morell et al. 2010). This response may be caused by the alteration of soil organic matter decomposition and microclimate after tillage. Tillage promotes aggregate disruption, exposes protected organic matter to decomposition (La Scala Jr et al. 2008), and contributes to the mixing of plant residues with soil that in turn increase soil microbial activity. Reicosky et al. (1997) related the increase in $R_s$ after tillage (burst effect) to release of gases entrapped in soil pores from previous microbial activity. Small differences in $R_s$ between tillage and no-tillage treatments were observed by Reicosky and Lindstrom (1993) and Ball et al. (1999), in the first weeks after tillage. However, there is no information on mid-and long-term effects of tillage practice on $R_s$. Moreover, no clear relationship between grazing and $R_s$ had been observed in semiarid grassland ecosystem (Milchunas and Lauenroth 1993; Reeder and Schuman 2002), whereas a decline in $R_s$ with grazing has been observed (Bremer et al. 1998; Cao et al. 2004; Raiesi and Asadi 2006). Some authors have reported variations in $R_s$ in response to rates of nitrogen input from cattle excreta (Jones et al. 2006) and reductions in root respiration rate and organic matter decomposition alteration due grazing activities (Bremer et al. 1998; Detling et al. 1979; LeCain et al. 2002). However, the complexity of the mechanism involved in herbivore effects on soil processes makes predicting the direction and magnitude of these effects often very difficult (Bardgett et al. 1998; Mazancourt et al. 1998; Stark et al. 2000). As a result, in semiarid grassland ecosystems, the effects of grazing management on the biogeochemical processes that
control the exchange of C between the soil and atmosphere are not well understood (Reeder and Schuman 2002).

Together, the type, frequency, intensity and timing of these management practices (tillage, grazing), may, in addition to environmental controls, modify the $R_s$ dynamics in dehesa systems. Understanding the response of $R_s$ to management is crucial to our ability to predict the impact of current and management regimes on regional C exchange; and to establish strategies to help mitigate greenhouse gases emissions.

The objectives of this study were to (1) quantify the effects of pasture and tillage practices on $R_s$; (2) determine the influence of the management practices on soil C and N stocks and the response of $R_s$ to soil microclimate, and (3) determine the influence of tree canopy (Quercus ilex) on $R_s$, soil C and N stock in a dehesa ecosystems situated in the center of Spain (Toledo).

### 5.2 MATERIAL AND METHODS

#### 5.2.1 Study area

The experiment was conducted from July 2008 to February 2010 in a dehesa ecosystem, Dehesón del Encinar in the center of Spain (Oropesa, Toledo) (39°59´N, 5°8´W). The site is located at an approximate altitude of 350 m asl, occupies about of 714 ha, and has moderate slopes.

The climate is Mediterranean pluviseasonal oceanic with a mean annual precipitation of 572 mm and a mean annual temperature of 15.3 °C (Rivas-Martínez and Rivas-Saenz 2010). Meteorological data during the experiment were obtained from the meteorological station installed in the Dehesón del Encinar (Figure 5.1). The vegetation in the experimental area is composed of herbaceous stratum and a tree canopy stratum of evergreen oak Quercus ilex L. subsp. ballot (Desf.) Samp. Mean tree density was around 32 trees/ha. Trees had a mean crown radius of about 4.3 ± 1 m, height 8.3 ± 2 m and diameter 46.8 ± 19.1 cm. The grass layer vegetation is comprised of annual subnitrophilous pastures of the Stellarietea mediae and Sisymbrietalia officinalis (López-Carrasco et al. 2012). The majority of pasture growth in these annual
communities occurs in April–May around 70% of the annual yield, according to Olea et al. (1990-1991), and herbaceous plants generally dry between mid June and October.

![Graph showing monthly temperatures and accumulated rainfall from meteorological station in Dehesón del Encinar during the experiment (2008-2010).](image)

**Figure 5.1** Monthly temperatures and accumulated rainfall from meteorological station in *Dehesón del Encinar* during the experiment (2008-2010).

Soils are classified as Haplic luvisol and Haplic cambisol (IUSS 2007; Simón et al. 2012). They are acidic, sandy and poor in nutrient and organic matter content (Gea-Izquierdo et al. 2009). Most common activities in *Deheson del Encinar* are research, livestock grazing with native and artificial intercropping pasture and more marginal activities are firewood, cork production and hunting. In the farm areas used in the study the management system was grazing by sheep (2-3 Talaverana heads/ha) from mid April to June and grazing by cattle (6-8 Avileña heads/ha) during summer months.
5.2.2 Experimental layout

Two grazed and two non-grazed areas of around 1.5 ha each were established in the dehesa. The distances between the four experimental areas were from 600 m to 3 Km. Within each area, eight trees of Quercus ilex were randomly selected (at about fifty meters of distance between them), four of which trees were tilled in April 2008 using conventional deep (20-25 cm) tillage of mouldboard ploughing around the trunk; the other four trees remained untitled in each area. Soils in the dehesa had not been tilled since 1987. Thus a total of four different treatments were obtained: Non grazed-non tilled (NGNT); non grazed-tilled (NGT); grazed-non tilled (GNT) and grazed-tilled (GT) with two replicates per treatment (consisting of four trees per treatment rep).

PVC collars were installed (diameter 10 cm x height 4.5 cm) in all the treatments into the soil at 2.5 cm depth to limit root severing, at least 1 week prior to investigation to the first Rs measurements to prevent an overestimation of soil fluxes. Four collars were installed under tree canopy (UC) in the N, S, E, and W orientations under subset of four trees in all treatments. To evaluate the effect of canopy cover, one additional collar out of the influence of canopy in open area (OA) were installed only in non tilled treatments (NGNT and GNT). The average distances to the tree trunks were about 3 m UC and 11 m in OA. A total of 144 collars were installed during the experiment in all treatments. Rs was measured at nine different times from July 2008 to February 2010 using a closed dynamics system LI-6400 coupled to an LI-6400-9 soil chamber (LI-COR inc., Lincoln, NE, USA). All measurements during the experiment were made between 10 a.m and 5 p.m. Inside the collars, the emerging ground vegetation was trimmed without altering the soil surface and removed in conjunction with coarse materials before inserting the soil chamber. The measurement of Rs consisted of placing the chamber on the collar, scrubbing the CO2 to sub-ambient levels and measuring the flux rate as it rose from 15 ppm below to 15 ppm above the atmospheric value according to LI-6400 owner manual. Three measurements of Rs were made on each collar at each observation point. In addition, Ts and volumetric Ms were recorded in three points around each collar at the same time of Rs with a thermocouple sensor (Omega Engineering, Stamford, CT) and time-domain reflectometry system (TRIMEGM, IMKO GmbH, Ettlingen, Germany).
annual C emission was calculated from average \( R_s \) values in each of treatments (\( \mu \text{mol m}^{-2} \text{s}^{-1} \)), extrapolated to a year and expressed as g C m\(^{-2}\) yr\(^{-1}\).

5.2.3 Soil sampling and analysis

At the end of the experiment, two soil cores samples were taken beneath the collars used in the \( R_s \) measurements, after removal of plant debris from the surface. One of the core samples (diameter 6 cm x height 5 cm) was used to calculate soil bulk density, and the other to calculate C and N content into the soil. The samples were kept separately in plastic bags and transported on ice in a dark cooler to the laboratory. Bulk density (\( B_d \)) was calculated as mass of the oven-dry soil (110ºC for 48h) divided by the core volume. The soil samples for C and N analysis were air dried at room temperature for 2-3 days, sieved through a 2 mm mesh then ground and homogenized using a mortar and C and N content determined using a LECO TruSpec CHN elemental analyzer (LECO Corp., St. Joseph, USA). Soil C and N stocks (Mg ha\(^{-1}\)) were calculated based on soil C and N concentration of the fine fraction (g/kg) a depth of 5 cm and soil bulk density (g/cm\(^3\)).

5.2.4 Statistical analysis

Data were checked for homogeneity of variances and for normal distribution using Levene and Kolmogorov-Smirnov test respectively. Two-way ANOVA was used to evaluate (1) the effects of grazing and tilling on \( R_s, T_s, M_s, C \) and N stocks in all treatments; (2) the effect of canopy on C and N stocks in non tilled treatments. A one-way ANOVA was used to test differences in \( R_s, T_s \) and \( M_s \) between NGNT and GNT in July and August 2008; and canopy influences on \( R_s, T_s \) and \( M_s \) in non tilled treatments. Post-hoc comparisons were tested using Tukey’s HSD test. A Spearman-rank correlation test was used to examine the relationship between \( R_s, T_s \) and \( M_s \) for each treatment combination. Apparent \( Q_{10} \) values were determined using the model described in Jannsen and Pilegard (2003).

Due to strong seasonality of Mediterranean ecosystems, the correlations between \( R_s \) and soil microclimate were performed across the whole data set in each treatment, and on data set from three broad climatologically periods of the dehesa, representing (1) dry
months, characterized by high temperatures and low moisture ($T_s > 25^\circ\text{C}$, $M_s 1\text{-}5\%$), hereafter called “hot, dry period” corresponds to the months of July 2008; August 2008; October 2008 and September 2009; (2) cold months, characterized by low temperature and high moisture ($T_s < 7^\circ\text{C}$, $M_s 8\text{-}28\%$), hereafter called “cold, moist period” corresponds to the months of December 2008 and February 2010; and (3) warmer months, characterized by mild temperature and moisture ($T_s > 11^\circ\text{C}$, $M_s 2\text{-}6\%$), hereafter called “mild period” corresponds to the months of November 2008, March 2009 and April 2009 (Table 5.1). All statistical analysis was performed using the Statistica 6.0 software package (StatSoft. Inc., Tulsa. USA) and Sigma Plot 6.0 (SystatSoft. Inc., San Jose, USA) using a 5% probability level for significance.

5.3 RESULTS

5.3.1 Soil respiration ($R_s$), soil temperature ($T_s$) and soil moisture ($M_s$)

5.3.1.1 Pasture and tillage treatments

Periodic mean values of $R_s$ ($\mu\text{molm}^{-2}\text{s}^{-1}$) obtained during the experiment in the different treatments are shown in (Figure 5.2a). The $R_s$ ($\mu\text{molm}^{-2}\text{s}^{-1}$) across the measurements ranged from 8.48 to 0.34 in NGNT, from 6.02 to 0.11 in NGT, from 5.71 to 0.30 in GNT and from 5.28 to 0.31 in GT.

Grazing significantly decreased $R_s$ by 26%, 48%, 24% and 38% ($p<0.05$) in July 2008, August 2008, April 2009 and September 2009, respectively. Tilling resulted in opposite responses: a 20% decrease in $R_s$ ($p<0.05$) in November 2008, and an increase of 32% ($p<0.05$) in February 2010. Also a significantly interaction effect between grazed and tilled treatment ($p<0.05$) was observed in September 2009 only. Grazing resulted in an increase in $T_s$ values ($p<0.05$) in July 2008, and a decrease ($p<0.05$) in October 2008, December 2008 and April 2009. Tilled treatments had lower $T_s$ values ($p<0.05$) in November and December 2008 and higher $T_s$ values ($p=0.1$) in February 2010 (Figure 5.2b). Grazed areas showed an increase of $M_s$ in November 2008, April 2009 and February 2010; and higher $M_s$ values were found in tilled treatments in
Figure 5.2 Mean values and standard errors of (a) soil respiration ($R_s$: μmol·m$^{-2}$·s$^{-1}$); (b) soil temperature ($T_s$: ºC); and (c) soil moisture ($M_s$: %) during the experiment in each treatment. NGNT: non grazed-non tilled; NGT: non grazed-tilled; GNT: grazed- non tilled; GT: grazed-tilled. Asterisks indicate significant effect of grazing and arrows indicate significant effect of tilled by measurement period. Different letters show interaction between grazing and tilled treatments.
December 2008, March 2009 and April 2009, respectively. However, the interaction between grazed and tilled treatments on $M_s$ values was significant in December 2008, indicating that the tilling effect was most pronounced in the ungrazed area (Figure 5.2c).

Across all data, the $R_s$ was 12% lower in grazed than in non grazed treatments, but no significant differences were found with tilling and there was no interaction between both treatments (grazing x tilling). Furthermore, we observed a positive effect of grazing and tilling treatments on $M_s$ and negative effect on $T_s$ by tilling. The estimates of annual C losses through $R_s$ from the different agrosilvopastoral practices using the whole data were 742 g·C·m$^{-2}$·yr$^{-1}$ for NGNT, 719 g·C·m$^{-2}$·yr$^{-1}$ for NGT, 640 g·C·m$^{-2}$·yr$^{-1}$ for GNT and 655 g·C·m$^{-2}$·yr$^{-1}$ for GT.

Mean $R_s$ rates ($\mu$mol·m$^{-2}$·s$^{-1}$) for each treatment across the entire experiment and for the different periods selected are shown in Table 5.1. The lowest $R_s$ values were observed in hot and dry period (Table 5.1). Using the data obtained from the whole experiment, Spearman rank correlation coefficients (Table 5.2) showed a weak negative relationship between $R_s$ and $T_s$ and weak positive relationship between $R_s$ and $M_s$. The correlations between $R_s$, $T_s$, and $M_s$ were slightly stronger in the grazed treatments; while the correlation with $M_s$ was more strongly positive in the tilled treatments. When the data were analyzed by climatic periods, different patterns emerged and the correlation between $R_s$ and $M_s$ became generally less pronounced. In the hot, dry period $R_s$ was uncorrelated with $T_s$ in all but the GNT treatments. Only in non-grazed treatments (NGNT- NGT) was there a positive correlation between $R_s$ and $M_s$. In the cold moist period, a positive correlation between $R_s$ and $T_s$ was detected in all treatments; whereas a positive correlation between $R_s$ and $M_s$ was observed only in the GT treatment. In the mild period, there was not a significant correlation between $R_s$ and soil microclimate, except in GNT where $R_s$ was positively correlated with $M_s$ and negatively with $T_s$. The apparent $Q_{10}$ was calculated only for the cold moist period, and $Q_{10}$ values were 5.68 for NGNT, 2.47 for NGT, 2.88 for GNT and 4.12 for GT.
Table 5.1 Mean values and standard error of soil respiration ($R_s$: µmol m$^{-2}$ s$^{-1}$), soil temperature ($T_s$: ºC) and soil moisture ($M_s$: %) across the entire experiment (2008-2010) and for the different periods in each treatment NGNT: non grazed-non tilled; NGT: non grazed- tilled; GNT: grazed- non tilled; GT: grazed-tilled. Values in parenthesis indicate number of data points.

<table>
<thead>
<tr>
<th>Time</th>
<th>Treatment</th>
<th>$R_s$</th>
<th>$T_s$</th>
<th>$M_s$</th>
</tr>
</thead>
<tbody>
<tr>
<td>All experiment</td>
<td>NGNT</td>
<td>1.96 ± 0.08 (187)</td>
<td>16.63 ± 0.50 (187)</td>
<td>4.41 ± 0.23 (165)</td>
</tr>
<tr>
<td></td>
<td>NGT</td>
<td>1.90 ± 0.07 (138)</td>
<td>12.79 ± 0.55 (138)</td>
<td>6.47 ± 0.47 (133)</td>
</tr>
<tr>
<td></td>
<td>GNT</td>
<td>1.69 ± 0.06 (227)</td>
<td>16.43 ± 0.48 (227)</td>
<td>6.29 ± 0.46 (166)</td>
</tr>
<tr>
<td></td>
<td>GT</td>
<td>1.73 ± 0.07 (177)</td>
<td>12.36 ± 0.43 (177)</td>
<td>7.65 ± 0.56 (177)</td>
</tr>
<tr>
<td>Hot, dry period</td>
<td>NGNT</td>
<td>1.66 ± 0.15 (83)</td>
<td>22.11 ± 0.34 (83)</td>
<td>3.04 ± 0.25 (69)</td>
</tr>
<tr>
<td></td>
<td>NGT</td>
<td>0.88 ± 0.11 (23)</td>
<td>23.05 ± 0.73 (23)</td>
<td>1.70 ± 0.28 (23)</td>
</tr>
<tr>
<td></td>
<td>GNT</td>
<td>1.17 ± 0.07 (95)</td>
<td>23.82 ± 0.41 (95)</td>
<td>2.45 ± 0.25 (34)</td>
</tr>
<tr>
<td></td>
<td>GT</td>
<td>0.90 ± 0.15 (31)</td>
<td>20.44 ± 0.64 (31)</td>
<td>1.93 ± 0.19 (31)</td>
</tr>
<tr>
<td>Cold, moist period</td>
<td>NGNT</td>
<td>2.14 ± 0.17 (37)</td>
<td>7.19 ± 0.23 (37)</td>
<td>9.39 ± 0.50 (29)</td>
</tr>
<tr>
<td></td>
<td>NGT</td>
<td>2.24 ± 0.14 (45)</td>
<td>6.27 ± 0.21 (45)</td>
<td>13.59 ± 0.66 (40)</td>
</tr>
<tr>
<td></td>
<td>GNT</td>
<td>2.05 ± 0.14 (40)</td>
<td>6.69 ± 0.22 (40)</td>
<td>14.05 ± 1.23 (40)</td>
</tr>
<tr>
<td></td>
<td>GT</td>
<td>2.25 ± 0.16 (47)</td>
<td>6.17 ± 0.21 (47)</td>
<td>16.84 ± 1.32 (47)</td>
</tr>
<tr>
<td>Mild period</td>
<td>NGNT</td>
<td>2.24 ± 0.10 (67)</td>
<td>13.80 ± 0.36 (67)</td>
<td>3.69 ± 0.18 (67)</td>
</tr>
<tr>
<td></td>
<td>NGT</td>
<td>2.02 ± 0.09 (70)</td>
<td>13.62 ± 0.48 (70)</td>
<td>3.97 ± 0.19 (70)</td>
</tr>
<tr>
<td></td>
<td>GNT</td>
<td>2.06 ± 0.10 (92)</td>
<td>13.02 ± 0.25 (92)</td>
<td>4.33 ± 0.19 (92)</td>
</tr>
<tr>
<td></td>
<td>GT</td>
<td>1.73 ± 0.06 (99)</td>
<td>12.76 ± 0.38 (99)</td>
<td>5.07 ± 0.21 (99)</td>
</tr>
</tbody>
</table>

Hot, dry period corresponds to the months of July 2008; August 2008; October 2008 and September 2009.

Cold moist period corresponds to the months of December 2008 and February 2010.

Mild period corresponds to the months of November 2008; March 2009 and April 2009.
Table 5.2 Spearman correlation coefficients between soil respiration (Rs), soil temperature (Ts) and soil moisture (Ms) with all data obtained during the experiment (2008-2010) and for the different periods in “Dehesón del Encinar” in each treatment NGNT: non grazed-non tilled; NGT: non grazed- tilled; GNT: grazed- non tilled; GT: grazed-tilled. Parenthesis means number of data.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>All experiment</th>
<th>Hot, dry period</th>
<th>Cold, moist period</th>
<th>Mild period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rs-Ts</td>
<td>Rs-Ms</td>
<td>Rs-Ts</td>
<td>Rs-Ms</td>
</tr>
<tr>
<td>NGNT</td>
<td>-0.33 (187)</td>
<td>0.31 (165)</td>
<td>n.s</td>
<td>0.51 (59)</td>
</tr>
<tr>
<td>NGT</td>
<td>-0.29 (138)</td>
<td>0.41 (133)</td>
<td>n.s</td>
<td>0.38 (23)</td>
</tr>
<tr>
<td>GNT</td>
<td>-0.43 (227)</td>
<td>0.37 (166)</td>
<td>0.29 (99)</td>
<td>n.s</td>
</tr>
<tr>
<td>GT</td>
<td>-0.39 (177)</td>
<td>0.45 (177)</td>
<td>n.s</td>
<td>n.s</td>
</tr>
</tbody>
</table>

All bold coefficients values show in the table are significant (p < 0.05). (n.s) indicated no significant values.

Hot, dry period corresponds to the months of July 2008; August 2008; October 2008 and September 2009.

Cold moist period corresponds to the months of December 2008 and February 2010.

Mild period corresponds to the months of November 2008; March 2009 and April 2009.

5.3.1.2 Influence of tree canopy

The periodic mean Rs values (µmol m⁻² s⁻¹) were higher (p < 0.05) under tree canopy than in open areas (Figure 5.3,4a) during most of the experiment, irrespective of grazing treatment. Annual C losses through Rs were on average 33% and 18% less in OA than UC in NGNT and GNT treatment, respectively. Canopy cover had an attenuating effect on Ts, which was generally lower (p <0.05) under canopy compared to open locations (Figure 5.3,4 b) except during the coldest months, when Ts was higher below the tree canopy. Differences in Ms were generally less pronounced, but when they occurred, higher Ms (p <0.05) were observed in OA than in UC in most of the cases (Figure 5.3,4 c).

Under the canopy no significant differences in Rs were found due to orientation. These aspects differed significantly in soil microclimate, with higher Ts values and lower Ms values (p <0.05) in the S compared to the N across all treatments.
Figure 5.3 Influence of canopy on (a) soil respiration ($R_s$: µmol m$^{-2}$ s$^{-1}$); (b) soil temperature ($T_s$: °C); and (c) soil moisture ($M_s$: %) in NGNT: non grazed-non tilled. Asterisks (*) indicate significant differences between tree canopy and open areas.
Figure 5.4 Influence of canopy on (a) soil respiration ($R_s$: $\mu$mol m$^{-2}$ s$^{-1}$); (b) soil temperature ($T_s$: °C); and (c) soil moisture ($M_s$: %) in GNT: grazed- non tilled. Asterisks (*) indicate significant differences between tree canopy and open areas.
5.3.2 Soil carbon and N stocks

Mean values of bulk density (g/cm$^3$), C and N stocks (Mg/ha) and C/N ratio obtained during the experiment shown in Table 5.3. No significant differences in $B_d$ data were found due to canopy or management treatment (Table 5.3). Grazing treatments increased soil C and N stocks ($p < 0.05$) while tilled treatments decreased these stocks ($p < 0.05$). Furthermore, there was a significant interaction ($p < 0.05$) between grazing and tilling effects on soil C and N stocks (Mg ha$^{-1}$) such that increases in C and N stock with grazing were most pronounced in untilled sites and diminished with tilling. Mean C and N stocks values (Mg ha$^{-1}$) under tree canopy were two to three times the value ($p < 0.05$) of those in open areas resulting in higher C/N values under canopy of GNT treatment compared to OA (Table 5.3).

5.4 DISCUSSION

5.4.1 Grazing influences

Grazing clearly decreased $R_s$ values in our experiment when the whole experimental data was considered. This effect was fundamentally driven by differences obtained in the months of July 2008, August 2008, September 2009 and April 2009, three of which belong to the hot dry period, coinciding with cattle grazing. Decreases in $R_s$ with grazing have been found previously (Bremer et al. 1998; Cao et al. 2004; Raiesi and Asadi 2006) in similar ecosystems. However, $R_s$ increases (Lecain et al. 2000; Liebig et al. 2013) or a lack of a clear relationships between grazing and $R_s$ have been observed in other studies (Milchunas and Lauenroth 1993; Reeder and Schuman 2002).
Table 5.3 Mean values and standard errors of bulk density (Bd), C and N stock and C/N ratio in the top 10 cm of the soil for all treatments. NGNT: non grazed-non tilled; NGT: grazed-tilled; GNT: grazed-non tilled and GT: grazed-tilled obtained during the experiment (2008-2010) in Dehesón del Encinar. Lower letters indicate significant differences between treatments at under canopy (UC) locations; capital letters indicate significant treatments differences within open area (OA) and bold number indicate significant differences between under canopy and open area within a given treatment.

<table>
<thead>
<tr>
<th></th>
<th>NGNT UC</th>
<th>NGNT OA</th>
<th>NGT UC</th>
<th>NGT OA</th>
<th>GNT UC</th>
<th>GNT OA</th>
<th>GT UC</th>
<th>GT OA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bd (g/cm³)</td>
<td>1.07 ± 0.02a</td>
<td>1.16 ± 0.12A</td>
<td>1.11 ± 0.03a</td>
<td>–</td>
<td>1.05 ± 0.03a</td>
<td>1.02 ± 0.08A</td>
<td>1.03 ± 0.05a</td>
<td>–</td>
</tr>
<tr>
<td>C stock (Mg/ha)</td>
<td>9.57 ± 0.5b</td>
<td>3.76 ± 0.30A</td>
<td>8.71 ± 0.6b</td>
<td>–</td>
<td>15.8 ± 1.08a</td>
<td>6.52 ± 1.30b</td>
<td>9.29 ± 1.1b</td>
<td>–</td>
</tr>
<tr>
<td>N stock (Mg/ha)</td>
<td>0.68 ± 0.03b</td>
<td>0.29 ± 0.01A</td>
<td>0.71 ± 0.06b</td>
<td>–</td>
<td>1.32 ± 0.07a</td>
<td>0.79 ± 0.14b</td>
<td>0.68 ± 0.09b</td>
<td>–</td>
</tr>
<tr>
<td>C/N</td>
<td>14.10 ± 0.27a</td>
<td>14.97 ± 0.66A</td>
<td>12.73 ± 0.39b</td>
<td>–</td>
<td>11.87 ± 0.3c</td>
<td>8.10 ± 0.86b</td>
<td>13.71 ± 0.31ab</td>
<td>–</td>
</tr>
</tbody>
</table>
The lower $R_s$ values in grazed sites cannot adequately be explained by simple changes in microclimate even though temperature and water availability have been found to be the most important factors controlling autotrophic and heterotrophic respiration (Mielnick and Dugas 2000; Raich and Schlesinger 1992). In our data set, $R_s$ was correlated with temperature and moisture, but not always and not consistently so. Higher temperatures resulted in reduced $R_s$ rates across the entire data set with the negative correlation somewhat stronger in the grazing treatments. However, the relationship between temperatures and $R_s$ was inconsistent across seasons. In Mediterranean and semiarid ecosystems, soil moisture often plays a more important role in controlling $R_s$ especially during the warmer growing season. Results found during our experiment are in agreement with other authors in Mediterranean systems (Hussain et al. 2009; Ma et al. 2007). Soil moisture conditions can constrain the $R_s$ responses to $T_s$ and may account for large differences in $R_s$ between wet and dry periods (Davidson et al. 2000; Olsen and Van Miegroet 2010; Sulzman et al. 2005). Only during cold moist periods did $T_s$ emerge as an important positive driver of $R_s$ indicated by the $Q_{10}$, which ranged from 2.5 to 6. It is interesting to note that the undisturbed locations (NGNT) showed the highest sensitivity of $R_s$ to $T_s$ change, while this sensitivity declined with disturbance through either tilling or grazing.

The lower $R_s$ values found in the grazed treatment may be caused by lower inputs of decomposable soil organic matter (SOM) compared to the non grazed sites associated with export by grazing animals (Baron et al. 2002) and/or changes in plant species composition (Biondini et al. 1998; Raiesi and Asadi 2006; Taddese et al. 2002; Yates et al. 2000). In combination, the change in quality and quantity of litter input may have decreased soil microbial activity in the grazed sites. The reduction in belowground translocation of carbohydrates from removed photosynthetic tissue could also be responsible for the reduction on soil root respiration in grazed places (Bremer et al. 1998). As well, herbage removal has been noted to result in considerable reduction in weight of roots (Johnson and Matchett 2001) and thus in root exudates, which probably contributed to lower $R_s$ in grazed treatment.

We cannot ascribe the grazing induced changes in $R_s$ to soil compaction, as our result did not shown any change in soil bulk density after grazing. In general, the changes in bulk density due to grazing depend on their intensity and soil characteristics.
Light to moderate livestock grazing on well drained pastures (Greenwood and McKenzie 2001), rangelands (Gifford and Hawkins 1978; Trimble and Mendel 1995) and semiarid steppe (Steffens et al. 2008) contributes little to long-term overall soil compaction as measured by soil bulk density or water infiltration rate. Heavy grazing (Linnartz et al. 1966; Mapfumo et al. 1999) or grazing on wet soils (McNabb et al. 2001; Proffitt et al. 1993) often reduces water infiltration rates and soil moisture status, reduces air-filled porosity, and increases soil bulk density, and surface soil hardness (Jia et al. 2007).

On the other hand, our results show an average increase of 3 Mg/ha of C and 0.3 Mg/ha of N stocks due grazing effects, consistent with results found by different authors in similar ecosystems (Bauer et al. 1987; Conant et al. 2005; Chaneton and Lavado 1996; Reeder and Schuman 2002; Schuman et al. 1999; Simón et al. 2012). However other author observed no changes (Barger et al. 2004; Binkley et al. 2003) or decreases of soil C and N stocks (Abril and Bucher 1999; Frank 2005; Neff et al. 2005; Raiesi and Asadi 2006; Steffens et al. 2008) in response to grazing intensities. These differences in the responses of soil C and N stocks to grazing, possibly result from differences in climate, soil characteristics, landscape position, vegetation community, grazing management practices (Milchunas and Lauenroth 1993; Reeder and Schuman 2002); seasonal grazing frequency, intensity and duration (Reeder and Schuman 2002). In addition, variability in livestock defecation and urination effects (Stark et al. 2000), or in soil sampling methodology could account for the different findings among reported studies (Schuman et al. 1999).

In addition to soil microclimate and C stock changes, the observed reduction of $R_s$ in the grazed sites could also be related to differences in SOM characteristics (Reeder et al. 2004). Raisei and Asadi (2006) found a decline in labile SOM due to grazing activities. Furthermore, Brady and Weil (2008) and Follett et al. (2001) found that water holding capacity in grazed sites of grassland ecosystems produces more stable aggregates of SOM, converting SOM in the grazing treatments into more stable, less decomposable soil C and N reservoirs. From our results, we conclude that light grazing in our dehesa ecosystem did not have negative effects on the soil C and N reservoir capacity.
5.4.2 Tillage influences

The superficial tillage utilized in this experiment caused slight responses in $R_s$ that were significant only during two of the measurement times and differed with time of the year. The decrease in $R_s$ in November 2008 and increase in February 2010, coincided with similar variations in $T_s$ in those months, combined with somewhat higher $M_s$ content, again indicating the importance of $M_s$ in this ecosystem (Joffre and Rambal 1988). The pronounced increase of $R_s$ values in February 2010 in the tilled sites might be caused by the high precipitation during that month, where the lower values on $R_s$ in non tilled treatment may have resulted from restriction of the soil macroporosity by the precipitations, reducing soil air-filled pore space and respiration (Ball et al. 1999).

When all data were considered, tilling had no significant effect on $R_s$, a result also found by other authors (Alvarez et al. 1998; Ball et al. 1999; Reicosky and Lindstrom 1993) in similar ecosystems. Other studies have described higher $R_s$ values under no-till than under ploughed soils (Franzluebbers et al. 1995; Jackson et al. 2003; Kessavalou et al. 1998) related to labile C accumulation in no-till soil and the subsequent C mineralization (Alvarez et al. 1998). In other situations, the lower $R_s$ values occurred in non tilled soils (Alvarez et al. 2001; Álvaro-Fuentes et al. 2007; Bono et al. 2008; Ellert and Janzen 1999; Morell et al. 2010; Reicosky et al. 1997) have been attributed to a more intense C turnover in plowed soils (Franzluebbers 2002) or a slower residue decomposition rate under no-till (Fortin et al. 1996).

Our data reveal that tilling decreases C and N stocks on average by 3.5 Mg/ha and 0.3 Mg/ha, respectively, with stock declines due to tilling most pronounced in the grazed sites. The observed interactions between grazing and tilling treatment could indicate that positive grazing effect on C and N stocks might counteract the negative tilling effect on C and N stocks, where both treatment coincide. Bono et al. (2008) also reported a higher level of soil C for the no-till treatment than for the tillage treatment up to 15-cm depth. The superficial tilled most likely expose soil aggregates which, together with increases in $M_s$, cause a significant initial loss of organic matter. This loss was not detected in $R_s$ changes due the time elapsed between tilling and $R_s$ measurements. Lopez-Garrido et al. (2009, 2011) reported reductions in soil C stocks in the first year after tillage and increases two years later. They related the initial C loss to an increase in
microbial activity and $R_s$ resulting from increased aggregate destruction and exposure with soil inversion.

The absence of significant differences in $B_d$ (g/cm$^3$) between non tilled and tilled treatments may be related with the sandy texture of dehesa soil, the shallow depth of ploughing, and the lag time between application and soil measurements.

Although different authors have reported increases in soil organic C under conservation tillage (reduced-minimum tillage, no-tillage) in Spain (Álvaro-Fuentes et al. 2008; Bescansa et al. 2006; Hernanz et al. 2002; López-Bellido et al. 1997), our observations show a slightly negative influence of superficial tilling on soil C and N stocks in dehesa systems.

5.4.3 Tree and grazing interactions

The low $R_s$ average values ($\mu$mol m$^{-2}$s$^{-1}$) in open areas relative to those under tree canopy agree with findings in a dehesa system (Casals et al. 2009) and in a Mediterranean forest (Almagro et al. 2009) in Spain. The values of the soil C and N stocks and C/N ratio were similar to those found by other authors in dehesa ecosystem in Spain (Casals et al. 2009; Moreno and Obrador 2007; Moreno et al. 2007; Schuman et al. 1999; Simón et al. 2012). The greater accumulation of C and N in organic matter below the tree canopy (Breman and Kessler 1995; Gallardo 2003; Gallardo et al. 2000; Sharrow and Ismail 2004) could explain the higher $R_s$. Several authors have shown an improvement in fertility and nutrient availability beneath tree canopy (Escudero 1985; Gallardo 2003; Gallardo and Merino 1998; Gallardo et al. 2000; Joffre and Rambal 1993; Menezes et al. 2002; Moreno and Obrador 2007) caused by differences in biomass production (Gea-Izquierdo et al. 2009) and a change in microbial community Saetre and Baath (2000) between below canopy and open areas.

Grazing decreases the differences in $R_s$ between below canopy and open areas in the dehesa. Animals attracted to tree shelter could contribute more to the redistribution of organic matter and nutrients in the soil profile. No tree effects were detected in grazed sites in a Spanish dehesa (Casals et al. 2009). Thus, it can be difficult to separate tree canopy effects from grazing effects on soil properties in silvopastural systems.
The presence of a tree canopy affected soil microclimate in our experiment, with changes in $T_\text{s}$ more evident changes in $M_\text{s}$. Lower $T_\text{s}$ beneath canopy was found by Moreno et al (2007, 2005) in similar ecosystem, and has been attributed to the shading effect of the tree canopy (Gea-Izquierdo et al. 2009). However in the coldest months (November 2008, December 2008 and February 2010) the highest $T_\text{s}$ values were found beneath canopy, likely because the vegetation cover reduced heat loss from the soil. The effect of trees on $M_\text{s}$ is variable and depends on the ecosystem type. Our observed decrease in $M_\text{s}$ under canopy is in agreement the study results by Cubera and Moreno (2007) and Gea et al. (2009) in the same ecosystem; however, they contradict the higher soil moisture under canopy found in the proximity of trees by Gallardo et al. (2003).

5.5 CONCLUSIONS

Results from this study show that light grazing and superficial tilling practices used in the studied dehesa system in Spain had a slight but non-consistent impact on soil respiration and soil microclimate during the study period. Moreover, divergent responses in soil C and N stocks to grazing or tilling management were observed, with grazing increasing and tilling reducing soil C and N stocks. Furthermore, the grazing and tilling effect on the $R_\text{s}$ response to $T_\text{s}$ and $M_\text{s}$ depended on the climatic period. Soil moisture was important in constraining the temperature sensitivity of soil respiration, indicating that water availability is one of the major ecological factors in dehesa system.

An important contribution of tree canopy ($Quercus ilex$, L) was observed on soil respiration, soil microclimate as well as soil C and N stocks storage ability. This reflects the relevance of conservation of woodland and forestation (since natural regeneration is usually absent or scarce) in order to maintain the sustainability and improve C and N reservoir potential in dehesa ecosystem.

The conclusions of this study reveal that grazing and tree canopy had a positive influence in the ability to storage soil C and N stocks while superficial tilling show a negative effect on soil C and N store capacity in this system. Thus, maintaining the beneficial practices and improving tilling applied in this area may have important consequences in carbon sequestration capacity in this dehesa system.
5.6 ACKNOWLEDGEMENTS

This research was conducted in the framework of the Spanish SUM2006-00034; CGL2006-02922 and CGL2009-07031. The authors would like to express their gratitude to agricultural research center team “Dehesón del encinar” and especially Celia López Carrasco for her support. We also appreciate the assistance of Dr. Agustín Rubio and Dr. Eugenio Diaz-Pines.

5.7 REFERENCES


Chapter 5

Pasture, tillage and canopy effects on carbon dioxide fluxes in a Spanish dehesa


General Discussion
GENERAL DISCUSSION

Due to the uncertainties associated with the estimation of greenhouse gas fluxes and soil sink capacity both at regional and global scale, the development of data bases of trace gas fluxes for different ecosystems, which could be used to parameterize and validate biogeochemical models, has become increasingly necessary (Parton et al. 2001; Potter et al. 1996). Within this context, the objective of this thesis is to contribute towards furthering our knowledge of greenhouse gas exchange between forest and agroforestry soils and the atmosphere in Mediterranean ecosystems. Particular emphasis has been placed on soil carbon dioxide flux in order to determine the potential carbon storage capacity of the soil in the studied ecosystems. Hence, soil respiration and its interaction with physical-chemical and biological soil parameters in different Mediterranean ecosystems has been analysed.

Understanding the spatial and temporal variability of soil CO₂ efflux (soil respiration, Rₛ) within an ecosystem is critical to the improvement of modeling tools and affords us a clearer understanding of ecosystem behavior, thus allowing the likely consequences of climatic change to be predicted with greater accuracy. High spatial and temporal variability in soil respiration has been reported (Janssens et al. 2003a; Raich et al. 2002; Xu and Qi 2001) in different ecosystems throughout the world, although numerous factors may underlie such variability, such as differences in species composition, stand age, management practices, climatic and edaphic conditions, perturbations etc. Spatial variability in soil respiration occurs at various scales, from a few square centimeters to several hectares up to global scale. It is necessary to understand the spatial variability of soil CO₂ efflux within small geographical areas in order to make a representative estimate of the regional C budget. This involves establishing natural gradient studies (e.g., latitudes, altitudes, topography, and successional ages), to examine variations in soil respiration (Simmons et al. 1996, Conant et al. 1998; Rodeghiero and Cescatti 2005; Schindlbacher et al. 2010). However, identifying the environmental factors which control the variability of Rₛ continues to be a task plagued with uncertainties, especially in the case of forest soils in mountainous regions. Temporal and spatial changes are particularly important since they affect the numerous factors that directly or indirectly influence soil respiration. Changes in soil microclimate, soil respiration and the response of soil respiration to temperature
conditions were observed in a small Mediterranean forest catchment in the Valsaín mountains (Chapter 2), where there was a marked spatial effect along the elevation gradient; both soil respiration values and apparent $Q_{10}$ and soil moisture ($M_s$) increasing with altitude whereas soil temperature ($T_s$) values decreased (Garten Jr and Hanson 2006; Rodeghiero and Cescatti 2005; Wang et al. 2010). The influence of soil moisture on the $R_s$ in a small catchment depends on the site. In some plots, the temporal variation of $R_s$ was mainly controlled by soil moisture, while in others the interaction between $T_s$ and $M_s$ explained most of the variability observed. The elevation factor alone may not explain the $R_s$ sensitivity. The topography produces spatially heterogeneous $M_s$ and therefore soil respiration. In the Valsain study (Chapter 2), more mesic north-facing slopes showed higher $R_s$ relative to less mesic south-facing slopes, despite higher $T_s$ on south-facing slopes. In addition to soil temperature and moisture, soil carbon and nitrogen stocks as well as soil microbial biomass carbon (C-MBT) regulate soil respiration and may contribute towards explaining differences among sites. Temporal changes were also observed during the experiment, indicating that seasonal variation in soil respiration was mainly controlled by soil temperature and moisture. In the wetter seasons with milder temperatures, the highest values for soil respiration, apparent $Q_{10}$ and soil moisture were observed, while lower soil respiration and apparent $Q_{10}$ values were observed during the hotter, drier seasons.

Another of the challenging questions addressed in this thesis is the manner in which GHG exchange responds to disturbances. Forest ecosystems are subjected to natural and anthropogenic disturbances, which can lead to substantial changes in the ecosystems affected. These changes may have an impact on GHG dynamics as regards exchanges between soils and the atmosphere. Fire is one of the main forms of ecosystem disturbance in Mediterranean forest ecosystems (Lavorel et al. 1998; Naveh 1990; Syphard et al. 2009) and there is concern that climate change may alter the frequency and intensity of forest fires (Conard and Solomon 2008; IPCC 2007; Westerling et al. 2006). Therefore, it is becoming increasingly important to identify the effects of wildfires on the characteristics of forest soils and on soil-atmosphere GHG exchange. Six to eight years after a severe wildfire, the forest ecosystems studied in Chapters 3 and 4 present alterations in carbon dioxide ($CO_2$), nitrous oxide ($N_2O$) and methane ($CH_4$) exchange between soils and the atmosphere.
The long-term effects of wildfires on soil CO₂, N₂O and CH₄ fluxes depend on soil characteristics, forest site and seasonal climatic conditions. Several changes were detected in the physical, chemical and biological soil characteristics in burned-forest areas during the experiment, as reported in previous studies (Boone et al. 1998; Borken et al. 2006). These changes involved a reduction in exchangeable cations, pH and fine roots, alterations in the mineralogical composition and changes in SOC quality (indicated by a drop in the C/N ratio and labile carbon (DOC; C-MBT) and a relative increase in refractory SOC forms (a shift in the HA/FA composition)). Additionally, soil temperatures were higher and soil moisture content lower. The impact of wildfires on tree vegetation as well as on the physical and chemical soil parameters is addressed in Chapter 3, as are the main causes underlying the reduction in annual C loss (10–43 %) through soil respiration at burned sites.

The decreased post-fire C/N ratio is a phenomenon frequently cited for several types of post-fire soils (Fernández et al. 1999; González-Perez et al. 2004; Ryu et al. 2009). Although no statistically significant differences in SOC were observed between burned (B) and non burned (NB) sites in this thesis, the differences in the C/N ratio may be due to differences in the C and N consumption and/or differences in the recovery of C vs. N pools, the fire causing a disproportionate loss in N compared to C pools (Certini 2005; Neary et al. 1999). The lower pH in B plots accompanied by a depletion in exchangeable bases may be related to greater erosion of the ash layer or leaching by rainwater (Ulery et al. 1993). The decrease in soil pH in B plots may also encourage the growth and proliferation of soil microbes and affect both soil respiration and Q₁₀ values since soil pH regulates not only chemical reactions but also a number of enzymes in microorganisms which are pH-dependent (Luo and Zhou 2006).

The lack of post-fire plant recovery at burned sites caused a decline in root biomass (less autotrophic respiration), and carbon inputs (e.g litterfall), which subsequently lowered microbial populations (less heterotrophic respiration) and overall soil respiration. Furthermore, the shift in SOC quality at burned sites from more easily decomposable forms towards more recalcitrant SOC compounds (which are more resistant to chemical and biological breakdown), might contribute to the formation of stable SOC pools. It may also partially explain the fact that burned sites tend to emit less CO₂.
The different parameters used this study (Chapter 3) to characterize SOC quality indicate a preferential loss of more labile SOC and a greater preponderance of recalcitrant SOC forms, including a decline in DOC and C-MBT and a shift in the HA/FA composition. The increase in SOC recalcitrance is indicated by the HA (formed by condensed structures) accumulation in the superficial layer of B soils, which are considered an index of humification intensity (Certini 2005; Van Miegroet and Olsson 2011). The increment in the HA/FA ratio after fire could be due to the decrease in FA fractions that were transformed into an acid-insoluble macromolecule (HA) by aromatization and condensation phenomena (Vergnoux et al. 2011). The E₄/E₆ ratios obtained in this study (3.8 to 4.8) are all similar and characteristic of humic acids (Khan and Schnitzer 1972; Kononova 1962). However it is difficult to interpret the differential post-burn responses in terms of fire-induced changes in humification as fire influence on the labile fractions of SOC depends on fire type and severity (Choromanska and DeLuca 2002; Walstad et al. 1990) as well as on the time elapsed since the fire (Boerner et al. 2004; Döckersmith et al. 1999; Gundale et al. 2005; Rutigliano et al. 2007).

The size of the soil microbial community (Dilustro et al. 2005; Inclán et al. 2010) was lower at the B sites, presenting C-MBT values (138-280 mg/kg) similar to those previously reported (Chapter 2). Lower C-MBT values for long-term post-fire soils have also been reported by Dumontet et al.(1996) and Litton et al.(2003). This lower C-MBT at B compared to NB sites suggests a reduced heterotrophic contribution to total soil respiration, explaining part of the Rs diminution (Mabuhay et al. 2006). Furthermore, the lower fine root biomass content in B plots might have contributed to the observed declines in Rs in B plots, associated with the removal of trees by fire and lack of post-fire re-establishment of trees in B plots (Inclán et al. 2007; Irvine et al. 2007; Litton et al. 2003; Ryu et al. 2009; Sullivan et al. 2011).

The Q₁₀ values and the correlation between Rs and Ts obtained for NB plots (Chapter 3) were higher than for B plots when all the data were considered, revealing that Rs displays less sensitivity to Ts following fire. This lower sensitivity might be related to the loss of labile fraction of SOC ((Hernández et al. 1997; Marschner and Kalbitz 2003; Van Miegroet and Olsson 2011) combined with lower Ms conditions (Conant et al. 2004; Liski et al. 1999) in the B plots.
The absence of trees and the presence of shrub vegetation in the burned areas indicates lower C assimilation capacity in comparison to non-burned areas and implies that B sites continue to be a C source 5-8 years after the fire. Several authors state that the post-fire forest recovery rate is slow (Dore et al. 2008; Savage and Mast 2005) and it is likely that the burned forest areas studied in Chapter 3 continue to act as C sources and will not shift to C sinks until the forest has fully regenerated.

The impact of fire on the fluxes of N\textsubscript{2}O and CH\textsubscript{4} differed from one ecosystem to another and from one season to another (Chapter 4). A clear effect of fire on soil N\textsubscript{2}O fluxes was only observed in the *Quercus pyrenaica* (QP) forest, where the decreases in N\textsubscript{2}O fluxes in burned areas may be related to the significant reduction in the soil microbial population due to fire, in combination with changes in vegetation cover, soil moisture and clay content. The absence of post-fire differences in soil N\textsubscript{2}O flux for the other ecosystems studied could be related to the time elapsed since the fire (the most recent fire being that of the *Quercus ilex* (QI) forest), because there may be a temporal transient increase in N content. Furthermore, in the case of the *Pinus sylvestris* (PS) burned plot, the N input resulting from the invasion of the site by the N-fixing leguminous plant *Adenocarpus hispanicus* following the fire may have replaced the lost N. In other forest and woodland soils, the presence of N fixing vegetation has been shown to increase soil N\textsubscript{2}O production (Fest et al. 2009; Pérez et al. 2004).

The burned sites showed higher CH\textsubscript{4} oxidation in *Q. ilex* stands, and lower oxidation rates in *P. sylvestris* stands. The increases in CH\textsubscript{4} uptake cumulative fluxes in the *Quercus ilex* stand might be related to the increases in the soil factors that favored soil aeration (high proportion of coarse particles and lower soil moisture), which in turn improve the methane oxidation. In *Pinus sylvestris* stands, the decrease in CH\textsubscript{4} uptake may be due to the increase in inorganic N in the soil as a consequence of the N-fixing species present in this plot. Increasing NH\textsubscript{4}\textsuperscript{+} content in the soil should lead to a reduction in methanotrophic activity because NH\textsubscript{3} may be a competitive substrate for methane monooxygenase (Castaldi and Fierro 2005). Low CH\textsubscript{4} oxidation values in PSB could also be explained by the low pH values observed in this plot. Negative effects of pH on CH\textsubscript{4} oxidation were noted by Hütsch et al.(1994). However, no clear differences were found in the *Quercus pyrenaica* forest between burned and unburned areas, probably due to organic N stability after fire, despite the declining microbial population.
in the soil at burned sites. Additionally, the combination of higher temperature, less structured soil and smaller quantities of soil nutrients could be detrimental to the methanotrophic population of these soils and their CH$_4$ oxidation capacity.

Mean fluxes of CO$_2$, N$_2$O and CH$_4$ were highest in Scots pine and Pyrenean oak stands (Chapter 3 and 4). The differences identified between the GHG emissions from the different forest soils studied can most likely be explained by differences in soil properties and soil moisture. Differences in N$_2$O efflux among species (Chapter 4) were also identified in terms of the quantity of C and N on the forest floor, which can be explained by interspecific differences in litter production and quality (Finzi et al. 1998; Pilegaard et al. 2006). Furthermore, shifts in the dominant vegetation type may alter soil C availability as a result of differences in the quantity and quality of plant detritus inputs (Carrera et al. 2007), particularly in semiarid ecosystems where soil organic C and N pools are small. Our study revealed a negative correlation between log-N$_2$O total mean emission and the C/N ratio, which is in accordance with the findings of Pilegaard et al. (2006). A similar negative relationship has been identified between net nitrification and C/N ratio in forests throughout Europe (Persson et al. 2000). In PS stands, where N$_2$O fluxes were higher, the low pH values could favour N$_2$O production from both autotrophic and heterotrophic nitrifiers (Martihainen and Boer 1993). The average values obtained during the experiment showed that Rs were positively related with total SOC stocks and C-MBT (Chapter 3). The results agree with those of Priess and Fölster (2001), who highlighted the relationship between the decomposition process and hence microbial respiration and increased SOC. Various authors suggest that soil C may be one of main determinants of Rs, particularly at large spatial and temporal scales (Davidson and Janssens 2006; Giardina and Ryan 2002; Ryan and Law 2005). However, during dry months, the response mechanism of Rs to water scarcity will cause changes in the correlations between Rs and C-MBT (Borken and Matzner 2009). CH$_4$ absorption increases with soil DOC content and C/N ratio whereas it decreases with clay content (Chapter 4). CH$_4$ uptake appears to be strongly affected by soil texture (Born et al. 1990) and soil moisture (Borken et al. 2000) due to their effect on the diffusion velocities of CH$_4$ and O$_2$ (Potter et al. 1996 a).

The soil GHG exchange in the forest ecosystem studied in Chapter 3 and 4, showed a clear seasonal variation. The higher Rs values found in spring and in autumn, as in
other Mediterranean ecosystems (Almagro et al. 2009; Asensio et al. 2007; Inclán et al. 2007; Inclán et al. 2010; Joffre et al. 2003), could be due to the optimal soil temperature and soil moisture conditions that stimulate both plant and microbial activity during these seasons (Dickmann et al. 1996; Xu and Baldocchi 2004).

The strongest correlation between $R_s$ and $T_s$ in the ecosystems studied in Chapter 3, were obtained in winter and autumn in all the forest areas and the weakest correlation was found in summer. $T_s$ and $M_s$ explained most of the variation in $R_s$, but their relative roles changed according to the season. A similar pattern has been observed in numerous other studies conducted in seasonally dry areas (Almagro et al. 2009; Asensio et al. 2007; Inclán et al. 2010; Marañón-Jiménez et al. 2011; Olsen and Van Miegroet 2010; Rey et al. 2002; Xu and Qi 2001). $M_s$ had two, very different effects on $R_s$ depending on the season: When $M_s$ was greater than 5%-10%, during the wet seasons, $R_s$ responded positively to $T_s$ and negatively to $M_s$. However, when $M_s$ was below this threshold during dry season, $R_s$ responded with a weaker correlation (or negatively) to $T_s$. High water content can slow down the diffusion of $O_2$, which impedes decomposition and $CO_2$ production, while low soil water content can inhibit soil microbial activity and root respiration (Davidson et al. 1998). $Q_{10}$ exhibits a seasonal pattern regardless of site, with $Q_{10}$ values consistently greater in winter and autumn. A number of authors also point to $T_s$ and $M_s$ as important explanatory factors for the seasonal variation in $Q_{10}$ (Borken et al. 1999; Cernusak et al. 2006; Conant et al. 1998; Davidson et al. 2000). Janssens and Pilegaard (2003) further stated that $Q_{10}$ is not simply a reflection of temperature sensitivity, but rather expresses the combined response to fluctuations in temperature, root biomass, moisture conditions, and perhaps other variables that directly or indirectly have been affected by fire (Boone et al. 1998). The $Q_{10}$ model explained part of the variation in soil respiration in each ecosystem, but the application of a linear model that includes soil moisture allowed a clearer understanding of the effects of $T_s$, $M_s$ and wildfires on $R_s$.

The forest soils (Chapter 4) were weak $N_2O$ emitters and temporary sinks for atmospheric $N_2O$ fluxes (Castaldi et al. 2006; Rosenkranz et al. 2006). Seasonal differences indicated that in wet months, $N_2O$ fluxes were high and in dry months $N_2O$ fluxes declined. The parameters that best explain the seasonal variation in $N_2O$ fluxes in our forest ecosystems were soil moisture and previous day’s rainfall, followed by soil
General Discussion

Hourly N\textsubscript{2}O fluxes in all ecosystems are negatively correlated with soil temperature and positively correlated with soil moisture, following a pattern typical to seasonally dry ecosystems and underlining the fact that soil water content is a limiting factor in N\textsubscript{2}O fluxes. The dry and warm summer conditions favor soil aeration and are therefore thought to be unfavorable for N\textsubscript{2}O production by denitrification (Castaldi 2000) or nitrifier denitrification. Moreover, these conditions limit the activity of soil microorganisms, thus reducing the amount of N cycled in the ecosystem, which in turn could limit the rate of both nitrification and denitrification. The significant increase in N\textsubscript{2}O fluxes in wet months observed in our study might be explained, at least in part, by the positive correlation found between N\textsubscript{2}O fluxes and the previous day’s rainfall. The first rainfall on dry soil produces pulses of N\textsubscript{2}O, caused by the accumulation of inorganic N in dry soils and the reactivation of water-stressed bacteria upon wetting, which then metabolize the available inorganic N (Davidson 1993). The increment in NO\textsubscript{3}\textsuperscript{−} detected in the experiment might also explain the increases observed in N\textsubscript{2}O fluxes during these months. This increment might be due to N-deposition (Butterbach-Bahl et al. 2002), or NO\textsubscript{3}\textsuperscript{−} accumulation by microbial N immobilization, limited by C availability (Dividson et al. 1990).

The net negative N\textsubscript{2}O fluxes measured in our study fall within the range reported in the literature on forests (see Chapter 4). Most N\textsubscript{2}O uptake has been reported to occur under conditions of low mineral nitrogen availability and high soil moisture (Chapuis-Lardy et al. 2007), during denitrifying processes, which mainly occur under prevailing anoxic conditions (in high soil moisture conditions), and could explain the negative fluxes observed in our experiment under these soil conditions. The sink effect observed in the summer months has been reported in a number of previous studies (Rosenkranz et al. 2006, Goldberg and Gebauer 2009), although the underlying causes have not yet been identified. Negative fluxes in Mediterranean forest soils were linked to very low N availability and high soil C content, considering aerobic denitrification by heterotrophic nitrifiers as a possible pathway (Rosenkranz et al. 2006), and also to high temperatures and low soil moisture content.

The forest soils in the ecosystems studied in the area surrounding Madrid (Chapter 4) were mostly significant sinks for atmospheric CH\textsubscript{4} although they were net sources of
Greenhouse gas flows in forest and agroforestry soils in the center of the Iberian Peninsula

CH₄ in wet months. Well aerated soils are known to be significant sinks for atmospheric CH₄ (Merino et al. 2004; Rosenkranz et al. 2006; Castaldi and Fierro 2005; Castaldi et al. 2006). The shift from CH₄ sink to CH₄ source coincided with the periods of higher soil respiration and soil water content. In medium and light-textured, well-aerated soils this shift has been linked to the formation of anaerobic microsites in zones of intense microbial activity facilitated by the presence of easily decomposable fresh organic matter and a sufficiently high water content in the soil such that O₂ consumption by respiration occurs at a faster rate than O₂ diffusion from the surrounding areas, thus reaching anaerobic conditions and CH₄ production (Butterbach-Bahl et al. 2002; Castaldi et al. 2006; Castaldi and Fierro 2005; Sullivan et al. 2008). Although the ecosystems studied in Chapters 3 and 4 appeared to act as methane sinks, the C sequestered through CH₄ is insignificant in comparison to the C emitted through soil respiration.

The use of appropriate silvopastoral practices in dehesa systems in Spain can contribute to their sustainability, improve soil quality and productivity, and reduce the CO₂ flux from the soil to the atmosphere. The type, frequency, intensity and timing of these management practices (tillage, grazing etc.) may, along with environmental controls, modify the Rₛ dynamics in dehesa systems. Understanding the response of Rₛ to management is crucial to our ability to predict the impact of current management regimes on regional C exchange, and to establish strategies to help mitigate greenhouse gas emissions (Chapter 5).

In the work undertaken in the Dehesón del Encinar, when all the experimental data was considered, grazing was found to decrease Rₛ values according to the differences obtained for a month belonging to the hot dry period, coinciding with cattle grazing. Decreases in Rₛ with grazing have been reported in previous studies (Bremer et al. 1998; Cao et al. 2004; Raiesi and Asadi 2006) of similar ecosystems. Most of the lower Rₛ values at grazed sites cannot adequately be explained by simple changes in microclimate, even though temperature and water availability have been identified as the most important factors controlling autotrophic and heterotrophic respiration (Mielnick and Dugas 2000; Raich and Schlesinger 1992). In our data set, for example, Rₛ was correlated with temperature and moisture, although not always and not consistently so. As previously highlighted in this discussion, in Mediterranean and
semiarid ecosystems, soil moisture often plays a more important role in controlling $R_s$, especially during the warmer growing season (Hussain et al. 2009; Ma et al. 2007). Soil moisture conditions can constrain the $R_s$ response to $T_s$ and may account for large differences in $R_s$ between wet and dry periods (Davidson et al. 2000; Olsen and Van Miegroet 2010; Sulzman et al. 2005). Only during cold moist periods did $T_s$ emerge as an important positive driver of $R_s$, as indicated by the $Q_{10}$. It is interesting to note that the undisturbed locations (NGNT) showed the highest sensitivity of $R_s$ to $T_s$ change, while this sensitivity declined with disturbance through either tilling or grazing.

The lower $R_s$ values found in the grazed treatment may be caused by lower inputs of decomposable soil organic matter (SOM) compared to the non-grazed sites associated with export by grazing animals (Baron et al. 2002) and/or changes in plant species composition (Biondini et al. 1998; Raiesi and Asadi 2006; Taddese et al. 2002; Yates et al. 2000). In combination, the change in quality and quantity of litter input may have decreased soil microbial activity at the grazed sites. Also, the reduction in belowground translocation of carbohydrates from removed photosynthetic tissue could also be responsible for the reduction in soil root respiration at grazed sites (Bremer et al. 1998). Furthermore, it has been reported that herbage removal results in a considerable reduction in the weight of roots (Johnson and Matchett 2001) and therefore in root exudates, which probably contributed to lower $R_s$ in the grazed treatment.

An average increase of 3 Mg/ha of C and 0.3 Mg/ha of N stocks due to the effects of grazing in the studied dehesa is consistent with results reported by various authors for similar ecosystems (Bauer et al. 1987; Conant et al. 2005; Chaneton and Lavado 1996; Reeder and Schuman 2002; Schuman et al. 1999; Simón et al. 2012). This can be explained by the decline in labile SOM due to grazing activities (Raiesi and Asadi 2006), along with an increase in more stable aggregates of SOM and less decomposable soil C and N reservoirs (Brady and Weil 2008; Follett et al. 2001).

The superficial tillage employed in the experiment led to slight responses in $R_s$, which were only significant at two of the measurement times. Moreover, when all data were considered, tilling was found to have no significant effect on $R_s$, a result supported by the findings of other authors for similar ecosystems (Alvarez et al. 1998; Ball et al. 1999; Reicosky and Lindstrom 1993). Our data reveal that C and N stocks decrease on average by about 3.5 Mg/ha and 0.3 Mg/ha respectively as a result of tilling; the most
pronounced declines in stock (due to tilling) occurring at grazed sites. The interactions observed between grazing and tilling treatments appear to indicate that the positive effect of grazing on C and N stocks might counteract the negative effect of tilling where both treatments coincide. Decreases in soil C stocks after tillage were also reported by Bono et al. (2008) and Lopez-Garrido et al. (2009, 2011) who related the initial C loss to an increase in microbial activity and R, resulting from increased aggregate destruction and exposure due to soil inversion. However, this loss was not detected in the R, changes in our experiment, probably due to the time elapsed since tilling and R, measurements.

The low R, average values obtained in open areas of the Dehesa relative to those under tree canopy agree with findings by (Casals et al. 2009) in a Dehesa system and those of Almagro et al. (2009) for a Mediterranean forest in Spain. Similarly, the values for soil C and N stocks and C/N ratio were similar to those reported by other authors in dehesa ecosystems in Spain (Casals et al. 2009; Moreno and Obrador 2007; Moreno et al. 2007; Schuman et al. 1999; Simón et al. 2012). The greater accumulation of C and N in the form of organic matter below the tree canopy (Breman and Kessler 1995; Gallardo 2003; Gallardo et al. 2000; Sharrow and Ismail 2004), the improvement in fertility and nutrient availability (Escudero 1985; Gallardo 2003; Gallardo and Merino 1998; Gallardo et al. 2000; Joffre and Rambal 1993; Menezes et al. 2002; Moreno and Obrador 2007) and in biomass production beneath the tree canopy (Gea-Izquierdo et al. 2009), in conjunction with changes in the microbial community (Saetre and Bååth 2000) could explain the higher R, detected in the experiment. Grazing decreases the differences in R, between below-canopy and open areas in the Dehesa. Animals attracted to tree shelter could further contribute to the redistribution of organic matter and nutrients in the soil profile. No tree effects were detected at grazed sites in a Spanish Dehesa (Casals et al. 2009). Thus, it can be difficult to separate tree canopy effects from grazing effects on soil properties in silvopastoral systems.

The results of this thesis highlight the sensitivity of greenhouse gas fluxes (CO2, N2O and CH4) to changes in microclimate, soil organic component and soil parameters in the studied forests and agroforestry systems, not only as a result of natural conditions but also due to the impact of wildfires, land use and management practices. Different responses of GHG emission to climatic shifts affect the carbon sequestration capacity.
and C and N dynamics of these ecosystems. It is hoped that the information obtained through this research will contribute towards improving our understanding of the dynamics and balance of C in Mediterranean systems, and help predict the impact of climate change on the exchange of C between forest and agroforestry ecosystems and the atmosphere.

REFERENCES


Luo Y and Zhou X 2006 Soil Respiration and the Environment. San Diego, CA.


Syphard A D, Radeloff V C, Hawbaker T J and Stewart S I (2009) Conservation Threats Due to Human-Caused Increases in Fire Frequency in Mediterranean-Climate Ecosystems


General conclusions
GENERAL CONCLUSIONS

- The spatial and temporal changes in soil respiration observed in a small Mediterranean pine forest in the center of Spain, highlighted the necessity of including changes in soil parameters over small scales when modelling landscape level soil respiration.

- C flux from soils is altered in the long-term by wildfires due to their impact not only on tree vegetation but also on the physical and chemical parameters of the soil in *Quercus ilex, Quercus pyrenaica* and *Pinus sylvestris* forests near Madrid. Soil chemical and biological factors, such as fine root biomass, soil microbial biomass, C/N and SOC quality co-vary with soil temperature and explain the decreases in soil respiration at burned sites in these ecosystems.

- The different responses of C emission to climatic shifts many years after the occurrence of wildfire highlight the need to include these shifts in C dynamics in future research in order to more accurately assess the C balance in Mediterranean forests and better predict the impact of climatic change on the exchange of C between forest ecosystems and the atmosphere.

- Forest soils in the area surrounding Madrid were mostly weak N₂O emitters and significant sinks for atmospheric CH₄. The nutrient status in forest soils had an effect on the fluxes of N₂O and CH₄. Mean fluxes of N₂O and CH₄ were highest in Scots Pine and Pyrenean oak stands. A weak N₂O uptake from the atmosphere into the soil was observed in fall and summer. A shift from CH₄ sink to CH₄ source was observed in the wetter months. Seasonal variations in N₂O and CH₄ were mainly related to soil water availability.

- The impact of fire on the fluxes of N₂O and CH₄ differed from one ecosystem to another and from one season to another. The burned sites showed higher CH₄ oxidation in *Q. ilex* stands, and lower oxidation rates in *P. sylvestris* stands. Fire decreases N₂O fluxes in *Q. pyrenaica* stands. The impact of fire on CH₄ and N₂O fluxes appears to depend heavily on climatic seasonal patterns, ecosystem type and the main soil characteristics.
- Silvopastoral practices such as light grazing and superficial tilling, both of which are used in the studied dehesa system, have a slight but non-consistent impact on soil respiration and soil microclimate.

- Different responses to grazing or tilling management were observed in the soil C and N reservoir capacities in the Dehesa system studied, where light grazing practices improved the soil C and N stocks while tilling practices reduced them.

- The presence of a tree canopy of *Quercus ilex* species had an important effect on soil respiration, soil microclimate and soil C and N reservoir capacities, reflecting the importance of woodland conservation in the maintenance and sustainability of the Dehesa ecosystem, thereby improving their potential as C and N reservoirs.

- In the Mediterranean and semiarid ecosystems studied, soil moisture plays an important role in the response of soil respiration to soil temperature. Soil respiration is largely controlled by soil temperature, but only if sufficient soil moisture is available. Hence, soil moisture is critical to C dynamics and C source/sink strength in Mediterranean ecosystems.
Agradecimientos
AGRADECIMIENTOS

A pesar de los sentimientos de desazón que inevitablemente afloran a lo largo de una tesis doctoral, realizarla supone un reto personal importante del que se aprende y madura a todos los niveles, además del científico. El camino recorrido a lo largo de esta tesis no hubiera sido posible sin el apoyo de los que más quiero, de la cantidad de gente buena que he encontrado en el camino y de los momentos pretéritos en soledad y compartidos que han hecho que esto valga muchísimo la pena.

GRACIAS a los compañeros de proyecto y trabajo del CIEMAT María Ángeles Clavero, Ramón Morante, Ana Mª Fernández, Ana Melón, Ana Cardeña, Miguel Sánchez, Rosa Pérez, Oscar Ballesteros, Manolo Fernández y David Manrique, a tod@s ell@s las GRACIAS por la colaboración y ayuda en este trabajo. Gracias especiales a Lola por la dedicación, el entusiasmo y el buen humor dedicado en todos los procesos de este hijo científico. GRACIAS a todos los estudiantes y becarios que han participado de alguna manera en los distintos estados de esta tesis por su valioso trabajo, a Carlos, Mercedes, Lucía, Laura, Pedro y Raúl. GRACIAS también a mis compañero@s del grupo de ecotoxicología Nacho, Hector, Rocío, Viki, Susana, Isaura, por los ánimos en la recta final, los ratitos de descanso compartidos y sobre todo las rosquillas de Javier que venían al dedillo siempre😊.

GRACIAS al personal de la ETSI de Montes, al Dr. Alfredo Blanco por su participación y siempre buena disposición. Al Dr. Agustín Rubio tutor de la tesis, por toda la ayuda recibida durante la elaboración de la misma. Y la Dra Sonia Roig por habernos dado la oportunidad de participar en el proyecto del Dehesón.

GRACIAS también a los diferentes gestores de los lugares en que se ha realizado la investigación en Campo. Agradecer a Javier Donés y Marisol Redondo del Área de Conservación del Centro de Montes y Aserradero de Valsaín por toda la ayuda prestada para la realización de las investigaciones del Capítulo 2 de esta Tesis. A Belén Vacas del Área de Conservación de Montes de la Comunidad de Madrid su colaboración para posibilitar los trabajos en el Monte de Pinus sylvestris nº40 de los Molinos, Madrid. A Alfonso Huidobro de Patrimonio Nacional por su colaboración en la investigación en el Monte de la Herrería, de El Escorial; agradecer también a los Guardas Forestales así como a los responsables de jardines de la Herraria por todo el apoyo prestado. A José
Antonio Oyarburu, por su generosidad al habernos permitido trabajar en la finca El Canto del Pico; agradecer también al guarda de la finca Ángel (que en paz descansse) su paciencia infinita estando siempre dispuesto a abrirnos la puerta a cualquier hora del día. A Celia López y el personal del Dehesón del encinar. También a Ricardo Ruiz del Departamento de Selvicultura y Gestión de Sistemas Forestales del INIA por su ayuda con el cálculo de los stocks de carbono. Y a Antonio Vallejo y Laura Sánchez por su ayuda y colaboración en el capítulo 4 de la Tesis. No hubiera sido posible este trabajo sin todo el apoyo prestado.

GRACIAS a mi directora Rosa Inclán por haberme dado la oportunidad de realizar esta tesis y adentrarme en el mundo científico, por su confianza, ayuda y apoyo a lo largo de la misma. GRACIAS a Helga Van Miegroet codirectora de esta tesis por su entusiasmo, eficacia y colaboración en este trabajo y por haberme dado la posibilidad de hacer una estancia inolvidable en Utah.

GRACIAS al Dr. Robert Jandl por su disposición siempre a colaborar, por la invitación al Centro de Investigación Forestal de Austria (BFW), su simpatía y por presentarnos a Helga!

GRACIAS a todos los amig@s y compañer@s con los que he tenido el placer de compartir este tiempo, Clara, Kike Mariquilla, Natalia, Lucía, Kiko, Elena, Cristina, Luisillo, Alberto, Henar, Cristobal, Irene....etc. No me imaginaba viviendo en una ciudad tan ciudad como Madrid y sin embargo ha sido una época inolvidable. Me siento sumamente afortunada y AGRADECIDA por todo lo vivido y aprendido. Gracias a tod@s y mucha suerte en vuestros proyectos vitales, especialmente aquell@s que debido a la situación actual habéis tenido que salir fuera. Y a ti Natali, suerte en tu nuevo proyectazo de mamá con Candela, ojala todo salga en casa lo bien que tiene que salir.

GRACIAS a Marco y Dani por la ayuda con el formato de la tesis que se iba y venía pa to los laos cada vez que tocaba el índice, vaya murga os he dado y cuanta paciencia habéis tenido; Marquito aunque no has estado físicamente presente durante este tiempo, te he imaginado muchas veces como Pepito Grillo, dando ánimos y fuerzas como siempre haces, eres un crack y un gran amigo. GRACIAS por ser como eres. Danielo tú tienes un arte que se refleja en todo lo que haces y encima siempre estás dispuesto a
echar una mano, muchas gracias niño. Mucha SUERTE a los dos en lo que hagáis, que todo os salga lo bien que merecéís.

GRACIAS especiales a Montse y May, ha sido una suerte encontrarnos y compartir hogar con vosotras durante 3 añitos inolvidables. Vaya momentos bonitos y divertidos que nos hemos pegado en la prospe. Gracias por hacer de público del DEA a las tantas de la noche y por soportar los momentos “pico de sierra” de la tesis tan bien. Vuestra amistad es uno de los mejores regalos que me llevo de esta etapa.

GRACIAS familia, los que estás cerca y los que no pero daís fuerza a la distancia. Gracias tía Rosa por los ánimos y empuje en la recta final de la tesis cuando creía que se acababan las fuerzas y me recordabas que ya no quedaba na, y a ti prima Adri por el calorcito y la alegría que traes siempre contigo; a Lilian y Pablito por sumaros al hogar. Es genial compartir con todos vosotr@s lo bueno y lo malo también.

GRACIAS Padre por el amor y respeto transmitido por la vida y los seres con los que compartimos este planeta a mis herman@s y a mi. El amor por la Ciencia y el Medioambiente te lo debo a ti. A ti madre, por esa mezcolanza tan bonita que tienes de ternura y fuerza con la que nos has educado, tu confianza en nosotros es siempre un REGALO y tu ejemplo un motor de empuje. Gracias a mi hermano Pablo por su continua preocupación y apoyo incluso a control remoto y a su compañera Sini por cuidarlo y quererlo y haber traído a Inkeri, la LUZ de la casa; a mi hermana Nandi por ser un ejemplo de tesón y coraje y esforzarse tanto para llegar a ser lo que siempre ha querido, mucha suerte en este inicio de vida profesional hermana, estoy segura que serás una gran veterinaria.GRACIAS familia por vuestro apoyo incondicional, vuestro amor y vuestro ejemplo. Teneros es una verdadera FORTUNA. Este trabajo está especialmente dedicado a vosotros.
Annexes
CONTENT

8.1 ANEXE I: Sketch maps of experimental devices........................................ 233
8.2 ANEXO II: Profile description table (pit) ................................................... 239
8.3 ANEXO III: Study areas location............................................................... 240
8.1 ANEXE I: Sketch maps of experimental devices

EL ESCORIAL
PARCELA NO QUEMADA

Leyenda

- Acumulador
- Arbol
- Desfronde
- Hobbo

Calicata
Camara Gases
Cerco

Escala 1:110
EL ESCORIAL
PARCELA QUEMADA

Leyenda

- Acumulador
- Pluviometro
- Desfronnde
- Hobbo
- Calicata
- Camara Gases
- Cerco
- Dosimetros

Escala 1:100
Greenhouse gas flows in forest and agroforestry soils in the center of the Iberian Peninsula
LOS MOLINOS
PARCELA NO QUEMADA

Leyenda

- Acumulador
- Arbol
- Desfronde
- Hobbo
- Calicata
- Camara Gases
- Cerco

Escala 1:125
TORRELODONES
PARCELA NO QUEMADA

Leyenda

- Acumulador
- Arbol
- Desfronde
- Hobbo
- Calicata
- Camara Gases
- Cerco

Escala 1:100
TORRELODONES
PARCELA QUEMADA

Leyenda

- Acumulador
- Pluviometro
- Desfrontería
- Hobbo
- Calicata
- Camara Gases
- Cerco
- Dosimetrometros

Escala 1:100
8.2 ANEXO II: Profile description table (pit)

**Table 8.2.1. Quercus ilex forest ecosystem**

<table>
<thead>
<tr>
<th>Horizon</th>
<th>Depth</th>
<th>SOM%</th>
<th>OC%</th>
<th>N%</th>
<th>C/N</th>
<th>pH</th>
<th>Fe$_2$O$_3$</th>
<th>K (ppm)</th>
<th>Moisture%</th>
<th>Fine soil%</th>
<th>Clays%</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non burned</td>
<td>A</td>
<td>0-23</td>
<td>3.80</td>
<td>2.21</td>
<td>0.27</td>
<td>8.32</td>
<td>5.70</td>
<td>0.79</td>
<td>30.7</td>
<td>3.3</td>
<td>45</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Aw1</td>
<td>23-40</td>
<td>1.37</td>
<td>0.80</td>
<td>0.14</td>
<td>5.83</td>
<td>5.82</td>
<td>0.71</td>
<td>30.3</td>
<td>1.6</td>
<td>36</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Aw2</td>
<td>40-62</td>
<td>1.55</td>
<td>0.90</td>
<td>0.14</td>
<td>6.24</td>
<td>5.92</td>
<td>0.44</td>
<td>25.2</td>
<td>1.6</td>
<td>41</td>
<td>5</td>
</tr>
<tr>
<td>Burned</td>
<td>A</td>
<td>0-23</td>
<td>1.40</td>
<td>0.80</td>
<td>0.14</td>
<td>5.75</td>
<td>5.56</td>
<td>0.68</td>
<td>22.1</td>
<td>1.8</td>
<td>45</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Bw1</td>
<td>23-48</td>
<td>0.86</td>
<td>0.50</td>
<td>0.11</td>
<td>4.51</td>
<td>5.54</td>
<td>0.87</td>
<td>20.2</td>
<td>2.6</td>
<td>46</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Bw2</td>
<td>48-62</td>
<td>0.63</td>
<td>0.37</td>
<td>0.09</td>
<td>3.96</td>
<td>5.49</td>
<td>0.7</td>
<td>12.5</td>
<td>2.0</td>
<td>22</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>67-97</td>
<td>0.37</td>
<td>0.22</td>
<td>0.10</td>
<td>2.21</td>
<td>5.79</td>
<td>0.44</td>
<td>14.0</td>
<td>2.8</td>
<td>32</td>
<td>7</td>
</tr>
</tbody>
</table>

**Table 8.2.2 Quercus pyrenaica forest ecosystem**

<table>
<thead>
<tr>
<th>Horizon</th>
<th>Depth</th>
<th>SOM%</th>
<th>OC%</th>
<th>N%</th>
<th>C/N</th>
<th>pH</th>
<th>Fe$_2$O$_3$</th>
<th>K (ppm)</th>
<th>Moisture%</th>
<th>Fine soil%</th>
<th>Clays%</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non burned</td>
<td>Au1</td>
<td>0-20</td>
<td>7.85</td>
<td>4.56</td>
<td>0.42</td>
<td>10.68</td>
<td>6.58</td>
<td>0.42</td>
<td>50.4</td>
<td>3.0</td>
<td>62</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>20-51</td>
<td>2.40</td>
<td>1.40</td>
<td>0.19</td>
<td>7.25</td>
<td>6.83</td>
<td>0.51</td>
<td>60.4</td>
<td>1.8</td>
<td>54</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>E/Bs</td>
<td>51-75</td>
<td>0.76</td>
<td>0.45</td>
<td>0.13</td>
<td>3.38</td>
<td>6.68</td>
<td>0.35</td>
<td>77.7</td>
<td>1.4</td>
<td>69</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Bs</td>
<td>75-105</td>
<td>0.73</td>
<td>0.42</td>
<td>0.11</td>
<td>3.62</td>
<td>6.16</td>
<td>1.02</td>
<td>39.4</td>
<td>1.8</td>
<td>75</td>
<td>8</td>
</tr>
<tr>
<td>Burned</td>
<td>Au1</td>
<td>0-22</td>
<td>3.92</td>
<td>2.28</td>
<td>0.27</td>
<td>8.20</td>
<td>5.9</td>
<td>1.02</td>
<td>27.7</td>
<td>1.8</td>
<td>59</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Au2</td>
<td>22-40</td>
<td>3.63</td>
<td>2.12</td>
<td>0.18</td>
<td>11.42</td>
<td>5.8</td>
<td>0.82</td>
<td>17.9</td>
<td>2.6</td>
<td>32</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Bw1</td>
<td>40-68</td>
<td>3.53</td>
<td>2.05</td>
<td>0.18</td>
<td>10.85</td>
<td>5.78</td>
<td>1.04</td>
<td>19.2</td>
<td>2.5</td>
<td>39</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Bw2</td>
<td>68-98</td>
<td>2.80</td>
<td>1.65</td>
<td>0.20</td>
<td>8.20</td>
<td>5.88</td>
<td>0.95</td>
<td>18.6</td>
<td>1.8</td>
<td>49</td>
<td>7</td>
</tr>
</tbody>
</table>

**Table 8.2.3. Pinus sylvestris forest ecosystem**

<table>
<thead>
<tr>
<th>Horizon</th>
<th>Depth</th>
<th>SOM%</th>
<th>OC%</th>
<th>N%</th>
<th>C/N</th>
<th>pH</th>
<th>Fe$_2$O$_3$</th>
<th>K (ppm)</th>
<th>Moisture%</th>
<th>Fine soil%</th>
<th>Clays%</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non burned</td>
<td>A</td>
<td>0-20</td>
<td>6.88</td>
<td>4.00</td>
<td>0.33</td>
<td>11.85</td>
<td>5.14</td>
<td>0.31</td>
<td>14.2</td>
<td>3.4</td>
<td>68</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Bw</td>
<td>20-45</td>
<td>2.27</td>
<td>1.32</td>
<td>0.19</td>
<td>6.83</td>
<td>5.17</td>
<td>0.36</td>
<td>13.8</td>
<td>2.6</td>
<td>53</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Bw/C</td>
<td>45-75</td>
<td>0.46</td>
<td>0.27</td>
<td>0.13</td>
<td>1.95</td>
<td>5.57</td>
<td>0.41</td>
<td>7.5</td>
<td>2.2</td>
<td>73</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>75-105</td>
<td>0.28</td>
<td>0.16</td>
<td>0.08</td>
<td>1.89</td>
<td>5.38</td>
<td>0.71</td>
<td>5.1</td>
<td>2.2</td>
<td>92</td>
<td>7</td>
</tr>
<tr>
<td>Burned</td>
<td>A</td>
<td>0-25</td>
<td>4.25</td>
<td>2.47</td>
<td>0.36</td>
<td>6.76</td>
<td>4.33</td>
<td>0.71</td>
<td>18.8</td>
<td>2.1</td>
<td>73</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Bw</td>
<td>25-46</td>
<td>1.18</td>
<td>0.69</td>
<td>0.13</td>
<td>4.96</td>
<td>4.6</td>
<td>0.93</td>
<td>6.1</td>
<td>1.2</td>
<td>71</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Bw/C</td>
<td>46-75</td>
<td>0.39</td>
<td>0.23</td>
<td>0.08</td>
<td>2.66</td>
<td>4.69</td>
<td>0.71</td>
<td>10.0</td>
<td>1.3</td>
<td>60</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>75-105</td>
<td>0.43</td>
<td>0.25</td>
<td>0.71</td>
<td>0.35</td>
<td>4.75</td>
<td>0.97</td>
<td>11.2</td>
<td>1.4</td>
<td>58</td>
<td>8</td>
</tr>
</tbody>
</table>
8.3 ANEXO III: Study areas location

Figure 8.3.1 Location of the experimental plots along an elevation gradient in Valsaín Forest (Chapter 2); (a) Orthoimage geometric perspective, 3D representation; (b) 2D representation. (ArcMap). Geometric Process and Cartography designed by Raul Martinez Garrido.
Figure 8.3.2 Location of the experimental study areas in Sierra de Guadarrama, Madrid (Spain) (Chapter 3 and 4). Geometric Process and Cartography designed by Raul Martinez Garrido.
Figure 8.3.2 Location of the experimental treatments in *El Dehesón del Encinar* (Oropesa, Toledo) (Chapter 5). Geometric Process and Cartography designed by Raul Martinez Garrido