UNIVERSITÀ DEGLI STUDI DELLA TUSCIA

Dipartimento di Scienze dell'Ambiente Forestale e delle sue Risorse

DOTTORATO DI RICERCA

in

ECOLOGIA FORESTALE XVIII CICLO

AGR 05

Responses of soil biological processes to elevated atmospheric [CO_2] and nitrogen addition in a poplar plantation

Candidato

ALESSANDRA LAGOMARSINO

Tutori

Prof. PAOLO DE ANGELIS

Prof. STEFANO GREGO

Coordinatore

Prof. Paolo De Angelis

INDEX

1. GENERAL CONTEXT OF RESEARCH

AND STATE OF THE ART

1.1 The global carbon balance and the increase of atmospheric CO_2 concentration

The global C biogeochemical cycle consists of four principal C pools that are important in the time frame of decades to centuries: atmosphere, oceans, reserves of fossil fuels, and terrestrial ecosystems, including vegetation and soils. (fig. 1.1; Houghton, 2003).

The oceanic and the geological pools are the largest, with 38,000 and 5000-10000 Pg C (1Pg = petagram = 1 x 10^{15} g) respectively. Most of the oceanic carbon is in intermediate and deep waters; only 700–1,000 PgC are in the surface layers of the ocean, that part of the ocean in direct contact with the atmosphere. The C present in reactive ocean sediments has a slow turnover, and sediments are not generally considered as part of the active, or short-term, carbon cycle, although they are important in determining the long-term concentration of CO_2 in the atmosphere and oceans.

The terrestrial biosphere includes the soil C and the biotic pool of vegetation. The amount of carbon contained in the living vegetation of terrestrial ecosystems (550 Pg) is somewhat less than that present in the atmosphere (780 Pg). Soils contain 2–3 times that amount in the top meter as soil organic C (SOC). In addition to the SOC there also exists soil inorganic carbon (SIC) pool estimated at 695 to 748 Pg to 1–meter depth (Batjes, 1996; Eswaran et al., 1995). The SIC pool is an important constituent of the soils of the arid and semiarid regions and similar to the SOC, the dynamics of SIC pool is also influenced by soil degradation and anthropogenic perturbations (Lal and Kimble, 2000).

Each year the atmosphere exchanges ~ 120 Pg C with terrestrial ecosystems through photosynthesis and respiration. The uptake of carbon through photosynthesis is the gross primary production (GPP). At least half of this production is respired by the plants (autotrophic respiration) leaving a net primary production (NPP) of ~ 60 Pg C/year (recent estimates of global terrestrial NPP vary between 56.4 Pg C/year and 62.6 Pg C/year; Saugier et al., 2001). Most of the NPP is consumed by animals or respired by decomposer organisms in the soil (heterotrophic respiration). A smaller amount (~ 4 Pg C/year globally) is oxidized through fires. In steady state the net flux of carbon between terrestrial ecosystems and the atmosphere (net ecosystem production (NEP)) is approximately zero, but year-to year variations in photosynthesis and respiration (including fires) may depart from this long-term balance by as much as 5–6 Pg C/year.

Since the industrial age, human activities are perturbing the climate/biogeochemical systems and in particular the global carbon cycle directly by changes in carbon fluxes and indirectly through changes in atmospheric chemistry.

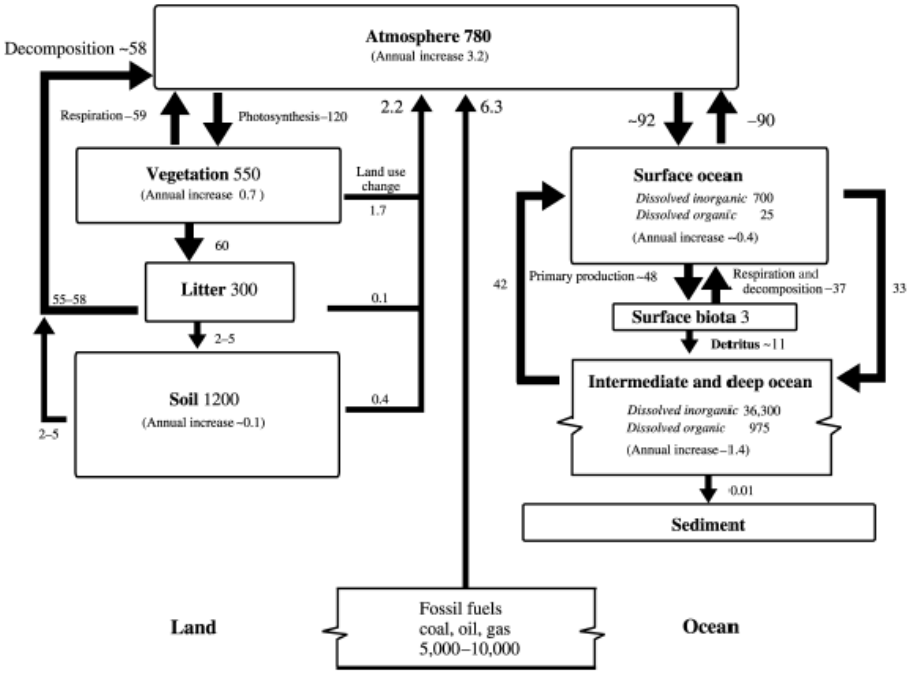

Figure 1.1. The contemporary global carbon cycle. Units are Pg C or Pg C yr^{-1} (Houghton, 2003).

The importance of atmospheric CO_2 concentration and the other greenhouse gases (GHGs) on the global climate changes is related to the influence of the radiation balance by permitting the short wave radiation to enter the Earth's atmosphere but capturing a fraction of the long wave radiation emitted by the Earth. This imbalance is expressed in terms of "radiative forcing", which is defined as the change in net irradiance (w m^{-2}) at the tropopause after allowing the stratospheric temperatures to readjust to radiative equilibrium (Ramaswamy, 2001). The radiative forcing of GHGs has led to an increase in the average global surface temperature of 0.6 °C since the late 19th century (Folland and Karl, 2001). Consequently, changes in the amount, distribution and intensity of rainfall/precipitation are also expected to occur (IPCC, 2001).

Annual emissions of CO_2 from fossil fuel combustion are small relative to the natural flows of carbon; nevertheless, these anthropogenic emissions are the major contributor to increasing concentrations of CO_2 in the atmosphere and above all they represent a transfer

of carbon from the slow carbon cycle to the active carbon cycle. From 1850 to 1998, 270 ± 30 Pg C were emitted from fossil fuel burning and cement production (Marland et al., 1999). During the same period, emissions from land use change are estimated at 136 ± 55 Pg C (Houghton et al., 1999). These last were related to deforestation, biomass burning, conversion of natural to agricultural systems and the ploughing of soils; world soils historically have been major source of atmospheric enrichment of CO_2: until the 1950s more C was emitted into the atmosphere from land use change and soil cultivation than from fossil fuel combustion (Lal, 2003). Presently, about 20% of the global emissions come from land use change (IPCC, 2001).

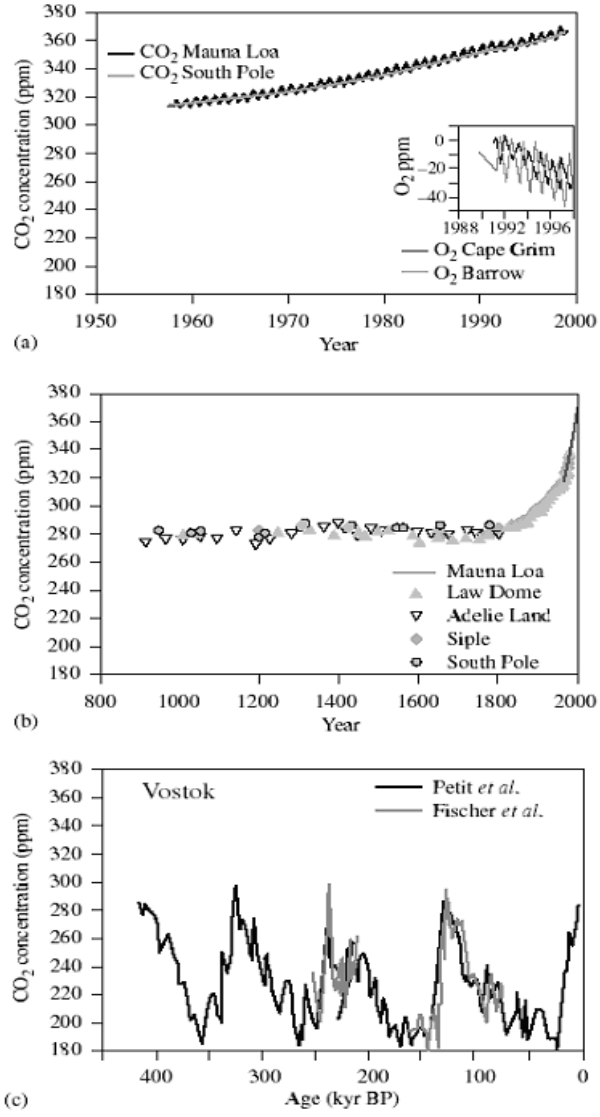

Figure 1.2. Concentration of CO_2 in the atmosphere: a) from 1960 to 2000, b) over the last 1000 years, and c) over the last 4 x 10^5 years. IPCC, 2001.

The concentration of CO_2 in the atmosphere has increased from 280 parts per million (ppm) in 1750 to 367 ppm in 1999 and currently is increasing at the rate of 1.5 ppm/year or 3.3 Pg/year (IPCC, 2001, fig. 1.2). Ice cores from Greenland and Antarctica show that the pre-industrial concentration of CO_2 was between 275 ppm and 285 ppm and over the last 1,000 years the concentration of CO_2 in the atmosphere has varied by less than 10 ppm (IPCC, 2001; Fig. 1.2(b)). The increase between 1750 and 2000, therefore, has been ~ 85 ppm, equivalent to ~ 175 PgC, or 30% of the pre-industrial level.

Recently the anthropogenic emissions included 5.4 ± 0.3 Pg C/year by fossil fuel combustion and cement manufacture and 1.7 ± 0.8 Pg C/year by land use change for the decade of 1980s, while 6.3 ± 0.4 Pg C/year by fossil fuel combustion and cement manufacture and 1.6 ± 0.8 Pg C/year by land use change for the decade of 1990s (IPCC, 2001). The increase in atmospheric CO_2 concentration, however, occurred at the rate of 3.3 ± 0.2 Pg C/year for the 1980s and of 3.2 ± 0.1 Pg C/year for the 1990s (Prentice, 2001). Actually, in the 1980s the absorption by ocean was 2.0 ± 0.8 Pg C/year and the residual terrestrial sink, also called "missing sink", was 1.9 ± 1.3 Pg C/year, while in the 1990s the absorption by ocean was 2.3 ± 0.8 Pg C/year and the terrestrial sink was 2.3 ± 1.3 Pg C/year (tab. 1.1).

	1980 - 1990 Pg C/year	1990 – 1999 Pg C/year
Anthropogenic CO_2 emissions		
From Fossil Fuel and Cement	5.4 ± 0.3	6.3 ± 0.4
From Land Use Change	1.7 ± 0.8	1.6 ± 0.8
Allocation in C pools		
Atmospheric increase	3.3 ± 0.2	3.2 ± 0.1
Ocean uptake	- 2.0 ± 0.8	- 2.3 ± 0.8
Terrestrial sink	- 1.9 ± 1.3	- 2.3 ± 1.3

Table 1.1. Global C budgets for the 1980s and 1990s. Negative values indicate a withdrawal of CO_2 from the atmosphere. From IPCC, 2001.

Direct evidence for the importance of terrestrial metabolism (photosynthesis and respiration) can be seen in the effect it has on the atmospheric concentration of CO_2 (Fig. 1.2 (a)).

The cause of the oscillation is the metabolism of terrestrial ecosystems and the seasonal cycling of photosynthetic activity of land plants. The highest concentrations occur at the end of each winter, following the season in which respiration has exceeded photosynthesis and thereby caused a net release of CO_2 to the atmosphere. Lowest concentrations occur at the end of each summer, following the season in which photosynthesis has exceeded respiration and drawn CO_2 out of the atmosphere. The latitudinal variability in the amplitude of this oscillation suggests that it is driven largely by northern temperate and boreal ecosystems (IPCC, 2001).

These data indicate clearly that the carbon cycle is out of equilibrium as a result of human activities and that the terrestrial sink wiil have an influence on the atmospheric accumulation. The capacity of ecosystems to store C depends on the balance between NPP and heterotrophic respiration and it is evident that several interrelated feedbacks are occurring. Current understanding suggests that the primary direct ecosystem response to increase CO_2 concentration is an increase of NPP due to a "CO_2 fertilization effect" which is potentially a negative feedback on atmospheric CO_2 concentrations. Terrestrial NPP is not saturated by present atmospheric CO_2 concentrations. Consequently, rising levels of atmospheric CO_2 concentration may cause profound changes in the structure and function of terrestrial ecosystems and there is a large potential for forests to act as sinks for atmospheric CO_2 (Schimel, 1995). The mechanisms thought to be responsible for the terrestrial sink have included factors that enhance growth, such as CO_2 fertilization, nitrogen deposition, and the differential effects of climate variability on photosynthesis and growth relative to respiration and decay. Changes in land use may also lead to terrestrial C sinks through the regrowth of forests following agricultural abandonment or harvest, but the net effect of land-use change is estimated to have released C globally and, thus, does not explain the net terrestrial sink (Houghton et al., 2003).

1.2 Forest management for climate change mitigation

The intent of any mitigation option is to reduce atmospheric CO_2 relative to that which would occur without implementation of that option. Biological approaches to curb the increase of atmospheric CO_2 can occur by one of three strategies (IPCC, 1996):

conservation: conserving an existing C pool, thereby preventing emissions to the atmosphere; sequestration: increasing the size of existing carbon pools, thereby extracting CO_2 from the atmosphere; and substitution: substituting biological products for fossil fuels or energy-intensive products, thereby reducing CO_2 emissions.

The benefits of these strategies show contrasting temporal patterns. Conservation offers immediate benefits via prevented emissions. Sequestration impacts often follow an S-curve: accrual rates are often highest after an initial lag phase and then decline towards zero as C stocks approach a maximum. Substitution benefits often occur after an initial period of net emission, but these benefits can continue almost indefinitely into the future.

The general goal of sequestration activities is to maintain ecosystems in the sink phase. However, if the system is disturbed (a forest burns or is harvested, or land is cultivated), a large fraction of previously accumulated C may be released into the atmosphere through combustion or decomposition. When the system recovers from the disturbance, it re-enters a phase of active carbon accumulation.

Forest ecosystems constitute a major terrestrial C reservoir containing over 60% of all C stored in the terrestrial biosphere and more than 85% of the total plant C on the earth and between 60-70% of the total soil C (Dixon et al., 1994). Within the light of the changing global carbon balance, there is a large potential for forests to act as sinks for atmospheric CO_2 either through reforestation or through the enhanced tree growth rates (Ceulemans et al., 1999). It has been argued that conservation of forests by using good selvicultural practice and through tree planting can enhance strongly the C sink provided by terrestrial ecosystems (Baral, 2004).

Actually the Kyoto Protocol provides to take into account afforestation, reforestation and other forest activities in meeting CO_2 reduction targets and afforestation can be used by Annex I (industrial) countries to meet stipulated emissions reduction targets (IPCC, 2000). Increasing the amount of carbon held in forests might be achieved through at least six management options (Houghton, 1996; Kohlmaier et al., 1998): i) a reduction in the rate of deforestation (current rates of deforestation in the tropics are responsible for an an-

nual release of 1–2 Pg C); ii) an increase in the area of forests (afforestation and reforestation); iii) an increase in the stocks of carbon within existing forests; iv) an increase in the use of wood (including increased efficiency of wood harvest and use); v) the substitution of wood fuels for fossil fuels; and vi) the substitution of wood for more energy intensive materials, such as aluminium, concrete, and steel. Estimates of the amount of carbon that might be sequestered on land over the 55-year period 1995–2050 range between 60 Pg C and 87 Pg C (1–2 Pg C/year on average) (Brown, 1996).

Nabuurs *et a*l. (2000) estimated the potential of a broad range of forest-related activities (including protection from natural disturbance, improved silviculture, savannah thickening, restoration of degraded lands, and management of forest products) at 0.6 Pg C/yr over six regions in the temperate and boreal zone (Canada, USA, Australia, Iceland, Japan, and EU, fig. 1.3). According to their estimates, alternative forest management for C sequestration is technically feasible on 10% (on average) of the forest area in each region examined.

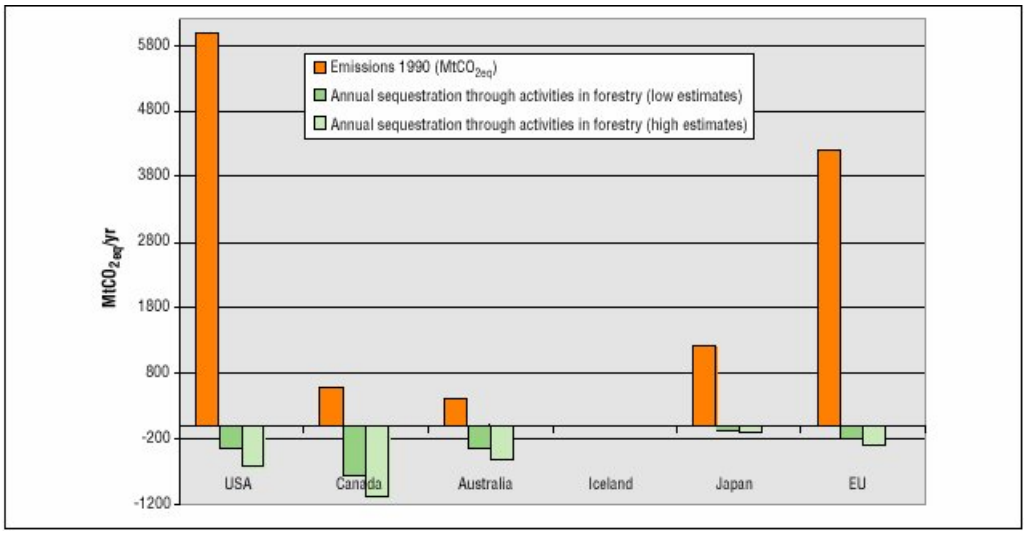

Figure 1.3: Indications of the magnitude of the carbon sink in case study countries for a set of forest management measures. The values for the three bars for Iceland are 2.6, 2.8, and 2.9, respectively. The figure is based on the forest part of the model "Access to Country Specific Data" (ACSD). IPCC, 2001

Managed forests and plantation are increasing in importance occupying today 130 Mha, with annual rates of establishment of 10.5 Mha (FAO, 1995). Agroforestry systems, as well as many natural tree based ecosystems, are perceived to improve or maintain soil fer-

tility and productivity, to promote soil conservation, reduce soil degradation and achieve sustainable production.

In particular in the future short-rotation forestry (SRF) may play an increasing role as a source of renewable energy and could be very important for yield estimates on the one hand and for C sequestration on the other hand (Zsuffa et al., 1996). Using short rotation forests might reduce C emissions in two ways. One is direct carbon sequestration by reforestation and afforestation that yields a stock of carbon in standing trees. The other is the use of forest products as substitutes for fossil fuels or fossil fuel intensive goods such as steel and concrete. In both instances, carbon offset occurs by preventing the emissions from fossil fuels, which would otherwise have been used (Baral, 2004). Furthermore the soil C pool may change following afforestation of arable land and while C sequestration occurs more slowly in soil than in biomass, C stored in soils would be more resistant to sudden changes in forest management than C stored in biomass. It is therefore essential to include changes in soil C estimates of C sequestration due to afforestation (Vesterdal et al., 2002).

Fig. 1.4 shows the pools and fluxes of carbon in an even-aged, single species plantation that is periodically harvested and replanted. Trees take atmospheric CO_2 during photosynthesis and fix it in woody (branches, stems, and woody roots) and non-woody (foliage and fine roots) parts. At the end of each rotation, a portion of biomass is transferred to wood products, or energy feedstock, that release carbon back to the atmosphere upon combustion, another portion could be stored in soil for longer term.

The wood products eventually decay to release the carbon either by aerobic or anaerobic decomposition. The forest floor receives dead woody and nonwoody litter continuously as tree components die because of natural mortality throughout each rotation and also at each thinning and harvest. A portion of litter is decomposed by microorganisms into CO_2 and the remaining litter is transferred to soil organic matter.

The amount of carbon that can be sequestered by forests depends on the biomass accumulation rate, rotation length, and the accumulation in litter and soil organic matter. For SRF, yields largely depend on genotype, cultural practices and site quality (Graham et al., 1992). Among SRF, silver maple, sweetgum, American sycamore, black locust, poplars, and eucalyptus have been identified as potential SRF species.

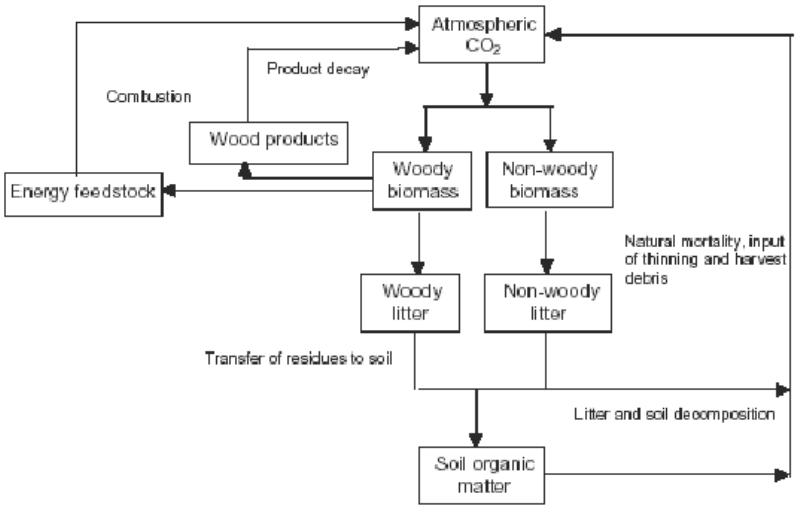

Figure 1.4. Carbon flows and pools in forests, which are regularly harvested and planted (Dewar and Cannel, 1992)

Hybrid poplar and eucalyptus have shown the greatest potential for very high growth rates in the USA. Because of their very fast growth rate and high productivity *Populus* species are especially well suited for plantation culture (Gielen and Ceulemans, 2001). The success of *Populus* is mainly due to the easy of vegetative propagation, fast growth, wide inter-specific crossability and plasticity and in the future SRF using poplar may play an increasing role as a source of renewable energy (Zsuffa et al., 1996).

The response of trees to the increase of atmospheric CO_2, will be crucial in determining the ability of woody plantations and natural forests to sequester C at the global scale (Jarvis, 1998). It remains unclear the implications of below-ground C allocation for long-term carbon storage in soil C pools with slow turnover: the response of trees and forests to enhanced [CO_2] will ultimately depend on the interactions connecting the different organisms that compose the complex trophic webs of such system (Scarascia Mugnozza et al., 2000).

To quantify accurately the effect of changes in forest management on the net transfer of C to the atmosphere, the whole system should be considered. Many earlier studies on SRF focused on the immediate results of forest management measures, e.g. the higher biomass growth rate following a silvicultural treatment or the protected stock of C if wildfire or logging is prevented. Global assessments based on these studies have limitations. Estimates, in terms of tC/ha or tC/ha/yr, leave unanswered the critical questions of the timing, security, and sustainability of these effects. Recently, more comprehensive studies indicate the importance of complete accounting for the whole C flows in and out of the system and

the analysis of long-term patterns. For example, Schlamadinger and Marland (1996) showed that the positive effect of short-rotation plantations for fossil fuel substitution is less than implied by the simple substitution of fossil fuels, because of the continued input of fossil fuels needed to operate the system. While the limitations of earlier studies are now evident, data for comprehensive analysis at the global scale are not yet available.

1.3 The role of soil in the global carbon balance and its potential to store carbon

The importance of soil in the global C balance depends on its potential to store C in pools with a slow turnover: it must be considered that the capacity of ecosystem C sequestration is strongly regulated by the residence time of C in terrestrial pools, that is defined as the time of C remaining in an ecosystem from entrance via photosynthesis to exit via respiration (Luo et al., 2001). If photosynthetically fixed C is largely cycled through fast pathways, e.g. root respiration and root turnover, the capacity of an ecosystem to store C is small. If fixed C is largely cycled through slow pathways with decadal or longer residence times, such as woody biomass and, mostly, soil organic matter (SOM), the C sequestration capacity is large (fig. 1.5).

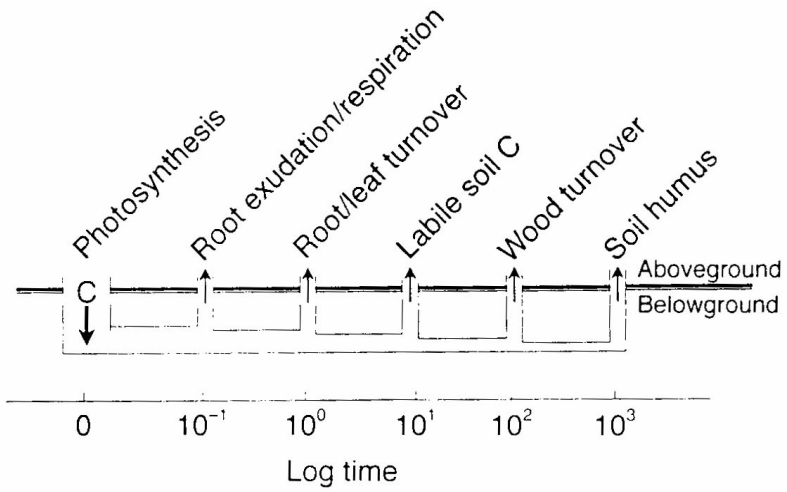

Figure 1.5. Schematic representation of rhizospheric processes and their temporal scale. Unit of measurement is 1 year (Luo et al., 2001).

Moreover, because of the large amounts of C involved (see above par. 1.1), if modifications induced by atmospheric CO_2 were to cause even small relative changes in soil organic carbon (SOC), it could constitute a significant feedback effect on GHGs in the atmosphere. A change by just 10% in SOC would be equivalent to all the anthropogenic CO_2 emitted over 30 years (Kirschbaum, 2000). Actually soil respiration is estimated to 77 Pg C/year and is a major flux in the global C cycle in terrestrial ecosystems, second in magnitude to GPP and equal to or greater than the estimated global terrestrial NPP (Schlesinger

and Andrews, 2000). In boreal and temperate forests the variability of the balance between GPP and respiratory losses, is mainly determined by the variability of soil respiration (Valentini et al., 2000). Soil respiration can be functionally divided into autotrophic (C released by respiration of roots) and heterotrophic (CO_2 released from microbial biomass and animals during decomposition of C substrates) respiration (fig. 1.6).

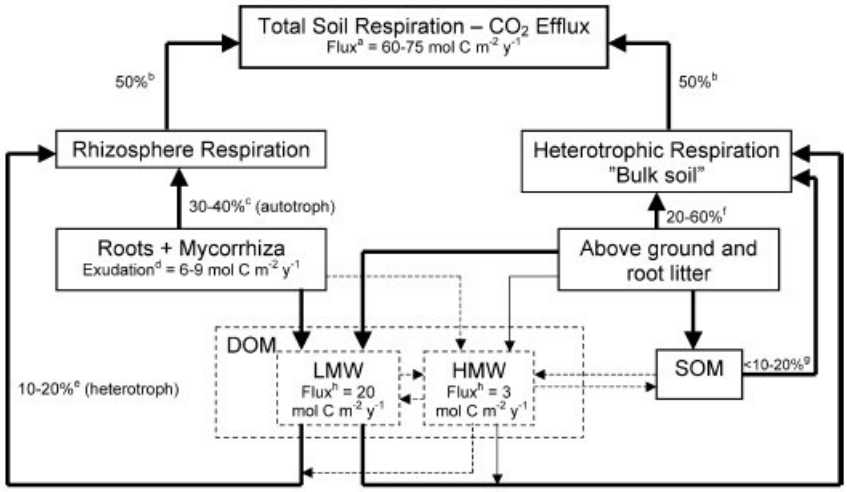

Figure 1.6. Schematic diagram of soil CO_2 fluxes and relative contributions from different sources. All percentage values refer to total soil respiration. Solid bold arrows represent major C fluxes, thin soil arrows small C fluxes and dashed arrows minor C fluxes. Solid boxes represent major C pools and dashed minor C pools. DOM: Dissolved Organic Matter; LMW and HMW : Low and High Molecular Weight compounds. Figure and values are taken from reference cited in van Hees et al., 2005.

Because the strict connection between roots and microorganisms, the rhizosphere respiration includes belowground autotrophic respiration and heterotrophic respiration of C substrates originating from newly assimilated C, e.g. root exudates and recent dead root biomass (van Hees et al., 2005). One of the main problems with predicting soil C losses, is that soil respiration it is influenced by a multitude of interacting factors as: soil temperature, moisture, soil C or litter quality, root density, microbial community structure and size, physical and chemical soil properties, vegetation type, nutrient status and growth rate (Raich and Tufekcioglu, 2000). Consequently, in most ecosystems the rate of soil respiration is highly temporally and spatially variable. Furthermore, the decomposition of organic

matter can be highly accelerated by human activities, including biomass burning, plowing, drainage, and low input farming (fig. 1.7, Lal, 2003).

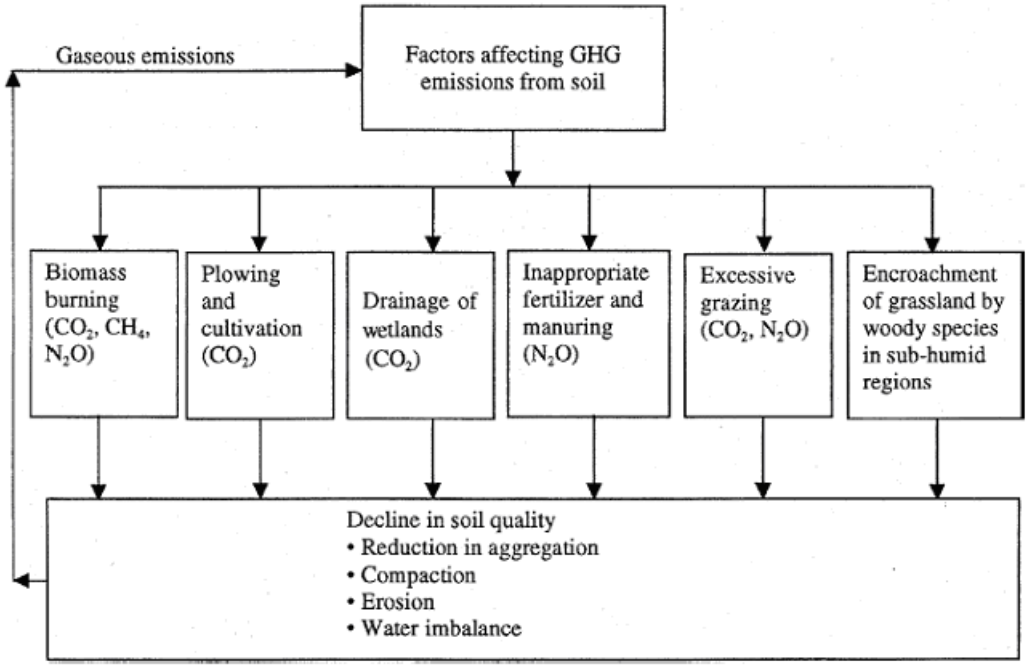

Figure 1.7. Agricultural practices affecting emissions of greenhouse gases into the atmosphere (Lal, 2003).

Conversion of natural to agricultural ecosystems increases the maximum soil temperature and decreases the soil moisture storage in the root zone, especially in drained agricultural soils, thus strongly impacting the SOC pool. Tillage, in addition to mixing and stirring of soil, breaks up aggregates and exposed organo-mineral surfaces otherwise inaccessible to decomposers (Post and Kwon, 2000). The mineralization rate of SOC due to intensive cultivation or continuous cropping may range for about 20% in 20 years in temperate climate to about 50% in 10 years in the tropics (Woomer et al., 1994).

The term "soil C sequestration" implies net removal of atmospheric CO_2 by plants and its storage as soil organic matter. Considering the fertilization effect of CO_2 on NPP, it has been speculated that soils could have a larger capacity to absorb the extra C released by plants therefore mitigating the increase in atmospheric CO_2. Nevertheless, the ability of soils to store C is highly controversial, because only small fractions of new C inputs into

soil will become SOC in the long-term, whereas the largest fraction will be respired back to the atmosphere: in aerobic soil conditions only 1% of what enters the soil accumulates in the stable, humic fraction (0.4 Pg C/year) (FAO, 2001). As showed in fig 1.8, in soils it can be distinguished three main C pools (active, slow and passive) of soil organic matter (SOM) with different residence time, ranging from years to decades or thousand of years (fig. 3.4; Brady and Weil, 1999).

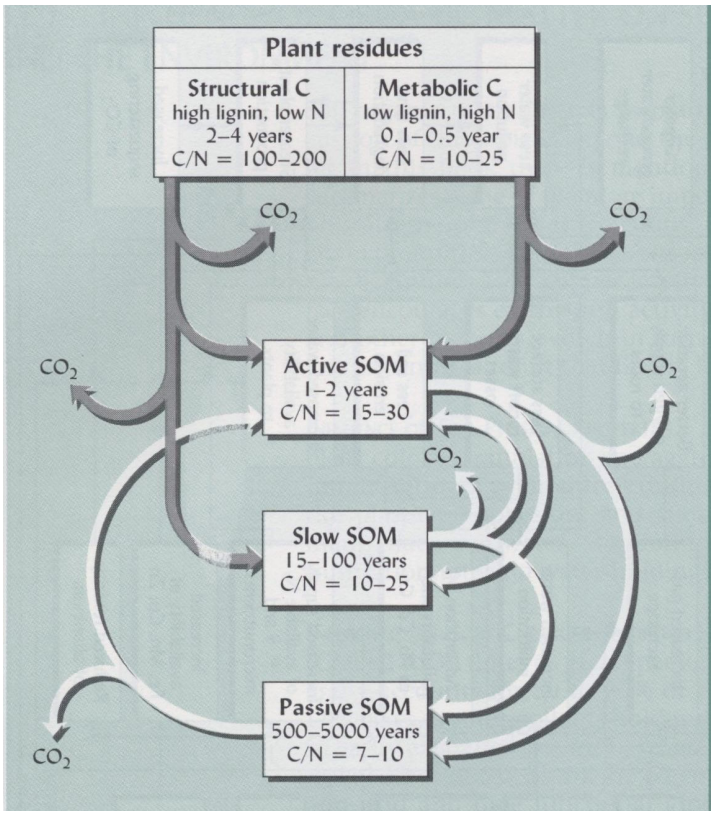

Figure 1.8. Different soil C pools with residence time and C/N ratio, derived from plant organic residues, Brady and Weil, 1999.

Active fraction: it consists of materials with relatively high C/N ratios and short half-lives. Components include the living biomass, some of the fine particulate detritus (Particulate Organic Matter, POM), most of the polysaccharides and other non-humic substances, and some of the more labile and easily decomposed fulvic acids. This fraction provides most of the readily accessible food for the soil organisms and most of the readily mineralizable N. Rarely comprises more than 10 to 20% of the total SOM. **Passive fraction**: it consists of very stable materials remaining in the soil for hundred or thousand of

years. Accounts for 60 to 90% of SOM and is most closely associated with the colloidal properties of soil humus. **Low fraction**: it has intermediate properties between the other two and includes substrates with high content in lignin and other slowly decomposable and chemically resistant components. It is an important source of mineralizable N and other plant nutrients, and is a food source for the steady metabolism of soil microbes.

The soil C accumulation occurs only when a significant portion of C inputs is stored in C pools that turn over slowly, like passive or slow fraction (Luo et al., 2001).

The process of C sequestration includes humification, aggregation, deep incorporation of C in the subsoil and calcification: the increase in amount of slow or inactive SOC pools is the main important factor in C sequestration, and the slow pool may be involved in aggregation and physical protection (Lal and Kimble, 1997). Humification involves conversion of plant and animal residues into complex humic substances which are stable and recalcitrant, but C is only one of the several blocks needed for this conversion: to sequester 10,000 Kg of C in humus, 833 Kg of N, 200 Kg of P and 143 Kg of S are needed (Himes, 1998). Thus, soil quality and fertility and the interaction with C inputs may strongly influence sequestration of C (Canadell et al., 1996). In this sense the input of SOM and thus the land uses can be important factors determining the amount of C stored in soils and the rate at which this storage could happen.

Post and Know (2000) indicated that there are many factors and processes that determine the direction and rate of change in soil organic content when vegetation and soil management practices are changed. Ones that may be important for increasing SOC storage include: i) increasing the input rates of organic matter; ii) changing the decomposability of organic matter inputs that in particular increase the light fraction organic carbon; iii) placing organic matter deeper in the soil either directly by increasing belowground inputs or indirectly by enhancing surface mixing by soil organisms; iv) enhancing physical protection through either intra-aggregate or organomineral complexes. Conditions favouring these processes occur generally when soils are converted from cultivated use to permanent perennial vegetation, such as from crop to pasture, plantation and secondary forest (Guo and Gifford, 2002).

1.4 The soil biological system; C/N interaction.

Soil is a dynamic, living matrix that is an essential part of the terrestrial ecosystem. It governs plant productivity of terrestrial ecosystems and it maintains biogeochemical cycles because microorganisms in the soil degrade, sooner or later, virtually all organic compounds, playing a critical role in maintaining soil health, ecosystem functions and production (Nannipieri et al., 2003).

The functions of soil biota are central to decomposition processes and nutrient cycling: microbial activity can affect plant growth and productivity, since plants use mainly inorganic nutrients and they rely on soil microorganisms to mineralise organic nutrients for growth and development (Salisbury and Ross, 1991). The soil microbial biomass consists of organisms living in soil that are generally smaller than approximately 5 x 10^3 μm^3 and it has been defined as the eye of the needle through which all organic matter needs to pass trough (Jenkinson et al., 1987). As a susceptible soil component, the biomass may be therefore a useful indicator and it has been suggested that the microbial biomass content is an integrative signal of the microbial significance in soils because it is one of the few fractions of soil organic matter that is biologically meaningful, sensitive to management or pollution and measurable (Powlson, 1994). Energy flux through microbial biomass is the driving force for the decomposition of residue and detritus material. This energy flux determines whether the system is building or depleting the SOM pool. The organic matter inputs to the soil, in the form of plant litter or roots exudates, are a primary source of both carbon and nitrogen for microbes and decomposition rates are intimately linked with the nutrient supply to these microorganisms (fig. 1.9). The organic compounds all contain carbon, which serves as an energy source for the soil microbes involved in the nutrient cycling. Organic molecules are the major source of energy for many of the organisms found in the soil that are a key factor in determining the flow of nutrients from relatively stable organic pools and more labile inorganic nutrient pools in soil. Availability of soil C for microbial growth can be an important factor regulating forest nutrient cycling, and energy supply is often viewed as the major factor limiting soil microbial activity because of the heterotrophic nature of decomposition (Richards, 1987; Tate III, 1995). However, other factors such as substrate quality, N availability, physical environment (i.e. temperature and

moisture), and physical protection by clays have been invoked as additional controls over microbial processing of soil organic matter (Tate III, 1995; Mary et al., 1996).

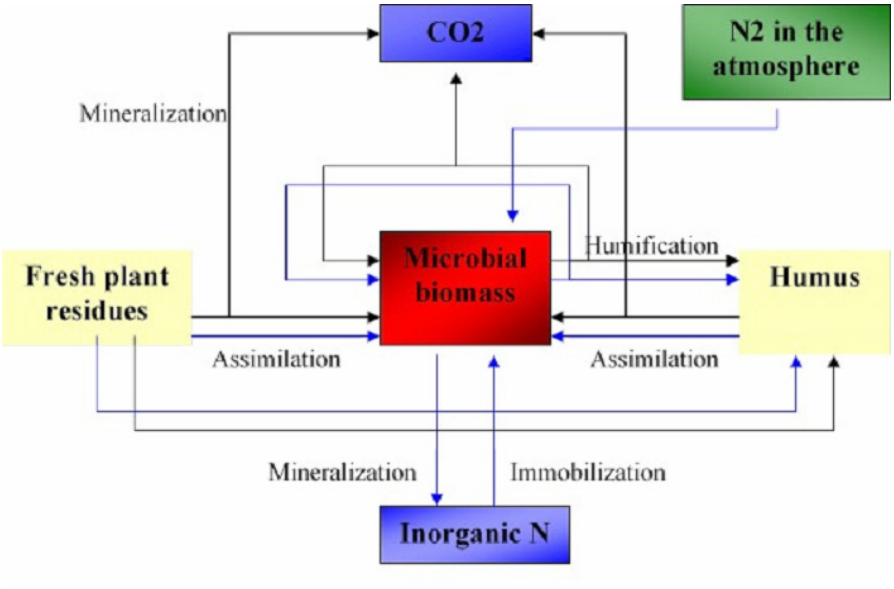

Figure 1.9. Soil biogeochemical nutrient cycle and the central role of microbial biomass (Magill and Aber, 2000).

Hodge and coauthors (2000) reported that the rate of SOM decomposition depends first on the C/N ratio of the substrate being decomposed and second on the decomposer community's need for N, relatively to its need for C (fig. 1.10).

Fungi generally assimilate substrate more efficiently than bacteria, which have a smaller C/N ratio and consequently a larger N demand per unit of C (Killham, 1994). If the C/N ratio of the substrate being decomposed exceeds that of the decomposers (after taking account of the respired CO_2) then those microorganisms will not release inorganic N during decomposition and might supplement their inorganic N requirements, reducing the availability of that pool to plants.

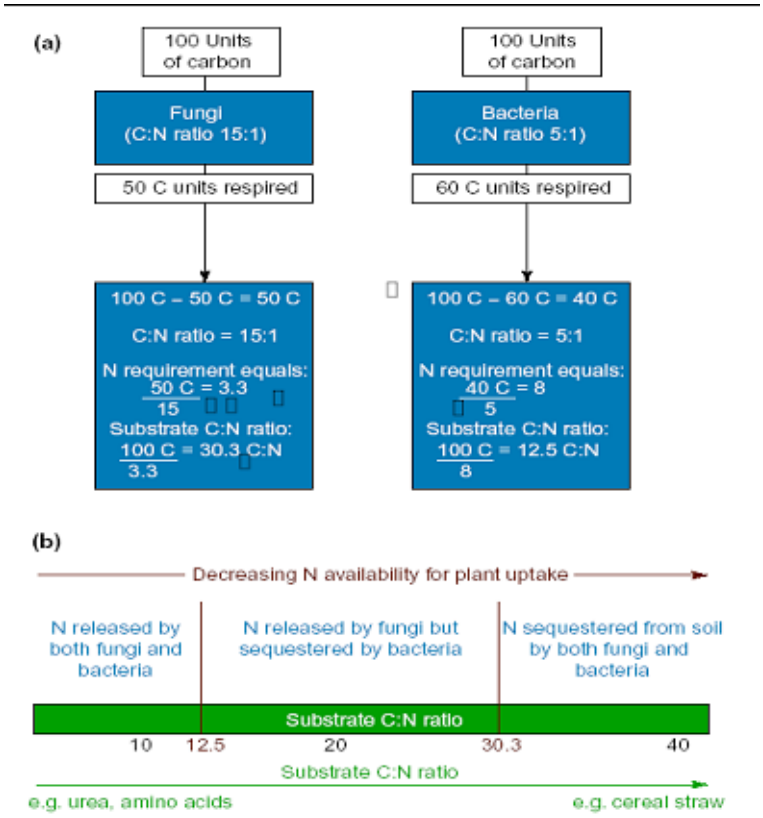

Figure 1.10. a) Influence of the type of decomposer (bacterial or fungal) on the minimum (or critical) C/N ratio for the decomposition of organic material at which net N mineralization is zero. Fungi have a greater substrate-assimilation efficiency than bacteria. b) Consequences of the relationships in a) on plant nitrogen availability. (From Hodge et al., 2000).

Conversely, if the C/N ratio of the substrate being decomposed is lower than that of the decomposers (after taking account of respired CO_2) then those microorganisms will add to the soil the mineralized N. Thus, at C/N ratio less than about 12:5, neither fungi nor bacteria will require additional N, and net mineralization will occur. At C/N ratio greater than about 30:1, both fungi and bacteria will require additional N and net N immobilization will occur. Nevertheless, the heterogeneous nature of soil organic matter and the presence of a range of decomposers in soil make the situation more complex and generally ensure that N mineralization and immobilization occur simultaneously.

One the main topics is that there is a huge diversity of microbial responses to substrate inputs by different processes and populations. Both laboratory and field substrate addition experiments showed that the microbial response to C limitation depends on the N availabil-

ity, the nature of C substrate, and factors associated with soil type (e.g. organic matter quality, pH, and probable differences in microbial community composition). French (1988) observed that decomposition is seldom limited by a single factor and concluded that enzyme activity is constrained directly by a combination of substrate (e.g. quantity and quality of C), environment, and physical access of enzyme to substrate. In addition, rates of enzyme production are influenced by availability of appropriate decomposer organisms, which may be restricted by climatic, soil, or substrate factors. Field and laboratory comparisons showed that organic matter quality, as modified by differences in the physical environment and associated plant community, is an important cause of community differences in microbial activity along a complex gradient in temperature and N availability. Moreover, even when community differences in environmental factors (i.e. soil temperature and moisture) were eliminated in laboratory incubations, it was evident that energy supply, in particular N, and other un-manipulated variables associated with organic matter quality or microbial community composition were important in controlling microbial activity. Thus, the simultaneous non-availability of labile C and N seems to limit microbial activity in soils (Vance and Chapin III, 2001).

The addition of different substances to the soil might also produce an interaction between the transformation of the added substances and the natural soil cycles of C and N: the mechanism is called *priming effect* and is defined as a short-term change in the turnover of SOM caused by comparatively moderate treatments of the soil (Kuzyakov et al., 2000). The *priming effect* is often supposed to result from an increase in the overall microbial activity (microbial respiration and co-metabolic enzymatic activity) due to the higher availability of energy and nutrients released from easily degradable organic matter (positive *priming effect*), but it could also happen a reduction of mineralization and thus an immobilization of the added C or N (negative *priming effect*) (fig. 1.11).

Kuzyakov et al. (2000) also distinguish between real and apparent *priming effects*, where the second is independent from the soil microbial biomass and appear typically after the addition of mineral fertilizers to the soil.

Interactions between soil microorganisms, soil fauna and plants are regarded as one of the keys to understanding *priming effects* (fig. 1.12). Plants increase the microbial activity in the rhizosphere trough rhizodeposition and thus can enhance the N mineralization in soil. The size of the extra N mineralization is dependent on the complexity of the food chains and on the N availability. Under N limited conditions a strong competition between plants and microorganisms is expected to take place and the further N release from the

SOM could be retarded or halted if the soil microbial biomass may immobilize large amounts of nutrients, while if N is taken up by plants, the turnover is increased and N-mobilization from the SOM may continue.

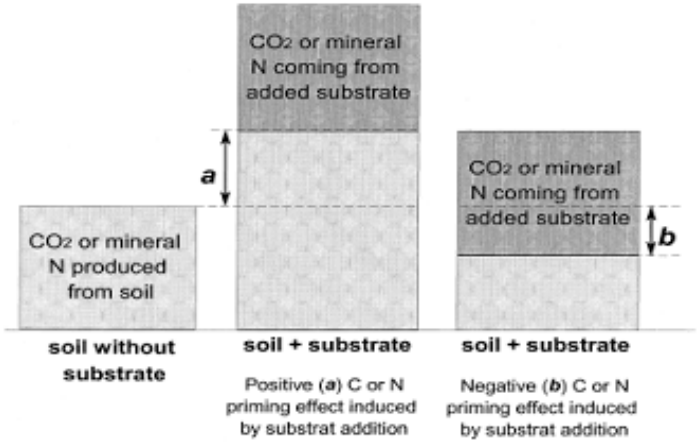

Figure 1.11. Schematisation of the *priming effect* – non-additive interactions between decomposition of the added substrate and of soil organic matter (SOM): a) acceleration of SOM decomposition – positive *priming effect*; b) retardation of SOM decomposition – negative *priming effect.* (From Kuzyakov et al., 2000).

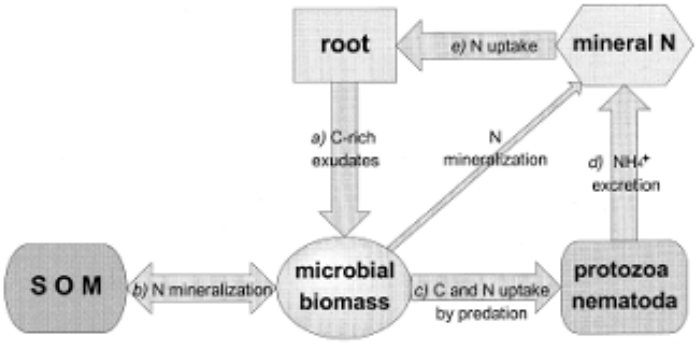

Figure 1.12. Schematisation of interactions in the rhizosphere caused by exudation of C rich substrates, which trigger the additional N mineralization from SOM: a) release of C rich substrates from the roots, microflora (particularly bacteria) takes up C; b) microflora starts to grow and increases the decomposition of SOM to receive additional N, c) protozoa (and other soil fauna) feed on the microbiota, and d) release mineral N; e) plants partly take up the mineral N (from Kuzyakov et al., 2000).

1.5 The likely impact of elevated [CO_2] and nitrogen fertilization on soil biological processes. State of the art

Since atmospheric CO_2 concentrations are negligible compared to those observed in the soil, it is highly unlikely that direct effects of CO_2 enrichment could occur on soil biota. Indirect effects, however, are expected through changes in biomass production, litterfall and rhizodeposition that could influence microbial communities.

Nearly every biological and biologically regulated process within the soil can be indeed strongly affected by increasing CO_2 levels (Bernston and Bazzaz, 1996). Soil microflora and fauna are dependent on plant-derived organic material and both quantity and quality of C moving to soil should be considered (Cardon, 1996). On the other hand microbial activity determines the rate of N mineralization, and changes in plant litter production in elevated [CO_2] could alter microbial N demand and amounts of N available for plant uptake, a response that could potentially feed back to modify plant productivity in a CO_2 enriched atmosphere (fig. 1.13).

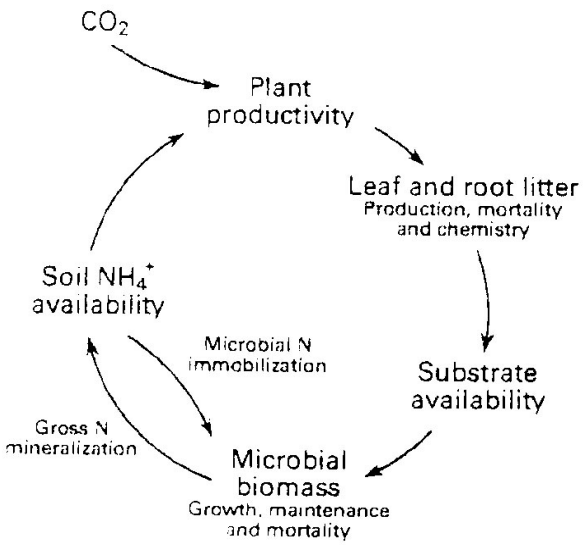

Figure 1.13. Conceptual model showing the linkages between plants and microbial activity in terrestrial ecosystems, and the ability of elevated [CO_2] to modify this relationship (Zak et al., 2000).

In their review Zak and co-authors (2000) hypothesized that plant-derived substrates entering soil that stimulate microbial growth (i.e. simple carbohydrates and organic acids)

create a biosynthetic demand for N, thus increasing microbial immobilization and potentially lowering the amount available for plant uptake, whereas substrates providing relatively small amounts of energy for microbial metabolism (i.e. lignin and tannins) reduce the biosynthetic demand for N and decrease the rate of microbial immobilization.

The plant-soil interaction depends on a number of belowground feedback such as alterations in the root system, stimulation of mycorrhizal association, increased C/N ratios of plant tissues, increase in belowground C inputs, alteration in plant water and nutrient uptake etc., that can affect both positively or negatively the decomposer community (Hu and Zhang, 2004). These belowground feedbacks have a fundamental role in release nutrients available for plant uptake and store C in slow and passive soil C pools.

In their review Tingey et al. (2000) reported an average increase of +52% of fine root production of coniferous trees under elevated [CO_2]. Similar results were reported for broadleaves by Kinney and Lindroth (1997). Root turnover of *Populus* spp. was also increased by elevated [CO_2] as reported by Kubiske et al. (1998) and Lukac et al. (2003). Elevated [CO_2] has also been found to accelerate mycorrhizal colonization of newly formed root tips and to stimulate formation of extraradical mycelium on *Populus* trees (see the review of Ceulemans et al., 1999). In two FACE experiments on woody plants the litter production was significantly higher under elevated [CO_2] (Hamilton et al., 2002; Cotrufo et al., 2005). In addition to quantitative changes, qualitative alterations in plant tissues raised in elevated [CO_2] have also been observed. Changes in tissues composition originate both from chemical changes in the green plant materials produced during growth in elevated [CO_2] and from the shifts in biomass partitioning resulting in a higher relative contribution of root litter and of mycorrhizal detritus (Ceulemans et al., 1999). The main change in chemical composition of tissues is the accumulation of total non-structural carbon (TNC) that would increase the production of C based secondary compounds such as lignin, tannins and other polyphenols, which have been shown to decrease decomposition rates (Gebauer et al., 1998). It is also widely accepted that plants grown under elevated [CO_2] have lower tissue N concentration, and thus a less quality (higher C/N ratio) (Cotrufo et al., 1998).

To conclude, it is clear that the quantity and quality of inputs from plants to soil are significantly affected by elevated [CO_2] and generally we can assume an increase of the flux of organic matter because of enhanced litter production and rhizodeposition and a probable decrease in the quality of these inputs (Pregitzer et al., 1995; Cardon, 1996; Hodge et al., 1998, Cheng, 1999). It remains uncertain how soil microorganisms can react to these

changes and the direction of feedbacks and interactions in the soil biota. Microbial biomass appears to be closely linked to aboveground plant productivity in many ecosystems (Zak et al., 1994), suggesting that the biomass of microbes depends directly on inputs of reduced carbon to the soil. Higher microbial biomass has been documented in grasslands (Hungate et al., 2000), crops (Rogers et al., 1994) and forests (Zak et al., 1993); however, other experiments showed no significant effects of elevated [CO_2] on soil microbial biomass (Allen et al., 2000; Berntson and Bazzaz, 1998; Wiemken et al., 2001). The relative change of microbial biomass under elevated [CO_2] beneath woody plants ranged from a 52% decline to a 121% increase and this high degree of variability seems to indicate that shifts in substrate availability under elevated [CO_2] can induce different changes in microbial biomass (Zak et al., 2000). Enhanced C inputs under elevated [CO_2] can stimulate microbial biomass and activities because soil microbes are usually C-limited (Paterson et al., 1997; Paul and Clark, 1996) and an increase of recent plant inputs are believed to be the main driver of increased rates of soil respiration at elevated [CO_2]. Trueman and Gonzalez-Meler (2005) found that the 90% increase of soil respiration was because of the oxidation of old C and suggested that the rapid oxidation of more stable SOM is dependent on the availability of a continuous supply of labile C to heterotrophs. Increased belowground C inputs as a result of increased root mass and root C turnover can therefore alleviate microbial C limitations and microbes can prompt the decomposition of previously unavailable recalcitrant SOM (i.e. *priming effect*).

Results from Allen and Schlesinger (2004) suggest that any additional inputs of labile C due to plant growth at elevated [CO_2] will result in increased soil respiration and microbial biomass C. These results are not consistent with the hypothesis that higher C:N ratios in litter will decrease decomposition rates, but are consistent with the hypothesis that microbial activity increases when the C supply to microbes is increased. Greater microbial respiration, degradative enzyme activity and metabolism of plant-derived substrates all indicate that soil microbial communities are more metabolically active under elevated [CO_2] (Zak et al., 2003). Nevertheless, the high degree of variability, and the not statistically significant results reported in literature, suggest that at the moment it is difficult to predict the extent to which microbial metabolism will change under elevated [CO_2].

In recent years, several hypotheses have been proposed regarding the role that soil microbes may play, through SOM decomposition dynamics, in constraining or facilitating sustained enhancement of NPP under elevated [CO_2] and in increasing the potential of soils to store C.

In earlier studies Diaz et al. (1993) found a significant increase in microbial biomass (C and N), while aboveground plant biomass was not significantly increased. From these results they suggested that elevated [CO_2] produced an increase in the total allocation of C into the soil stimulating the soil microbes that in turn were able to immobilize most of the available N, thereby resulting in plant growth constrain. This hypothesis was supported later by Hungate et al. (1997) and Cheng and Johnson (1998), which showed the combining effect of elevated [CO_2] and fertilization treatment in stimulating SOM decomposition.

On the contrary, Zak et al. (1993) found a significant increase in the biomass production of *Populus Grandidentata* as well as in microbial biomass in the rhizosphere and in the net N mineralization rates under elevated [CO_2], suggesting a positive feedback on growth enhancement via nutrient supplies. Hungate and Chapin (1995) and later on Cardon (2001) explained these conflicting results with the mineral nutrients availability in soils: if nutrients are abundant, microbes easily utilize C rich rhizodeposits and immobilize mineral N, depressing the degradation of more resistant SOM and causing a reduction in the nutrient availability for plants. If nutrients are limited, microbes utilize rhizodeposits as a C source, but break down more SOM in order to obtain nutrients that are mineralized and thus available for plants. Allen et al. (2000) resume these hypotheses considering the effect of the litter quality on the decomposition rates and in turn on the availability of N for plants grown at elevated [CO_2] as shown in fig. 1.14.

These different results indicated that the effect of elevated atmospheric [CO_2] on SOM decomposition was dependent on different plant-soil systems and was not mono-directional (Cheng and Johnson, 1998).

Cheng (1999) hypothesized that the extra input of labile root-derived C in elevated [CO_2] initially decreases SOM decomposition as a result of the increase in microbial growth and immobilization of mineral nutrients, but that later it stimulates SOM decomposition and nutrient release because of the turnover of this newly grown microbial biomass, depending on the quality of these root-derived substrates. Hu et al. (1999) found that the direction of microbial feedback to elevated [CO_2] changed in an annual grassland over six years. In the early years, when microbes were C-limited, enhanced C inputs stimulated microbial activities and CO_2 production. But, as elevated [CO_2] stimulated plant N uptake, and available soil N was translocated to less-available pools in the form of standing plant biomass and soil organic matter, N limitation became the major factor limiting microbial activities (Fig. 1.15).

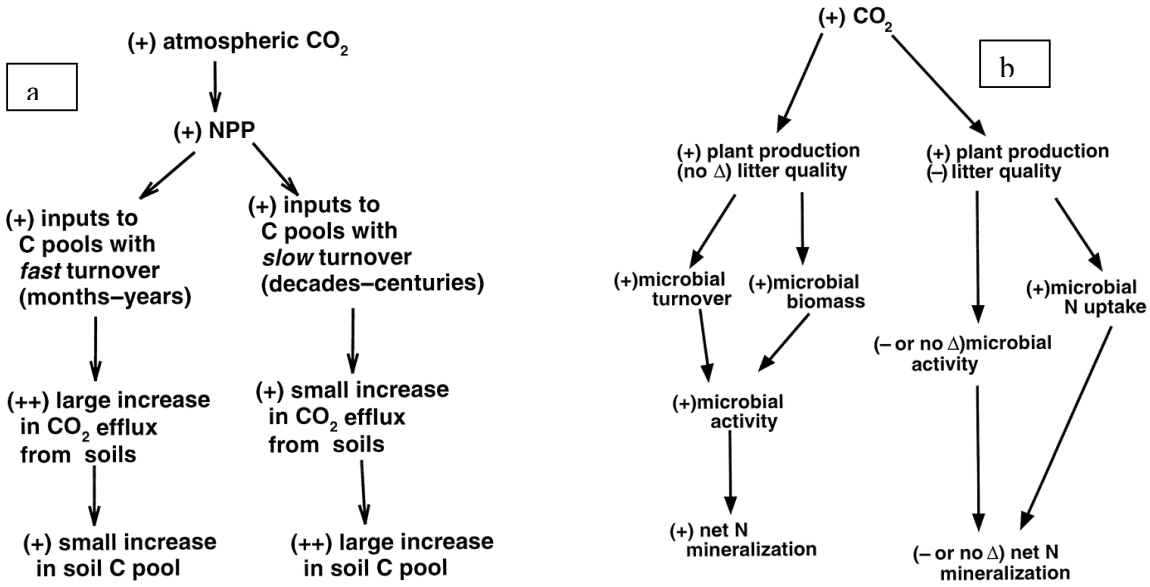

Figure 1.14. Two hypothesized pathways through which elevated atmospheric [CO_2] may affect (a) soil C storage and (b) soil N availability. (From Allen et al., 2000).

Furthermore, elevated [CO_2] can affect the soil biota also by altering soil physical conditions (such as soil moisture) and soil structure. Niklaus et al. (2003) found a decrease in soil aggregate sizes, most likely due to increased soil moisture in elevated [CO_2]. Ehnanced production of fine roots and fungal hyphae occurring under elevated [CO_2] could protect soil C by enhancing soil aggregation (Tisdall, 1994). Increased rhizodeposits under elevated [CO_2] can also stimulate the formation and stabilization of soil aggregates through the chemical interaction of clay minerals, metal oxides and SOM (Brady and Weil, 1999). Soil properties influence the decomposition of organic C in the soil, because they determine the living conditions for microbes and protect the soil C, while the impact of atmospheric CO_2 enrichment on C dynamics in soils can be small in comparison with the large differences between soil types (Hagedorn et al., 2001).

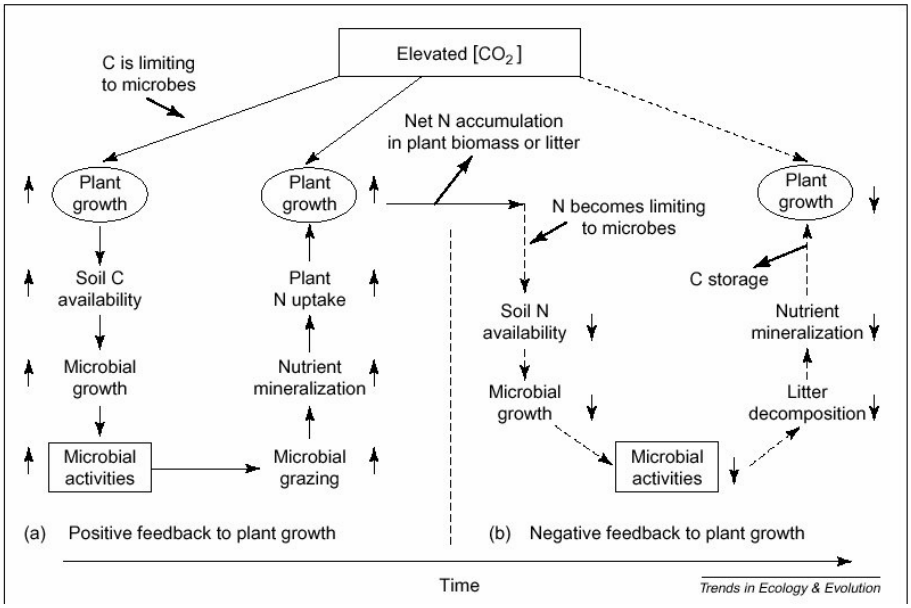

Figure 1.15. Elevated [CO_2] concentration alters the relative availability of C and N for microbes over time, which affects microbial feedbacks to further plant growth. (a) When C is limiting to microbes, enhanced C inputs caused by elevated [CO_2] concentration stimulate microbial activities and increase N availability for plant growth (unbroken lines). (b) As N accumulates in less labile pools, N deficiency limits microbial growth and activities, and retards N mineralization, negatively feeding back growth (dashed line). The dashed arrow from elevated [CO_2] to plant growth means that elevated [CO_2] does not have effects on plant growth because nutrient limitation constraint. (From Hu et al., 1999).

Resuming, responses on SOM decomposition dynamics can be envisaged depending on the:

1. substrate quantity and quality (C:N ratio of organic matter inputs to soil as litter and rhizodeposition)
2. relative differences in the nutritional quality of soil-derived and plant-derived organic matter
3. plants and microbial requirement for nutrients (i.e. competition)
4. soil structure.

Regarding these remarks, we can observe three main processes:

a. increased SOM decomposition
b. decreased SOM decomposition
c. a combination of both over time

1.6 Objectives

This thesis was realize in the frame of the POPFACE – EUROFACE project: an integrated research activity at European scale on the role of forest tree plantations to mitigate the impact of greenhouse gases in support of the Kyoto protocol, under conditions of changing climate.

The main objective of POPFACE experiment was "*to determine the functional responses of a multi-clonal poplar plantation to actual and future atmospheric CO_2 concentrations, and to assess the interactive effects of this anthropogenic perturbation with the other natural environmental constrains on key biological processes and structures. Additionally, this project will yield data relevant to assess the potential for increasing the C sequestering capacity within the European Union, using such forest tree plantation*" (Scarascia Mugnozza et al., 2000).

This thesis focused on the impact of elevated [CO_2] on soil biological processes, mediated through plant-microbes interactions, and on their role in determining the potential for terrestrial ecosystems to mitigate the increase of CO_2 concentration in the atmosphere

Two main topics need to be answered to define the possibility of terrestrial ecosystem to mitigate atmospheric CO_2 increase: i) if the increase in NPP will be sustained by an adequate nutrient supply and ii) if the increase in C input will raise the capability of soils to store C in the long term.

The effect of elevated [CO_2] on soil is in fact mediated by plants inputs, from litter and rhizodeposition, and changing quantity and quality of these inputs will affect the soil biota and its metabolism, that in turn can create a feedback on the atmospheric CO_2 concentration. To understand the directions of this feedback, we need to study in depth the processes that lead the plant-soil interactions.

The experimental design offered also the opportunity to take into account the role of soil nutrient *status* in determining the directions of changes in soil biological processes as affected by CO_2 enrichment, thus the study of the interactions between elevated [CO_2] and N fertilization was included.

At the same time, the land use change from crop to plantation and the poplar species composition, as well as the vegetative activity, were considered as determinant factors affecting soil biological processes.

The temporal changes on chemical and biological soil properties were also thoroughly analysed as sensible indicators of processes modifications in the long term.

In particular the work was structured in specific topics, according to the following different objectives:

1. **The modifications induced by the treatments with elevated [CO_2] and nitrogen fertilization on soil nutrient availability and processes**. Hypotheses to be tested: increased plant growth under elevated [CO_2] is supposed to modify the nutrients needs of plants, N in particular, thus perturbing plants-microbes relationships and competition. This, in turn, can influence the N mineralization-immobilization processes that regulate N availability in soils.
2. **The impact of treatments on soil organic C fractions and in C storage.** Hypotheses to be tested: modifications in above- and belowground plant activity under elevated [CO_2] can result in a different quantity and quality of inputs to soil. Labile C fractions, representing the net result of plant input and microbial decomposition activity, can early indicate the direction of changes in the soil C storage.
3. **The use of microbial indices as bio-indicators of environmental changes**. Hypothesis to be tested: microorganisms have a central role in determining soil activity and to combine microbial activity and biomass can provide sensitive indications on the modifications induced by the treatments on soil processes.
4. **The impact of treatments on the structural and functional diversity of soil microorganisms**. Hypotheses to be tested: changes in the availability of C and N can affect the structural composition and determine changes in species number. At the same time, the functional diversity of microbial communities can be differently affected by treatments, indicating modifications in the microbial physiological activities.
5. **The impact of treatments on microbial decomposition of soil C: mineralization activity and its determinants**. Hypothesis to be tested: changes in the quantity and quality of decomposable substrates can affect organic C decomposition and mineralization rates. Microbial composition, nutrient availability, labile and recalcitrant C fractions, as affected by treatments, can determine changes on microbial activity, and thus on the capacity of soil to store C.

6. **The impact of the treatments on annual and interannual patterns of soil respiration: mechanisms for increased soil C emissions under elevated [CO_2].** Hypothesis to be tested: the greater photosynthetic activity under elevated [CO_2] can increase the flux of C from plant to soil, thus influencing both root and microbial activity, determining higher CO_2 efflux rates and/or changes in the soil respiration response to temperature.
7. **The impact of treatments on rhizo-microbial respiration and the influence of temperature and labile C substrates**. Hypothesis to be tested: the increase of belowground biomass and activity, through the release of soluble C compounds, can affect the heterotrophic respiration in the rhizosphere, and its response to temperature changes.
8. **Separating the autotrophic and heterotrophic contribution on soil CO_2 efflux, and determining the impact of treatments on components respiration in a long-term field experiment**. Hypotheses to be tested: the treatments can modify the relative contribution of auto- and heterotrophic components to soil respiration, due to the role played by living roots in determining the response of soil CO_2 efflux to elevated [CO_2].

2. MATERIALS AND METHODS

2.1 Experimental site

The POPFACE experimental site has been established near the city of Tuscania (VT, Italy), on a 9 ha former wheat field, 150 m above the sea level. The field had been under forest until about 1950. Since then a variety of agricultural C3 crops have been cultivated until the establishment of the POPFACE plantation. The poplar plantation was realized in spring 1999 utilising about 40000 cuttings of *P. x euramericana* at a commercial spacing of 2 m x 1 m. At the same time, within the plantation, 6 experimental plots were planted each with 900 cuttings of three different poplar species, *Populus alba* (clone 2AS-11), *Populus nigra* (clone Jean Pourtet) and *Populus x euramericana* (*Populus deltoides* x *Populus nigra*, clone I-214) (Fig. 2.1).

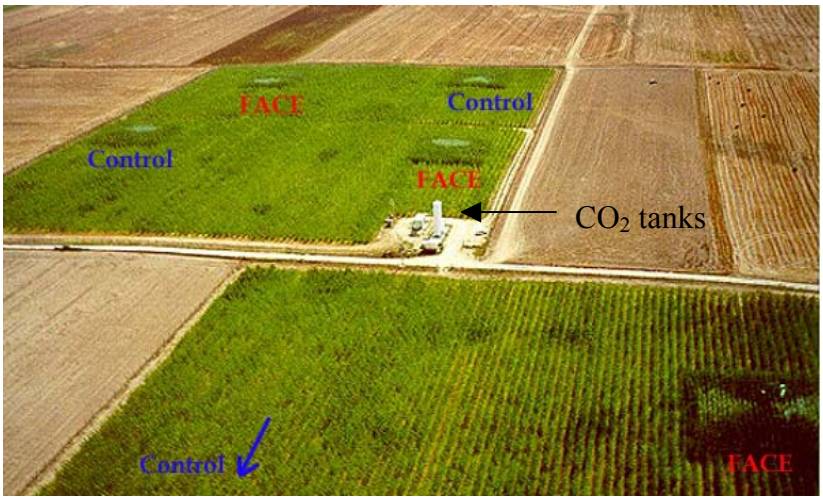

Figure 2.1. Aerial view of the plantation. 5 of the 6 experimental plots are visible, as well as the CO_2 container.

The plots were 30 m x 30 m in size and the trees were spaced at 1 m x 1 m to facilitate early canopy closure. Each of the plots was divided into 3 sections, every one of which was planted out with trees of a single species. Each plot was further split into two halves with a 1m deep barrier to allow for high and low fertilization treatments. In this way each plots had six sectors (three genotypes, 2 N-treatments) yielding 52 plants per sector and 24 assessment plants per sector and per clone (fig. 2.2). Thus, the experiment was a complete block design with three blocks, each including a FACE and a Control plot.

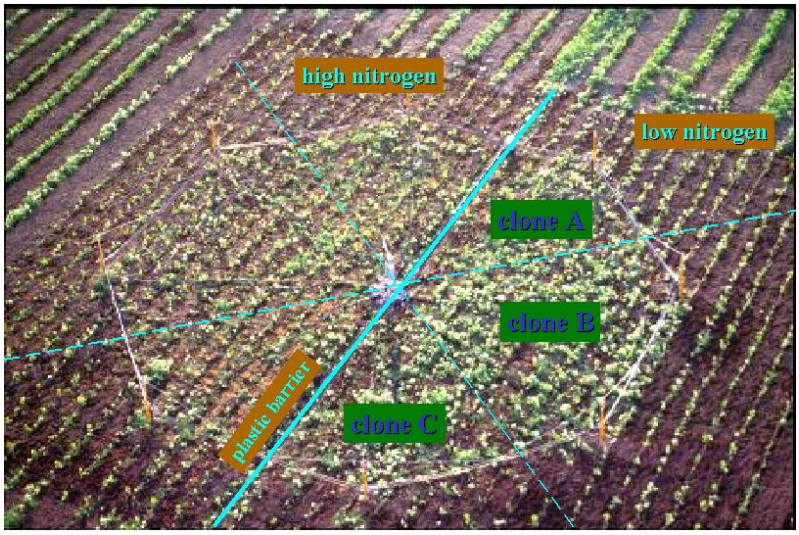

Figure 2.2. Schematic design of a plot. The six symmetrical sectors are evident.

Within each clonal sector a permanent growth plot made of six trees was selected to closely monitor, by means of non destructive measures, the growth and the crown development of the trees in the plantation. Three of these plots were treated at 550 ppm of CO_2 concentration, the forecasted concentration for the middle of the next century, whereas the remaining plots were at ambient CO_2 concentration. The six experimental plots were equally spaced in order to avoid enrichment pollution of ambient CO_2 plots with air blown in from enriched CO_2 plots.

The whole plantation was drip irrigated at 6 to 10 mm of water per day during the growing season, starting approximately at the beginning of April until the beginning of November, by a series of dripping tubing 50 km long in total. The amount of water was sufficient to avoid water stress even during the hottest days of a growing season. For further details see Scarascia Mugnozza et al., 2000. The site was equipped with two containers (20 and 30 tons) for liquid CO_2 and with 3 vaporizers; CO_2 was supplied every 3 – 4 days with a truck, from a natural source located about 120 km away. The system provides CO_2 directly in the atmosphere through FACE (Free Air CO_2 Enrichment) technology as described in Miglietta et al., 2001. FACE is a technique for exposing crops, forest plantations and natural vegetation to elevated atmospheric CO_2 concentrations without an enclosing structure, and FACE experiments are almost unanimously considered to provide the best opportunity to expose patches of managed or unmanaged vegetation to conditions of elevated atmospheric concentrations with minimal alteration of the natural environment where plants are growing. Nevertheless, FACE systems also suffer from some experimental limitations such

as the presence of substantial infrastructure, the unavoidable presence of CO_2 concentration gradients along the wind direction and short-term fluctuations in CO_2 concentration.

In the POPFACE system, pure carbon dioxide was released to the atmosphere through a very large number of small gas jets, at high velocity. The design of the FACE system was optimized by the use of a gas dispersion model based on the principles of Computational Fluid.Dynamics (CFD). Such innovation was introduced because the theory of fluid mechanics establishes that when a gas jet reaches sonic velocity, a shock wave is created at the jet outlet and the air-CO_2 mixing is greatly enhanced (Munson et al., 1990). The effect of different weather conditions, of canopy height and distribution of the CO_2 releasing point on the gas dispersion was simulated and the model determined the vertical wind profiles within and above the canopy, it calculated turbulent kinetic energy created by the vegetation and turbulence dissipation and computed gas dispersion from any given CO_2 mass source. Each of the FACE plots was enclosed in an area of nearly 350 m^2. The octagonaly shaped ring was designed to provide an internal area with equal distribution of CO_2 with a diameter of 22 metres. Each ring had polyethylene pipes (diameter 25 mm) releasing pure CO_2, suspended at various heights to facilitate vertical distribution of CO_2 within the canopy. At the beginning of the experiment, immediately after planting, an initial single layer of pipes was located at approximately 50 cm above ground. Later on second and third layer of pipes were added, reaching a maximum height of 12 m. An automatic monitoring station at the centre of each plot collected meteorological data that was used to control the directional release of gas in the FACE plots. Daytime CO_2 enrichment was provided from bud burst to leaf fall starting from spring 1999 to fall 2004.

In winter 2001-2002, after 3 years of growth, the whole plantation, including the experimental plots, was coppiced. All biomass was chipped and used to generate electricity in a newly constructed power plant. All trees were cut at the base of the stem (5-8 cm above ground), resulting in stools with many new re-sprouting shoots in spring 2002.

The fertilizer treatment started in the spring 2002: hydraulic pumps (Ferti-injector Amiad, IMAGO srl, Italy), installed outside each plot, added the fertilizer in the fertilized half of each plot. In the first year the fertilizer was Navarson 20 (16 ammoniacal and 4 nitric)-6-6 N, P, K + microelements was supplied weekly (16 weeks, starting on July 8) in constant amount through the drip-irrigation system, providing a total concentration of 212 kg N ha^{-1}. In the 2003-2005 growing seasons the fertilizer Ammonium Nitrate 34 (17 ammoniacal and 17 nitric) was supplied weekly in amounts proportional to the growth rate for 20 weeks and provided a total amount of 290 kg N ha^{-1}.

2.2 Soil sampling and handling

Soil cores 10 cm wide, 20 cm long were collected inside each of the three sectors in each plot after removal of litter layer (1-2 cm depth). Following the soil profile description of Hoosbeck et al. (2004), in all plots the depth 0-20 was included in the A horizon. After sampling, the soil cores were immediately sieved (<2mm), the roots were removed and soils were stored at 4 °C prior analysis. For all biochemical analysis, soil water content was adjusted to the 60% of the water holding capacity. This ensures similar conditions for the microbes as concerns the availability of water, which is crucial for their growth and metabolic activity. From October 2000 to October 2001 two soil cores per genotype were collected in not fertilized sub-plots, for a total of 36 soil cores. From June 2002 to October 2003 two soil cores per genotype were collected in fertilized and not fertilized sub-plots, for a total of 72 soil cores. In June 2002 soil samples were collected also in fertilized sub-plots although the addition of nitrogen started the following month, then data related to these samples are not considered in the calculation of the fertilization effect. In June and October 2004 and July 2005 one soil core was collected in fertilized and not fertilized sub-plots, for a total of 36 soil cores. July 2005 samples were used only for DGGE and CLPP analyses.

In the second cycle of the plantation the fertilization treatment started in July 2002. The first sampling date of June 2002 was considered as t_0 of fertilization treatment and reported in the graphs, but was excluded from statistical analysis to detect the effect of fertilization and its interaction with the others factors of variation.

2.3 Statistical analysis

Analysis of variance (ANOVA) was performed to evaluate the main effects of elevated [CO_2], fertilization, species and their interaction on parameters analysed. Data were tested for normality with the Shapiro-Wilk statistic. A randomised block design was applied using the general linear model procedure of Systat 11.0 statistical software package (SPSS Inc.), with elevated [CO_2], fertilization and species as factors of variation. The two replicates for each plot were averaged and plot (3 control plots and 3 FACE plots) was the unit of replication. The significance of FACE effect was determined in not fertilized plots (years 2001-2004, n = 36). The significance of fertilization and its interaction with FACE was determined in fertilized and not fertilized plots from 2002 to 2004 (n = 72). When there were no significant variations due to the different poplar species, data from different poplar genotypes were pooled together. When interactions were not significant they were excluded from analysis.

Bonferroni post Hoc test was performed to determine which pair of means differ significantly, using the Pairwise comparison command of the general linear model of Systat 11.0 statistical software package (SPSS Inc.).

In the results section the effect of elevated [CO_2] and/or fertilization treatments has been reported as percentage variation with respect to the control. FACE effect it has been calculated and reported as average of not fertilized and fertilized sub-plots. Fertilization effect it has been calculated and reported as average of FACE and Control soils. Both FACE and fertilization effects referred to all sampling dates from October 2002 to October 2004: June 2002 is, in fact, not included since fertilization was started the following month.

Multivariate analysis of variance (MANOVA) was performed to evaluate the main effects of FACE, fertilization, clones and their interaction on MicroResp data using the general linear model of SPSS 11.0 statistical software package (SPSS Inc.). ANOVA was performed for each substrate using CO_2, fertilization and clones as factor of variation.

Principal Component Analysis was performed using correlation method and no rotation.

DGGE Gels were analyzed and lanes were normalized using GelCompar II software (Applied Maths, Ghent, Belgium). A curve-based dendrogram was constructed by using the Pearson Correlation Index for each pair of lanes within the gel.

2.4 Soil analysis

Physical analysis

Soil water content

5 g field-moist soil samples were weighed in ceramic pots. Soils were placed in a stove at 100 °C. After 24 hours soils were removed from the stove and weighed again. Soil water content was referred to the fresh weight.

Water holding capacity

Soil samples were saturated with water in a cylinder. The cylinder was placed on an absorbent membrane until the excess water was drawn away by gravity. When equilibrium was reached, the water holding capacity was calculated based on the weight of the water held in the sample vs. the sample dry weight.

Chemical analysis

Soil pH

Soil pH or soil reaction is an indication of the acidity or alkalinity of soil and is measured in pH units. Soil pH is defined as the negative logarithm of the hydrogen ion concentration. Sieved soil was suspended in a solution of deionised water (to determine active acidity) or in KCl 1N (to determine exchangeable acidity) in 1:2.5 ratio. The pH was measured in the surnatant with a pH meter (pH 211, Hanna Instruments).

Cation exchange capacity

(Gillman, 1979)

The cation exchange capacity (CEC) of a soil is simply a measure of the quantity of sites on soil surfaces that can retain positively charged ions (cations) by electrostatic forces. Cations retained electrostatically are easily exchangeable with other cations in the soil solution and are thus readily available for plant uptake. Soil CEC is normally expressed in units of charge per weight of soil (meq/100 g).

2 gr of soil were saturated with a 10% $BaCl_2$ solution pH 8.1. After 2 hours shaking, samples were centrifuged and solution was decanted. 0.1N $MgSO_4$ was then added to replace Ba with Mg. After shaking, samples were centrifuged and solution was decanted. 10ml of supernatant were mixed with NH_4Cl pH 10 and then titrated with EDTA 0.05N with a potentiometric titration.

Total Organic Carbon (TOC)

(Springer, U. and Klee, J., 1954).

The methods we used relies on the reduction of $Cr_2O_7^{2-}$ by organic C compounds present in the soil and subsequent determination of unreduced $Cr_2O_7^{2-}$ by oxidation-reduction titration with Fe^{2+}.

$$2Cr_2O_7^{2-} + 3C^0 + 16H^+ = 4Cr^{3+} + 3CO_2 + 8H_2O$$

The potentiometric titration uses the different electric potential between the calomel electrode and the solution.
Weigh 1 g of soil in 100 ml volumetric flasks and add 10 ml H_2SO_4 and 10 ml $K_2Cr_2O_7$. Then incubate for 90 minutes at 100 °C. After the incubation cool the flasks with ice and bring to volume with deionized water. Add 5 ml of H_2SO_4 to 20 ml of oxidized C solution, and titrate with Ferrous ammonium sulphate 0.2 N (TT 85 Titrator, Radiometer Copenhagen). Two controls without C are also analyzed.

Total Extractable Carbon (TEC)

(Springer, U. and Klee, J., 1954).

The procedure consists in an extraction with a strong basic extracting solution. After the extraction, the principle of the method is the same as TOC.

4 g of soil were weighed in 250 ml plastic flasks and 100 ml of 0.1 M $NaOH/Na_4P_2O_7$ were added. After an 48 hours incubation at 65 °C, the extracts were filtered with filter paper Whatman 42 and an aliquot was transferred in volumetric flasks, 10 ml or less depending on the C content (between 5 and 25 mg of C). The rest of the procedure is the same as for TOC.

Water Soluble Carbon (WSC)

(Burford J.R., Bremner J.M., 1975)

15 g of soils were extracted in 30 ml of cold deionized water for 30 min at 250 rpm. After a centrifugation at 4000 rpm extracts were filtered with filter paper Whatman 42.

The oxidation of 10 ml of extracts followed the method of TOC, with 1 ml of $K_2Cr_2O_7$ 0.4N, 10 ml of H_2SO_4 96%, 5 ml of H_3PO_4 85%. After 1 hour incubation at 100 °C, samples were cooled with ice and deionized water was added to reach the final volume of 100 ml. 25 ml of extract, and two controls with water, were tirated with 0.0333N Ferrous ammonium sulphate with an automatic titrator (TT 85 Titrator, Radiometer Copenhagen).

Total nitrogen

(Bremner and Mulvaney, 1982)

Total N is mainly constituted by organic N. The Kjeldahl procedure involve two steps: i) digestion of the sample to convert organic N to N-NH_4^+ and ii) determination of the N-NH_4^+ in the digest. The digestion is performed by heating the sample with sulphuric acid (Heater unit BD-40, Technicon Instrument Corporation). Selenium acts as a catalyst of the reaction.

1 g of soil was weighed in digestion tubes 10 ml of H_2SO_4 concentrate (95%) and 1.1 of selenium catalyst (K_2SO_4, $CuSO_4$, Selenium at 100:10:1 ratio) were added.

After digestion all nitrogen is in the form of ammonium and the detection method is the same as for ammonium, using only 0.1 ml of digested.

Inorganic nitrogen

Inorganic N is the sum of ammonium and nitrate in the soil solution. Inorganic N is supplied by the mineralization of organic compounds or added to soil as fertilizers.

Ammonium

(Anderson and Ingram, 1993)

Ammonium was extracted in 1M KCl with a 1:4 extraction ratio. For the determination of ammonium we used a colorimetric method.

The calibration curve was made with standards of ammonium sulphate containing 0, 5, 10, 15, 20, 25 μg/ml NH_4^+ -N.

In test tubes 1 ml of each standard and sample, 5ml of reagent 1 (25 g sodium citrate, 0.12 g sodium nitroprusside, 34 g sodium salycilate, 25 g sodium tartrate in 1l) and, after 15 min, 5 ml of reagent 2 (30 g sodium hydroxide, 10 ml sodium hypochlorite solution 5% in 1l) were added. After 1 hour of incubation for full colour development, the absorbance was read at 655 nm with a spectrophotometer.

Nitrate

(Cataldo et al., 1975)

Nitrate was extracted in 0.5M K_2SO_4 with a 1:2 extraction ratio. For the determination of nitrates we used a colorimetric method.

The calibration curve was made with standards of potassium nitrate containing 0, 2, 4, 6, 8, 10 μg/ml NO_3^- -N.

In test tubes 0.5 ml of each standard and sample, 1ml of salicylic acid and, after 30 min, 10 ml of NaOH were added. After 1 hour of incubation for full color development, the absorbance was read at 410 nm with a spectrophotometer.

In situ net nitrogen mineralization

(Eno, 1960)

Two adjacent soil cores were collected (10 cm in diameter and 20 cm depth) in each sector. One core was replaced in the field in a thin plastic bag making sure water cannot enter and incubated for 1 month. The other core was immediately returned in laboratory, sieved and the nitrate and ammonium content were measured with above described methods. After 1 month the other core (final) was removed from the field and treated as for the first core (initial).

Net mineralization, net ammonification and net nitrification were calculate as follows:

$Final_{(N\text{-}NH4+ + N\text{-}NO3-)} - Initial_{(N\text{-}NH4+ + N\text{-}NO3-)}$ = net N mineralization

$Final_{(N\text{-}NH4+)} - Initial_{(N\text{-}NH4)}$ = net ammonification

$Final_{(N\text{-}NO3-)} - Initial_{(N\text{-}NO3-)}$ = net nitrification

Biochemical analysis

Microbial Biomass C

(Vance et al., 1987).

Several methods have been used to estimate microbial biomass in soil. In this work was used the Fumigation Extraction method. The chloroform fumigation of soil kills and lyses microbial cells with the release of cytoplasm into the soil environment; thus the cell material can be extracted from soil.

Two portions of moist soil (20 g oven-dry soil) were weighed, the first one (non fumigated) was immediately extracted with 80 ml of 0.5M K_2SO_4 for 30 min by oscillating shaking at 200 rpm and filtered with filter paper (Whatman n. 42); the second one was fumigated for 24h at 25 °C with ethanol-free $CHCl_3$ and then extracted as described above. Organic C in the fumigated and non fumigated extracts was determined after oxidation with 0.4 N $K_2Cr_2O_7$ at 100 °C for 30 min. The remaining procedure is the same as for WSC.

To calculate the microbial C the formula is the follow:

$$\mu gMBC = ((\mu gC_F) - (\mu gC_{NF})) * 2.64$$

The conversion factor of 2.64 was calculated by relating the results of fumigation incubation and fumigation extraction method.

Microbial Biomass Nitrogen

(Joergensen and Brooks, 1990)

On the same extracts used to determine the microbial biomass C it is possible to estimate the microbial biomass N. The colorimetric method uses the ninhydrin reagent: ninhydrin

forms a purple complex with molecules containing α-amino nitrogen and with ammonium and other compounds with free α-amino groups such as amino acids, peptides and proteins. The amount of ninhydrin reactive compounds, released from the microbial biomass during the CHCl3 fumigation and extracted by K2SO4, is strongly related to the initial soil microbial biomass C content.

The calibration curve was made with standards of L-Leucine containing 0, 2, 4, 6, 8, 10 μg/ml NH_4^- -N.

1 ml of K_2SO_4 extracts (fumigated and non fumigated) were placed in 10 ml test tubes. 0.5 ml of Ninhydrin reagent solution were added and test tubes were mixed thoroughly. Samples were then heated to 100 °C for 25 min. After heating, an ethanol: water mixture was added, the solutions were mixed thoroughly again and the absorbance was read at 570 nm (UV mini 1240, Shimadzu).

The calculation for the ninhydrin reactive N (NRN) in the microbial biomass is the following:

$$\gamma NRN = (\mu gN_F) - (\mu gN_{NF})$$

The calculation for the microbial biomass N is the following:

$$\mu gMBN = \gamma NRN * 5$$

Microbial potential respiration

(Badalucco et al., 1992)

The metabolic activity of soil microorganisms can be quantified by measuring the CO_2 production or O_2 consumption. The temperature of incubation is about 28 °C, a not limiting temperature for microbial activity. The soil respiration without added substrates can be followed for long periods of time. From 2000 to 2003 we followed the CO_2 production for 10 days, while in 2004 for 28 days. The CO_2 evolved during the incubation of soil in a closed system is trapped in an NaOH solution, which is then titrated with HCl.

40 g of sieved soil (60% WHC) were weighed in a baker and placed in the bottom of a 1l jar. In another baker inside the jar 2 ml NaOH were placed. The CO_2 evolved was trapped, during the incubation period, in 2 ml 1M NaOH. The reaction was stopped with 4 ml of $BaCl_2$ 0,75 N to precipitate C in $BaCO_3$. The excess of NaOH that did not react with the CO_2 was determined by titration with 0.1M HCl.

The CO_2 evolved during the 10^{th} day of incubation was used as the basal respiration value because, after that period, the soil reached a relatively constant hourly CO_2 production rate.

In June and October 2004 the C mineralization kinetics up to 28 days was performed following Riffaldi et al. (1996), using the first order kinetic model:

$$C_m = C_0 (1-e^{-kt})$$

where

C_m = cumulated mineralized C during the incubation time

t = incubation time (28 days)

C_0 = potentially mineralizable C

k = kinetic constant

Microbial indices

Microbial indices were calculated as follows:

Cmic:Corg = µg of Biomass C µg total organic carbon^{-1} (Anderson & Domsch, 1989);

qCO_2 = (µg C-$CO_{2\ basal}$ h^{-1} x µg Biomass C^{-1})10^3 (Dilly and Munch, 1998);

qM = µg C-$CO_{2\ cumulative}$ µg total organic carbon^{-1} (Pinzari et al., 1999);

qC = µg biomass C - loss µg biomass C^{-1} d^{-1} (modified by Anderson & Domsch, 1990).

Acid phosphatase activity

(Garzillo et al., 1996)

Phosphatases belongs to the group of phosphoric monoester hydrolases and catalyse the hydrolysis of phosphate esters, releasing inorganic phosphorus which can be taken by plants. Acid phosphatase it has been detected in microorganisms, plants and animals.

Acid phosphatase assay is carried out near neutral pH (6.5-7.0) and the optimum temperature has been found to range from 40 to 60 °C. Artificial substrates such as phenyl phosphate or p-nitrophenyl phosphate are low molecular weight esters that are more rapidly hydrolysed than natural substrates. The reaction is the follows:

p-nitrophenyl phosphate + $H_2O \rightarrow$ p-nitrophenol + H_3PO_4

The method is based on the colorimetric determination of p-nitrophenol (pNP) released after the incubation of soil with p-nitrophenyl phosphate (pNPP) for 20 min at 37 °C.

The calibration curve was made with standards containing from 0 to 50 ppm of p-nitrophenol. The absorbance was read at 410 nm with a spectrophotometer (UV mini 1240, Shimadzu).

To avoid the determination of autohydrolitic reactions of substrate that are independent by phosphatase activity and the interference of chromophores already presents in the soil, two series of controls were made: one without the soil but with all reagents as the samples and one with soil but without the substrate.

β-glucosidase activity

(Eivazi e Tabatabai, 1988)

β-glucosidase is the rate limiting enzyme in the microbial degradation of cellulose to glucose. The enzyme catalyses the hydrolysis of glucosides. It has been detected in micro-organisms, plants and animals. The method is based on the determination of the released p-nitrophenol after the incubation of soil with p-nitrophenyl glucoside solution for 1 hour at 37 °C.

The calibration curve was made with standards containing from 0 to 50 ppm of p-nitrophenol. The absorbance was read at 400 nm with a spectrophotometer (UV mini 1240, Shimadzu).

To avoid the determination of auto-hydrolytic reactions of substrate that are independent by β-glucosidase activity and the interference of chromophores already presents in the soil, two series of controls were made: one without the soil but with all reagents as the samples and one with soil but without the substrate.

Chitinase (N-acetyl-β-D-glucosaminidasi) activity

(Badiane et al. 2001)

Chitin, a homopolymer of N-acetyl-glucosamine, is the major organic element in the exoskeleton of insects, crustaceans, and many species of fungi and other organisms. Chiti-

nases are produced by bacteria, fungi and plants. Significant correlation between chitinase activity and nitrogen content has also been found. The method is based on the determination of the released p-nitrophenol after the incubation of soil with p-nitrophenyl glucoside solution for 2 hours at 37 °C.

The calibration curve was made with standards containing from 0 to 50 ppm of p-nitrophenol. The absorbance was read at 400 nm with a spectrophotometer (UV mini 1240, Shimadzu).

To avoid the determination of auto-hydrolytic reactions of substrate that are independent by β-glucosidase activity and the interference of chromophores already presents in the soil, two series of controls were made: one without the soil but with all reagents as the samples and one with soil but without the substrate.

Arylsulphatase activity

(Elsgaard et al., 2002)

Sulphatases catalyse the hydrolysis of organic sulphate esters in phenol e SO_4^{2-} following the irreversible reaction:

$$R\text{-}OSO_3^- + H_2O \rightarrow R\text{-}OH + H^+ + (SO_4)_2^-$$

Arylsulphatases has been detected in microorganisms, plants and animals. In soil, the sulphate esters represent a large fraction (25-93%) of total sulfur, but the role of arylsulphatase in sulphur mineralization is not clear. The inorganic sulphur (SO_4^{2-}), S(IV) e S (VI), the PO_4^{3-}, AsO_4^{3-}, MoO_4^{2-} and WO_4^{2-} inhibite the enzymatic activity. The activity is extracellular for the 45%, and the remaining 55% is associated with the microbial biomass.

The method is based on the determination of the released p-nitrophenol after the incubation of soil with p-nitrophenyl glucoside solution for 1 hour at 37 °C.

The calibration curve was made with standards containing from 0 to 50 ppm of p-nitrophenol. The absorbance was read at 400 nm with a spectrophotometer (UV mini 1240, Shimadzu).

To avoid the determination of auto-hydrolytic reactions of substrate that are independent by arylsulphatase activity and the interference of chromophores already presents in the

soil, two series of controls were made: one without the soil but with all reagents as the samples and one with soil but without the substrate.

Denaturing Gradient Gel Elettrophoresis (DGGE)

DNA was extracted with a bead beating procedure using the BIO 101 Fast DNA Spin Kit for soil of October 2004 and the MO BIO PowerSoil DNA Isolation kit for soil for July 2005. The effectiveness of extraction was checked on ethidium bromide agarose gels. DNA extracts of July 2005 were purified prior the PCR with the Wizard DNA Clean up Kit (Promega). 18S rDNA gene fragments were amplified with PCR using fungal primers (FR1 GC and FF39, Vainio and Hantula, 2000). The reaction mixture consisted of 1 µl of template DNA, 1x DNA polymerase buffer, 2.5mM $MgCl_2$, 4% (v/v) dimethyl sulfoxide, 0.1µg μl^{-1} Bovine serum albumin (BSA), 0.2 µM of each primer, 0.2 mM desoxynucleotide triphosphates, 0.025 U μl^{-1} BioTherm^{TM} DNA polymerase (GeneCraft) and sterilized water to reach the final volume of 25 µl. After 8 min of denaturation at 94 °C, 35 thermal cycles of 30 s at 94 °C, 45 s at 48 °C, and 3 min at 72 °C were performed, followed by an extension step at 72 °C for 10 min. DGGE was performed with the INGENY phorU system. The PCR products (2 µl) were loaded onto 8 % (w/v) polyacrylamide gels containing a linear denaturing gradient ranging from 30% to 70% (100% denaturant corresponds to 7 M urea plus 40% (v/v) of deionized formamide) and DGGE was performed in a 1xTAE buffer at 60 °C at a constant voltage of 100V for 18 h. After electrophoresis, gels were stained with an automated gel stainer (Hoefer) using silver staining (Felske et al., 1996).
DGGE Gels were analyzed and lanes were normalized using GelCompar II software (Applied Maths, Ghent, Belgium). A curve-based dendrogram was constructed by using the Pearson Correlation Index for each pair of lanes within the gel.

Community Level Physiological Profiling (CLPP)

Analysis of CLPP was performed using the MicroResp method as described in Campbell et al. (2003). Carbon Substrates were selected depending on their ecological relevance to soil and their solubility in water. In particular rhizospheric C sources (carboxylic acids and carbohydrates) were chosen considering the importance of root inputs for microbial metabolism. Nitrogen was added in the form of NH_4NO_3 to check the role of the N availability in

the C utilization. Each C source was dissolved in deionised water and added to soils to deliver 30 mg C g soil water^{-1}. N-acetyl-glucosamine was delivered at 7.5 mg C g soil water^{-1} because of its poor solubility. The C sources were dispensed before the soil was added to ensure that the soil contacted the C source at the same time for all wells. Soil was placed in 96 deep-well plates volumetrically by using another 300 µl well plate from which the bottom had been removed. Approximately 0.4 g of soil was added to each well. Three lab replicates were used for each field replicate. The deep-well plate was then sealed with the detection plate.

To detect the evolved CO_2, a colorimetric method relying on the change in the pH of a gel-based solution of bicarbonate. The absorbance at 595 nm was read with a Zenyth multimode reader (Anthos Labtech, Wals, Austria) immediately before and after 6 h of incubation at 22 °C. The absorbance after 6 h was normalized for any differences recorded at time zero and then converted to the headspace CO_2 concentration by using a calibration curve obtained with a infra-red based respiration measurement device (Heinemeyer et al., 1999) using 5 different soils with and without glucose addition (1% C). The best fit for the calibration curve, using the difference of absorbance between t_0 and t_1 (after 6 h) was: $y = 0.1229 \ln(x) + 0.2091$, $R^2 = 0.835$.

Temperature function of rhizo-microbial respiration

In February and in May 2004, 24 soil samples were collected in the 12 sub-plots planted with *P. x euramericana* (2 samples x 3 replicates x 2 CO_2 level x 2 nitrogen), with cylinders of 5 cm of diameter in the 0-5 cm soil layer. After the sampling, the intact soil cores were kept at 4 °C for about 24 hours.

The soil CO_2 efflux (SR) was measured in the laboratory using an open gas exchange system (CMS 400, WALZ) connected to an IRGA (BINOS 100/4P, Leybold AG). Soil cores were placed in a cuvette under controlled temperature and humidity conditions.

Preliminary measurements were performed to check the effectiveness of the method: further 8 soil cores were collected at POPFACE site and CO_2 efflux measurements at 5 °C started immediately after the sampling and continued for the following 24 hours. During this period, the CO_2 efflux evolved from soil cores is expected to reach an equilibrium with the air flow in the cuvette. Within few hours (2-3) soil respiration achieved a steady state that was maintained for the whole period of measurement. This equilibration period was observed for all the CO_2 efflux measurements.

The temperature response of soil respiration was estimated on the 24 intact soil cores by rising gradually temperature from 5 to 25 °C (±0.05) with steps of 2.5 °C. For every step the soil was allowed to acclimate to the measurement temperature and respiration rate was allowed to stabilize for about 20 minutes before it was recorded. The measurement of CO_2 efflux in the 20 °C interval required on average 6-8 hours for each soil core. Over the range of measurement temperatures, relative humidity was maintained stable at 80% (±10).

The steady state values were used to estimate the parameters of the first-order exponential function:

$$SR = a \cdot e^{kT} \qquad \text{(eq. 1)}$$

where SR is the measured soil CO_2 efflux, *a* is the value of SR at 0 °C (intercept), *k* is the rate of the SR increase (slope) and T is the soil temperature in °C.

The Q_{10} values were calculated with the function:

$$Q_{10*} = \frac{R_{(T+10)}}{R_T} \qquad \text{(eq. 2)}$$

To examine the possibility of a residual CO_2 efflux coming from roots after the 24 hours of incubation we sampled 12 soil cores (3 replicates x 2 CO_2 level x 2 fertilization) with cylinders of 5 cm of diameter in the 0-5 cm soil layer. The intact soil cores were treated as described above and after 24 hours at 4 °C, fine roots were extracted from the samples. The root segments were immediately hand washed, dried and placed inside the cuvette for respiration measurement at 18 °C. Respiration rate was allowed to equilibrate before it was recorded (Janssens et al., 1998). During this period the CO_2 efflux reached a *plateau* value. This CO_2 efflux rate (μmol CO_2 g^{-1} s^{-1}) was considered as the residual root respiration for each treatment and was subtracted from the CO_2 efflux rate of the intact soil cores. The first order exponential function was then corrected as follow:

$$SR = \alpha \cdot e^{kT} \qquad \text{(eq. 3)}$$

were α = a – a*root fraction/100, and the root fraction was the value of the root residual respiration for each soil core.

In situ measurement of soil respiration and temperature function

Soil respiration was measured every two weeks or monthly during years 2003-2005. Three semi-permanent plastic collars were installed into the soil surface for each sector of the plantation. Early tests indicated that the use of the collars did not affect measurements. Measurements were made manually with an infrared gas analyzers (IRGA) operated in the closed path mode. The environmental gas monitor (EGM-4, PP systems, UK) used was equipped with the SRC-1 soil respiration cuvette engaged on the soil collars. Each measurement was made for twice the time requested for the rate of soil CO_2 efflux to become stable (usually within 2 min). A quadratic model was fitted to the concentration in the chamber and time. At the time of measurement, soil temperature was recorded using a manual digital thermometer to a depth of 3-5 cm.

To analyze the dependence of soil CO_2 efflux on temperature, soil respiration values were used to estimate the parameters of the first-order exponential function:

$$\mathrm{SR} = a \cdot e^{kT}$$

where SR is the measured soil CO_2 efflux, a is the value of SR at 0 °C (intercept), k is the rate of the SR increase (slope) and T is the soil temperature in °C.

The Q_{10} values were calculated with the function:

$$Q_{10*} = \frac{R_{(T+10)}}{R_T}$$

In situ separation of soil respiration components

In April 2003 108 bottomless pvc tubes 10cm ∅, 50 cm long were installed in the experimental plots, 3 tubes for each sector. About 47 cm of the tubes extended belowground and the top rim of the tubes extended about 3 cm from the soil surface, and worked as collars for CO_2 efflux measurements. In each sector three soil conditions were produced, one for each tube:

- **Soil-h:** Soil cores with the dimension of the tubes were dug in each sector. Soil cores were sieved and fine and coarse roots were removed. The tubes were inserted in the holes and the soil without roots was placed back in the tubes in re-

verse order of removal. Soil was compacted to try to reach previous condition of bulk density;

- **Soil-d:** The tubes were inserted in the ground and, in this way, roots were trenched. The tubes itself prevented living roots inclusion;
- **Soil –r:** The tubes were inserted in the ground and, in this way, roots were trenched. In this case the tubes had openings which made possible new living roots inclusion. The openings were large enough to allow the inclusion of fine and coarse roots (around 5 x 3 cm).

Measurements of CO_2 efflux were made monthly in the three Soil types with an environmental gas monitor (EGM-4, PP systems, UK) equipped with the SRC-1 soil respiration cuvette engaged on the top of the tubes.

At the end of the experiment, in February 2005, soil cores were collected in the top layer of the soil types h and r in the not fertilized sectors of *P. nigra* and *P. x euramericana* with cylinder 5 cm x 5 cm. Sample soils were sieved at 2 mm, roots were removed from soil-r and root biomass was measured.

The following analyses were performed in the sampled soil cores:

TOC, Microbial biomass C, Water Soluble C, K_2SO_4 Extractable C, and the Community Level Physiological Profile (CLPP) using different C and N substrates.

3. SOIL NUTRIENTS AVAILABILITY AND PROCESSES: AN ANALYTICAL APPROACH USING CHEMICAL AND BIOLOGICAL INDICATORS

3.1 Introduction

Soil nutrient availability is an important regulator in the response of plants and microorganisms to elevated [CO_2] and of ecosystem capability to sequester C. In fact, nutrients and carbon cycles are strictly related within plants and metabolism of nitrogen and other nutrients is regulated by signals that are derived from carbon metabolism. When plants are exposed to elevated [CO_2] concentrations, they often exhibit enhanced growth with increased biomass accumulation, which in the long-term can result in an increased nutrient uptake from the soil.

Short term experiments indicate that mineral N availability in the soil can decrease, increase or exhibit no significant change beneath temperate trees grown under elevated atmospheric CO_2 (Zak *et al.,* 2000). N mineralization under elevated [CO_2] presents a high degree of variability within and between soils under graminoid, herbaceous and woody plant species (Zak et al., 2000). Explanations for lower soil N availability under elevated [CO_2] include decreased leaf N concentrations, leading to litter that is more recalcitrant to decomposition (Cotrufo et al., 1998) and increased labile C exudates by a higher fine root biomass, causing increased microbial N assimilation (Janssens et al., 1998). However, some studies suggests that N mineralization could increase in response to enhanced labile C supply from roots (Zak et al., 1993).

Microbial biomass has an important role in determining the availability of nutrients to plants in a [CO_2] enriched atmosphere. On the one hand, in the absence of adequate N availability the oxidisation of old organic matter can be increased (*priming-effect*) in order to obtain mineral N to build up new biomass (Zak *et al.,* 1993; Cardon, 1996; Allen *et al.,* 2000); on the other hand, easily decomposable substrates from plants because of faster root turnover or increased production of root exudates (i.e. simple carbohydrates and organic acids exuded by fine root biomass) can stimulate microbial growth and N immobilisation, thus reducing plant available N (Diaz et al., 1993; Cardon et al.,1996; Cheng, 1999; Schortmeyer et al., 2000).

Changes in plant above- and belowground litter production and quality under elevated [CO_2] could indeed alter microbial N demand and the amounts of N available for plant uptake (Zak *et al.,* 2003). The carbon nutrient balance hypothesis (CNBH) predicts that under conditions favouring C uptake, as in elevated [CO_2] environments, C not needed for growth can be stored as non structural carbohydrates (tannins, starch or phenolics). These can subsequently be used in the synthesis of C-based secondary compounds that alter litter

chemistry (increases in C:N or lignin:N ratio) thus affecting decomposition rates (Allen et al., 2000; Hartley et al., 2000; King et al., 2001, Tuchman et al., 2002).

The biochemical composition of the organic inputs through its influence on C decomposition rate in the soil has also an important effect on the Cation Exchange Capacity (CEC) (Tian et al., 1992; Vanlauwe et al., 1996). Readily plant-available base cations are located on exchange sites of SOM and mineral surfaces, where they are released to the soil water by cation exchange, and absorbed by plants or microbes or transported to groundwater. The CEC is often related to stable aggregate (Dimoyiannis et al., 1998) thus improving soil structure, that in turn is a key factor in the functioning of soil, its ability to support plant growth and to increase C sequestration (Bronick and Lal, 2005). SOM is the most important contributor to the CEC and has a main role in determining the sign and magnitude of the net charge and therefore in the retention of plant nutrients (Oorts et al., 2003). Although it is well known that the dominant sources of the exchangeable cations are from primary-mineral dissolution and atmospheric deposition (Chadwick et al., 1999), how contemporary increases in atmospheric [CO_2] are affecting cation exchange, soil acidification, and mineral dissolution is practically unexplored (Andrews and Schlesinger, 2001, Oh and Richter, 2004). Soil biophysical models that simulate how soil respiration controls the concentrations of soil CO_2 suggest that increasing soil respiration by twofold, approximately doubles the belowground concentration of CO_2 (Cerling, 1984, Amundson et al., 1998). Soil CO_2 dissolves and reacts with soil water forming carbonic acid. This provides protons that stimulate cation exchange and mineral dissolution releasing base cations to soil water, therefore lowering pH and increasing the cation concentration in soil solution (Allen and Schlesinger, 2001; Oh and Richter, 2004).

Little is also known on how phosphorus availability and demand are affected by elevated [CO_2]: Harley et al. (1992) suggested that the inorganic phosphorus could become a limiting factor for photosynthetic activity and could provoke plants down-regulation responses under elevated [CO_2].

Elevated [CO_2] and fertilization treatment can also alter the extracellular enzyme activity, changing the utilization of plant derived substrates (Phillips et al., 2002). Decomposition processes are in fact the result of the collective activity of microbial communities driven by the acquisition of nutrients released by extracellular degradation of detritus (Sinsabaugh and Moorhead, 1994). Soil enzymatic activities are closely related to microbial activity or biomass as they catalyse biochemical reactions and nutrient cycling in the soils. The concentration of substrates induces soil microbial synthesis of extracellular enzymes and as-

saying the substrates degradation is one approach of assessing physiological capabilities of microbial communities (Larson et al., 2002).

Soil enzymes have been widely referred as early indicators of ecosystem stress and candidate "sensors" of changes in soil management, in soil health, in microbial activity patterns, in soil ecological stress, in soil fertility (Sinsabaugh et al., 1994; Bandick and Dick, 1999; Aon et al., 2001; Badiane et al., 2001; Vepsäläinen et al., 2001). The use of soil enzymatic activities can provide important information to support other parameters related to soil microbiota responses to elevated [CO_2] (Moorhead and Linkins, 1997; Mayr et al., 1999; Moscatelli et al., 2001; Larson et al., 2002: Ebersberger et al., 2003). In this context measuring the activity of several soil enzymes could be useful to understand the organic matter turnover and the availability of inorganic nutrients and could give indications on the function and quality of an ecosystem and on the interaction among subsystems (Dick and Tabatabai, 1993).

The aims of this work were:

1) to detect the modifications induced by the treatments with elevated [CO_2] and nitrogen fertilization on soil capacity to sustain plant production providing exchangeable cations and other nutrients (mineral N and P) through microbial processes of mineralization and immobilization
2) to evaluate if soil enzymes, as indicators of microbial and plant nutrient acquisition activity, were modified by the treatments.

3.2 Results

Active and exchangeable pH

POPFACE soil presented a pH of 6.38 on average in the two sampling dates (2000 and 2004), a low acid – neutral soil in the U.S.D.A. classification (fig. 3.1). The three clones and the treatments with elevated [CO_2] and N fertilization did not induce any significant change in the active acidity (pH_{H2O}), that was stable in the years from 2000 to 2004.

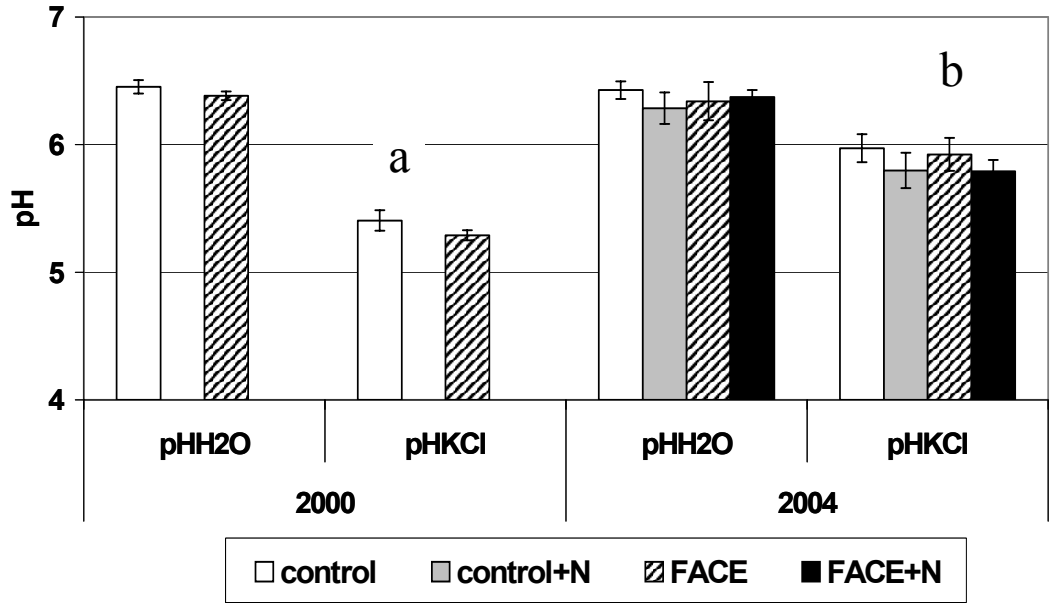

Figure 3.1. Active and exchangeable acidity in Control and FACE not fertilized soil in the year 2000 and in Control and FACE fertilized and not fertilized soils in the year 2004. Values from the three clones were pooled together since no significant differences were found. Different letters for pH_{KCl} mean a statistically significant difference between the average values of the two sampling dates ($p<0.001$).

The pH_{KCl} is an estimate of soil exchangeable acidity and gives indirect information on soil exchange capacity. It increased significantly in 2004 with respect to four years before (fig. 3.1). This increase was similar for the three clones and no modifications induced by the treatments or by the interaction between time and CO_2 enrichment were found.

Cation Exchange Capacity (CEC)

The Cation Exchange Capacity (CEC) was measured in three years (2000, 2002 and 2004). CEC was similar for the three clones in each sampling date and the average values were presented (fig. 3.2). The temporal variation from 2000 to 2002 and to 2004 was significant. The significant increase observed in 2004 with respect the initial values is reported in tab. 3.1.

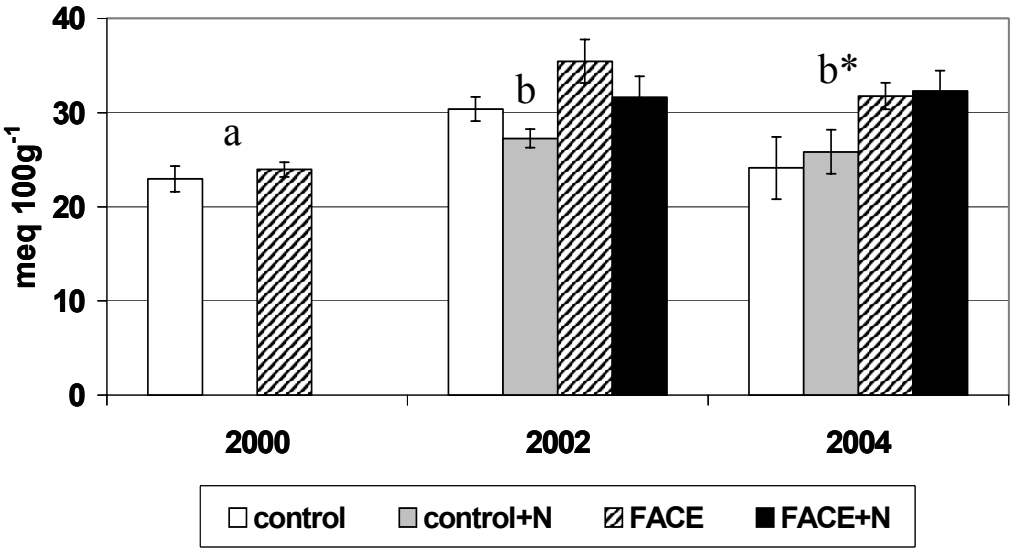

Figure 3.2. Cation Exchange Capacity in Control and FACE not fertilized soil in the year 2000 and in Control and FACE fertilized and not fertilized soils in the year 2002 and 2004. Values from the three clones were pooled together since no significant differences were found. Different letters mean a statistically significant difference between sampling dates. For the year 2004 the * means a statistically significant difference between FACE and Control soils ($p<0.001$).

Elevated [CO_2] treatment induced a significant increase in CEC in 2004 in all clones independently by the N supply (fig. 3.2). Moreover the temporal increase observed from 2000 to 2004 was significantly higher in FACE with respect to Control plots (tab. 3.1).

	pH_{H2O}	pH_{KCl}	CEC
Control [n]	-0.4%	+10.5%	+5.0%
FACE [n]	-0.7%	+12.0%	+32.6%
	Analysis of Variance		
time	n.s.	0.000	0.000
CO_2	n.s.	n.s.	0.009

Table 3.1. Temporal variation of pH_{H2O}, pH_{KCl} and CEC in Control and FACE not fertilized soil. The relative change is expressed in percentage with respect to the values of the year 2000. The Analysis of Variance is reported for time and CO_2 enrichment as factors of variation. p values <0.05 are reported. No significant interactions were removed from the analysis.

Total nitrogen

The total N content of POPFACE soils was the 0.15% on average. The temporal fluctuation did not show a clear trend, but an increase in 2004 was observed (fig. 3.3). The elevated [CO_2] treatment decreased significantly the total soil N content in the 2nd cycle of the plantation, independently by the poplar clones and by the N fertilization (tab. 3.2). The N fertilization induced an increase of N content particularly evident in the first year of the treatment. The largest increase was observed in *P. alba*, and the interaction between N addition and the poplar clones was significant (tab. 3.2).

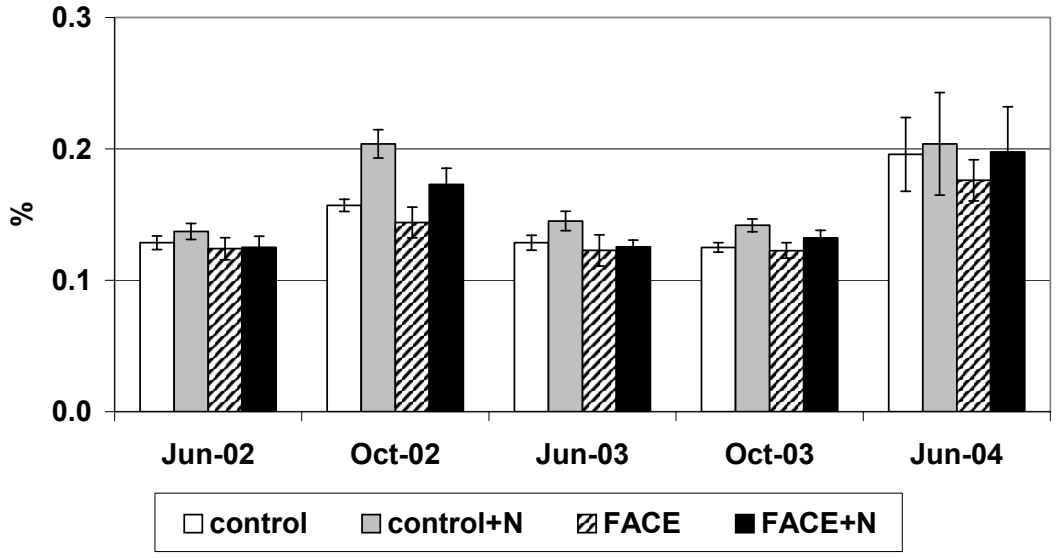

Figure 3.3. Total nitrogen content of Control and FACE fertilized and not fertilized soils. Values from the three clones were pooled together since no significant differences were found.

Microbial Biomass N (MBN)

MBN is the living pool of nitrogen. The N amount in the microbial cells was determined in the period October 2002 – October 2004, and presented a seasonal fluctuation linked to the vegetative period (June or October, fig. 3.4). Values were on average higher at the end of the vegetative season, independently of the poplar clones (tab. 3.2). The effect of the treatments was instead more evident in June, in comparison to October. The elevated [CO_2] treatment induced in June a not significant increase, and the fertilization induced an increase of microbial N immobilization only in June, as demonstrated by the significant interaction between fertilization and the season (tab. 3.2).

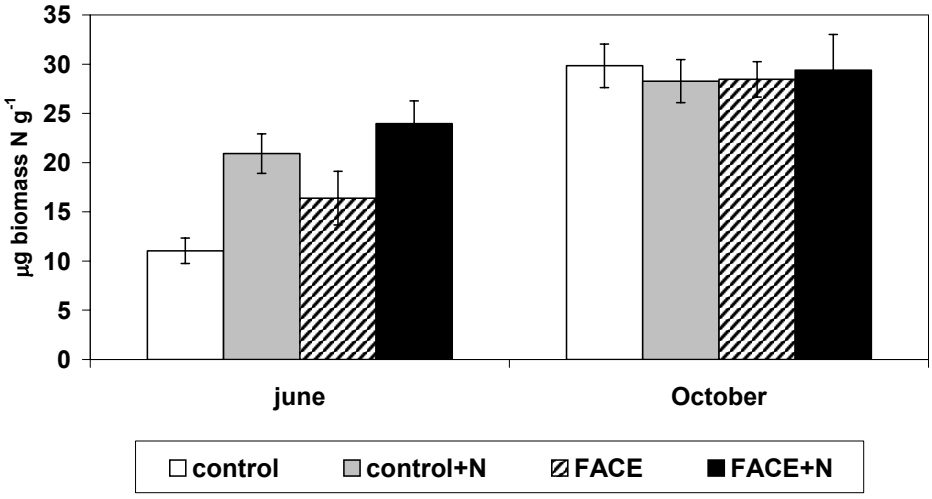

Figure 3.4. Microbial Biomass N (MBN) in Control and FACE not fertilized and fertilized soils. Data are presented as average values of June and October samplings (2002-2004). Values from the three clones were pooled together since no significant differences were found.

Ratio of microbial biomass N to total N (MBN/TN)

The living fraction of the N pool, expressed by the ratio MBN/TN, did not show a clear temporal trend. Values never reached the 2% and were on average around 1.4%. The FACE treatment induced a significant increase in this ratio all over the period of measurement, both in fertilized and not fertilized soils (fig. 3.5 and tab. 3.2). The fertilization treatment caused a significant increase of the MBN/TN ratio only in June 2003, as demon-

strated by the significant interaction between fertilization and time (tab. 3.2), while in the other dates no significant effects were observed.

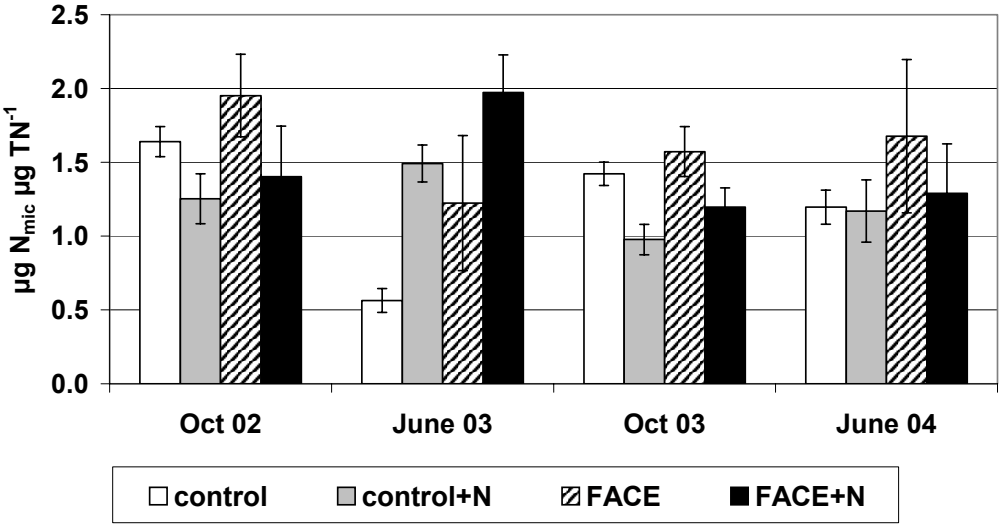

Figure 3.5. MBN/TN ratio in Control and FACE not fertilized and fertilized soils. Values from the three clones were pooled together since no significant differences were found.

	TN	MBN		MBN/TN
		June	October	
FACE	-8%	+26%	0	+27%
Nitrogen	+11%	+64%	-1%	-4%
	Analysis of Variance			
time	0.000	0.000		n.s.
CO_2	0.0420	n.s.		0.009
nitrogen	0.0093	n.s.		n.s.
species	n.s.	n.s.		n.s.
nitrogen x species	0.0335	n.s.		n.s.
nitrogen x time	n.s.	0.000		0.000

Table 3.2. Percentage effects of FACE and fertilization treatments on the total N soil content (TN), the microbial biomass N (MBN) and the ratio MBN/TN. Analysis of variance is reported for each factor of variation and their interactions. p values <0.05 were reported, n.s. not significant. The no significant interactions were removed from the analysis.

Inorganic N (ammonium and nitrates)

Inorganic nitrogen was analysed separating the two forms of ammonium and nitrates. Measurements were performed twice a year from October 2000 to October 2004. The temporal variation of the two forms at the end of the first rotation cycle is shown in fig. 3.6.

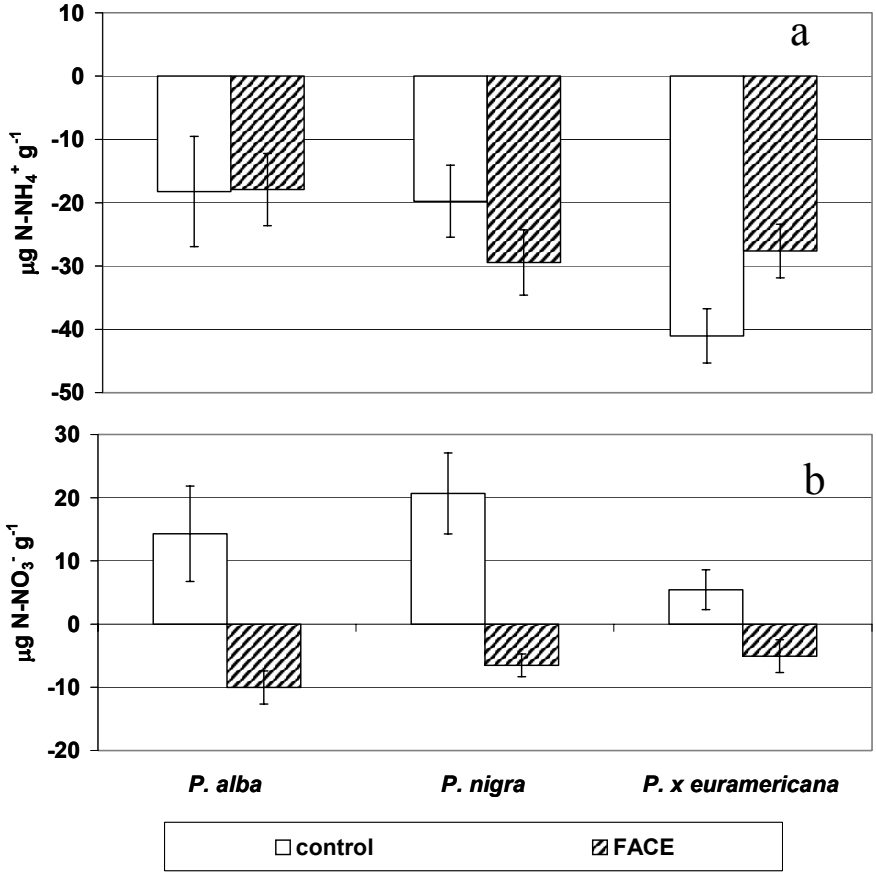

Figure 3.6. Net changes of ammonium and nitrates in Control and FACE not fertilized soils occurred between October 2000 and October 2001 for *P. alba*, *P. nigra* and *P. x euramericana.*

From October 2000 to October 2001 there was a significant depletion of $N\text{-}NH_4$ independently by the CO_2 treatment (tab. 3.3). The decrease was similar in the three clones, although in *P. x euramericana* the stronger depletion was detected (fig. 3.6a).

$N\text{-}NO_3$ amount showed instead values not significantly different between October 2000 and October 2001. Elevated [CO_2] treatment determined a significant decrease of nitrates in the 1st rotation cycle (fig. 3.6b and tab. 3.3).

In fig. 3.7 and 3.8 are reported the amount of $N\text{-}NH_4$ and $N\text{-}NO_3$ pools of POPFACE soils in the second cycle of the plantation (years 2002-2004). Ammonium and nitrate content in not fertilized soils remained approximately around the same values recorded at the end of 2001, even if in 2004 an increase was evident for the two inorganic N forms.

Ammonium showed a peak in June 2004 and a following decrease in October to reach the previous values (fig. 3.7). Regarding the FACE treatment, a decrease was observed in *P. alba*, particularly in not fertilized sub-plots, while no effects were detected for the other two species. The N fertilization did not affect significantly the $N\text{-}NH_4$ fraction, excluding a strong increase at the end of the first year of treatment as indicated by the significant interaction between time and fertilization (fig. 3.7 and tab. 3.3). No interactions with $[CO_2]$ enrichment were found.

Nitrates presented large fluctuations (between 2 and 30 µg $N\text{-}NO_3$ g^{-1}) depending on time, treatments and species. *P. x euramericana* showed the lower values, never exceeding 20 µg $N\text{-}NO_3$ g^{-1}. The elevated $[CO_2]$ treatment induced a significant reduction of $N\text{-}NO_3$ content in soil, unrelated to the clones, but depending on time (tab. 3.3): the larger decrease due to the treatment was in the first and second year after the coppicing in not fertilized plots, while in 2004 this effect was no more evident. The effect was signifcant only in not fertilized soil (Bonferroni post Hoc test, p=0.001). *P. nigra* showed even an increase in $N\text{-}NO_3^-$ amount in FACE fertilized soils and the interaction between elevated $[CO_2]$ and fertilization was significant (fig. 3.8 and tab. 3.3).

The nitrogen fertilization induced a strong increase of nitrates in soil. This increase reached the higher effect in October 2002, at the end of the first year of fertilization, and was higher in Control than in FACE plots. After this peak, the positive effect of fertilization on $N\text{-}NO_3$ remained stable until the end of 2004 (fig. 3.8).

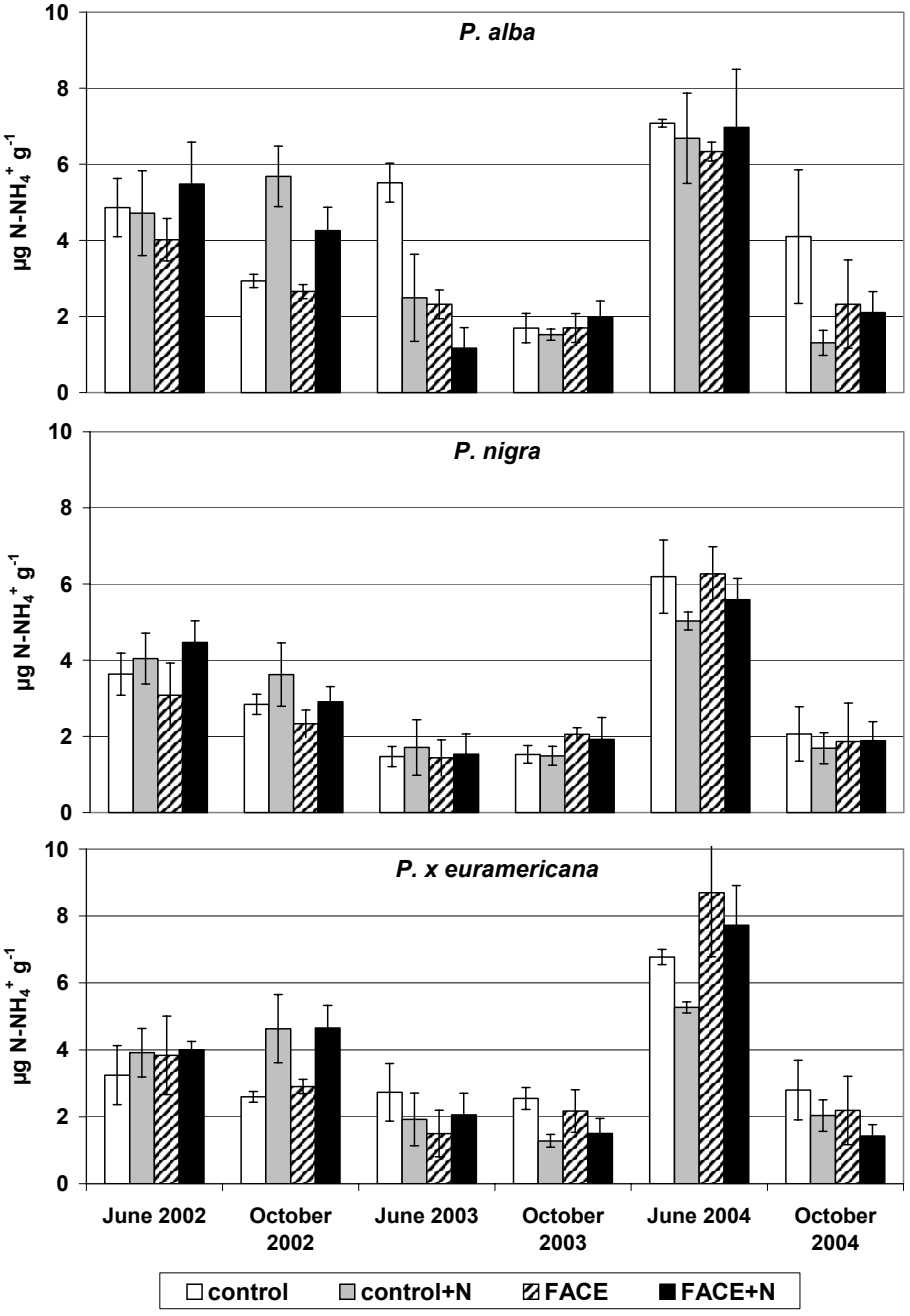

Figure 3.7. N-NH_4 content of Control and FACE fertilized and not fertilized soils for *P. alba*, *P. nigra* and *P. x euramericana.*

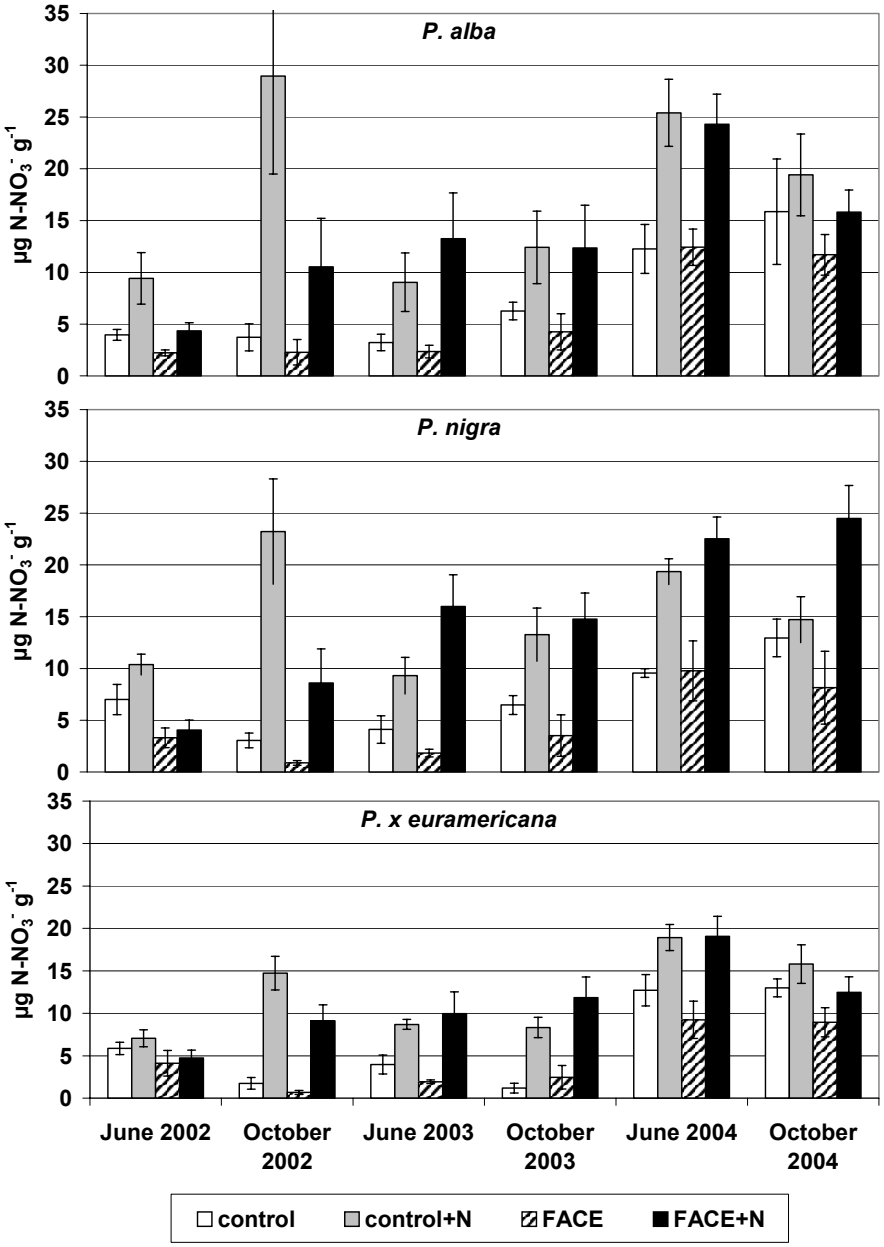

Figure 3.8. N-NO_3 content of Control and FACE fertilized and not fertilized soils for *P. alba*, *P. nigra* and *P. x euramericana.*

	$N\text{-}NH_4$				$N\text{-}NO_3$			
	Average '00-'01	Average 2002-2004			Average '00-'01	Average 2002-2004		
	poplar spp.	*P. alba*	*P. nigra*	*P. x euramericana*	poplar spp.	*P. alba*	*P. nigra*	*P. x euramericana*
CO_2 [n]	-7%	-28%	-1%	0	-31%	-20%	-33%	-29%
CO_2 [N]		-7%	2%	15%		-20%	+8%	-6%
Nitrogen [C]		-17%	-4%	-14%		+130%	+121%	+104%
Nitrogen [F]		+8%	-1%	-1%		+131%	+256%	+169%
	Analysis of Variance							
time	0.000	0.000			n.s.	0.000		
CO_2	n.s.	n.s.			0.006	0.000		
nitrogen	-	n.s.			-	0.000		
species	n.s.	n.s.			n.s.	0.002		
CO_2 x time	n.s.	n.s.			0.0013	0.004		
CO_2 x nitrogen	-	n.s.			-	0.008		
time x nitrogen	-	0.0377			-	0.000		

Table 3.3. Treatment effects on $N\text{-}NH_4$ and $N\text{-}NO_3$ content are reported for years 2000-2001 in not fertilized soils as average of the three clones and for years 2002-2004 for fertilized and not fertilized soils for *P. alba*, *P. nigra* and *P. x euramericana,* starting from October 2002. Analysis of variance was reported for time, FACE, fertilization and species and their interactions. p values <0.05 were reported, n.s. not significant. The not significant interactions were removed from the analysis.

N mineralization

The nitrification was the main process determining N mineralization in the plantation. Ammonification was null or negative, and was not affected by treatments (fig. 3.9). Nitrification presented a significant seasonal trend, with the highest values in June 2004. The three clones showed similar values, and did not induce modifications in the nitrification activity (fig. 3.9 and tab. 3.4). The elevated [CO_2] treatment decreased significantly both nitrification and mineralization in the two sampling dates (tab. 3.4). The effect for nitrification was a 46% and a 4% decrease in not fertilized and fertilized soils respectively, and for mineralization a 49% and a 5% decrease in not fertilized and fertilized soils respectively. No significant interactions between elevated [CO_2] N fertilization were however detected (tab. 3.4). The N fertilization did not induce any significant change neither on nitrification nor on N mineralization, although in FACE soil the effect was a 58% increase.

	Nitrification		N Mineralization	
	June	October	June	October
FACE	-23%	-32%	-35%	-24%
Nitrogen	+19%	+11%	-5%	+8%
	Analysis of variance			
time	0.0022		n.s.	
CO_2	0.0382		0.0564	
nitrogen	n.s.		n.s.	
species	n.s.		n.s.	
nitrogen x time	n.s.		n.s.	

Table 3.4. Percentage effects of FACE and fertilization treatments as average of the three clones in June and October 2004 for the nitrification and mineralization processes. Analysis of variance is reported for each factor of variation and their interactions. p values <0.05 are reported, n.s. not significant. The not significant interactions are removed from the analysis.

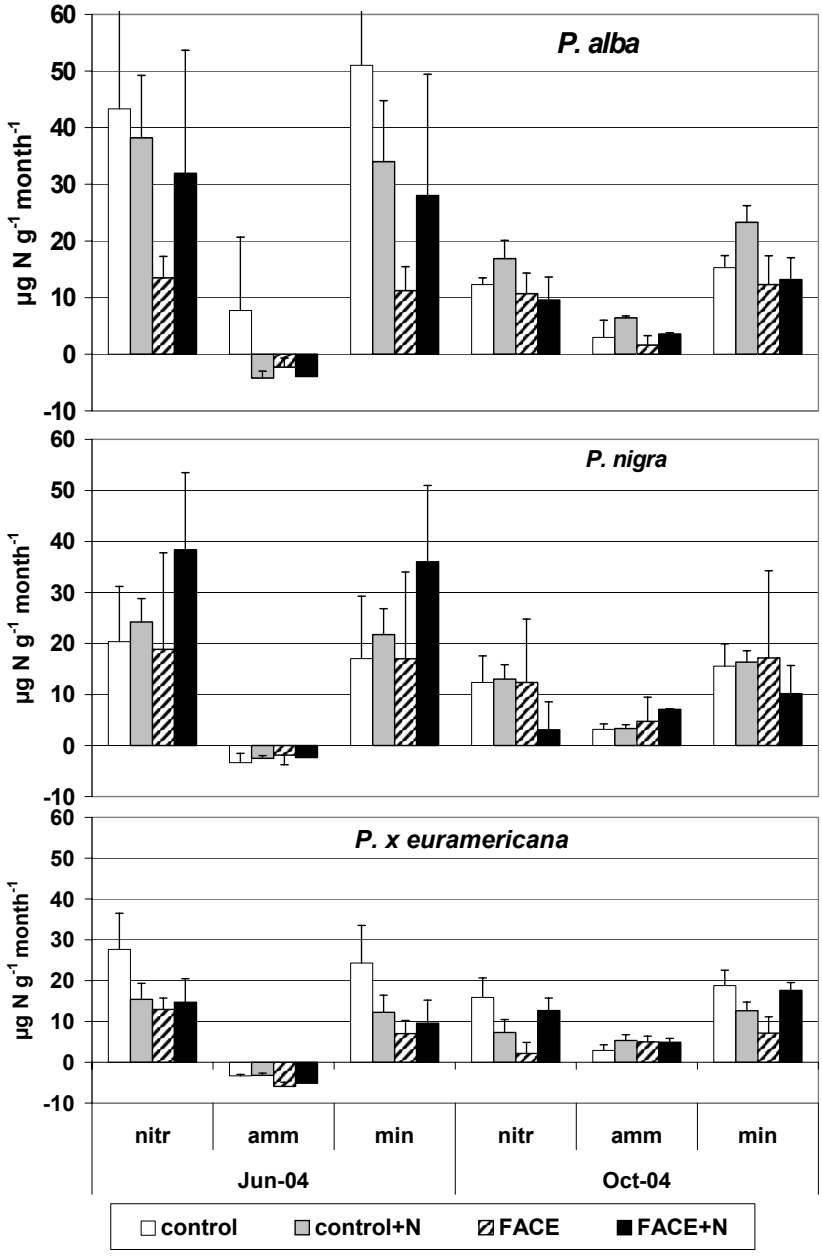

Figure 3.9. ***In situ*** **N mineralization (min), calculated from nitrification (nitr) and ammonification (amm) rates in Control and FACE fertilized and not fertilized soils for** ***P. alba*****,** ***P. nigra*** **and** ***P. x euramericana.***

Extractable phosphorus and acid phosphatase

Values of extractable phosphorus in the first cycle of the plantation (data collected between October 2000 and October 2001) assessed around 10 μg g^{-1} and were generally higher in FACE plots with an average effct of +17% ($p<0.05$, fig. 3.10). Acid phosphatase, in the same period, followed a similar trend and increased significantly under elevated [CO_2] with a 18% on average (fig. 3.10). The polar clones in the first cycle did not influence neither P content nor the enzymatic activity.

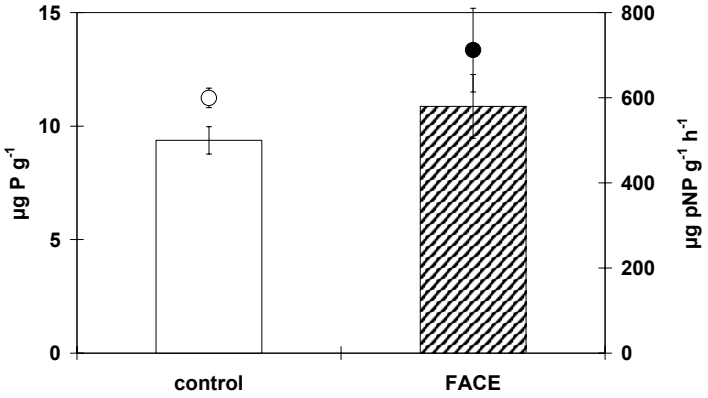

Figure 3.10. Extractable phosphorus (bars) and acid phosphatase activity (circles) in Control and FACE plots as average of the three clones in the period 2000-2001.

After the coppicing of 2002, in the second cycle of the plantation, extractable phosphorus reached the highest values (20 μg g^{-1} on average, fig. 3.11). A negative trend was however evident after June 2003, particularly in FACE plots. Indeed the elevated [CO_2] treatment in the second cycle induced a decrease of extractable P availability: in 2004 in not fertilized soils of *P. alba* and *P. x euramericana* and for the whole period of the second cycle (2002-2004) in fertilized soils, as confirmed by the significant interaction between FACE and fertilization (tab. 3.5). The N fertilization induced a significant increase in the phosphorus amount in soil, but this increase was dependent on time: the greater effect was observed in October 2002 in Control plots and in June 2003 in Control and FACE plots, while afterwards only a slight increase was observed (fig. 3.11). The poplar clones did not influence significantly the P amount neither in the first nor in the second cycle.

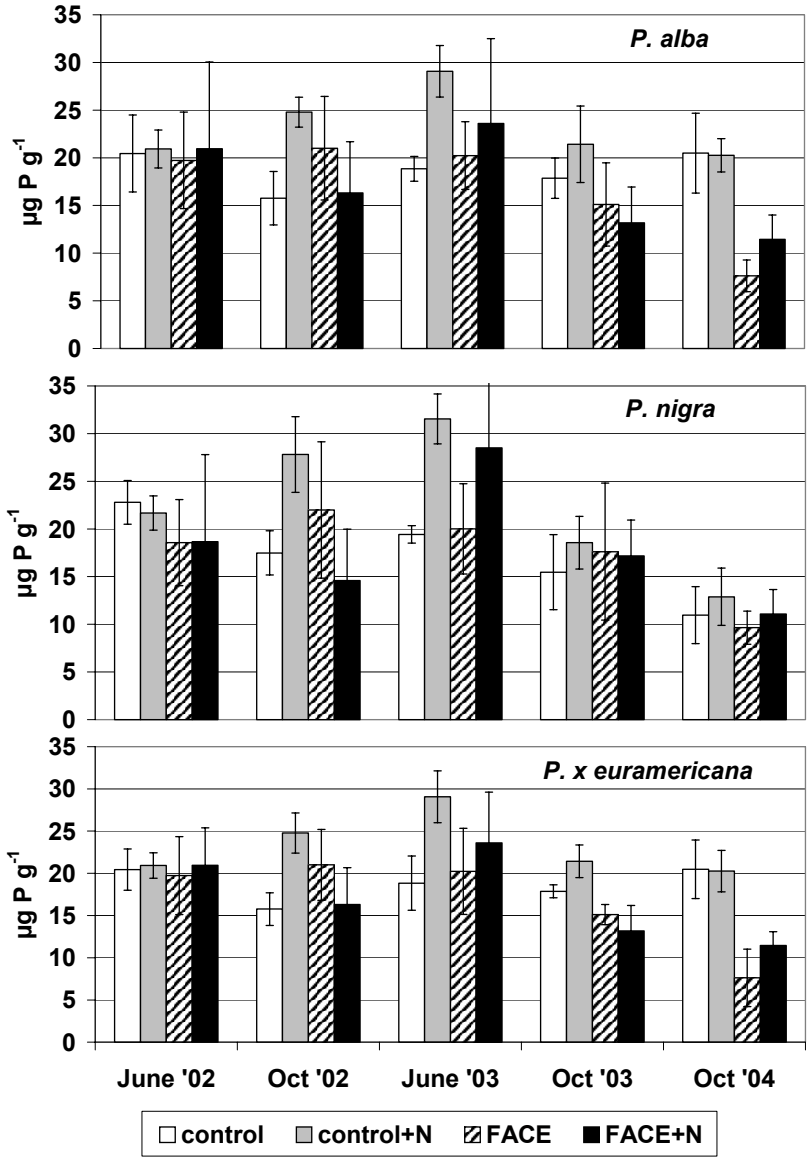

Figure 3.11. Extractable phosphorus in Control and FACE not fertilized and fertilized soils of *P. alba*, *P. nigra* and *P. x euramericana*.

Acid phosphatase activity, in the second cycle, showed a significant positive trend, starting from the minimum values of June 2003 (fig. 3.12). *P. nigra* reached, on average, the highest values, while *P. x euramericana* maintained lower values. The FACE treatment induced a significant increase, unrelated to the soil N level and to the clones (tab. 3.5). The N fertilization induced a consistent increase at the end of the first year (October 2002), while no effects were detected in the following years (fig. 3.12).

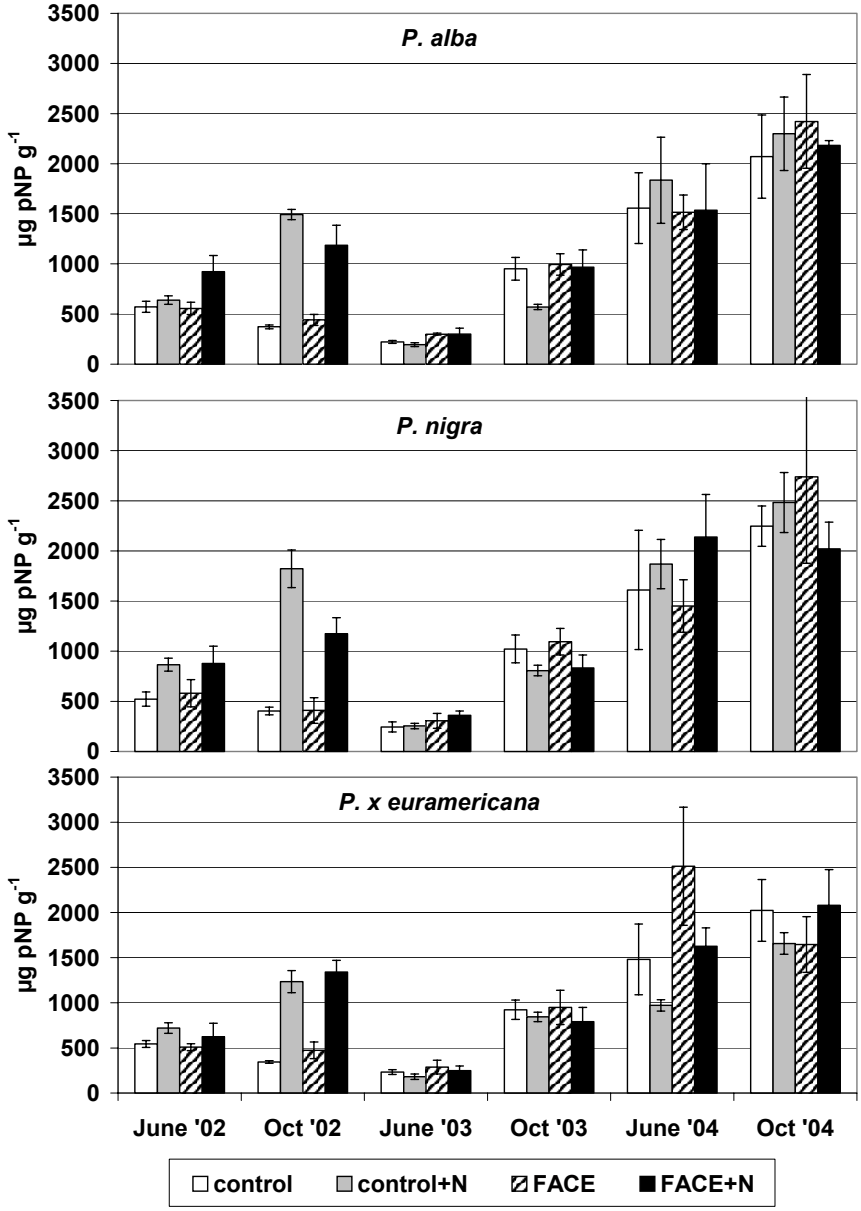

Figure 3.12. Acid phosphatase in Control and FACE not fertilized and fertilized soils of *P. alba*, *P. nigra* and *P. x euramericana*.

Extractable phosphorus and acid-phosphatase were significantly inversely correlated in the whole period of study (2000-2004) independently by the treatments, as shown in fig. 3.13.

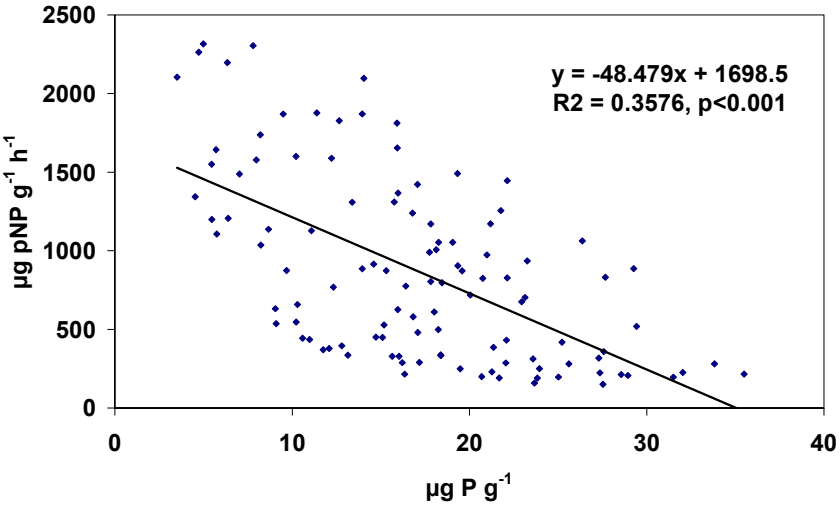

Figure 3.13. Linear regression between extractable phosphorus and acid phosphatase for the period 2000-20004.

	Phosphorus	Acid phosphatase
	µg P g^{-1}	µg pNP g^{-1}
FACE	-10%	+5%
Nitrogen	+18%	+12%
	Analysis of Variance	
time	0.000	0.000
CO_2	0.035	0.030
nitrogen	0.015	0.000
species	n.s.	0.035
nitrogen x CO_2	0.034	n.s.
nitrogen x time	n.s.	0.000

Table 3.5. Percentage effects of FACE and fertilization treatments as average of the three clones on extractable phosphorus and acid phosphatase for the years 2002-2004. Analysis of variance was reported for each factor of variation and their interactions. p values <0.05 were reported, n.s. not significant. The not significant interactions were removed from the analysis.

Arysulphatase

Arysulphatase showed a seasonal fluctuation with maximum levels in June (particularly June 2004) and minimum levels in October (fig. 3.14). The poplar clones did not influence significantly arylsulphatase, neither in interaction with time nor with treatments (tab. 3.6).

The elevated [CO_2] treatment induced a significant increase of the enzymatic activity all over the period of study while no interactions with N fertilization were found (tab. 3.6). On the contrary, the N fertilization decreased the enzymatic activity, in particular in the period October 2003 - June 2004 as confirmed by the significant interaction between time and fertilization (fig. 3.14).

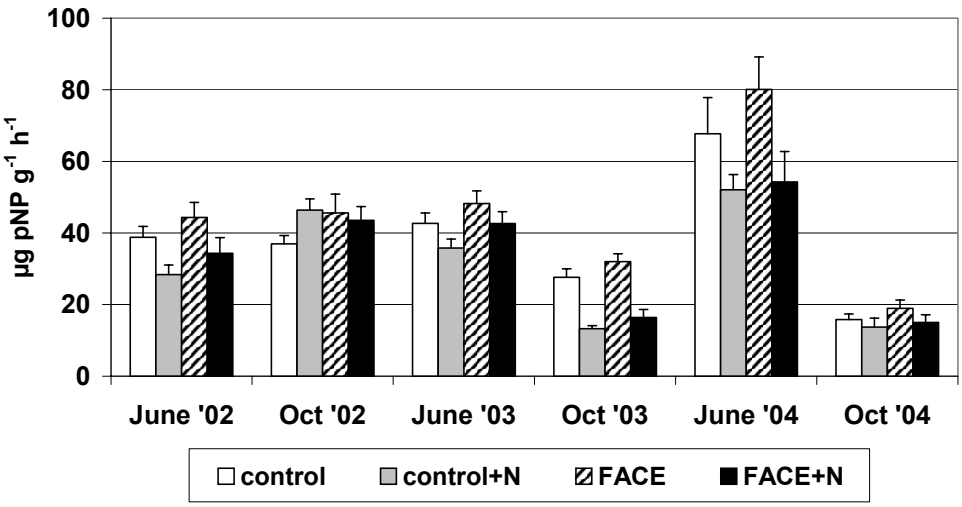

Figure 3.14. Arylsulphatase activity in Control and FACE not fertilized and fertilized soils of *P. alba*, *P. nigra* and *P. x euramericana*.

Chitinase

Chitinase (N-acetyl-glucosaminidase) is one of the three enzymes involved in the degradation of the chitin. The activity of chitinase was similar to that of arylsulphatase with the highest values in June and the lowest in October (fig. 3.15). The FACE treatment induced an increase in the enzymatic activity without interactions with time, with fertilization or with the poplar clones (tab. 3.6). Neither the fertilization treatment, nor the species influenced significantly this enzyme.

Arylsulphatase and chitinase activity were significantly correlated (fig. 3.16).

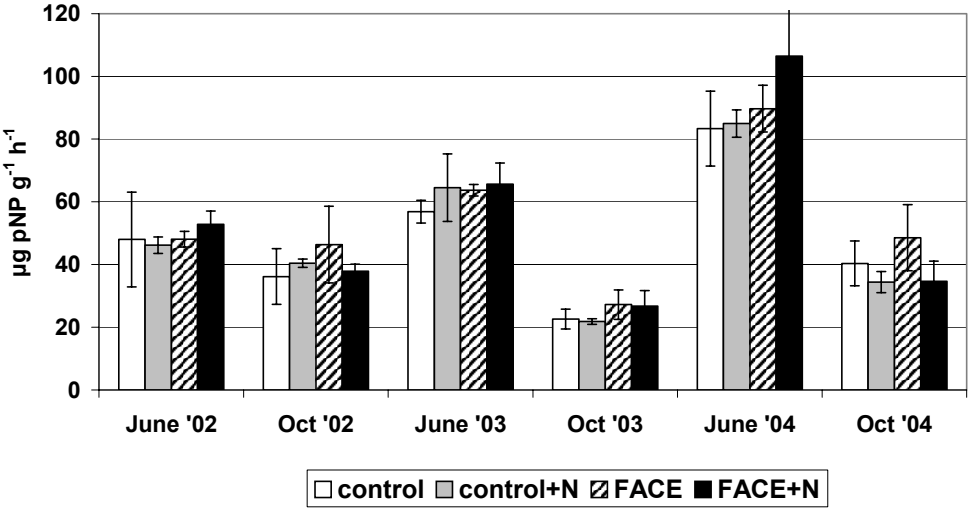

Figure 3.15. Chitinase activity in Control and FACE not fertilized and fertilized soils of *P. alba*, *P. nigra* and *P. x euramericana*.

	Arylsulphatase	Chitinase
	µg pNP g^{-1}	
FACE	+13%	+13%
Nitrogen	-20%	+1%
	Analysis of Variance	
time	0.000	0.000
CO_2	0.017	0.016
species	n.s.	n.s.
nitrogen	0.000	n.s.
nitrogen x time	0.000	n.s.

Table 3.6. Percentage effects of FACE and fertilization treatments as average of the three clones on arylsulphatase and chitinase activity over the whole period of study. Analysis of variance was reported for each factor of variation and their interactions. p values <0.05 were reported, n.s. not significant. The not significant interactions were removed from the analysis.

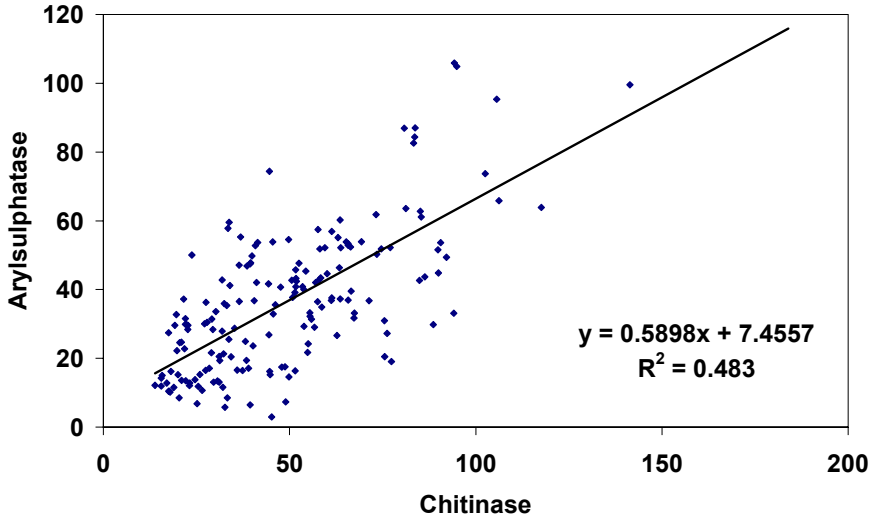

Figure 3.16. Linear regression between arylsulphatase and chitinase activity for the period 2002-20004.

3.3 Discussion

Soil pH and CEC

Soil CO_2 and H_2CO_3 are recognized as primary weathering agents that over geologic time scales control atmospheric CO_2 concentration (Holland et al., 1986). However, a number of studies have considered carbonic acid as being self-limiting in its ability to acidify soils because of its weak acid character, contributing fewer protons to soils as soil pH is reduced (Reuss and Johnson, 1986, Dahlgren et al., 1990) and Carnol et al. (2002) found an increase of soil pH after four years of CO_2 enrichment. At the same time, soil acidification caused by N fertilization has been reported in different parts of the world (Wallace, 1994).

In our work, POPFACE soil pH is close to neutrality and soil capacity to provide nutrients available for plants should be high. After five years of CO_2 enrichment and two of N fertilization, we did not find any change either in soil active (pH_{H2O}) or in soil exchangeable (pH_{KCl}) acidity due to the treatments (fig. 3.1 and tab. 3.1). We can hypothesize that 1) the short time of the treatments did not allow a detectable modification of soil reaction, and 2) the buffering capacity of the plantation soil might prevent changes in soil pH. This last hypothesis is also confirmed by the high cation exchange capacity (around 25 meq $100g^{-1}$), the clay contents of the site (18% on average, Hoosbeck et al., 2004) and the significant increase, regardless the treatments, of both exchangeable acidity and CEC in four years (fig. 3.2 and tab. 3.1). Exchangeable acidity is an indicator of soil capacity to exchange cations and it is known that an important CEC-related property is soil buffering capacity, that is the resistance of a soil to changes in pH: the higher the CEC, the higher is the soil resistance to changes in pH.

After five years of treatment, we found an increase of CEC under FACE. This result is in agreement with the work of Carnol (2002) that found an increase of some exchangeable basic cations under elevated [CO_2].

Generally, SOM is responsible for 25-90% of the total CEC of surface horizons of mineral soils (Van Dijk, 1971; Oades et al., 1989) and Drake and Motto (1982) found the CEC of SOM to be six times higher than the CEC of clay. Oorts et al. (2000) demonstrated also that the biochemical composition of litter had a significant effect on the CEC: the higher the C/N or lignin/N ratio of the litter, the higher was the CEC of the SOM, indicating that more resistant organic residues seemed to induce better charge characteristic. The CEC is related to stable aggregates too (Dimoyiannis et al., 1998) that in turn are indicators of soil structure (Six et al., 2000). Soil structure and chemical properties of SOM can determine

the charge and the complexation capacities thus influencing decomposition rates (Schulten and Leinweber, 2000). Furthermore, i) the microbially derived carbohydrates tend to be resistant to the decomposition and were associated with improved structure of soils (Sheperd et al., 2001; Debosz et al., 2002); ii) the mucilage and other compounds from lignin decomposing fungi can contribute to soil aggregation (Caesar-Ton That, 2002) and iii) plant roots and rhizosphere have many positive effects on soil aggregation (Bronik and Lal, 2005). All these evidences support the following hypotheses: the higher C/N ratio of plant litter (Cotrufo et al., 2005), the greater root and mycorrhizal fungal biomass (Lukac et al., 2003), and the greater rhizosphere activity (see sect. 9) found under elevated atmospheric CO_2 in POPFACE soils, had a positive impact on CEC, probably creating a positive feedback on soil aggregation and soil structure. In fact, an increase of macroaggregates was found in FACE soils by Del Galdo et al. (2004), that hypothesized an enhanced aggregate formation under FACE, possibly due to increased input of litter residues belowground. This, in turn, can have important consequences both in the retaining of nutrients for plants and in the storage of C in the longer term, resulting in greater potential capability for SRF to sequester C in a CO_2 enriched world.

Fertilizer application generally improves soil aggregation, but was also found an adverse effect of NH_4^+ (Haynes and Naidu, 1998). FACE and fertilization did not interact in our site and fertilization did not induce any difference in CEC. This could be due either to a shorter period of fertilization treatment (three years) and to the lack of effects of N addition to the N-NH_4^+ soil content.

Soil nutrient availability

Changes in plant litter production and quality under elevated [CO_2] could alter microbial N demand and the amount of N available for plant uptake (Zak et al., 2003). N depletion, observed in soils of long-term [CO_2] enrichment experiments, is often the advocated reason why plants should acclimate with respect to growth enhancement to elevated [CO_2] in the long-term (Luo *et al.,* 2004). [CO_2] enrichment induced a decrease of N concentration in all plant tissues and in all species, but the hypothesized Progressive Nitrogen Limitation was not occurring in the first cycle (Calfapietra et al., in preparation). Nevertheless, in the 2nd cycle of the plantation the total N content was significantly lower in FACE soils indicating that an effective higher loss of N from the system took place under elevated [CO_2]. A better efficiency in the N retranslocation before leaf fall from the canopy to the perennial tissues (Calfapietra et al., submitted), and a consequent reduction in the N input via litter

fall (Cotrufo et al., 2005), both occurred in the first cycle, caused a reduction of the soil N content that became significant in the 2nd cycle.

Regarding the mineral N, more easily absorbed by plants, we observed a great depletion of soil ammonium, unrelated to the FACE treatment, during the third growing season of the first cycle, the most productive one, whereas nitrate declined in FACE but not in control soils. We can suppose three main mechanisms for ammonium depletion in the plantation soil: i) the interlayers clay fixation following the dry-rewetting cycles during years (Paul and Clark, 1989), ii) a less expensive poplar uptake, with respect to nitric form, iii) a very rapid turnover because of high soil nitrification activity. The hypothesis that poplar trees could discriminate between the two forms of mineral nitrogen is confirmed by Rothstein et al. (2000) who reported, in studies with excised roots of *Populus tremuloides*, a more rapid kinetics of NH_4^+ uptake than of NO_3^-. Considerable more energy is generally required by plants for uptake and assimilation of NO_3^- than of NH_4^+, largely due to the fact that NO_3^- must be metabolically reduced to ammonia before it can be assimilated. Regarding the last hypothesis, net nitrification and net N mineralization were equivalent in the plantation, and nitrification was the main process influencing the inorganic N amount in soil, therefore reducing greatly and rapidly the amount of ammonium in the soil, while nitrate was the dominant inorganic-N form after the first year of the experiment.

Both poplar uptake and microbial nitrification can also explain the significant decrease of nitrates in FACE soils found all over the period of study and the lack of a FACE effect on ammonium soil content (fig. 3.6, 3.7 and 3.8), also showed by Barnard et al. (2004) in European grasslands. Under elevated [CO_2], availability of respiratory carbon substrates can enhance root energy status and could thus positively influence NO_3^- uptake rates (BassiriRad et al, 1997). The same authors reports in fact enhancement of nitrate uptake in seedlings of *Pinus* spp. under elevated [CO_2].

Nevertheless, Calfapietra et al. (submitted) did not find any modification in the N uptake by poplars, and the main mechanism for nitrates reduction in the plantation under elevated [CO_2] was the decrease of nitrification rate. Other studies reported unaltered or even decreased net N mineralization rate after carbohydrates amendements to temperate perennial grassland (Jonasson et al., 1996) as well as agricultural (Rutherford and Jume, 1992) and silvicultural system (Turner and Olson, 1976). On the contrary, Carnol et al. (2002) reported an increase of nitrification under elevated [CO_2], related to a decrease in microbial biomass and activity that reduced competition for NH_4^+. This is not the case of POPFACE soils, where the size of microbial biomass increased (see sect. 4 and 5) and where the com-

petition for ammonium was supposed to be high in the plantation (Calfapietra et al., 2003). Microrganisms, besides plants, are generally known to prefer NH_4^+; when both NH_4^+ and NO_3^- forms are present at the same time, the soil biomass apparently assimilates NH_4^+ considerably more rapidly than NO_3^-, because less energy is required for the assimilation of the former (Haynes, 1986). Mycorrhizal fungi in particular are known to prefer NH_4^+ to NO_3^- and can also use a wide range of relatively simple organic compounds such as amides, amino acids and nucleic acids (Smith, 1980). Thus, as Verstraete (1981) indicated, it seems possible that mycorrhizae may act as agents of biological control of nitrification under vegetated conditions.

Hungate et al. (1999) found that elevated [CO_2] depressed gross mineralization and ammonium consumption, and caused an increase in the specific rate of ammonium immobilization by soil microorganisms. Several theories on the role of microbial biomass in determining the amount of N available for plants asserted that the increase of easily degradable C may stimulate microbial activity and nutrient cycling, but a decrease in litter quality (high C/N ratio) may slow the rate of nutrient cycling and increase N immobilization (Allen et al., 2000; Williams et al., 2000). In the plantation, the microbial biomass N and the N mineralization rate showed an opposite seasonal pattern between the first part and the end of the growing season (fig. 3.4 and 3.9). The mineralization process prevailed at the beginning of the growing season, which is the period of more intense plant activity and nutrient uptake, with respect to the late autumn. Under elevated [CO_2] microbial immobilization was the dominant process, as demonstrated by the increase in the ratio MBN/TN, furthermore suggesting a greater microbial demand for N (fig. 3.5). Results of Magill and Aber (2000) indicated that, in the presence of a labile C source, available soil N was rapidly immobilized and it is possible that easily decomposable substrates derived from plants (i.e. simple carbohydrates and organic acids from a higher fine root biomass), stimulating microbial growth and immobilization, reduced soil N availability (Janssens et al., 1998). The labile C increased significantly in the FACE plots, and a higher C/N ratio of labile SOM was also observed (par. 4), inducing therefore microbes to immobilize N. This trend was in agreement with what observed in their review by Van Groenigen et al. (2005), who postulated that microbial N immobilisation is the main mechanism involved in N retention in soils under elevated [CO_2] especially when they become nutrient poor.

Indeed, the interaction between elevated [CO_2] and N addition acted in a positive way on nitrates soil content, in particular in *P. nigra*. We can argue that a double mechanism could have been occurred: i) a differentiated poplar uptake, and ii) a more intense microbial min-

eralization activity. Although no significant interaction between elevated [CO_2] and N addition was found on mineralization and nitrification rates, in June we observed a 62% activity increase on average in *P. nigra* FACE fertilized soil, suggesting that poplar clones can differentiate microbial communities physiology, mainly with adequate C and N supply, as showed also in sect. 6.

Nevertheless, other authors reported microbial immobilization of N even under rich nutrient conditions (Gorissen and Cotrufo, 1998, Vance and Chapin III, 2001). In our study, only N-NO_3^- was positively affected by fertilization for the whole period of study, while NH_4^+ only in the first year. Moreover, although nitrification was not affected by fertilization, the microbial immobilization of N increased during fertilizer addition (June). Vance and Chapin III (2001) also found an increase in microbial biomass N following N fertilization, and suggested an increased microbial N storage and/or a decreased fungi/bacteria ratio, as well as a strong microbial competitive pressure for N. In our site we can suppose that: i) microbes were strongly limited by nitrogen in June, when the competition with plants was higher; ii) the conversion of NH_4^+ to nitrates occurred rapidly, after a first year of adaptation; iii) the microbial biomass and its C/N ratio were highly dependent on ammonium, more than on nitrates (sect. 4 and 5 and Paul and Clark, 1989) and iv) a modification of microbial populations took place following the addition of fertilizer (see sect. 6). Moreover Rothstein et al. (2000) found for *P. tremuloides* that the fertilization treatment resulted in decreases in specific NH_4^+ and NO_3^- uptake rates of approximately 50% compared with the rates measured in the low-N treatment, and suggested a down regulation of NH_4^+ uptake system in the response to improved N status. Therefore microbes could take advantage of this regulation overcoming plants in the competition for N uptake. This can in part explain the lack of a positive effect of fertilization and of elevated [CO_2] on plant production in fertilized soils and the positive effect of the interaction of elevated [CO_2] and N fertilization in some cases (Liberloo et al., 2004). In addition, considering the total N content of fertilized soils, this increased greatly because of the mineral N addition, suggesting that microbial immobilization probably enhanced the transformation of big amounts of N into organic forms less accessible to plant uptake. Abiotic fixation of NH_4^+ in soils has also been well documented (Nommik and Vahtras, 1982; Axelsson and Berg, 1988; Schimel and Firestone, 1989, Trehan, 1996) and may be another important mechanism in the plantation soil.

To conclude, even if total N uptake by trees did not change under elevated [CO_2] as compared to the ambient, the microbial immobilization process could cause a more intense N

limitation over a longer-term period in not fertilized soils. The fertilization treatment probably favoured microbes with respect to plants in the competition for NH_4^+ and the very rapid conversion of ammonium to nitrate favoured this form of mineral N. Moreover, a marked immobilization mechanism of N in organic forms, less accessible to plant uptake, occurred after the N addition, as demonstrated by the increase of total N amount.

Phosphorus availability can also limit plant production. In particular under elevated [CO_2] the plants need for phosphorus could increase since its importance in the photosynthetic process (Demetz and Insam, 1999; Syvertsen and Graham, 1999). The plant growth response under P limitation appears to be closely linked to the mycorrhizal colonization rates, which enhances P uptake benefit at low availability, but also invokes belowground C cost for mycorrhizal root growth and maintainance (Syvertsen and Graham, 1999). Elevated [CO_2] may reduce this relative cost of ectomycorrhizas, presumably by increasing belowground carbohydrate supply from increased photosynthesis (Morgan et al., 1994; Lewis and Strain, 1996). Mycorrhizal abundance, on the contrary, generally declines in response to N and P fertilization across studies (Treseder, 2004). In our site phosphorus amount is not limiting plant growth being present in a relatively high amount in POPFACE soils, but after four years from the plantation the P availability showed a negative trend (fig. 3.11). We found in addition an opposite effect of elevated [CO_2] on phosphorus availability between the first and the second cycle. While in the first cycle extractable phosphorus increased under elevated [CO2], after the coppicing we observed a significant decrease due to the FACE treatment (fig. 3.10 and 3.11). In FACE soil an increase in mycorrhizas was found by Lukac et al. (2003) in the 1st cycle. However, we have evidences of greater fungal biomass also in the 2nd cycle (see below and par. 4). It is known that phosphorus availability is under biotic control of enzymatic activity of plant and microorganisms, as confirmed by the negative relationship between phosphorus and acid phosphatase, widely reported in literature and also found in this work (fig. 3.13). We can assume that the plantation requested a great amount of phosphorus during years, that returned to the soil trough phosphorus-containing compounds derived from litter decay, and actively provided by roots mycorrhizas (Moorhead and Linkins, 1997). Since the plants need of P was presumably higher (Harley et al., 1992), and the litter decomposability decreased under elevated [CO_2] (Cotrufo et al., 2005), we can hypothesize that the treatment caused a progressive decrease that in the long term could be limiting for plant growth.

Regarding the N addition, it must be remembered that in 2002 phosphorus was supplied with the fertilization treatment, but in that year the increase due to the fertilization treatment was evident only in Control plots, while in the following year, when fertilization supplied only ammonium and nitrate, the extractable phosphorus increased in Control and FACE plots. The fertilization treatment, reducing mycorrhizal colonization, could even act as a negative feedback on phosphorus availability under elevated [CO_2] in nutrient rich soils, but could induce the P acquisition through an increase of enzymatic activity directly from plants and other microorganisms.

Soil nutrient acquisition activity

Both microorganisms and plants can release acid phosphatase in soil environment (Dick and Tabatabai, 1993). Elevated [CO_2] may increase the potential for plant nutrient acquisition by stimulating mycorrhizal colonization of roots and root exudation of phosphorus-mobilizing carbon compounds such as phosphatases (De Lucia et al., 1997). The significant enhancement of acid phosphatase under elevated [CO_2] might therefore reflects a microbial and/or plant demand for this nutrient. The requirement of P did not decrease with fertilization, and the positive effect of elevated [CO_2] was evident also in N-rich soils. Regarding the effect of fertilization, when also P was added with the treatment, an extra synthesis of the enzyme was observed, but without any immediate effect on soil extractable P. The effect on the nutrient availability was significant only in the following spring, when the fertilizer-inductive effect on acid phosphatase ended. We can suppose that mineral nutrients addition in soil induced microbes to an extra enzymatic synthesis. The effect was similar also for β-glucosidase activity and for short-term respiration rates in October 2002 and June 2003 (see sect. 7). It is known that the addition of easily decomposable C and N substrates could induce an acceleration of metabolic (respiration) and co-metabolic (enzymes) activity defined as *priming effect* (Kuzyakov et al., 2000). In addition, priming effects were found not only for C and N, but also for P (Fokin and Radzhabova, 1996). Therefore we can suppose that after the fertilization treatment of 2002 (providing also P) a possible *priming effect* on acid-phosphatase activity occurred, overcoming the natural regulatory control previously described and illustrated in fig. 3.13.

Chitinolitic and arylsulphatase enzymes are produced by both plants and microorganisms (Parham and Deng, 2000; Tabatabai and Bremner, 1970) and are indicators of N and S demand. Moreover, these enzymatic activities are also considered as indirect indicator of the presence of fungi population being esther sulphates (substrates of arylsulphatase) pre-

sent only in fungal cells (Bandick and Dick, 1999) and the chitin the main constituent of fungal cell wall (Miller et al, 1998). These last authors found a direct relationship of chitinase with two independent indicators of fungal biomass (Phospholipid Fatty Acid and Ergosterol analyses) and concluded that measuring chitinase activity may provide a sensitive measure of soil fungal biomass. The two enzymatic activities were also significantly correlated between themselves (fig. 3.15) and with the C/N ratio of microbial biomass (pearson correlation, $p<0.001$), that is an indicator of an increase of fungi/bacteria ratio in soils (Joergensen et al., 1995). The positive effect of FACE treatment on arylsulphatase and chitinase activity observed constantly at POPFACE during the period of study could therefore indicate an increase of fungal population under higher [CO_2], as well as an increase in the S and N acquisition activity. These data were consistent with Phillips et al. (2002) reporting an increase of ^{13}C-N-acetylglucosaminidase metabolisms under elevated [CO_2]. Moreover mycorrhizas colonisation of *Populus* fine roots has been recently reported to be positively affected by the elevated [CO_2], after three growing seasons in POPFACE experimental station, confirming a larger presence of fungi biomass induced by CO_2 treatment (Lukac et al, 2003). The lack of elevated [CO_2] effect on the structural diversity of fungal community showed in sect. 6 suggests in fact that the treatment influence was in the direction of a biomass increase, not in a population shift.

The fertilization treatment, instead, acted differently on the two enzymes, with a significant decrease on arylsulphatase and a lack of effect on chitinase activity. In POPFACE soils a significant effect of fertilization on fungal community composition and a decrease in the number of species was found (sect. 6), whereas Allen and Schlesinger (2004) suggested that N addition caused a shift in the microbial community dominance from microbes with a high C/N ratio (i.e. fungi) to microbes with a low C/N ratio (i.e. bacteria, Paul and Clark). Microbial biomass immobilized N in the early growing season (fig. 3.6) and the microbial biomass C/N ratio decreased in fertilized soils in the same period (sect. 4). These evidences suggested an effective shift towards fast growing bacterial communities, but chitinase activity did not support this hypothesis. We can however suppose that the N requirement in fertilized soils did not change with respect to not fertilized ones (see above) and that the nutrient acquisition activity reflected this need.

In conclusion, considering the overall soil nutrient acquisition activity, the significant influence of FACE treatment proved that all the enzymatic activities were induced by the CO_2 enrichment (fig. 3.10, 3.12, 3.14 and 3.15 and tab. 3.4 and 3.5). Acid phosphatase, chitinase and arylsulphatase are indicators of P, N and S acquisition activity, respectively;

in this study these enzymes showed significant positive increases due to elevated [CO_2]. Therefore this could point out that microbial responses to FACE treatment resulted in a general enhancement of soil biological activity in order to maintain a balanced supply of nutrients. The effect of fertilization was more specific and differed greatly between the three enzymes and because of a temporal variation.

Anyhow, enzymes proved to be sensible indicators of changes in energetic requirements of microbial communities, and useful indicators of microbial processes modifications.

4. SOIL ORGANIC C FRACTIONS AND THE ROLE OF MICROBES IN THE SOIL C STORAGE

4.1 Introduction

"One of the most important goals of research on elevated [CO_2] is to investigate whether increased net CO_2 uptake in a high-CO_2 world will result in increased long-term carbon storage in ecosystems, in particular in the soil component …" (Mooney et al., 1999).

Plant inputs to soil (as litterfall, roots and root exudates) may be affected in quantity and quality by the increase in plant biomass and by the biochemical modifications of plant tissues, both occurring in elevated [CO_2] environments (Paterson et al., 1997; De Angelis et al., 2000; King et al., 2001). The dynamic of SOM reflects the quantity and quality of plant inputs and the biological activity of soil, which can be strongly affected by an enrichment of atmospheric CO_2. The increase of NPP under elevated [CO_2] is widely demonstrated in many ecosystems (De Lucia et al., 1999; Hamilton et al., 2001; Gielen et al., 2005) and a shift in the C allocation from foliage to roots is also supposed to happen (Finzi et al., 2001). This can result in an increase in production and turnover of fine roots (Lukac et al., 2003) or root exudation (Thomas et al., 1999) and, in turn, in an increase of labile C belowground. At the same time, a decrease in the quality of litter was found under elevated [CO_2] (Cotrufo et al., 1998) thus lowering the microbial decomposition rates. The increase of C input to soil could induce at least three mechanisms acting in opposite ways and reviewed by Cheng (1999): 1) the "priming effect" hypothesis: a stimulation of microbial activity, particularly in nutrient poor soils, could enhance SOM decomposition; 2) the "preferential substrate utilization" hypothesis: given abundant mineral nutrient supply, the mineralization of native soil organic C might be retarded due to the preference of microbes for easily decomposable substrates; and 3) the "competition" hypothesis: the extra root derived C input decreases SOM decomposition under mineral nutrient-limited conditions and increases SOM decomposition under conditions of adequate mineral supply. These hypotheses are predicting opposite effects of elevated [CO_2] on soil C sequestration capacity, and several studies strengthen one or the other (Cardon, 1996, Hungate et al., 1997, Cheng and Johnson, 1998; Zak et al., 2000). However, all three mechanisms may operate simultaneously (Cheng, 1999).

The nutritional status of the soil influences all these processes: soil fertility may control the C inputs to soil, since CO_2 enrichment can stimulate plant growth only in soils with adequate nutrients, while microbial activity is strongly dependent on the N availability in soils and on C and N interactions (Stitt, 1991; Vance and Chapin III, 2001).

Soil organic matter (SOM) comprises different groups of constituents that vary in mass and rate of turnover, ranging from years to decades or thousand of years: the *active*, *slow* and *passive* SOM fractions (Brady and Weil, 1999). Models that incorporate these fractions of soil organic matter have proven to be very useful in explaining and predicting real changes in soil organic matter levels and soil properties. If plants input are stored in active soil C pools with short residence time, this C can soon return in the atmosphere as CO_2 emission; if C is stored in pools that turnover slowly, the capability of terrestrial ecosystem to sequester C will increase significantly (Allen et al., 2000).

In this work, we tried to separate those C fractions more relevant for microbial metabolisms and characterised by a different (increasing or decreasing) degree of degradability. The overall labile C (LC), representing the sum of microbial biomass C (MBC) and the C extracted in K_2SO_4 (ExC), was considered as an *active* organic fraction playing an important role in the substrate availability for microbes (Dumontet et al., 2001). The soil microbial biomass is the living fraction of soil, therefore responsible for organic matter and residue decomposition, but at the same time is considered as a component of the soil labile C pools being also a substrate for the soil organisms (Sparling and Ross, 1993) and it represents a sensitive and labile pool with a turnover time of<1 year (Rice et al., 1996). Inside this labile C, the Water Soluble C (WSC) plays an important role: it is the most immediate and easily degradable C source for microorganisms (Wang et al., 2003), and its availability is the main factor limiting microbial respiration in soils (Cheng et al., 1996). McGill et al. (1986) suggested that the flow of C through water soluble components supplies substrate for microbial biomass turnover. But the WSC is not only a C source for microorganisms, since its production is also believed to be microbially mediated (Christ and David, 1996).

To detect if changes in labile C substrates influenced the organic C storage in soils, the Total Extractable C (TEC) and the yearly variation of Total Organic C (TOC) were also analysed. The Total Extractable C (TEC) is a recalcitrant fraction of organic C extracted with alkali and is comprehensive of humic and fulvic acids but not of humin, that is the material most resistant to microbial attack (*passive* SOM). Humic substances comprise about 60 to 80% of the soil organic matter and belong to different fractions of organic matter.

In addition, the C/N ratio of organic matter, and therefore its quality, was analysed because of its importance on intense competition occurring among microorganisms for available soil N when plant residues having a high C/N ratio are added to the soil. The different fractions of SOM reported above have different C/N ratio that identify the organic pool and its residence time (Brady and Weil, 1999). C/N ratio of plant residues can vary widely and

ranges from 10 to 100, while in the mineral soil the *active* SOM have a C/N ratio of 15-30, that decrease in the *slow* (10-25) and in the *passive* SOM (7-10). Microbes, even if belonging to the active pool, have a lower C/N ratio different for fungi (10-15) and bacteria (5-10).

Aims of this work were to detect if changes in labile C substrates influenced the organic C storage in soils, verifying i) whether treatments with elevated [CO_2] and N fertilization induced changes in the amount and quality of labile C pools and in microbial C immobilization; ii) whether these changes provoked modifications in the soil capacity to store organic C.

4.2 Results

The poplar clones, or their interaction with other factors of variation, did not influence significantly any of the parameters examined. Values of all clones were therefore pooled together and average data were presented.

Labile fractions

The overall labile C fraction fluctuated significantly between and within years, with the highest values recorded in June 2003 (fig. 4.1). The effect of elevated [CO_2] was a significant increase of labile C in the four years of observation (fig. 4.1 and tab. 4.1). The increase was observed both in not fertilized and fertilized sub plots and no significant interactions between the FACE and N treatments were found (tab. 4.1). The N fertilization decreased significantly the labile C supply in soils (tab. 4.1).

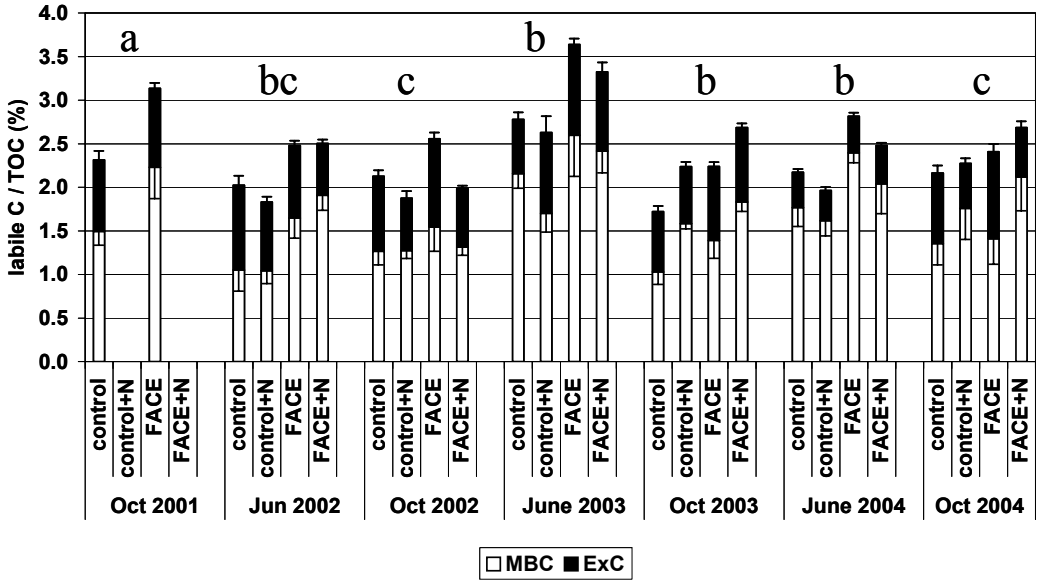

Figure 4.1. Overall Labile C (microbial biomass C + K_2SO_4 extractable C), expressed per unit of TOC. FACE and Control treatments for years 2001-2004 in not fertilized and for years 2002-2004 in fertilized sub-plots (N) are reported. Different letters represent significant difference between years ($p<0.05$).

The relative weight of the two fractions (MBC and Extr. C) of labile C did not change significantly during years and the FACE treatment did not influence it (fig. 4.1). On the contrary, the effect of the N fertilization was different in the two fractions: ExC decreased significantly in fertilized soils, while the MBC remained unaffected.

The most soluble fraction of soil C (WSC) is shown in fig. 4.2. The temporal variation among the years of measurement was significant (tab. 4.1). The increase of WSC under the FACE treatment was evident in all sampling dates and was higher in fertilized sub-plots, but the two treatments (FACE and fertilization) did not interact significantly (tab. 4.1). The N fertilization, on average, reduced significantly the soluble C in soil. The extent of this effect varied along years, although the interaction between time and N fertilization was not significant (tab. 4.1).

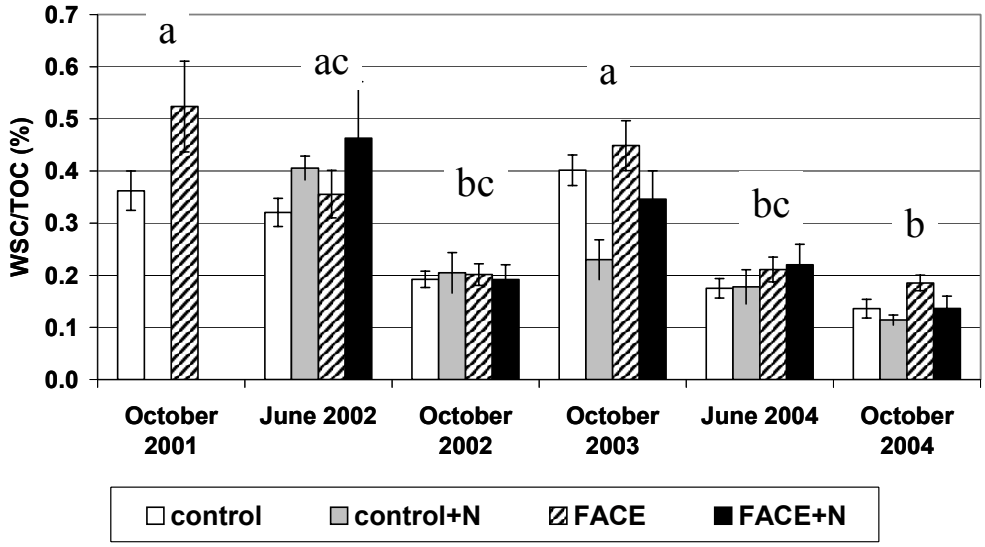

Figure 4.2. Water Soluble C, expressed per unit of TOC. FACE and Control treatments for years 2001-2004 in not fertilized and in fertilized sub-plots (N) for years 2002-2004 are reported. The three poplar clones were pooled together since no significant variations were recorded. Different letters represent significant difference between years ($p<0.05$).

	TEC	MBC	ExC	LC	WSC
FACE	+2%	+26%	+15%	+23%	+13%
Nitrogen	0	+7%	-19%	-2%	-26%
	Analysis of variance				
CO_2	n.s.	0.004	0.012	0.0190	0.0199
Nitrogen	n.s.	n.s.	0.012	0.0013	0.0104
Time	n.s.	n.s.	n.s.	0.000	0.000
Time x Nitrog.	n.s.	n.s.	n.s.	0.000	n.s.

Table 4.1. Mean percentage treatments effect on soil C fractions in the years 2002-2004. The effect of FACE treatment is reported for not fertilized (n) and fertilized (N) sub-plots. The effect of fertilization is reported for Control (C) and FACE (F) plots. The significance of treatments, time and their interactions are reported. The not significant interactions were excluded from the analysis. The p values < 0.05 are reported.

The C/N ratio of the living fraction of SOM (microbial biomass) and of the 0.5M K_2SO_4 extractable SOM, presented a seasonal fluctuation linked to the vegetative period (June - October) shown in fig. 4.3 and 4.4 respectively.

Regarding the C/N ratio of microbial biomass, the higher values, up to 20, were recorded in June not fertilized soils, whereas in October they varied to a limited extent and remained below 8 (fig. 4.3 and tab. 4.2). The elevated [CO_2] treatment increased the C/N ratio in June and October in both fertilized and not fertilized soils and no significant interactions between the treatments or between the CO_2 enrichment and the temporal variation were found, although a lower effect was evident in October in fertilized soils (tab. 4.2). The fertilization treatment infuenced the C/N ratio of microbial biomass differently in the two seasons, as indicated by the interaction between time and fertilization reported in tab. 4.2: the ratio decreased in June and increased in October in fertilized soil. Anyway, the N addition caused a decrease of microbial C/N values below 10 in both season, and below 8 considering only October (fig. 4.3).

The C/N ratio of 0.5M K_2SO_4 extractable SOM reached the highest values in June in not fertilized soils and varied from 14 to 18 in June and from 4 to 9 in October (fig. 4.4). The elevated [CO_2] treatment increased significantly the ratio in both seasons independently of the fertilization treatment. The largest increase was observed in October (tab. 4.2). The fertilization treatment induced only a slight not significant increase in the two seasons.

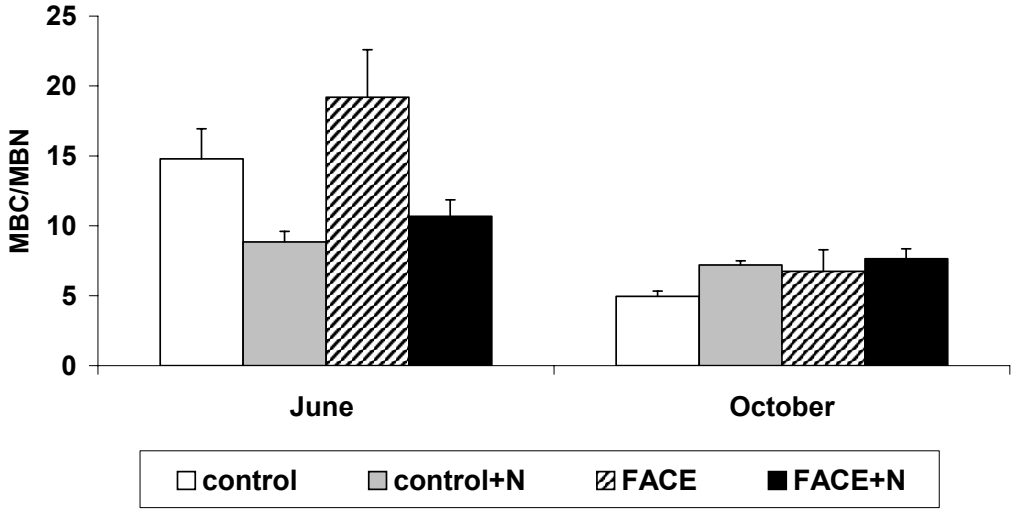

Figure 4.3. C/N ratio of microbial biomass in Control and FACE not fertilized and fertilized soils for the years 2002-2004 presented as average of June and October sampling dates. The three poplar clones were pooled together since no significant variations were recorded.

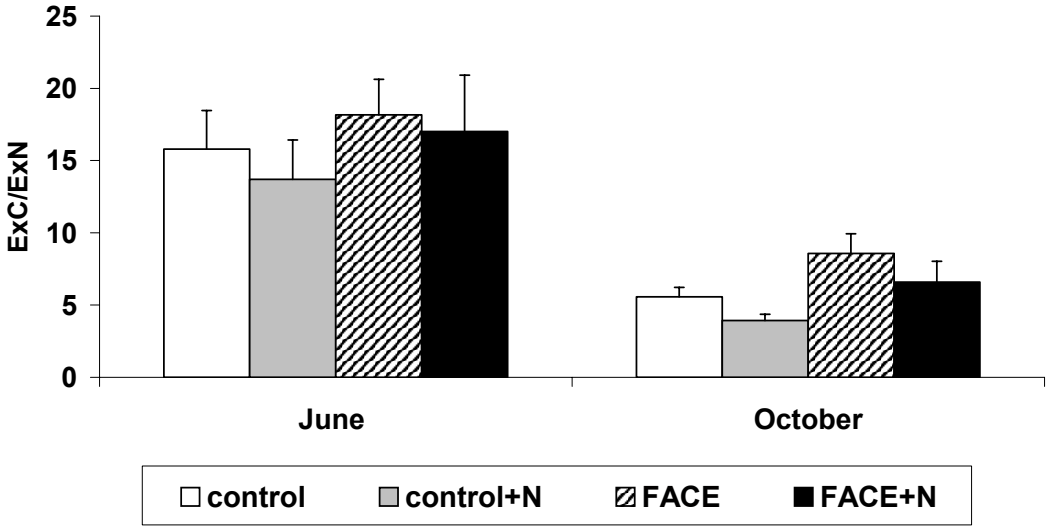

Figure 4.4. C/N ratio of 0.5M K_2SO_4 extractable SOM in Control and FACE not fertilized and fertilized soils for the years 2002-2004 presented as average of June and October sampling dates. The three poplar clones were pooled together since no significant variations were recorded.

	C/N microbial biomass		C/N K_2SO_4 extractable SOM	
	June	October	June	October
FACE	+26%	+18%	+19%	+59%
Nitrogen	-43%	+27%	-9%	-26%
	Analysis of variance			
CO_2	0.040		0.022	
Nitrogen	0.000		n.s.	
Time	0.000		0.000	
Time x Nitr.	0.000		n.s.	

Table 4.2. Mean percentage effects of treatments for soil C/N ratio of MBC and K_2SO_4 extractable SOM in the years 2002-2004. The significance of treatments, time and their interactions are reported. The not significant interactions were excluded from the analysis. The p values < 0.05 were reported.

Recalcitrant C

The slow fraction of C, represented by the Total Extractable soil organic C (TEC) did not fluctuate significantly with respect to TOC between the years of measurement and was stable around 65% of the total (fig. 4.5). The two treatments did not induce any significant change on this fraction (tab. 4.1).

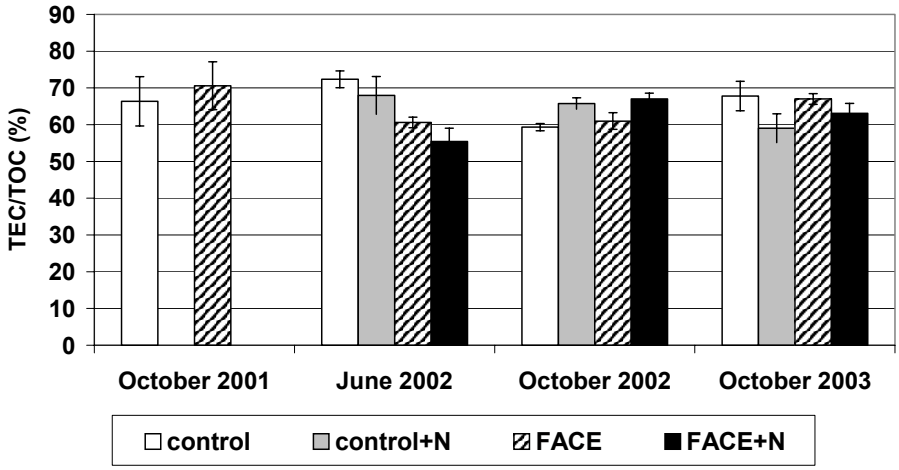

Figure 4.5. Total Extractable C, expressed per unit of TOC. FACE and Control treatments for years 2001-2003 in not fertilized and for years 2002-2003 in fertilized sub-plots (N) are reported. The three poplar clones were pooled together since no significant variations were recorded.

Total soil organic matter

The Total Organic C was presented as yearly variation, to avoid any bias due to the spatial variability. TOC showed significant variations between and within the years of measurement (tab. 4.3). Until October 2002 there was a significant increase, while in October 2003 the lowest values were registered. In the year 2004 there was a new, significant, increase of TOC (tab. 4.3). As a result, the organic C stock increased in soils independently from the treatments and the poplar clones and in the 2nd cycle a +23% increase was detected in the plantation. Elevated [CO_2] did not induce any significant change in the yearly variations of TOC soil content. The N addition induced a more pronounced decrease in 2003, with respect to not fertilized soil.

The C/N ratio of the soil organic matter varied slightly around 7, indicating a good quality of soil organic matter in the plantation, without a clear temporal trend (fig. 4.6). FACE treatment induced a not significant increase of 6% on average in fertilized and not fertilized soils. Fertilization did not induce any significant change.

	Total Organic Carbon (gC kg^{-1})			
	Δ 2001-2002	**Δ 2002-2003**	**Δ 2003-2004**	**Δ 2nd cycle (2002-2004)**
Control [n]	0.66 *(0.65)*	-0.37 *(0.23)*	3.12 *(0.45)*	2.53 *(0.62)*
FACE [n]	1.21 *(0.22)*	-0.38 *(0.29)*	2.02 *(0.87)*	1.96 *(0.69)*
Control [N]		-1.42 *(0.41)*	2.61 *(1.10)*	2.41 *(0.67)*
FACE [N]		-0.62 *(0.19)*	3.10 *(0.42)*	2.13 *(0.41)*
	Analysis of variance			
CO_2	n.s.	n.s.	n.s.	n.s.
Nitrogen	n.s.	0.049	n.s.	n.s.
Species	n.s.	n.s.	n.s.	n.s.
Time	0.000	0.000	0.000	0.000

Table 4.3. Yearly variation of Total Organic C, expressed as absolute change with respect to the previous year and as C accumulation in the 2nd cycle of the plantation (June 2002-October 2004). FACE and Control treatments for years 2001-2004 in not fertilized and for years 2002-2004 in fertilized sub-plots (N) are reported. The three poplar clones were pooled together since no significant variations were recorded.

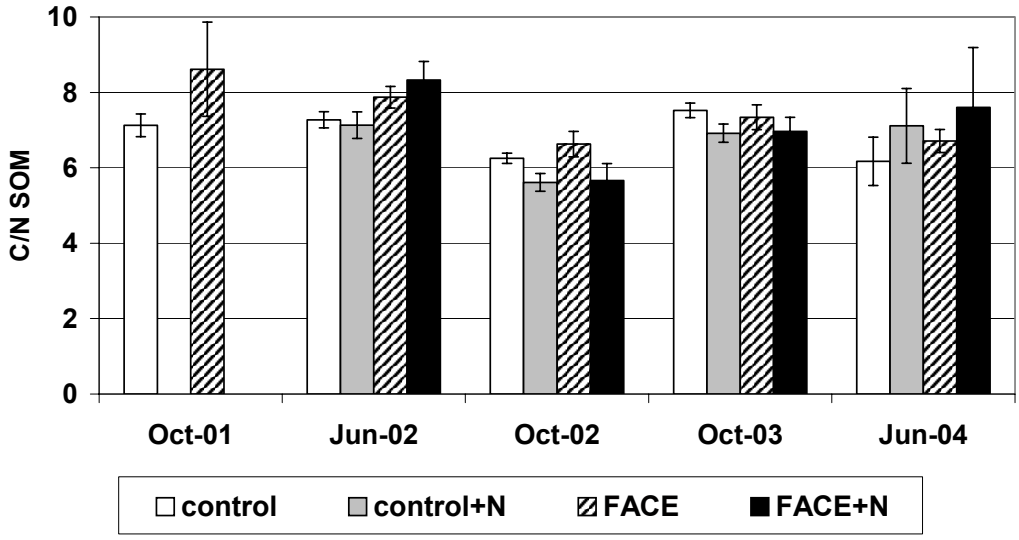

Figure 4.6. C/N ratio of soil organic matter in Control and FACE not fertilized soils for the year 2001 and not fertilized and fertilized soils for the years 2002-2004. The three poplar clones were pooled together since no significant variations were recorded.

4.3 Discussion

Temporal variation

The temporal variation of C fractions was strictly related to the microbial decomposition activity. In June 2003 we found, regardless the treatments, the higher C mineralization activity of the whole period of study (see sect. 7). As a consequence, at the end of 2003, there was a significant depletion of TOC amount in soil with respect to the previous year. Although the effect was more pronounced in fertilized soils, the C and N availability, and thus the FACE and fertilization treatments, did not influence this trend; the increase in mineralization activity seemed therefore linked also to some external factors. The year 2003 was particularly hot (Ciais, 2005) and the direct relationship between C decomposition and temperature is widely known (Kirschbaum, 1995; Pendall, 2004). Furthermore, at the beginning of 2002, the plantation was coppiced and new shoots resprouted in spring. In another study it was reported that soil C increased soon after coppicing when live root biomass diminished, but subsequently a decrease of TOC was observed, especially when high T induced tree re-growth (Trueman and Gonzales-Meler, 2005). In our site, the depletion of organic C occurred in the second year after coppicing, while in the first growing season a significant increase of C was shown. We can suppose that, regardless the treatments and the poplar clones, the loss of C from the system was due to a favourable combination of high temperature and high amount of material to decompose (i.e. dead fine roots that accumulated in soil, greater labile C availability), that might have induced higher C mineralization rates with a *priming effect* mechanism. The 23% increase of TOC in the plantation confirmed results from previous works. An increase in soil C after land use change from crop to plantation or secondary forest has been in fact reported as +18 and +52% respectively by Guo and Gifford (2002) in their review. The land use change from crop to plantation it was expected to promote soil C storage for 2 main reasons: i) after the initial ploughing, soil remained undisturbed in the following years, ii) it is reasonable to suppose a greater above and belowground C input in the plantation with respect to crop. These processes may contribute to an increase in soil C storage in the long term. This was not influenced by the treatments but the significant temporal increase suggested a good capacity for fast growing plantation to store C into soil.

Elevated [CO_2] treatment

The labile pool of C was increased by elevated [CO_2] at POPFACE site. The increase was not related either to seasonal or yearly fluctuations and to poplar clones, and was evident in both fertilized and not fertilized soils. Carbon inputs to soil may be increased by enhanced fine root production, turnover rates or increased root exudation (Heath et al., 2005). At the same time the soluble C is also a product of the microbial decomposition of organic matter, but in this case an increase of metabolic activity is expected to take place. The linkage between labile C inputs to soil, roots production and turnover as well as microbial activity can be explained by the mechanism of *priming effect* (Kuzyakov et al., 2000). Trueman and Gonzales-Meler (2005) found that the growth of *P. deltoides* at elevated atmospheric CO_2 enhanced belowground C inputs by increasing fine root biomass and the root C turnover. The increased C inputs to soils resulted in an increase in the rate at which decomposer oxidized old C, thus the amount of C in soil decreased. The high sand content of the soil, and a limited presence of macrofaunal communities during the length of the study may have prevented the stabilization of SOM in their study, as suggested by the authors themselves. Soil properties indeed influence the decomposition of organic matter in the soil, because they determine the living conditions for microbes and protect the soil C (Hagedorn et al., 2001). Protection of SOM against microbial decomposition typically occurs via chemical attraction to clay particles, that promotes soil aggregate formation (Muller and Hoper, 2004). At POPFACE experimental site the clay content is sufficiently high (see Hoosbeck et al., 2004) to protect chemically the soil C (Sollins et al., 1996). In FACE soils, where higher amounts of labile C were found, we did not observe a depletion either of TOC or of the extractable recalcitrant fraction (TEC). Another explanation for the lack of changes on organic C in the plantation soil could be a preference of microbes for the easily degradable C, where available, with respect to the more stable fractions, as hypothesized by Cardon (2001), or a competition for N with plants that could reduce the C metabolism (Cheng, 1999). Nevertheless, we did not detect any increase in the C mineralization activity neither under elevated [CO_2] nor in fertilized soils (see sect. 7). Depending on the different methodological approaches in measuring microbial contribution to soil respiration and the different meaning of what is really measured (potential vs. real, rhizospheric, specific respiration etc.), several authors reported changes in C mineralization rates in response to an enhanced flux of C from plants under elevated [CO_2] (Zak et al., 2000). At POPFACE plantation, the potential respiration of bulk soil microorganisms was measured without detecting any significant change due to the CO_2 enrichment, while an increase in

the C immobilized in the living fraction was found, suggesting a shift from catabolic activity towards cell biosynthesis. In the same site, on the other hand, an increase of rhizomicrobial respiration under [CO_2] enrichment was found, with no effects on microbial biomass C (see sect. 9). It is clear, therefore, that part of the C surplus from plant to soil was respired in the rhizosphere, while the residual fraction increased the labile C fraction of FACE soils. However, the TOC was unaffected by elevated [CO_2]. Two hypotheses can be drawn: i) the fast turnover characterising the labile C fractions affected by the FACE treatment could lead to a loss of C through the leaching of soluble C forms, as found by Bernard and co-authors (2004); ii) a 20% increase of a small fraction as labile C (on average the 3% of TOC) was not really appreciable considering the whole organic C in soil, and in this case we can not exclude that a longer term exposition to elevated [CO_2], in these experimental field conditions, could induce a build up of stable organic matter. The not significant effect of CO_2 enrichment on soil C has often been related to the difficulty of measuring changes in C against the background C-content of the soil (Ross et al., 1995; Hungate et al., 1996; Six et al., 2001).

Another consideration should be done on the quality of the organic fractions: the C/N ratio of part of the SOM considered as an active pool (Extr. SOM) was significantly increased by CO_2 enrichment, particularly in June when plants were more active, thus decreasing its quality and decomposability. Cotrufo et al. (2005) suggested that a bigger amount of the litter produced under elevated [CO_2] may be recalcitrant to microbial degradation, building up on the forest floor, and slowly entering into the SOM thus promoting C sequestration. As a confirmation, several studies reported slower decomposition rates of plant above and belowground residues under elevated [CO_2] (Gorissen et al., 1995; van Ginkel et al., 1996; Jastrow et al., 2000; Six et al., 2001). Moreover, the increase in C/N ratio of microbial biomass under elevated [CO_2] may indicate: i) lower energy availability to microorganisms and ii) an increase of fungal biomass with respect to bacterial biomass (Paul and Clark, 1989). Other experimental evidences confirm the last hypothesis with an increase in two indirect indicators of fungal biomass (arylsulphatase and chitinase activity, sect. 3). This preferential stimulation of fungi under elevated [CO_2] could be due to the increase in quantity but decrease in quality of plants input, because fungi usually grow on more complex substrates and are more efficient in converting substrate into biomass than bacteria (Couteaux et al., 1991). This may result in more soil carbon storage under elevated [CO_2] (Holland and Coleman, 1987).

Regarding the impact of soil N content on C storage under elevated [CO_2], at the end of the second rotation cycle TOC was similar in fertilized and not fertilized soils. The effect of elevated [CO_2] on the quantity and quality of labile SOM was in fact similar at both soil N content; on the other hand, the C/N ratio of total soil organic matter was not affected by elevated [CO_2] and N fertilization interaction, and the total N amount decreased under elevated [CO_2] in a similar way in fertilized and not fertilized soils (sect. 3). Several distinct populations exist within microbial biomass depending on the nutrient status of soils, as demonstrated by the different microbial C/N ratio in fig. 4.3 and for fungi in sect. 6. The relative importance of C and N limitation may differ among microbial functional groups or across time scale and it is possible that various feedbacks in the utilization of labile C compounds could interact also in relation to microbial communities (Vance and Chapin III, 2001; Allen and Schlesinger, 2004). As a confirmation, C substrates addition significantly increased N immobilization regardless soil N status also in the study of Vance and Chapin III (2001). The low quality of labile fractions could be therefore the main limiting factor in their utilization under elevated [CO_2], with and without N addition, and the microbial C and N immobilization processes seemed to be dominant in this system.

Fertilization treatment

The decrease of labile C fractions in fertilized soil confirmed results obtained by Okano et al. (1991), Ghani et al. (2003) and Hagedorn et al. (2001). N fertilization may in fact reduce C availability to microbes by condensing with soil humus (Nohrstedt et al., 1989) and may also repress fungal ligninolytic activity (Keyser et al., 1978) or reduce the production of enzymes to attack N-containing organic matter (Hu and van Bruggen, 1997). Indeed, the effect of fertilization was different between the living and the labile C fractions of soil, since no fertilization effects were found on MBC. A different fungal community in fertilized soils with a decrease in species number was instead found (sect. 6). N addition might also enhance microbial biomass turnover, which would assimilate relatively higher amounts of labile forms of C at a greater rate (Ghani et al., 2003).

In this study the fertilization treatment determined an increase in microbial N immobilization and in the total N content (see sect. 3) but did not influence the quality of labile fraction and SOM as well as the soil C storage. Soil organic C increased with N addition in several works (Blevins et al., 1983; McAndrew and Mahli, 1992) but Raun et al. (1998) found an increase only when N was applied at rates in excess of that required for maximum yield. On the other hand, we can suppose that during the first year of fertilization a *priming*

effect occurred in the plantation, determining higher losses of C in fertilized soils (tab. 4.3 and sect. 3 and 7). A following microbial selection probably favoured those microorganisms more dependent by nitrogen availability that tended to immobilize this nutrient. Community differences in microbial biomass pools are a consequence rather than a cause of contrasting organic matter accumulation (Vance and Chapin III, 2001). It is possible that a competition for nitrogen occurred in fertilized soils too also because of different populations. Neverthless, after a first year of adaptation, the N addition to the soil induced a rapid recovery of C in the soil. N addition induced therefore short-term changes in the soil C storage, dependent also on seasonal fertilizer applications, and the capacity for belowground C storage changed over time.

Final considerations

In the work of Hoosbeck et al. (2004) regarding the C storage in the first cycle of the plantation, the authors hypothesized a *priming effect* of the newly incorporated litter due to the FACE treatment. In this study, however, we have no evidences that this mechanism still occurred in the plantation. A first explanation could be the differences in methodological approaches. Another explanation could be a time dependence of the microbial response to elevated [CO_2], also supported by the sharp decrease in inorganic nitrogen occurred at the end of the first cycle (sect. 4). If microbial response depends on nutrient availability, negative and positive feedback might predominate at different temporal scales. Hu et al. (1999) in their review indicated that the direction of microbial feedback to elevated [CO_2] changed in an annual grassland over six years. In the early years, when microbes were C-limited, enhanced C inputs stimulated microbial activities and CO_2 production. But, as elevated [CO_2] stimulated plant N uptake and available soil N was translocated to less available pools, N limitation became limiting for microbial activities. In POPFACE plantation, even if N plant uptake was unaffected by CO_2 enrichment, the total N amount were significantly lower in FACE soils (see sect. 3). It is possible to argue that microbes did not utilize completely the C surplus in FACE soils and C and N microbial immobilization processes prevailed in the 2nd cycle of the plantation, suggesting a greater potential for FACE soils to store C.

5. SOIL MICROBIAL INDICES AS BIOINDICATORS OF ENVIRONMENTAL CHANGES

5.1 Introduction

Terrestrial ecosystems response to CO_2 fertilization is linked to the knowledge of below-ground processes and particularly those performed by the microbial pool (Zak et al, 2000). Soil microbial biomass, although it is very small on an ecosystem basis (1-3% of soil total organic carbon, TOC), plays a central role in understanding changes of C and N cycling, under elevated [CO_2]. It has often been referred to as the "eye of the needle" through which all organic matter must pass and, therefore, the measure of microbial biomass turn-over, providing useful information on soil responses to atmospheric CO_2 enrichment, could be a valid bioindicator and an "early warning" of changes in soil organic C; in addition it can help to predict nutrient availability for plant uptake and growth (Coleman and Crossley, 1996; Insam et al., 1999).

Microbiological parameters related to soil weight were often correlated or combined as an index in order to evaluate the significance of microbial populations and microbial activity in the cycling of elements in soils of different ecosystems (Nannipieri, 1994). Brookes (1995) recommends to combine microbial parameters in order to have an "internal control" such as biomass C as percentage of soil organic matter. The same author also reports that combining microbial activity and population measurements (biomass specific respiration or metabolic quotient) appears to provide more sensitive indications of soil pollution than either activity or population measurements alone (Dilly & Munch, 1998). Ecophysiological indices (metabolic quotients) are generated on the basis of physiological performances (respiration, growth/death, carbon uptake) on the total microbial biomass per unit time. Any environmental impact that will affect members of a microbial community should be detectable at the community level by a change of a particular total microbial community activity, which can be quantified (qCO_2, qD, etc) (Anderson, 2003).

The ratio of biomass C to soil organic C (Cmic:Corg) reflects the contribution of microbial biomass to soil organic carbon (Anderson & Domsch, 1989). It also indicates the substrate availability to the soil microflora or, in reverse, the fraction of recalcitrant organic matter into the soil (Brookes, 1995). After a change, the soil microbial biomass responds more quickly to the change than does the amount of organic matter in the soil, which is relatively slower. Thus, the Cmic:Corg ratio will increase for a certain time if the input of organic matter to a soil is increased, and decreases for a certain time if the input is decreased

(Anderson and Domsch, 1989). The qCO_2, (the community respiration per biomass unit or the metabolic quotient) has been widely used in literature and it was originally based on Odum's theory of ecosystem succession. Although its reliability as a bioindicator of disturbance or ecosystem development has been recently criticised by some authors, it is recognized to have valuable application as a relative measure of how efficiently the soil microbial biomass is in the utilization of C resources, and the degree of substrate limitation for soil microbes (Wardle & Ghani, 1995; Dilly & Munch, 1998). The *q*M (mineralization quotient) expresses the fraction of total organic carbon mineralized throughout the incubation time (Dommergues, 1960; Pinzari et al., 1999). The *q*C (microbial biomass change rate quotient) expresses the daily enrichment or loss of soil microbial C and is calculated based on *q*D as reported by Anderson and Domsch (1990). Positive or negative values indicate a daily loss or enrichment, respectively, of microbial biomass C in the ecosystem

Aim of this work was to assess the validity of the microbial indices as biondicators of microbial processes induced by the two treatments: FACE and N fertilization.

5.2 Results

The trend of microbial quotient (Cmic:Corg ratio), during the four years of observation, was a sharp decrease in the year 2001 (fig. 5.1), that parallels the trend of $N\text{-}NH_4^+$ reported in sect. 3, as also shown by the linear regression on these two parameters in Fig. 5.2. Cmic:Corg significantly decreased after the first year and assessed its value to less than 2% until the end of 2004 (fig. 5.1). However, although the contribution of microbial biomass to total organic carbon is very low in this soil, elevated [CO_2] treatment induced a significant increase of Cmic:Corg ratio in not fertilized plots (+35%, $p<0.001$) (tab. 5.1).

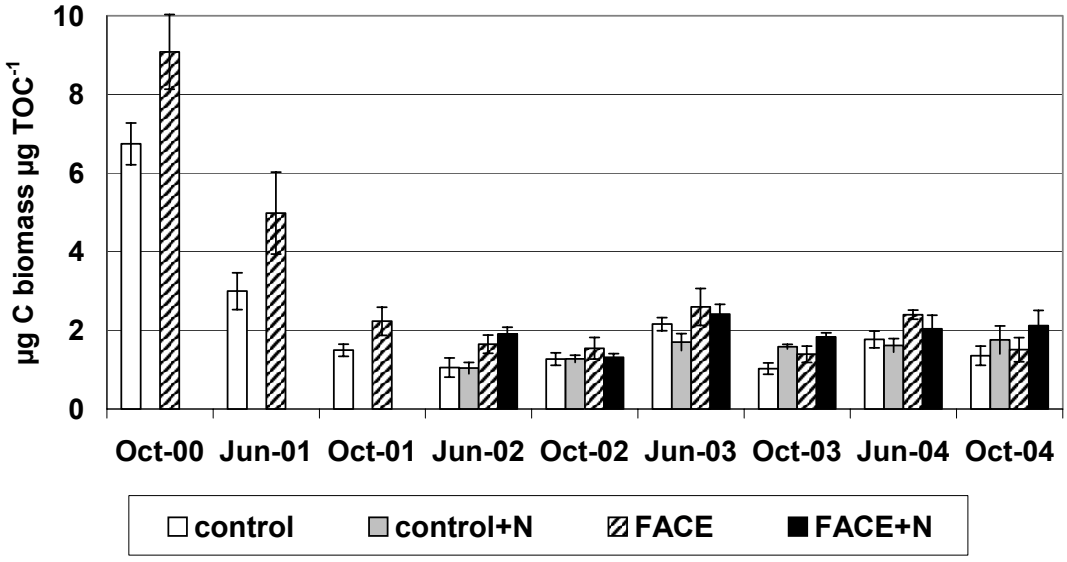

Figure 5.1. Microbial quotient Cmic:Corg measured in Control and FACE not fertilized soil in the years 2000-2004 and in Control and FACE fertilized soils in the years 2002-2004. Values from the three clones where pooled together since no significant differences were found.

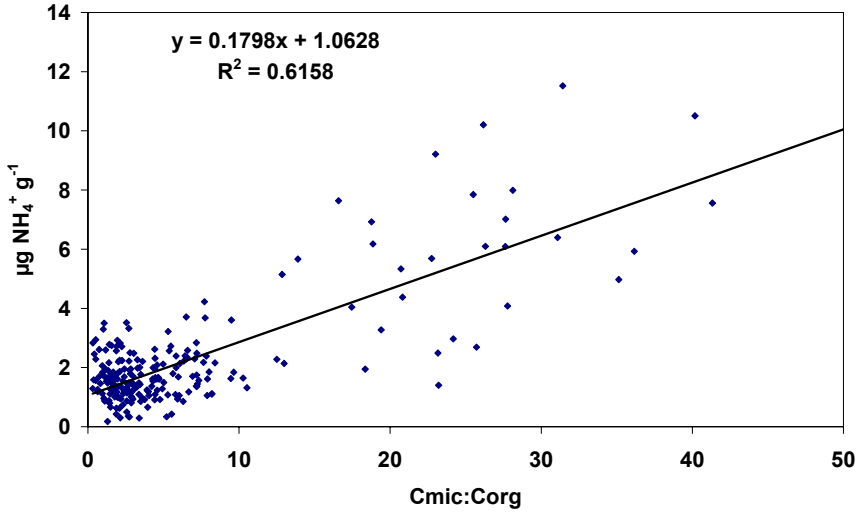

Figure 5.2, Linear regression between the N-NH_4^+ content and the Cmic:Corg in Control and FACE not fertilized soil in the years 2000-2004 and in Control and FACE fertilized soils in the year 2002-2004.

	Qmic	*q*C	*q*CO_2	*q*M
CO_2 [n]	+37%	+22%**	-14%	+8%
CO_2 [N]	+21%	+5%	-22%	0
Nitrogen [C]	+11%	-4%	-21%	-1%
Nitrogen [F]	+7%	-18%	-31%	-6%
	Analysis of Variance			
time	0.000	0.000	0.000	0.000
CO_2	0.000	n.s.	0.008	n.s.
nitrogen	n.s.	0.04	0.000	n.s.
species	n.s.	n.s.	n.s.	n.s.
nitrogen x time	0.026	0.007	0.000	n.s.

Table 2. Percentage effects of FACE and fertilization treatments on the microbial indices and the microbial respiration after 24 h. Analysis of variance is reported for each factor of variation and their interactions. p values <0.05 were reported, n.s. not significant. The not significant interactions were removed from the analysis. ** effect significant for p<0.01 (Bonferroni post Hoc test).

The microbial change rate quotient (*q*C) is reported in tab. 5.2. Microbial loss increased under elevated [CO_2] with respect to Control plots, significantly in not fertilized soils (Bonferroni post Hoc test, $p<0.01$). The N addition lowered the *q*C and therefore the decrease of microbial biomass.

The metabolic quotient did not show a clear temporal trend, but the lowest values were recorded at the beginning of the experiment. The qCO_2 was negatively and significantly affected by FACE and N fertilization treatments (fig. 3 and Tab. 2). Face lowered the qCO_2 in both fertilized and not fertilized soils and the addition of nitrogen caused a further decrease of this index in Control and FACE plots. Anyway, in general, the qCO_2 was lower than 2 ($\mu g\ C\text{-}CO_2\ h^{-1}\ \mu g\ C\ biomass^{-1}$)$10^3$ in fertilized soil.
An inverse correlation is generally observed between qCO_2 and Cmic:Corg ratio indicating a strict interdependency between microbial growth and maintenance. In this study the correlation coefficient between the two indices is $r = -0.388$ ($n=177$; $p<0.001$) and indicates that to a low qCO_2 corresponded a high Cmic:Corg ratio.

The C mineralization quotient (*q*M) provides information on the fraction of total organic carbon mineralized throughout the incubation time (10 days in this study) (Dommergues, 1960; Pinzari et al., 1999). It showed a maximum in June 2003 but was not affected by elevated [CO_2] or fertilization treatments (fig. 5 and tab. 2).

Period	Days	Control [n]	FACE [n]	Control [N]	FACE [N]
October 2000-March 2001	129	5.45 *(0.31)*	5.50 *(0.29)*		
March 2001-June 2001	82	-4.89 *(1.99)*	0.25 *(0.1)*		
June 2001-August 2001	98	1.22 *(0.94)*	2.13 *(1.13)*		
August 2001-October 2001	53	3.90 *(1.06)*	7.85 *(1.46)*		
October 2001-June 2002	240	0.78 *(0.35)*	0.98 *(0.29)*		
June 2002-October 2002	145	-2.75 *(1.60)*	0.47 *(0.64)*	-2.72 *(1.22)*	1.69 *(0.50)*
October 2002-June 2003	220	-2.20 *(0.75)*	-1.36 *(0.55)*	-3.82 *(1.18)*	-5.70 *(1.24)*
June 2003-October 2003	156	3.27 *(0.49)*	2.54 *(0.53)*	1.21 *(0.67)*	0.78 *(0.73)*
October 2003-June 2004	220	-3.83 *(1.06)*	-1.86 *(1.03)*	-2.66 *(1.10)*	-3.22 *(2.04)*
June 2004-October 2004	156	0.00 *(1.53)*	2.39 *(0.85)*	-1.12 *(0.85)*	-0.55 *(0.77)*
Average		-0.18	1.72	-1.80	-1.42

Table 5.2. Microbial biomass change rate quotient (*q*C) ((µg $Cmic_{t1}$ - µg $Cmic_{t2}$)/(µc $Cmic_{t1}$/(t2 – t1)) x 10^{-3} measured in FACE and Control not fertilized (Fall 2000 - Fall 2004) and fertilized plots (Spring 2002 - Fall 2004).

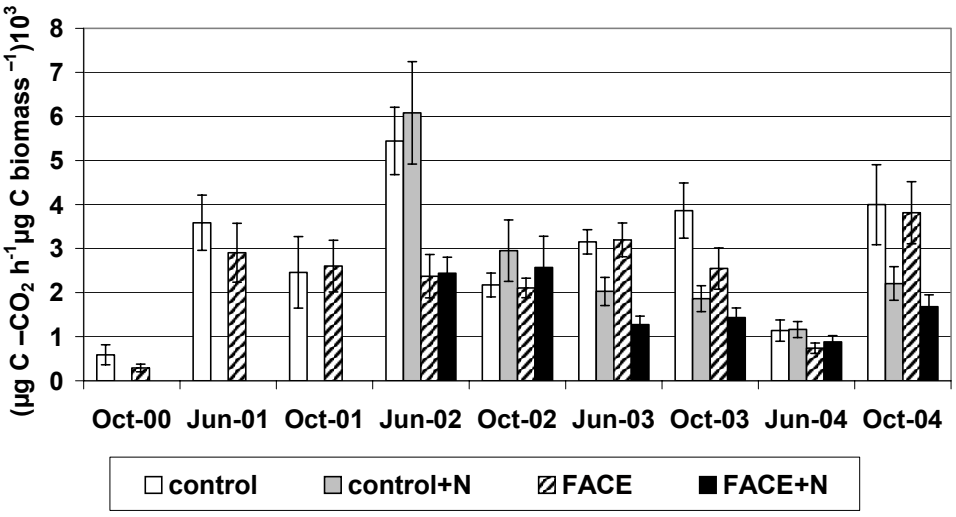

Figure 3. Metabolic quotient qCO_2 measured in Control and FACE not fertilized soil in the years 2000-2004 and in Control and FACE fertilized soils in the year 2002-2004. Values from the three clones where pooled together since no significant differences were found.

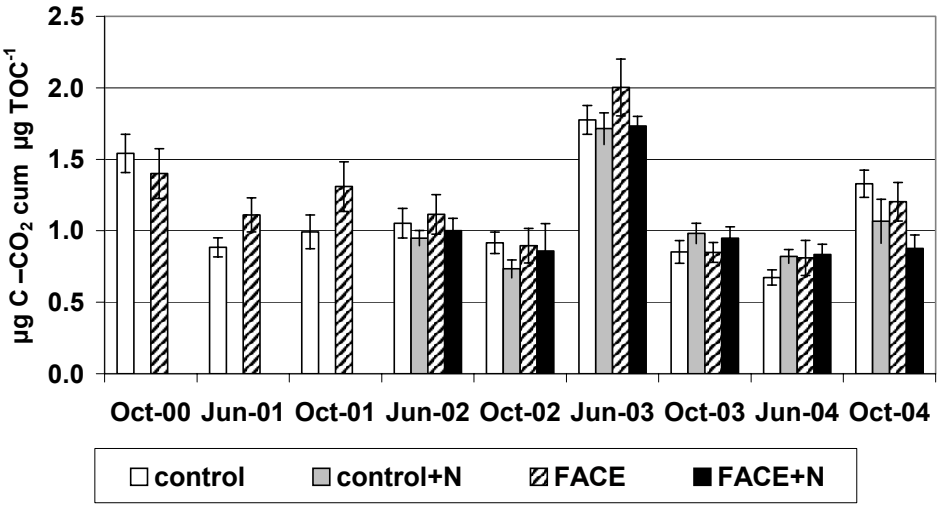

Figure 5. Mineralization quotient qM measured in Control and FACE not fertilized soil in the years 2000-2004 and in Control and FACE fertilized soils in the year 2002-2004. Values from the three clones where pooled together since no significant differences were found.

5.3 Discussion

In many studies microbiological parameters were correlated or combined as an index (Nannipieri, 1994). Neverthless ratios between microbiological parameters have often been used for evaluating the microbial ecophysiology implying an interlinkage between cell-physiological functioning under the influence of environmental factors (Anderson, 2003).

In this study the responses of Cmic:Corg ratio, qCO_2 (metabolic quotient) and qC (microbial change rate quotient) to FACE and nitrogen fertilization treatments, observed during three years, seemed to be strongly affected by the nutritional *status* of the soil, in particular by the $N\text{-}NH_4^+$ soil content. The microbial pool was strongly dependent on nitrogen and probably suffered from a competition with plants for this element. The depletion in inorganic nitrogen was, in fact, the driving variable of microbial physiological status. This nutritional "stress" could explain the decrease of Cmic:Corg ratio, in not fertilized plots, to values lower than 2.0 which is considered a critical threshold for soils with neutral pH (Anderson, 2003). Moreover, it is reasonable to assume that a nutritional unbalance between C and N may have altered the physiological state of microbes with changes in microbial death rates. The decrease of qC after the fertilization suggests an improvement of microbial nutritional conditions as nitrogen in easily available forms was provided.

Anderson (2003) refers to the same critical value, mentioned for Cmic:Corg, also with reference to qCO_2, affirming that metabolic quotient values >2.0 indicate an energetically less efficient microbial community. Changes in nutrient availability can modify microbial maintenance energy requirements. The low Cmic:Corg and the high qCO_2 reflect a less efficient use of organic substrates by microbial biomass (Anderson, 2003; Pinzari et al., 1999). Nutrients acquisition activity is an energetically expensive process particularly when microbes are forced to degrade stable SOM to get new available substrates. qCO_2 decreased due to $[CO_2]$ enrichment but this reduction was not enough to lower the index below the threshold of 2, whereas only in fertilized plots and especially in FACE fertilized soils the reduction was more pronounced. In fact, in FACE fertilized plots the concomitantavailability of C and N lowered the energetic requirement of microbial biomass. The C and N supply provoked probably a short-term selection inside the microbial community, confirmed by findings on fungal community (see sect. 6 and 7).

In elevated $[CO_2]$ environments it is assumed that, because of faster root turnover or increased production of root exudates, more C is available for microbes (Cardon et al.,

1996; Cheng, 1999; Schortmeyer et al., 2000). Elevated [CO_2] induced a significant increase of soil labile carbon fractions (see sect. 4) indicating a net flux of soluble C forms that could lead to the immobilization process observed. We can therefore hypothesize that, in our experimental conditions, the extra C made available for microbes has been used to build up more microbial biomass as the significant increase of microbial quotient under elevated [CO_2] suggests. Moreover, the decrease of metabolic quotient indicated that a more efficient microbial population was favoured under elevated [CO_2]. The sustaining of a greater microbial biomass was indeed allowed with an equal metabolic activity, avoiding in this way an increase of respired C with [CO_2] enrichment. These findings were confirmed by the lack of [CO_2] enrichment effect on the *q*M, or the potential C mineralization activity (measured under optimal conditions of temperature and humidity) as defined by Dommergues (1960). Conversely, the C surplus promoted microbial C immobilization determining a net increase of labilie C in the pantation under elevated [CO_2].

In conclusion, as far as the aim of this paper is concerned, microbial indices proved to be sensitive to changes occurred to soil processes under FACE and fertilization. We hypothesize that a competition for nitrogen between plants and microrganisms occurred in FACE soil, probably inducing a stress condition within microbial community. Elevated [CO_2] treatment provided C for microbial growth, but reduced nitrogen availability and increased microbial loss. Nitrogen fertilization, conversely, promoted soil microbial biomass enrichment, lowering nutritional stress.

Considering the indications provided by these indices, a not consistent change on carbon sequestration soil capacity has been observed between FACE and control soils probably because a limited use of the C resources surplus. Where N was added, the drawbacks to C utilization weren't so strong as in not fertilized soils, but we have evidence that a selection inside microbial communities could have changed the mechanisms dominating the C and nutrient balance in the soil towards a different equilibrium.

6. STRUCTURAL AND FUNCTIONAL DIVERSITY OF SOIL MICROBES

Introduction

The genetic composition of the microbial pool, its functionality, and the relationship between the two is an important question in studying the role of soil in the C cycle. Few studies have attempted to directly assess changes in the composition of microbial community in response to elevated [CO_2], and even fewer studies have documented changes in either the richness or evenness of microbial community composition (Zak et al., 1996; Griffiths et al., 1998; Klamer et al., 2002). In particular, fungi may be critical reservoirs of C in systems with elevated atmospheric CO_2 since fungi constitute the major part of microbial biomass in the soil system and are intimately involved in the flux of C channelled directly from the aboveground biomass (Klamer et al., 2002). To our knowledge however there are no studies on the influence of CO_2 enrichment and nitrogen fertilization on fungal genetic composition, using e.g. PCR-denaturing gradient gel electrophoresis (DGGE). Regarding the functional diversity of microbial communities, investigations have largely been based on extracting organisms from soil and determining their potential substrate utilization using community level physiological profiling (CLPP) with Biolog plates (Miller, 1995; Insam and Goberna, 2004). However, these approaches only provide information about some of the culturable organisms in soil, which may not be indicative of the greater soil microbial community and, relying on the growth of organisms in substrate-filled wells, discriminate against fungi and other slowly growing organisms with preference for more acidic conditions (Degens, 1999). The MicroResp method (Campbell et al., 2003), an advancement of the Degens (1999) approach, used in this work has several advantages over the Biolog-based CLPP approach. It reflects immediate activity in a short-term period and physiological information equivalent to that obtained from Biolog plates can be obtained without a prolonged lag phase (Campbell et al., 2003). Using the whole soil provides a direct measurement of substrate catabolism by microbial communities and reflects activity rather then growth because more immediate responses to substrates can be obtained. Moreover, the estimates of respiration in response to added substrates assumes that all components of the microbial biomass respond equally to the substrate, and that the initial activity is correlated to the actual size of the microbial population (Pennanen et al., 2004).
The aim of this work was to investigate the genetic composition of the fungal community and the functional diversity of soil microorganisms in a poplar plantation (POPFACE) grown under elevated [CO_2] with and without nitrogen fertilization. In the POPFACE experiment, indirect suggestions of a stimulation of fungal biomass and activity induced by

CO_2 treatment have been found (sect. 3 and 4). If elevated [CO_2] results in changes in the quantity and quality of plant inputs to soil, and in a stimulation of fungi, it could cause a shift within the microbial community, both on a structural and functional level. We hypothesized that a shift inside microbial community due to both FACE and fertilization treatments could occur (see also sect. 5), which we now want to confirm by PCR-DGGE and MicroResp based CLPP.

6.2 Results

Fungal community structure

The DGGE analysis showed few fungal species (between 3 and 15, fig. 6.1 and 6.2). In October the difference in the number of fungal species between not fertilized and fertilized soils in Control plots was significant, but not in July (fig. 6.1). In Control plots, there was a smaller number of bands in July than in October (fig. 6.1).

The cluster analysis (fig. 6.3) showed a very clear separation between not fertilized and fertilized soils for both seasons, and in particular for the October samples. Inside these two main groups, FACE and Control plots were also discernible, although less distinctly. The separation between FACE and Control soils was more clearly evident in fertilized subplots. No differences among the poplar clones were found.

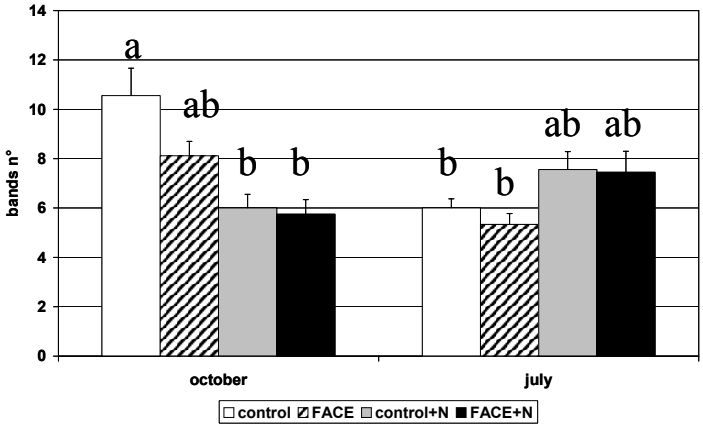

Figure 6.1. Number of bands recorded in the DGGE profiles for the two sampling dates in Control and FACE not fertilized plots and in Control + N and FACE + N fertilized plots. Bars with the same letter are not significantly different ($p < 0.05$).

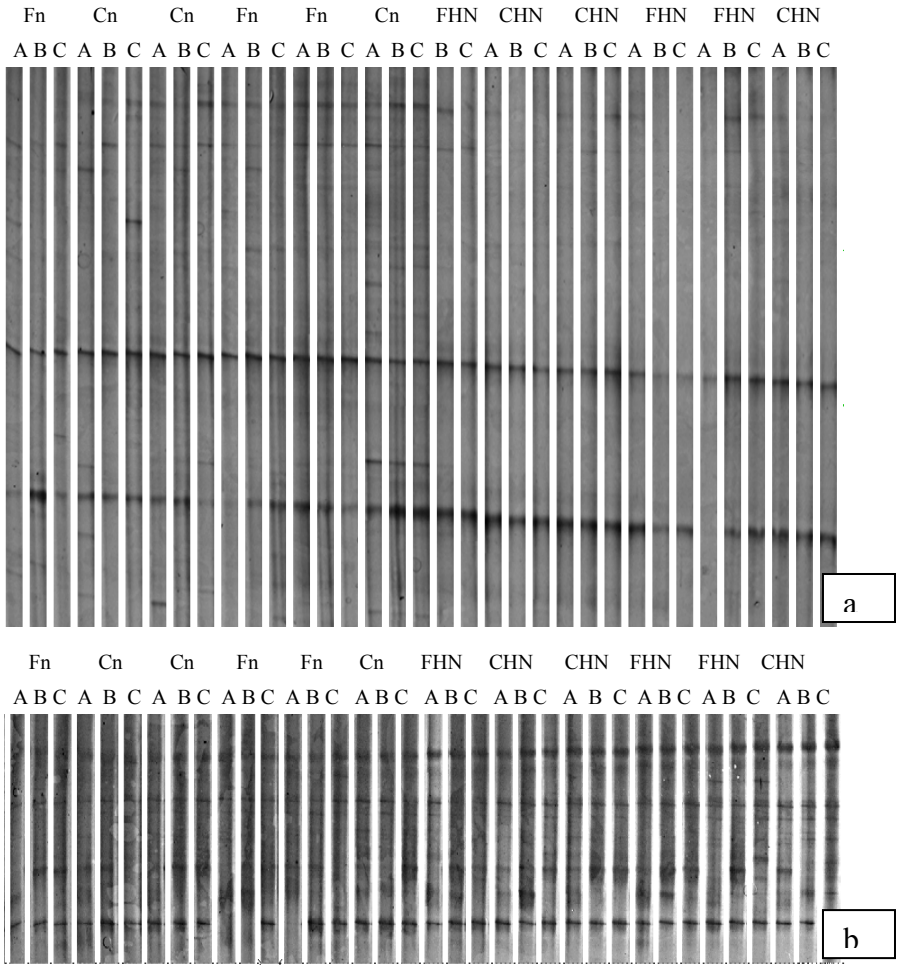

Figure 6.2. DGGE profiles of the fungal soil community of the poplar plantation for October (a) and July (b) samples. The labels identify Control (C) and FACE (F), not fertilized (n) and fertilized (HN) soils and the three poplar clones *P. alba* (A), *P. nigra* (B) and *P. x euramericana* (C).

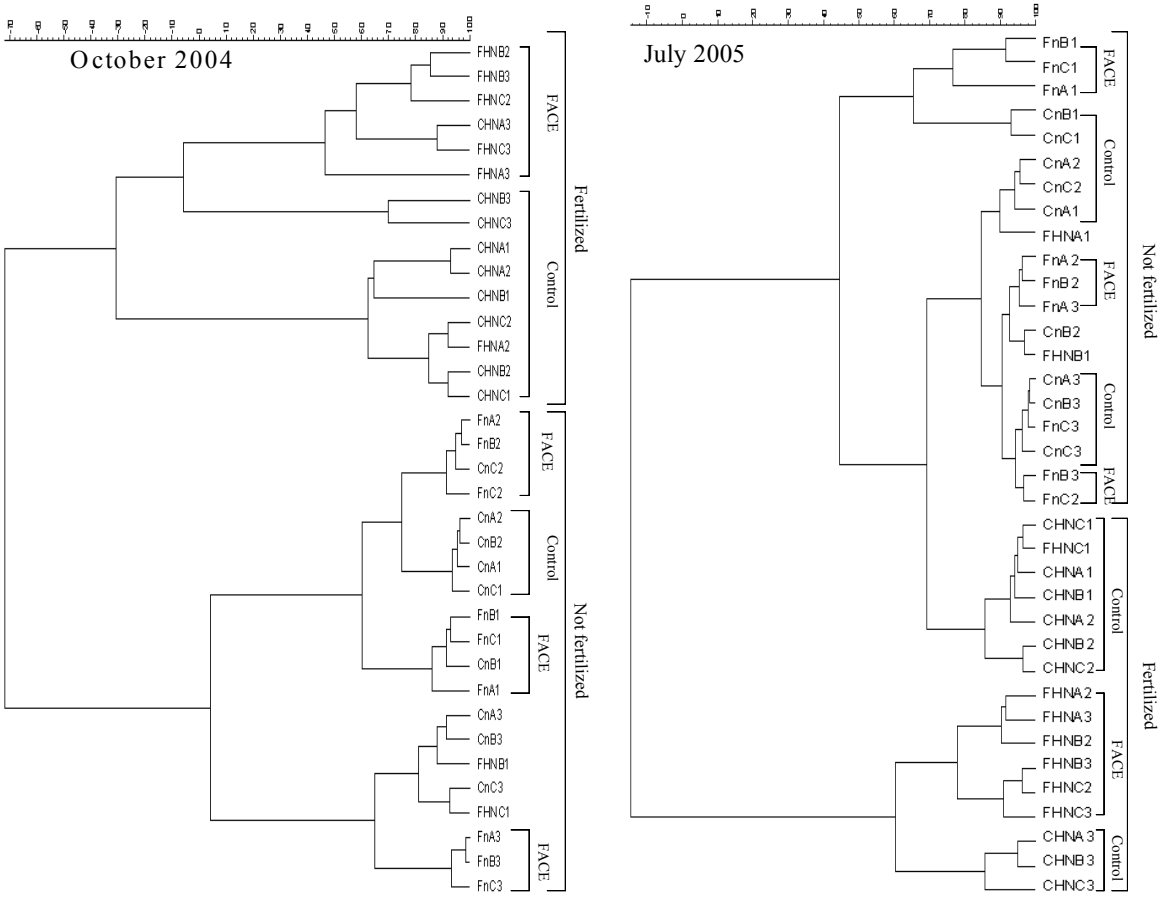

Figure 6.3. Curve based dendrogram generated from gel image data using Pearson correlation coefficients. (F = FACE, C = Control, n = not fertilized, HN = fertilized, A = *P. alba*, B = *P. nigra*, C = *P. x euramericana*).

Community level physiological profile

The PCA showed the separation between the three poplar clones, while the two treatments were not different at any sampling date (fig. 6.4).

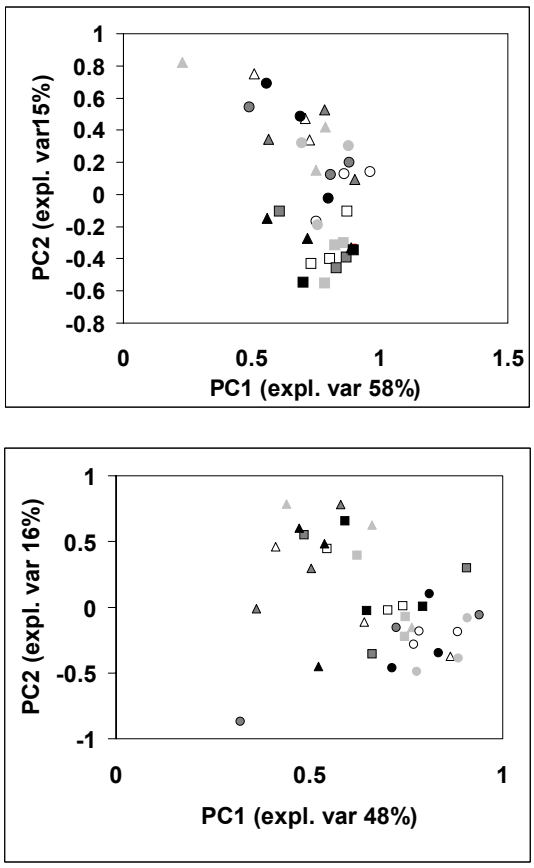

Figure 6.4. Principal Component Analysis of MicroResp data for October 2004 (a) and July 2005 (b) samples in Control (white) and FACE (dark grey) not fertilized plots and in Control + N (light grey) and FACE + N (black) fertilized plots for *P. alba* (□), *P. nigra* (Δ) and *P. x euramericana* (O).

The metabolic activity of soil microbes was similar at the two sampling dates, although lower in July with respect to October (fig. 6.5).

Nitrogen fertilization decreased respiration rates with a minimum of 1.3 μg CO_2 g^{-1} h^{-1} for *P. alba* in October. Malic and oxalic acids increased CO_2 production. According to MANOVA (tab. 6.1 and 6.2) the difference between the poplar clones was the main factor of variation for both sampling dates. Nitrogen treatment induced significant differences in October and, in interaction with the clones, in July. Fertilization resulted in an increase of CLPP (Community Level Physiological Profile), in particular in FACE fertilized soils in October, while in July only *P. x euramericana* followed this trend and *P. alba* showed a slight decrease (fig. 6.5). The CO_2 enrichment induced, in October, significant variations only in interaction with the fertilization treatment and the poplar clones (tab. 6.1), while in July we could not detect any effect (tab. 6.2). The FACE treatment, in October, resulted in a decrease of CLPP in not fertilized soils and an increase in fertilized soils for *P. nigra* and *P. x euramericana* (fig. 6.5). Regarding the response of microbes to the different substrates added, in October we recorded a higher number of substrates with significant effects than in July (tab. 6.1 and 6.2). Carbohydrates and carboxylic acids responded in a similar way in both treatments but the effect of elevated [CO_2] on microbial CLPP was evident only with substrates containing N (N-Acetyl-glucosamine and NH_4NO_3) or carboxylic acids (tab. 6.1 and 6.2).

Oxalic acid and NH_4NO_3 were the most responsive substrates (tab. 6.1 and 6.2).

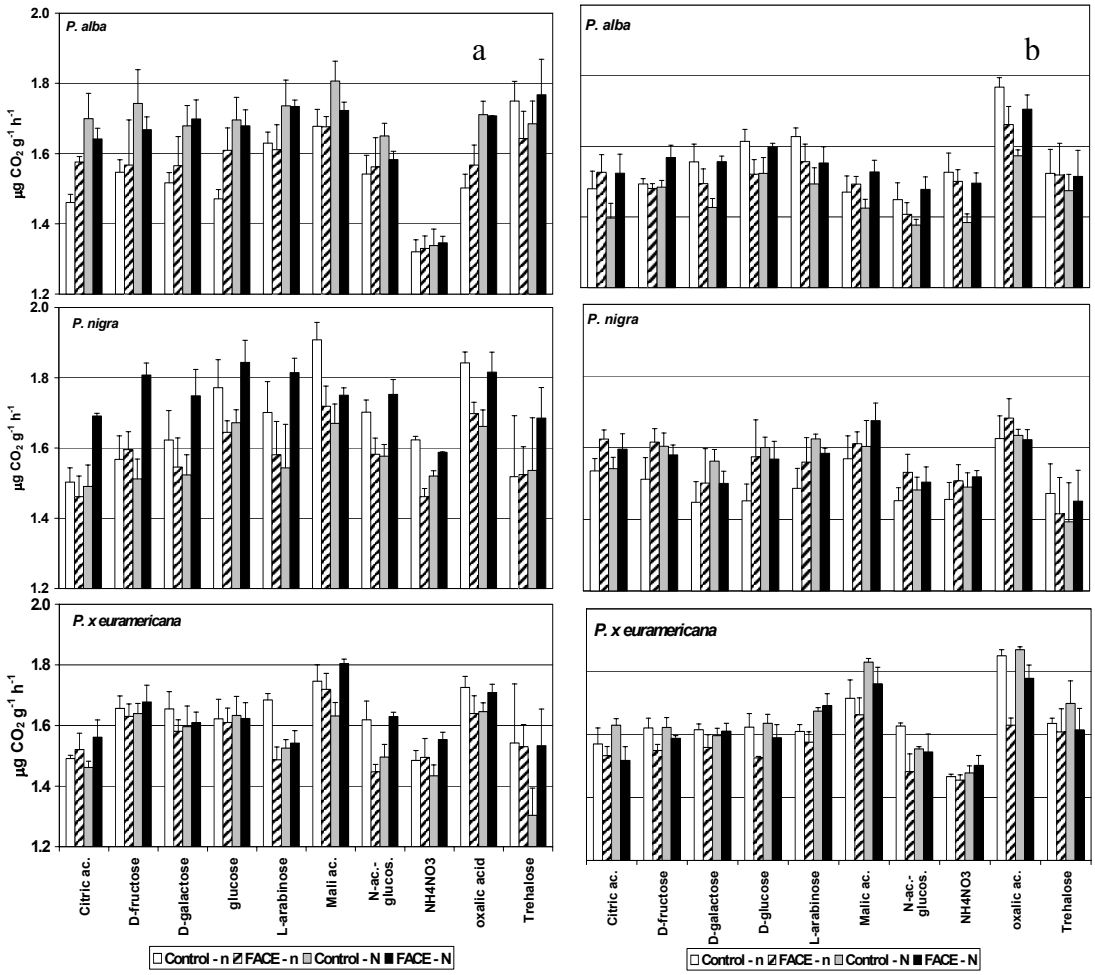

Figure 6.5. Community level physiological profiles measured with MicroResp for October 2004 (a) and July 2005 (b) samples in Control and FACE not fertilized plots and in Control + N and FACE + N fertilized plots for the three poplar clones.

	analysis of variance								
	October								
	MANOVA	Citric ac.	Fructose	Galactose	Glucose	Malic ac.	N-acetyl-gluc.	NH_4NO_3	Oxalic ac.
CO_2	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
species	0.000	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.000	0.003
nitrogen	0.046	0.001	0.014	0.033	0.041	n.s.	n.s.	n.s.	0.014
CO_2 x spp.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
CO_2 x nitrogen	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.031	n.s.
nitrogen x spp.	n.s.	n.s.	n.s.	n.s.	n.s.	0.018	n.s.	n.s.	0.002
CO_2 x nitrogen x spp.	0.035	0.007	n.s.	n.s.	n.s.	0.012	0.004	0.029	0.012

Table 6.1. Multivariate Analysis of Variance of FACE and fertilization treatments, the poplar clones and their interactions are reported for CLPP of October 2004 samples. ANOVA for single substrates utilization is also reported. The substrates without any significant effect were removed from the table. Values of p < 0.05 are given.

	analysis of variance							
	July							
	MANOVA	Citric ac.	Fructose	Arabinose	Glucose	Malic ac.	N-acetyl-gluc.	NH_4NO_3
CO_2	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
species	0.000	0.005	0.003	n.s.	n.s.	0.000	0.006	n.s.
nitrogen	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
CO_2 x spp.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
CO_2 x nitrogen	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
nitrogen x spp.	0.007	n.s.	n.s.	0.006	n.s.	n.s.	n.s.	0.021
CO_2 x nitrogen x spp.	n.s.	n.s.	n.s.	n.s.	0.035	n.s.	n.s.	n.s.

Table 6.2. Multivariate Analysis of Variance of FACE and fertilization treatments, the poplar clones and their interactions are reported for CLPP of July 2005 samples. ANOVA for single substrates utilization is also reported. The substrates without any significant effect were removed from the table. Values of p < 0.05 are given.

6.3 Discussion

To our knowledge, DGGE has rarely been used to study soil fungal communities (Kowalchuk, 1999; Gomes et al., 2003; Agnelli et al., 2004), and this is the first study using this approach in a [CO_2] enrichment experiment. Studies on fungal composition (using non-molecular techniques) gave contrasting results. Regarding the influence of elevated [CO_2] on fungi, there is evidence of an increase of fine roots and associated mycorrhizal fungi (Ineichen et al., 1995; Rouhier and Read, 1998; Wiemken et al., 2001; Lukac et al., 2003) and of fungal biomass in the bulk soil (Klamer et al., 2002). Klamer and co-workers, however, did neither find changes in fungal community composition nor in richness following [CO_2] enrichment. Effects of elevated [CO_2] on fungal community composition were evident mainly as interaction with the fertilization treatment where, in not N-limited soils, the FACE treatment selected a different microbial community (fig. 6.3). The interaction of N and FACE treatments provides in fact the two essential elements for microbial growth, differentiating the 18S rDNA without lowering the species richness (fig. 6.1). N addition had no significant effect on microbial composition (Johansson, 2001), while it changed the abundance of some fungal species (Sarathchandra et al., 2001). Wiemken et al. (2001) found an increase in mycorrhizal biomass after N fertilization only in a nutrient poor siliceous soil, but not in a nutrient rich calcareous soil. At the POPFACE site, not fertilized soil is low in mineral N (between 5 and 15 μg N g^{-1}) and a particularly strong depletion of N-NH_4^+ was recorded at the end of the third growing season (sect. 3); in sect. 5 the strong dependence of microbial pool on the nutritional *status* of the soil was demonstrated. Our results strengthen this conclusion: the fertilization treatment affected the fungal community composition independently of elevated [CO_2] or the poplar clones and, in October Control soils, lowered the species richness (fig. 6.1 and 6.2). The poplar clones did not affect the bulk soil fungal composition in any season, despite the known strong link of fungi and plant roots and their exudates. One possible explanation is that the extracted DNA did not reflect exactly the active fungal population receiving directly the plants inputs: fungal populations can differ between bulk and rhizosphere soils (Gomes et al., 2003) and high amounts of extracellular DNA were found in forest soils (Agnelli et al., 2004). Moreover, the two vegetative periods were similar in the treatment effect, even though the CO_2 enrichment lasted until the end of 2004 growing season. This suggests a slow change in the community structure.

Due to the functional redundancy of soil microorganisms, no relation exists between microbial diversity and microbial decomposition activity (Nannipieri et al., 2003). The immediate respiration response measured with the MicroResp was not supposed to change the community structure during the short incubation period and the results of Pennanen et al. (2004) suggested that within 8 h of incubation neither selective cell growth (favouring fast growing organisms like bacteria) nor activity limited to certain microbial sequence types occurred. Thus, we can assume that the MicroResp assay considered the whole active microbial population. This was directly influenced by the soil nutrients availability and was strictly dependent on plant inputs: the poplar clones significantly affected C utilization rates and the effects of FACE treatment were significant only until CO_2 enrichment was provided (October 2004). We found higher utilization rates of C compounds in soils without N limitation, although the increase of CLPP in fertilized soils was dependent on the clones and the seasons. Under elevated [CO_2], there was a decrease of CLPP in N limited soils whereas in *P.nigra* and *P. x euramericana* fertilized soils the substrate utilization was enhanced (fig. 6.4). Moreover, when N was added without C, microbial activity was, in general, lowered. It was suggested that the poor N supply (Van Veen et al., 1991) or the low availability of both mineral N and organic C (Niklaus and Körner, 1996) limits the microbial utilization of C compounds and therefore the microbial response under elevated [CO_2]. We can confirm a lower utilization of C input under elevated [CO_2] because of a lower N availability, as well to a lower quality of soil organic matter. Other authors, however, reported clear *priming effects* of N or C addition. Graystone et al. (1998) found that C utilization rates were stimulated by additional C flow at elevated [CO_2], mainly in soils with the lowest N application and Mayr et al. (1999) reported medium-term functional changes of the microbiota under elevated [CO_2] even if N availability was low. We can exclude that a *priming effect* of rhizospheric compounds addition did occur in FACE soil because of a microbial community strongly affected by the N competition with plants, that did not allow microbes to benefit from added substrates. In FACE soil the higher C utilization rates were observed only with the combined availability of added rhizospheric C compounds and mineral N, although the response was dependent on species and added substrates. The higher metabolic rates were observed in *P. nigra* with the addition of simple carboxylic acids and N containing compounds, thus confirming the role of substrate quality in determining C utilization.

It is evident that different factors influenced the microbial community at different levels: plant root exudates (assuming they are different among the three tree clones) affected func-

tional properties of the community, while the community composition (as seen with DGGE) remained unaffected. On the other hand, factors such as N or C availability had a strong impact on the community composition and functionality. Fungal community reflected the availability of N in soils, and the effect of elevated [CO_2] on community structure and functionality was evident only in not N-limited soils. The contemporaneous availability of C and N was therefore the main driving force acting on microbial diversity in this plantation.

MICROBIAL DECOMPOSITION OF SOIL C:

MINERALIZATION ACTIVITY AND ITS DETERMINANTS

7.1 Introduction

Soil C long-term storage is influenced by the balance among ecosystems net primary productivity (NPP), the rate of delivery of new organic matter to soil pools and the decomposition of soil organic matter (SOM) (Cardon et al., 2001). The driving force behind plant decomposition and organic matter formation is the microbial utilization of above and belowground residues for the production of metabolic energy, growth, biomass formation, along with some maintenance energy requirements (Smith et al., 1993). During decomposition, complex compounds are broken down to simpler forms that can be utilized by the microflora. Much of the material never breaks down completely, but remains in the soil as metabolic by-products and intermediate compounds that chemically combine to produce more resistant organic matter. The stability of soil organic C with respect to microbial respiration is determined by inherent recalcitrance of organic compounds, interaction with stabilizing substances and accessibility to microorganisms (Sollins et al., 1996). Energy supply is often viewed as the major factor limiting soil microbial activity (Richards, 1987; Tate III, 1995), however other factors such as substrate quality (C/N ratio, lignin/N ratio, presence of microbial-resistant plant material etc.), nitrogen availability, physical environment and physical protection by clays have been invoked as additional controls over microbial processing of soil organic matter (Swift et al., 1979; Richards, 1987; Tate III, 1995; Mary et al., 1996; Ryan and Law, 2005).

Under elevated [CO_2], many authors hypothesized that only with an adequate mineral nutrient supply the increased availability of rhizodeposits to soil microorganisms may reduce microbial dependence on older SOM as a carbon source (Kuikman et al., 1990; Allen et al., 2000; Cardon et al., 2001).

Insight can be gained from laboratory studies that measure microbial respiration in root-free soil under conditions conductive to microbial activity, as field capacity and not limiting temperatures. Such an approach can be useful for understanding changes in substrate availability under elevated [CO_2] that influence microbial respiration, and thus the loss of C from soil (Zak et al., 2000). Our measurements of microbial respiration and decomposition activity were made in laboratory-controlled conditions and therefore can be considered as a measure of potential microbial activity.

Moreover, the use of different parameters obtained from the microbial respiration measurements can be useful to integrate different information on the same process. The mathe-

matical description of the dynamics of C mineralization in incubation studies is of great interest for the prediction of the ability of soils in supplying potentially mineralizable organic C and, more generally, for the organic matter balance (Riffaldi et al., 1996). The microbially mediated decomposition and transformation reactions can be described by kinetic equations. A first-order model has been frequently used to describe the C-mineralization process of soil organic matter (Jones, 1984; Murwira et al., 1990). The equation assumes that the microbial biomass is constant and at a maximum throughout the process. Therefore, the rate of decomposition is dependent on the available substrate only (Smith et al., 1993).

Microbial SOM decomposition is linked to extracellular enzyme activity, as shown in several studies (Carreiro et al., 2000; Saiya-Cork et al., 2002; Michel and Matzner 2003; Gallo et al., 2004; DeForest et al., 2004; Sinsabaugh et al., 2005).

The extracellular enzymes involved in the degradation of soil organic matter are of particular interest for the organic matter decomposition and the consequent CO_2 emission from soil. The β-glucosidase activity is involved in the enzymatic degradation of cellulose and completes the hydrolysis process by catalysing the cleavage of cellobiose to release two moles of glucose per mole of cellobiose therefore regulating the supply of an important energy source for microorganisms (Turner et al., 2002). Moreover, this enzyme has a central role in soil organic matter cycling and provides an early indication of changes in organic matter status and turnover (Bandick and Dick, 1999; Debosz et al., 1999; Monreal and Bergstrom, 2000).

Aims of this work were:

i) to determine if the microbial potential C mineralization and decomposition activity was influenced by the treatments, therefore inducing changes in the loss of C from soil and

ii) to highlight some possible determinants of microbial activity.

7.2 Results

Short term and basal respiration

The microbial respiration after 24 hours of incubation and the basal respiration in laboratory-controlled conditions are shown in fig. 7.1 and 7.2 respectively. This was to emphasize the known difference between the flush of CO_2 following rewetting of soil and the basal respiration activity (Wang et al., 2003).

CO_2 production after 24 hours (MR_{24h}) was not modified by FACE treatment while the fertilization caused a significant increase of the initial flush in both FACE and Control plots, with the maximum peak in June 2003 (fig. 7.1 and tab. 7.1).

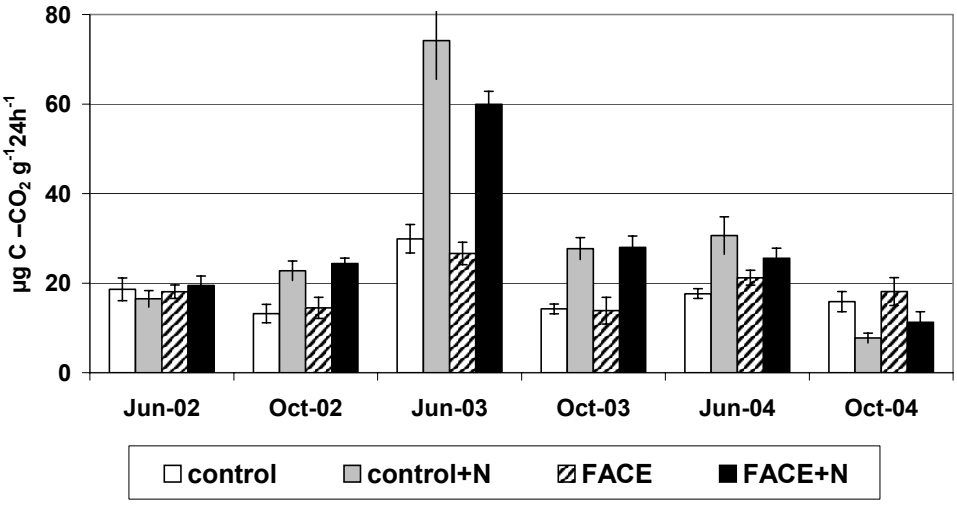

Figure 7.1. Microbial respiration after 24 hours measured in Control and FACE not fertilized and fertilized soils in the year 2002-2004. Values from the three clones where pooled together since no significant differences were found.

The CO_2 evolved during the 10^{th} day of incubation was used as basal respiration because, after that period, the soil reached a relatively constant hourly CO_2 production rate. The basal respiration did not show a clear temporal trend (fig. 7.2). The FACE treatment did not influence this parameter, neither in interaction with the N fertilization. The N addition decreased significantly the basal respiration, in particular in June 2003. A negative relationship between the initial flush and the basal respiration was observed (tab. 7.3).

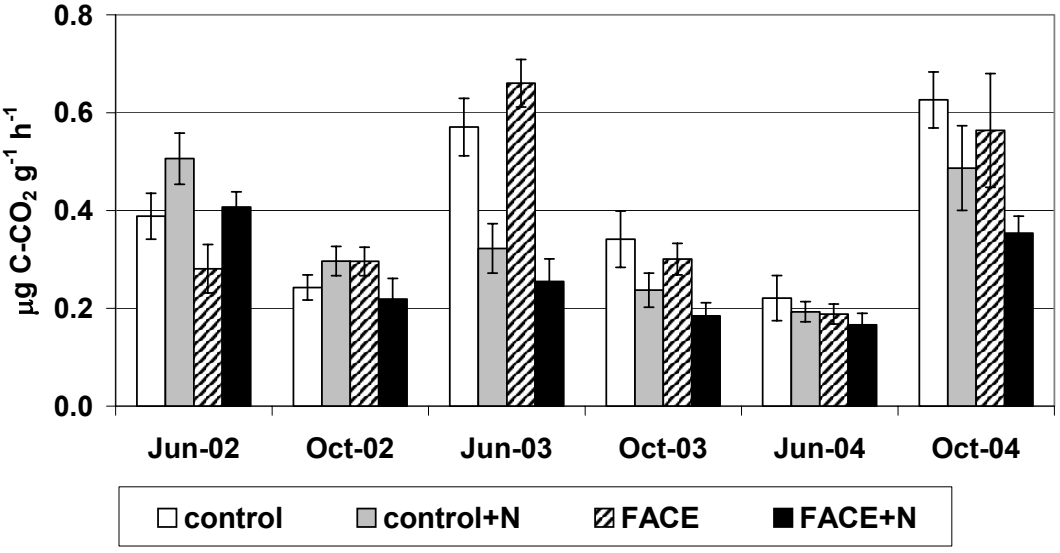

Figure 7.2. Microbial basal respiration after 10 days of measurements in Control and FACE not fertilized and fertilized soils in the year 2002-2004. Values from the three clones where pooled together since no significant differences were found.

β-glucosidase

β-glucosidase, which is responsible for the hydrolisis of cellobiose, showed a large seasonal fluctuation with the lowest values in June in not fertilized soils and in October 2003 in not fertilized and fertilized soils, and the highest in 2004 (fig. 7.3).

The CO_2 enrichment induced a significant increase of this enzymatic activity in both fertilized and not fertilized sub-plots. The interaction between [CO_2] enrichment and the poplar clones was significant (tab. 7.1): the hybrid poplar presented the greater positive effect (p= 0.021, Bonferroni post hoc test), while *P. nigra* showed a not significant decrease. The N fertilization induced a significant increase of β-glucosidase in October 2002 in Control plots and in June 2003 in FACE and Control plots (fig. 7.3, tab. 7.1). Afterwards this effect was no more detected.

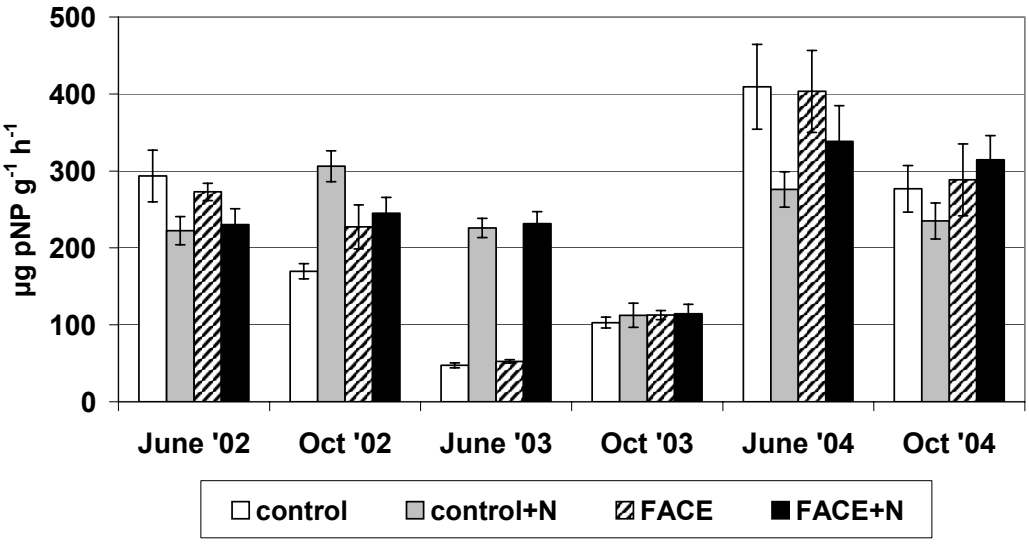

Figure 7.3. β-glucosidase activity in Control and FACE not fertilized and fertilized soils as average of *P. alba*, *P. nigra* and *P. x euramericana*.

	MR_{24h}	MR_{basal}	β-glucosidase		
	three spp.	three spp.	*P. alba*	*P. nigra*	*P. x euram.*
FACE	-4%	-10%	+13%	-12%	+30%
Nitrogen	+68%	-32%	+62%	+81%	+45%
	Analysis of Variance				
time	0.000	0.000	0.000		
CO_2	n.s.	n.s.	n.s.		
nitrogen	0.000	0.000	0.014		
species	n.s.	n.s.	n.s.		
nitrogen x time	0.000	0.000	0.000		
CO_2 x species	n.s.	n.s.	0.019		

Table 7.1. Mean percentage effect of treatments on microbial respiration in the first 24 hours (MR_{24h}), basal respiration (MR_{basal}) and β-glucosidase activity in the years 2002-2004. The significance of treatments, time and their interactions are reported. The not significant interactions were excluded from the analysis. The p values < 0.05 are reported.

C mineralization kinetics

In 2004 the time-course of organic C mineralization in the soil was analysed by fitting the experimental values with the first order equation $C_m = C_0 \times (1-e^{-kt})$ (Murwira et al., 1990; see sect. 2). The model provided a good description of the C mineralization kinetic and R^2 values ranged from 0.8783 to 0.9267 for the two sampling dates. As shown in fig. 7.4, cumulative mineralized C presented a curvilinear relationship with time over the 28-day incubation period in June and October 2004. All soils showed a similar pattern, with a larger initial release of CO_2 (highlighted in fig. 7.1) followed by a slower linear increase throughout the remaining period of incubation.

Regardless the treatments, values of microbial respiration were more than doubled in October with respect to June. Conversely, the effect of fertilization treatment acted clearly in an opposite way between the two seasons, with an increase in June and a decrease in October.

Kinetic parameters calculated according to the first order equation are shown in tab. 7.2. The sampling date was the factor of variation that influenced all parameters.

Potentially mineralizable C (C_0) values were almost three fold higher in October with respect to June. The FACE treatment did not influence this parameter either in June or in October and no interaction with fertilization treatment was found. The N addition caused a 25% increase in June and a 30% reduction in October on average, and the interaction between fertilization and time was significant (tab. 7.2).

C mineralization rate constant (k) was significantly lower in October with respect to June, but no effect of treatments was detected in the two seasons or in interaction with time (tab. 7.2).

The initial potential rate of C mineralization (C_0k) increased significantly in October with respect to June only in not fertilized soils (tab. 7.2). The FACE treatment decreased significantly this parameter by 14% on average. The fertilizer addition induced a significant decrease of this parameter only in October. C_0k was significantly related to the soil inorganic N content in June, but not in October (Fig. 7.5).

The mineralized C (C_m) followed the trend of C_0 with a significant increase in October (tab. 7.2). The FACE treatment did not influence significantly this parameter. The fertilization treatment induced a 18% increase in June and a 29% decrease in October on average.

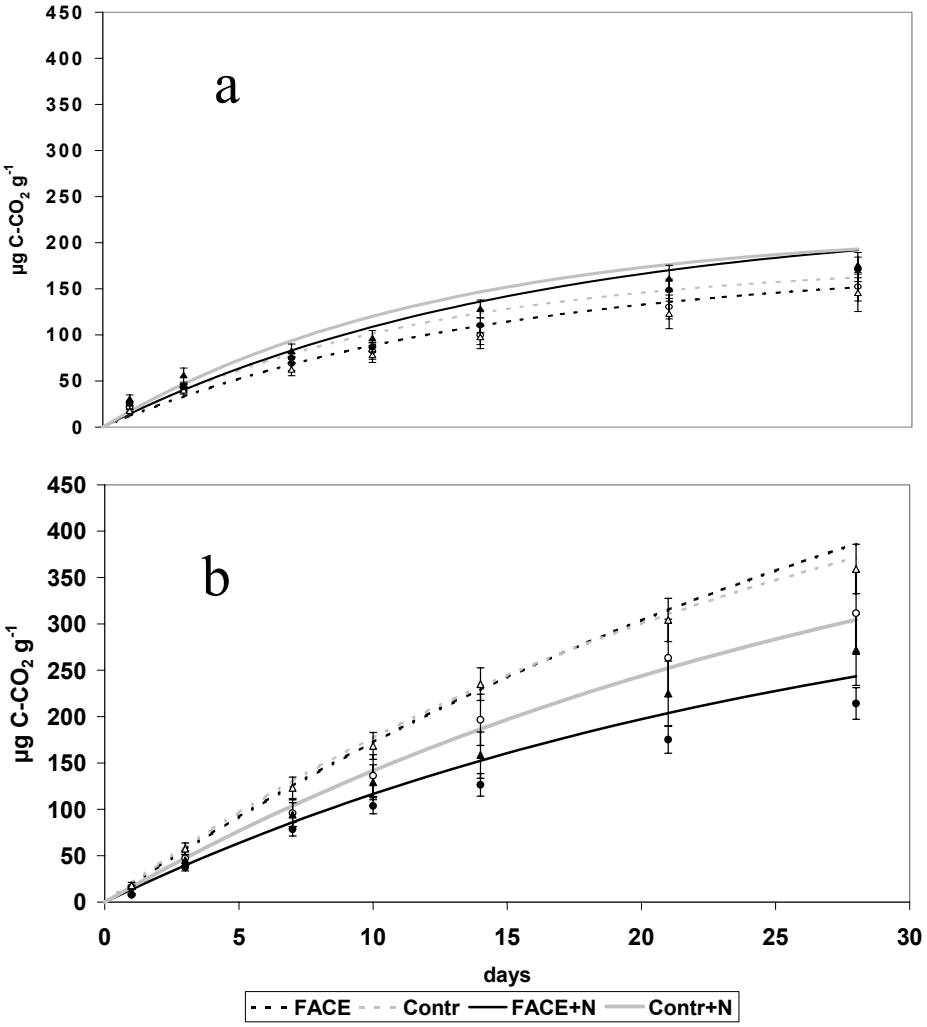

Figure 7.4. Cumulative microbial respiration in 28 days calculated from the first order equation [$C_m = C_0$ x $(1-e^{-kt})$] in June (a) and October (b) 2004. Mean values of experimental data are reported with standard error bars. Circle and triangle for FACE and Control soils, full and open for fertilized and not fertilized.

	C_0	k	C_0k	C_m	C_m/C_0
	µg C-CO_2 g^{-1}	day^{-1}	µg C-CO_2 g^{-1} day^{-1}	µg C-CO_2 g^{-1}	
June 2004					
Control - n	180 *(31)*	0.083 *(0.015)*	11.5 *(0.9)*	142 *(20)*	84.0 *(4.1)*
FACE - n	177 *(18)*	0.069 *(0.006)*	12.0 *(1.2)*	148 *(15)*	83.7 *(3.1)*
Control - N	215 *(25)*	0.082 *(0.009)*	15.9 *(1.6)*	174 *(14)*	85.5 *(5.9)*
FACE - N	230 *(35)*	0.064 *(0.008)*	12.6 *(0.7)*	168 *(14)*	79.2 *(5.3)*
October 2004					
Control - n	581 *(39)*	0.036 *(0.003)*	20.8 *(1.9)*	362 *(27)*	62.8 *(3.0)*
FACE - n	726 *(136)*	0.027 *(0.004)*	16.3 *(2.6)*	316 *(46)*	50.5 *(6.0)*
Control - N	507 *(92)*	0.033 *(0.004)*	14.8 *(2.3)*	271 *(40)*	57.8 *(5.0)*
FACE - N	378 *(53)*	0.037 *(0.006)*	12.2 *(1.3)*	212 *(17)*	61.1 *(5.5)*
	Analysis of variance				
time	0.000	0.000	0.014	0.000	0.000
CO_2	n.s.	n.s.	0.041	n.s.	n.s.
nitrogen	n.s.	n.s.	n.s.	n.s.	n.s.
species	n.s.	n.s.	n.s.	n.s.	n.s.
nitrogen x time	0.013	n.s.	0.002	0.002	n.s.

Table 7.2. Parameters estimated according to the first order equation. Values of Control and FACE not fertilized (n) and fertilized (N) soil are reported. Standard errors are in parenthesis. The significance of treatments, time and their interactions are reported. The not significant interactions were excluded from the analysis. The p values < 0.05 are reported.

The ratio between the effectively and the potentially mineralized C (C_m/C_0) can be a measure of the microbial capacity to mineralize the available C. This ratio was significantly lower in October with respect to June. No significant influence of the two treatments was detected. Considering the temporal variation, in October only the 60% of the potentially mineralizable C was respired in 28 days, while in June a greater fraction of C was utilized (fig. 7.6).

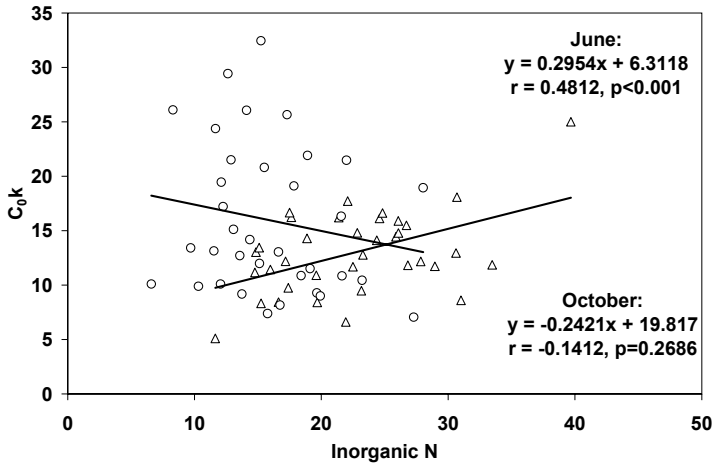

Figure 7.5. Linear regression between the initial potential rate of C mineralization in June (triangles) and October (circles). The significance of the regressions is reported in the figure.

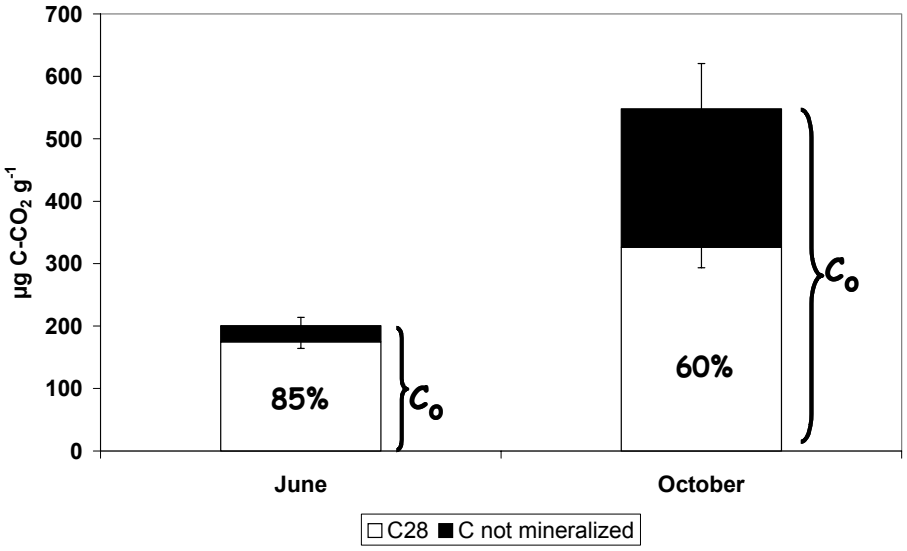

Figure 7.6. Mineralized and not mineralized C as fractions of potentially mineralizable C in June and October, reported as average values from the different treatments.

In tab. 7.3 are reported the Pearson correlation coefficient of examined parameters in relation to the main determinants of microbial respiration as quality and quantity of different C fractions, inorganic N content and metabolic quotient that is an estimates of microbial efficiency in the use of energy.

The significant correlations will be discussed in the following paragraph.

	C_0	k	C_0k	C_m	C_m/C_0	C/N_{mic}	C/N_{K2SO4}	$N\text{-}NH_4$	$N\text{-}NO_3$	Cmic:Corg	WSC	TOC	qCO_2	MR_{24h}
C_0	1													
k	-0.683***	1												
C_0k	0.542***	-0.043	1											
C_m	0.875***	-0.515***	0.837***	1										
C_m/C_0	-0.794***	0.896***	-0.013	-0.52***	1									
C/N_{mic}	-0.421***	0.408***	-0.174	-0.381**	0.491***	1								
C/N_{K2SO4}	-0.409***	0.412***	-0.196	-0.376**	0.435***	0.351**	1							
$N\text{-}NH_4$	-0.569***	0.520***	-0.182	-0.49***	0.6436***	0.668***	0.435***	1						
$N\text{-}NO_3$	-0.109	0.08	0.013	-0.113	0.125	-0.028	-0.041	0.019	1					
Cmic:Corg	-0.115	0.036	-0.167	-0.151	0.098	0.347**	0.143	0.128	0.023	1				
WSC	-0.187	0.095	-0.255*	-0.259*	0.167	0.340**	0.095	0.391***	0.146	0.345**	1			
TOC	0.298*	-0.202	0.360**	0.394**	-0.158	-0.208	-0.08	-0.141	0.046	-0.428***	-0.35**	1		
qCO_2	0.659***	-0.407***	0.4804***	0.660***	-0.515***	-0.46***	-0.341**	-0.43***	-0.201	-0.469***	-0.136	0.165	1	
MR_{24h}	-0.346**	0.368**	0.03	-0.241*	0.442***	0.322**	0.194	0.469***	0.329**	0.151	0.346**	-0.206	-0.286*	1
MR_{basal}	0.832***	-0.477***	0.728***	0.921***	-0.527***	-0.364**	-0.372**	-0.58***	-0.173	-0.141	-0.266*	0.369**	0.723***	-0.37**
β-glucos.	-0.091	0.031	0.047	0.027	0.167	0.189	0.242*	0.267*	-0.08	0.156	-0.019	0.339**	-0.186	0.072

Table 7.3. Matrix of Pearson correlation coefficients for the parameters of the microbial respiration kinetics (C_0, k, C_0k, C_m, C_m/C_0), the respiration in the first 24 hours (MR24h), the basal respiration (MRbasal), the C/N ratio of microbial biomass (C/N_{mic}) and the K_2SO_4 extractable SOM (C/N_{K2SO4}), the ammonium and nitrates content ($N\text{-}NH_4$ and $N\text{-}NO_3$), the ratio between microbial biomass and TOC (Cmic:Corg), the water soluble C (WSC), the metabolic quotient (qCO_2) and the β-glucosidase activity. Values of p<0.001 ***, p<0.01 ** and p<0.05 * are reported.

7.3 Discussion

Determinants of microbial respiration

The microbial respiration derives from the available organic material in soil and it is related to the amount of available carbon. It is also widely known that microbial activity is strictly dependent on the availability of nutrients, N in particular, which can determine the net balance between mineralization and immobilization.

A first observation can be made on the different relationships of the initial, the cumulative and the basal respiration with the organic C availability in soil. The cumulative respiration and the basal respiration (highly correlated each other, tab. 7.3) represent a measure of near steady-state microbial activity (Franzluebbers, 1999) and were significantly correlated to the TOC. The initial flush of respiration measured in the first 24 hours did not show instead this correlation, but was dependent on the most labile fraction of organic matter, the WSC. The first 24 hours represent an initial flush of mineralization attributed to soil sample handling and experimental artefacts such as temperature and moisture in not limiting conditions (Beauchamp et al., 1986). It is presumable that part of the most easily decomposable fraction has been made susceptible to rapid mineralization (Riffaldi et al., 1996). When an equilibrium was reached, this soluble C was preferably immobilized into microbial cells, as shown by the significant correlation between the WSC and the microbial pool, while the TOC was the decomposing substrate, as shown by the significant correlation between the TOC and the basal and cumulative respiration (tab. 7.3). Therefore it seemed that different types of substrates were mineralized throughout the incubation time and that other factors than the amount of labile C affected decomposition rates after the initial CO_2 flush.

In particular a different seasonal pattern in the C mineralization was evident, presumably linked to different substrate quality and to different communities involved in decomposition processes. The seasonal fluctuation of microbial respiration between June and October in not fertilized soils was similar to that of the N concentration in microbial biomass and in labile SOM, with lower values in June with respect to October (sect. 3 and 4). We can therefore argue that changes in substrate quality and nutrient availability occurring in the plantation at the beginning and at the end of the vegetative period influenced the microbial activity. This is also confirmed by the two parameters k and C_0k, that can be used as indicators of the degree of availability or of differences between the mineralized organic com-

pounds (Riffaldi et al., 1996): the significant seasonal variation found on both parameters confirmed the different quality of decomposing substrate (sect. 4). Furthermore, the relatively narrow range of K values between the treatments and the lack of significant effects suggested that microbes metabolised organic compounds, which were similar or had the same degree of degradability (Riffaldi et al., 1996).

C_0k can be a more precise estimate than the individual examined parameters (Stanford and Smith, 1972) and has proved to be the most effective index for identifying the relationship between the decomposition kinetics of different types of residues and their chemical composition (Saviozzi et al., 1993). In our soil this parameter was inversely correlated with the most labile fraction of soil C, whereas a positive correlation was found with the TOC (tab. 7.3). C_0k increase was also related to the specific respiration of microorganisms (qCO_2), therefore suggesting the presence of a less efficient community in the use of available energy.

The respiration process reflects the functionality of microorganisms and their efficiency in utilizing the available substrates. The functional behaviour of different microbial communities may influence C decomposition and storage (Myrold et al., 1989) and, in its turn, the organic matter quality is an important cause of community differences in microbial activity (Vance and Chapin III, 2001). The C_m/C_0 ratio and the C/N ratio of microbial biomass were negatively correlated with the specific microbial respiration (qCO_2) that is an index of microbial efficiency (Wardle & Ghani, 1995; Dilly & Munch, 1998). We found that the maximum efficiency (higher C_m/C_0, lower respired C) occurred whit the highest C/N ratio of microbial biomass and K_2SO_4 extractable C, thus suggesting that more efficient microorganisms (i.e. fungi, Paul and Clark, 1989) were predominant in soils with a lower quality.

$N\text{-}NH_4^+$ availability was the other factor determining microbial respiration rates in the plantation. Decomposition is usually limited by nitrogen availability (Hu et al., 2001) but in our soil the lowest basal and cumulative respiration was found when ammonium availability increased (negative correlation, tab. 7.3). We can postulate two hypotheses: i) "ammonium metabolite repression" that was observed as the inhibition of ligninolytic enzyme production (Keyser et al., 1978). Moreover, easily decomposable material such as polysaccharides may become unavailable to the microbial community when ammonium increases, thus resulting in decreased total activity (Thirukkumaran and Parkinson, 2000); ii) product inhibition by $N\text{-}NH_4^+$ availability on microbial mineralization and respiration activity: when ammonium soil content decreased, microbes were forced to degrade the stable or-

ganic matter (that in POPFACE soil has a low C/N ratio, about 7, see sect. 4), therefore increasing the CO_2 production, while when microbes could utilize the available N-NH_4^+, the mineralization was suppressed.

Elevated [CO_2] treatment

Microbial respiration at not limiting temperature and moisture conditions was not affected by elevated [CO_2]. Furthermore, the initial potential rate (C_0k) decreased under elevated [CO_2] suggesting a slower microbial community selection. Several studies reported a retarded decomposition of elevated [CO_2]-derived root material (Gorissen et al., 1995; Jongern et al., 1995; Van Ginkel et al., 1996; Van Ginkel et al., 2000). Moscatelli et al. (in preparation) in a work on the same site, confirmed that FACE treatment seemed to select a more "recalcitrant" microflora characterized by a slower metabolism and that the increase in microbial biomass C could be more the likely effect of a cell enlargement than a real proliferation process promoting therefore a dormant state in the microbial pool. Generally in soils there is an active population readily consuming substrates, and a sustaining population not utilizing available substrates, except for maintenance energy production (Smith et al., 1986). The lower quality of litter (Cotrufo et al., 2005) and of labile SOM (sect. 5) found in FACE soils may favour the increase of a sustaining population, or a modification of microbial biomass composition towards different functional groups. The relative importance of C and N limitation may differ among microbial functional groups (bacteria and fungi, Paul and Clarke, 1989) or across time scales (Norton and Firestone, 1996). All fungal biomass indices increased under elevated [CO_2] (sect. 3 and 4), and it is known that fungi have better access to recalcitrant N components of soil organic matter (Salamanca et al., 2002). Fungi are also more efficient in substrates decomposition and often present a slower activity in early stage of incubation (Paul and Clarke, 1999; Henriksen and Breland, 1999; tab. 7.2 and 7.3). The C surplus found in FACE soils (sect. 4) was therefore probably immobilized and used for microbial cell enlargement, as confirmed by the positive relationship between Cmic:Corg and the WSC, and by the significant increase of the living fraction under elevated [CO_2]. Anyway, we did not find any difference at the end of the incubation in both basal and cumulative respiration suggesting that the treatment did not induce modification on the whole activity.

We suggest that the lack of increase of microbial respiration under elevated [CO_2] might be ascribable to i) a lower quality of labile pools, and ii) a more efficient microbial biomass dominated by fungal populations.

The positive correlation between basal and cumulative respiration and the TOC suggested also that the preferential decomposing substrate was the stable SOM, favoured by the lower C/N ratio of stable SOM with respect to labile fractions. Anyhow, the C lost through SOM decomposition was balanced by the increased input to soil, suggesting a dynamic equilibrium between C input and losses from soil, resulting in a similar TOC contents in FACE and Control soils after 6 years of treatment (sect. 4).

Regarding the β-glucosidase activity, several studies have suggested that C mineralizing enzymes (β-glucosidase, xylanase or cellulases) may not respond to elevated [CO_2] (Mayr et al., 1999; Moorhead and Linkins, 1997), because C flow from roots is rich in easily assimilable C (Graystone et al., 1996). In our experimental site, the response of this enzymatic activity to FACE treatment was dependent on the clone, suggesting different quality of plant-derived material. Cotrufo et al. (2005) in the same site reported different litter quality among the three species, with the highest N concentrations measured in the leaf litter of *P. x euramericana* and the highest lignin concentration in *P. nigra*. These data are highly consistent with β-glucosidase values, and the negative effect of [CO_2] enrichment recorded in *P. nigra* could be due to an effective lower input quality. However, confirming the studies of Eivazi and Tabatabai (1990), Debosz et al. (1999) and Turner et al. (2002), the best predictor of β-glucosidase activity was the TOC (tab. 7.3). The lack of clear effects of elevated [CO_2] on this enzymatic activity can therefore be related to the similar organic C content of FACE and Control soils.

Fertilization treatment

Fertilizer effects on belowground soil microbial processes have often yielded variable results with suppression (Foster et al., 1980; Smolander et al., 1994; Arnebrant et al., 1996; Thirukkumaran and Parkinson, 2000), enhancement (Fog, 1988; Allen and Schlesinger, 2004), or no effects (Flanagan and Van Cleve, 1983). In our work the microbial respiration response to N fertilization was dependent on the season and varied with the different measured respiration parameters.

Regarding the seasonal effect, we observed a positive effect in June and a negative effect in October on the mineralization kinetic curves. Since the fertilization treatment lasted in September, we can argue that the positive effect was present only until the fertilizer was added, while after the end of supply, the effect was a decrease of C mineralization. Inorganic N pools have very fast turnover, being consumed in 1 or few days by microorgan-

isms (Hodge et al., 2000), suggesting a prompt dependence of microbial activity on the N addition, and a very fast utilization with a short-term effect on microbial activity. The regression between inorganic N and C_0k reported in fig. 7.5 in fact showed a positive relationship between the initial potential mineralization rate and inorganic N only with fertilizer supply, while in October other factors of variation influenced the mineralization rates. Moreover, considering both seasons, $N\text{-}NO_3^-$ content was positively related only with the initial flush of mineralization (MR_{24h}), while the $N\text{-}NH_4^+$ pool significantly affected the steady-state respiration parameters.

The response of microbial activity to nitrogen fertilization reached a maximum in June 2003 evident in the initial flush of respiration (MR_{24h}) and in β-glucosidase activity. It is well known that a sudden increase of CO_2 output from soil is generally observed after the addition of easily available organic substrates or of inorganic nitrogen fertilizers to the soil. This phenomenon, the so-called *priming effect*, is due to an increase of microbial activity resulting in an acceleration of soil organic matter mineralization as substrate and energy source (Kuzyakov et al, 2000). The effect found on microbial respiration in the first 24 hours and β-glucosidase was similar to that found on others enzymatic activities in the same period (sect. 3). We can hypothesize that the N addition induced a positive feedback on microbial decomposition activity that determined a greater microbial activity in the short term, mainly ascribable to an increase in nutrient and energy demand. This process was confirmed by the TOC decrease observed at the end of 2003, that was significantly greater in fertilized soils. Nevertheless, both basal and specific (qCO_2, sect. 5) respiration rates were negatively influenced by the fertilizer addition in the three years of treatment.

In conclusion, N fertilization determined short term increases in microbial decomposition activity, probably due to a *priming effect* during the fertilizer application, and longer-term prediction on C storage in fertilized soil might be difficult because of a large temporal variability of microbial responses.

ANNUAL AND INTER-ANNUAL PATTERNS OF SOIL RESPIRATION: MECHANISMS FOR INCREASED SOIL C EMISSIONS UNDER ELEVATED [CO_2]

8.1 Introduction

CO_2 emissions from soils exceed all other terrestrial-atmospheric carbon exchanges with the exception of gross photosynthesis (Raich and Schlesinger, 1992) and almost 10% of the atmospheric CO_2 passes through soils each year, that is more than 10 times the CO_2 released from fossil fuel combustion (Raich and Potter, 1995). The carbon balance of European forests was demonstrated to be determined by the ecosystem respiration (Valentini et al., 2000). Identifying the environmental factors that control soil CO_2 emissions, and their effects on C emission rates, is thus a necessary step in assessing the potential impact of environmental changes on C exchange between the terrestrial biosphere and the atmosphere (Raich and Tufekcioglu, 2000).

Critical factors reported to influence rates of soil respiration include (Rustad et al., 2000) i) temperature, ii) soil moisture, iii) vegetation and substrate quality, iv) net ecosystem productivity, v) the relative allocation of NPP above- and belowground, vi) population and community dynamics of the aboveground vegetation and belowground flora and fauna, vii) land-use and/or disturbance regimes, including fire.

The processes controlling the C exchange between terrestrial ecosystems and the atmosphere can be strongly influenced by the increase of CO_2 concentration in the atmosphere. Soil respiration is a multi-organismal network of oxidation pathways and the root/rhizosphere and heterotrophic components may respond to elevated [CO_2] in contrasting ways (Trueman and Gonzalez-Meler, 2005). Measurements of soil respiration, indeed, have great potential as an indicator of ecosystem metabolism but less as indicator of changes in ecosystem carbon storage (Ryan and Law, 2005). Nevertheless, the flux of CO_2 from soil can presage a change in the belowground flow of C in soil that has the potential to alter substrate availability for microbial metabolism, depending on how plants allocate the additional photosynthates acquired under elevated [CO_2] to the production and maintenance of fine roots and mycorrhizae (Zak et al., 2000).

It is known that plant metabolism or the decomposition of recently produced organic material generates most of the soil respiration (Ryan and Law, 2005). About 35-80% of the C fixed through the photosynthesis is transferred belowground for root production and respiration, mycorrhizae and root exudates (Raich and Nadelhoffer, 1989; Davidson et al., 2002; Giardina et al., 2003; Ryan et al., 2004). Rhizosphere respiration uses photosynthates as substrate, and will therefore depend on both primary production and C partition-

ing in plants, and may be controlled by current photosynthesis with short reaction time (van Hees et al., 2005). Substrate availability, regulated by NPP, will ultimately limit the response of soil CO_2 efflux to altered conditions: photosynthesis supplies C substrate for root and microbial metabolism, and a decrease in substrate supply can decrease soil respiration within days (Hogberg et al., 2001). In addition, the fraction of photosynthesis used belowground can vary with nutritional status, soil moisture, forest age, species and phenology (Ryan and Law, 2005).

Experimental evidences from a wide variety of ecosystems show that rising atmospheric [CO_2] will increase the rates of soil respiration (Janssens and Ceulemans, 2000; Zak et al., 2000; King et al., 2004). Multiyear data from four forest FACE experiments representing six distinct forest communities showed a consistent stimulation of soil respiration by growth under elevated [CO_2], ranging from +12% to +41% increase, persistent for up to 6 years (King et al., 2004). The authors found a significant correlation between the relative CO_2 response of coarse roots and the soil respiration, suggesting that plant size and productivity are primary determinants of soil CO_2 efflux. Janssens et al. (2001) reached the same conclusion and demonstrated that forest productivity overshadows temperature in controlling soil respiration in European forests. The strong link between plant productivity and soil respiration is supposed to be due to both auto- and eterotrophic components of soil respiration (Zak et al., 2003; King et al., 2004).

Aims of this section were to analyze in detail i) annual and inter-annual variations of soil C emissions, ii) the main mechanisms for increased soil respiration under elevated [CO_2] also in interaction with the fertilization treatment, and iii) the impact of the treatments on the temperature response of soil respiration.

8.2 Results

Year 2003 – Mean values of soil respiration

Average values of soil CO_2 effluxes in not vegetative and vegetative period, and in the whole year are reported in fig. 8.1. Regardless the treatments, the increase of soil respiration in the vegetative season was more than threefold higher the respiration rates of the dormant season (fig. 8.1 and tab. 8.1). FACE treatment induced a significant increase of soil CO_2 efflux for the whole year with similar effects in the two periods considered with a 56% effect on average (fig. 8.1 and tab. 8.1). Neither the fertilization treatment nor the poplar clones influenced significantly the soil respiration at any time. No interactions between the two treatments and with the three clones were observed (tab. 8.1).

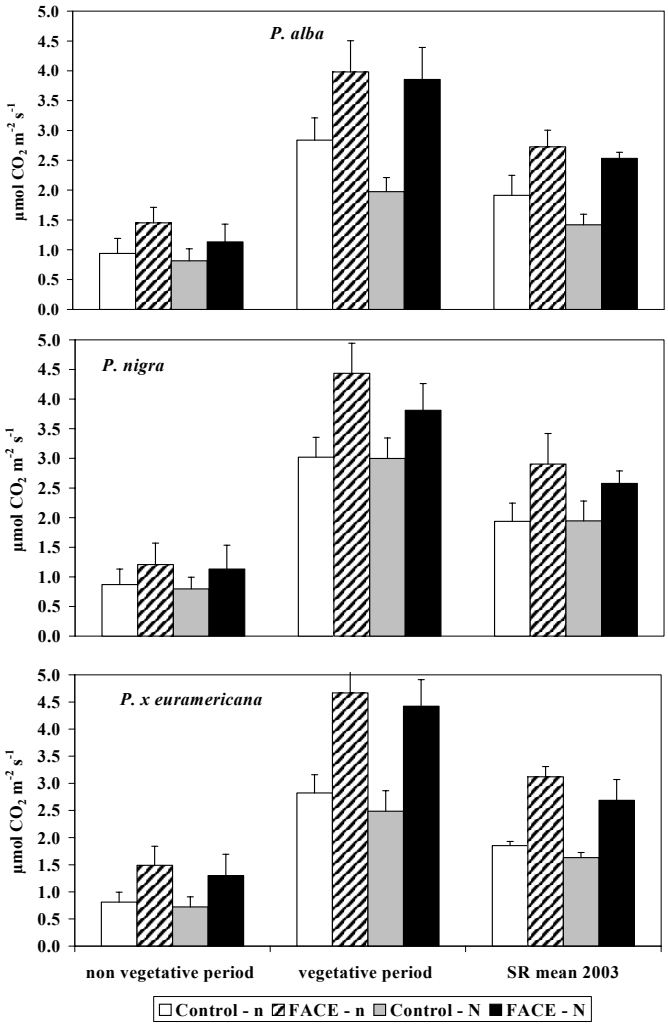

Figure 8.1. Mean values of soil respiration in the non vegetative and vegetative periods and as average of the year 2003, for Control and FACE not fertilized and fertilized soils of *P. alba*, *P. nigra* and *P. x euramericana*.

Year 2003 – Temperature functions

Temperature functions of soil respiration were estimated by a first order exponential equation: SR = $a \cdot e^{kT}$ (see material and methods section).

Values of the 0 °C intercept of soil respiration were significantly lower in *P. nigra*, with respect to the other clones (fig. 8.2 and tab. 8.1). FACE and fertilization treatments acted in opposite ways on *a*, with an increase under elevated [CO_2] and a decrease due to nitrogen addition (fig. 8.2 and tab. 8.1). The interactions between the two treatments were in fact significant and caused a lower FACE-induced increase of the CO_2 efflux at 0 °C in fertilized soils, shifting the +131% increase in not fertilized soils to a +36% increase in fertilized soils.

The slope of the temperature function (k) indicates the response of soil respiration to the temperature increase as also showed by the related Q_{10} values. Both parameters were significantly higher in *P. nigra* with respect to the other two clones. FACE and fertilization treatments did not influence the temperature sensitivity of soil respiration, but their interaction increased significantly both k and Q_{10} and a +10% and a +16% increase respectively was observed in fertilized soils under elevated [CO_2].

At not limiting temperature, the soil CO_2 efflux was similar for the three clones (fig. 8.2 and tab. 8.1). SR_{18} was significantly influenced only by elevated [CO_2] with a +60% increase on average and no interaction with N fertilization was observed (tab. 8.1).

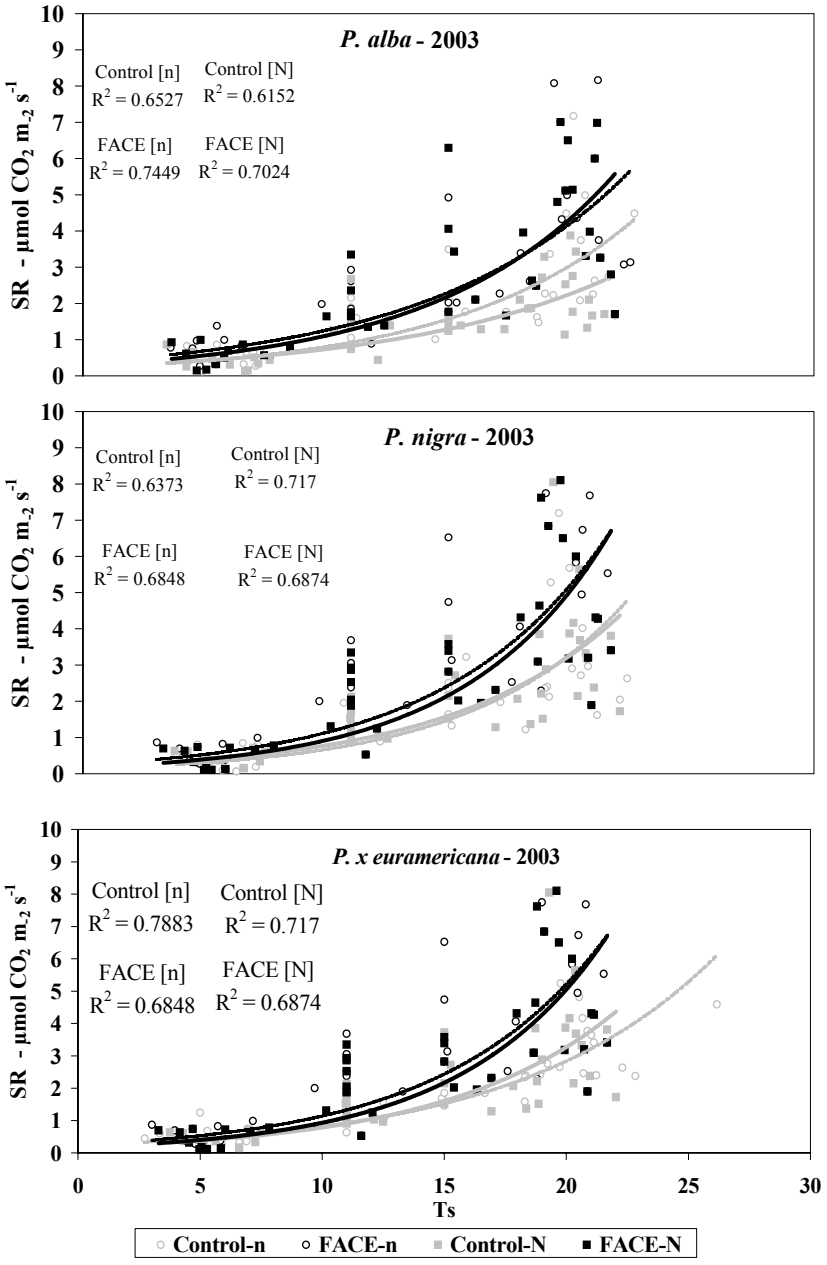

Figure 8.2. Temperature functions of soil respiration for the year 2003 in Control (grey) and FACE (black) not fertilized (dashed lines) and fertilized (solid lines) soils of *P. alba*, *P. nigra* and *P. x euramericana*.

2003	*a*	k	SR_{18}	Q_{10}	SR mean	SR vegetative	SR non vegetative
	µmol CO_2 m^{-2} s^{-1}	*°C^{-1}*	*µmol CO_2 m^{-2} s^{-1}*		*µmol CO_2 m^{-2} s^{-1}*	*µmol CO_2 m^{-2} s^{-1}*	*µmol CO_2 m^{-2} s^{-1}*
Control [n]	0.20 *(0.03)*	0.14 *(0.01)*	2.42 *(0.11)*	4.17 *(0.51)*	1.90 *(0.02)*	2.89 *(0.06)*	0.87 *(0.04)*
FACE [n]	0.47 *(0.09)*	0.12 *(0.01)*	3.74 *(0.12)*	3.39 *(0.44)*	2.92 *(0.11)*	4.36 *(0.20)*	1.38 *(0.09)*
Control [N]	0.20 *(0.03)*	0.14 *(0.01)*	2.21 *(0.23)*	3.96 *(0.38)*	1.66 *(0.15)*	2.49 *(0.30)*	0.78 *(0.03)*
FACE [N]	0.26 *(0.05)*	0.15 *(0.01)*	3.66 *(0.11)*	4.53 *(0.53)*	2.60 *(0.05)*	4.03 *(0.20)*	1.19 *(0.06)*
	Effects %						
FACE	+82%	-4%	+60%	-3%	+55%	+56%	+56%
Nitrogen	-30%	+9%	-5%	+12%	-11%	-10%	-13%
	Analysis of variance						
Nitrogen	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
CO_2	***	n.s.	***	n.s.	***	***	***
species	*	***	n.s.	***	n.s.	n.s.	n.s.
CO_2 * nitrogen	*	*	n.s.	*	n.s.	n.s.	n.s.

Table 8.1. Mean values of *a* (0 °C intercept), k (slope of the temperature function), SR_{18} (soil respiration at 18 °C), Q_{10}, SR mean (mean values of soil respiration in the year 2003), SR vegetative (mean values of soil respiration in the vegetative period) and SR non vegetative (mean values of soil respiration in the non vegetative period). FACE effect is reported as average of not fertilized and fertilized soils. Nitrogen fertilization effect is reported as average of Control and FACE soils. Analysis of variance of FACE and N treatment and of poplar species is also reported. The not significant interactions were removed from the analysis. The values of p are reported: * p<0.05, ** p<0.01, *** p<0.001.

Year 2004 – Mean values of soil respiration

The increase of soil respiration in the vegetative season with respect to the not vegetative period was significantly higher in FACE than in Control soils (3.7 vs. 2.5 times respectively, fig. 8.3 and tab. 8.2). In the dormant season the interaction between FACE and fertilization treatments was significant and the respiration increased significantly under elevated [CO_2] leading to a +48% in fertilized soils (Bonferroni post Hoc test, p=0.005, tab. 8.2). In the vegetative season the FACE treatment induced the highest increase, without interacting with the N addition (fig. 8.3 and tab. 8.2) and similar effects were observed considering the whole year. Either the N fertilization or the poplar clones did not modify any of the considered parameters.

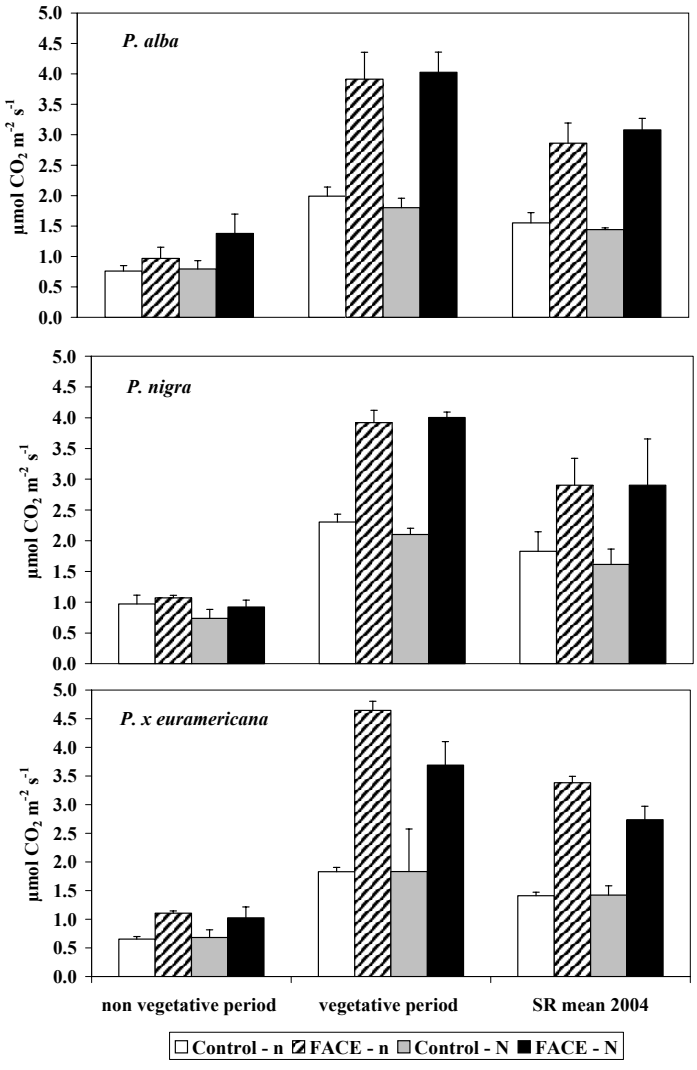

Figure 8.3. Mean values of soil respiration in the non vegetative and vegetative periods and as average of the year 2004, for Control and FACE not fertilized and fertilized soils of *P. alba*, *P. nigra* and *P. x euramericana*.

Year 2004 – Temperature functions

Soil respiration at 0 °C was not affected by any treatment, nor by the poplar clones. Elevated [CO_2] consistently increased the slope of the temperature function of soil respiration and both k and Q_{10} were significantly higher in FACE soils with an increase of 38% and 46% respectively (fig. 8.4 and tab. 8.2). No interaction with fertilization was detected, and neither poplar clones, nor N addition significantly affected the temperature response (tab. 8.2). At 18 °C the effect of elevated [CO_2] on soil respiration was significant and a 95% increase was observed both in fertilized and not fertilized soils (fig. 8.4 and tab. 8.2). The fertilization treatment and the poplar clones did not modify the soil CO_2 efflux at not limiting temperature.

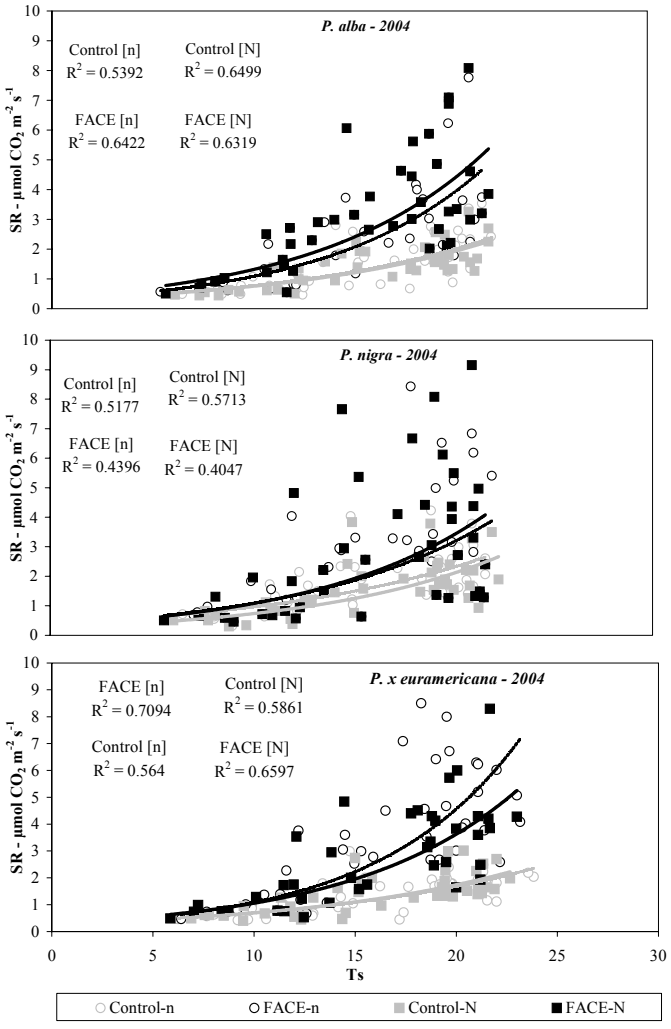

Figure 8.4. Temperature functions of soil respiration for the year 2004 in Control (grey) and FACE (black) not fertilized (dashed lines) and fertilized (solid lines) soils of *P. alba*, *P. nigra* and *P. x euramericana*.

2004	a	k	SR_{18}	Q_{10}	SR mean	SR vegetative	SR non vegetative
	µmol CO_2 m^{-2} s^{-1}	*°C^{-1}*	*µmol CO_2 m^{-2} s^{-1}*		*µmol CO_2 m^{-2} s^{-1}*	*µmol CO_2 m^{-2} s^{-1}*	*µmol CO_2 m^{-2} s^{-1}*
Control [n]	0.32 *(0.03)*	0.09 *(0.00)*	1.70 *(0.17)*	2.54 *(0.03)*	1.60 *(0.12)*	2.04 *(0.14)*	0.79 *(0.09)*
FACE [n]	0.28 *(0.01)*	0.14 *(0.00)*	3.25 *(0.16)*	4.11 *(0.53)*	3.05 *(0.17)*	4.16 *(0.24)*	1.05 *(0.04)*
Control [N]	0.27 *(0.01)*	0.10 *(0.01)*	1.59 *(0.09)*	2.70 *(0.17)*	1.49 *(0.06)*	1.91 *(0.09)*	0.74 *(0.03)*
FACE [N]	0.33 *(0.03)*	0.12 *(0.00)*	3.16 *(0.12)*	3.57 *(0.10)*	2.91 *(0.1)*	3.91 *(0.11)*	1.11 *(0.14)*
	Effects %						
FACE	+2%	+38%	+95%	+46%	+93%	+104%	+41%
Nitrogen	+1%	-4%	-4%	-6%	-5%	-6%	0
	Analysis of variance						
Nitrogen	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
CO_2	n.s.	***	***	***	***	***	***
species	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
CO_2 * nitrogen	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*

Table 8.2. Mean values of a (0 °C intercept), k (slope of the temperature function), SR_{18} (soil respiration at 18 °C), Q_{10}, SR mean (mean values of soil respiration in the year 2004), SR vegetative (mean values of soil respiration in the vegetative period) and SR non vegetative (mean values of soil respiration in the non vegetative period). FACE effect is reported as average of not fertilized and fertilized soils. Nitrogen fertilization effect is reported as average of Control and FACE soils. Analysis of variance of FACE and N treatment and of poplar species is also reported. The not significant interactions were removed from the analysis. The values of p are reported: * p<0.05, ** p<0.01, *** p<0.001.

Year 2005 – Mean values of soil respiration

In this year, the CO_2 fumigation was interrupted, so the results on FACE plots are consequence of the previous years fumigation. Regardless the treatments, the mean values of soil respiration were almost threefold higher in the vegetative season with respect to the not vegetative season (fig. 8.5). In the plots previously exposed to elevated [CO_2] a significant increase of soil respiration was observed, greater in the not vegetative season (fig. 8.5 and tab. 8.3). In the vegetative season higher values of soil respiration for *P. x euramericana* were observed, and the difference between poplar clones was significant also considering the mean values of soil CO_2 efflux in 2005. The N addition did not modify the respiration rates and no significant interaction between treatments was found.

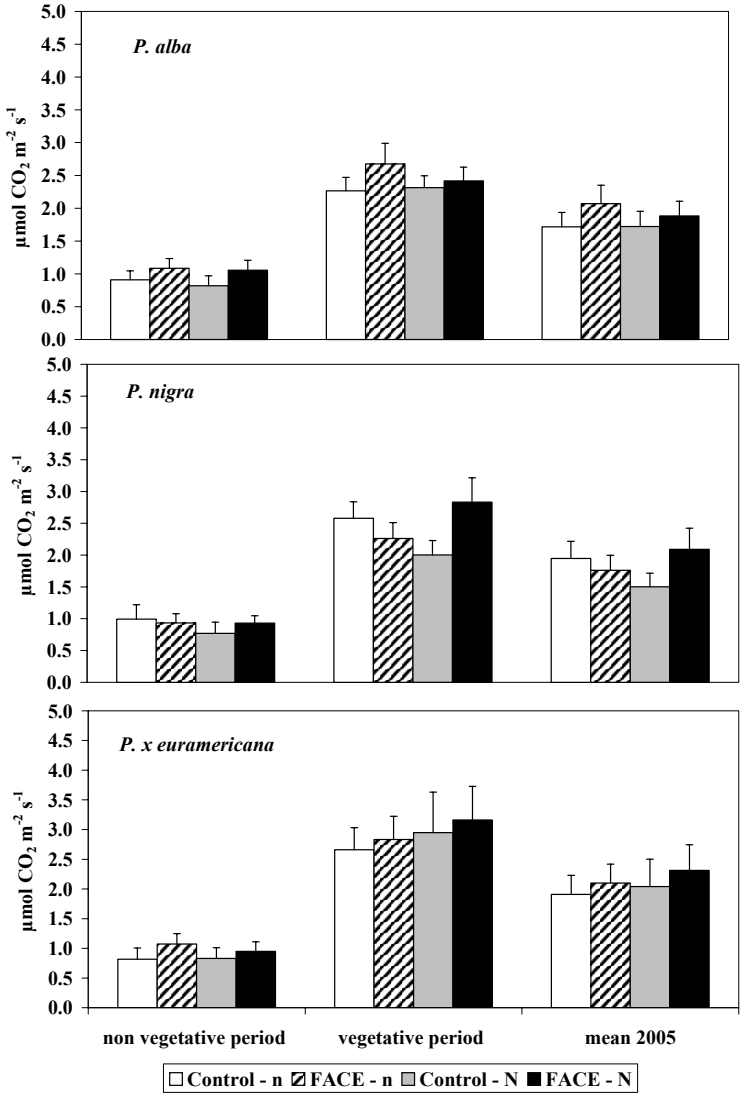

Figure 8.5. Mean values of soil respiration in the non vegetative and vegetative periods and as average of the year 2005, for Control and FACE not fertilized and fertilized soils of *P. alba*, *P. nigra* and *P. x euramericana*.

Year 2005 – Temperature functions

The 0 °C intercept (*a*) showed a significant 43% increase due to elevated [CO_2] in both fertilized and not fertilized soils (fig. 8.6 and tab. 8.3). The slope of the temperature function (k) and the Q_{10} were instead both negatively influenced by elevated [CO_2] and presented a -13% significant decrease (tab. 8.3). At 18 °C the respiration rates were higher under elevated [CO_2] but the 15% increase was smaller to that reported for *a* (tab. 8.3). A positive interaction between FACE and fertilization treatments was also found (tab. 8.3) and the increase under elevated [CO_2] was greater (+28% in fertilized soils with respect to +5% in not fertilized soils) and significant only in fertilized soils (Bonferroni post Hoc test, p=0.018). The three clones and the N fertilization did not induce any modification on the temperature function parameters (tab. 8.3).

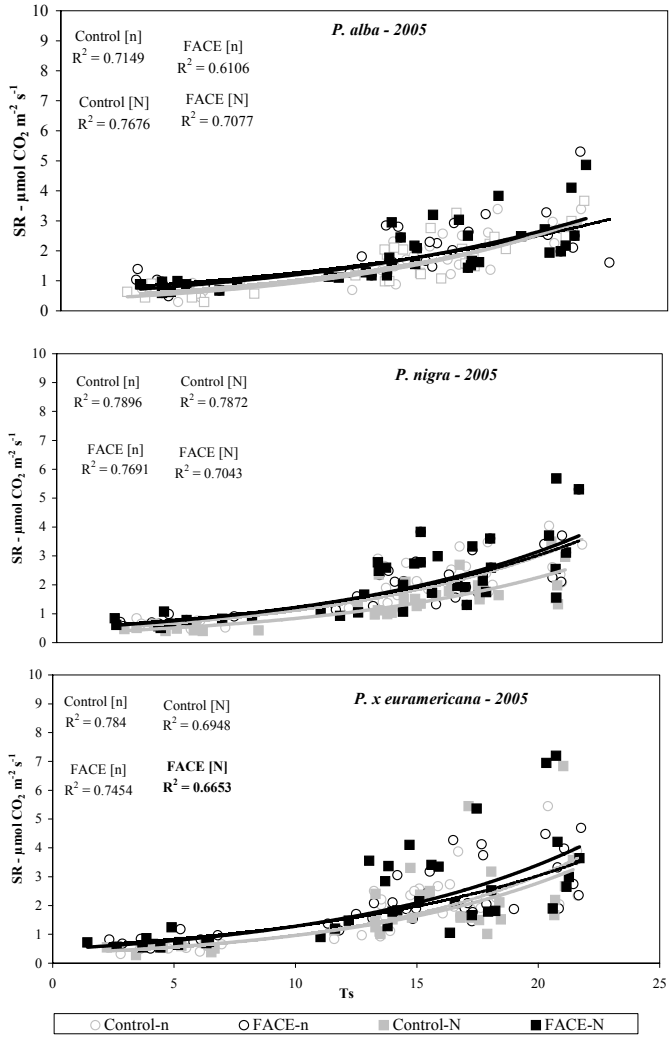

Figure 8.6. Temperature functions of soil respiration for the year 2005 in Control (grey) and FACE (black) not fertilized (dashed lines) and fertilized (solid lines) soils of *P. alba*, *P. nigra* and *P. x euramericana*.

2005	a	k	SR_{18}	Q_{10}	SR mean	SR vegetative	SR non vegetative
	µmol CO_2 m^{-2} s^{-1}	*°C^{-1}*	*µmol CO_2 m^{-2} s^{-1}*		*µmol CO_2 m^{-2} s^{-1}*	*µmol CO_2 m^{-2} s^{-1}*	*µmol CO_2 m^{-2} s^{-1}*
Control [n]	0.40 *(0.05)*	0.10 *(0.01)*	2.35 *(0.10)*	2.78 *(0.20)*	1.79 (0.07)	2.50 *(0.12)*	0.91 *(0.05)*
FACE [n]	0.55 *(0.04)*	0.08 *(0.00)*	2.45 *(0.11)*	2.33 *(0.03)*	1.97 (0.12)	2.59 *(0.17)*	1.03 *(0.05)*
Control [N]	0.34 *(0.02)*	0.10 *(0.00)*	2.05*(0.10)*	2.81 *(0.05)*	1.68 (0.19)	2.42 *(0.28)*	0.81 *(0.02)*
FACE [N]	0.51 *(0.03)*	0.09 *(0.01)*	2.63 *(0.14)*	2.51 *(0.16)*	2.10 (0.11)	2.80 *(0.22)*	0.98 *(0.04)*
	Effects %						
FACE	+43%	-13%	+15%	-13%	+13%	+10%	+17%
Nitrogen	-10%	+4%	-3%	+4%	0	+3%	-8%
	Analysis of variance						
Nitrogen	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
CO_2	***	*	*	*	**	*	**
species	n.s.	n.s.	n.s.	n.s.	*	0.06	n.s.
CO_2 * nitrogen	n.s.	n.s.	0.07	n.s.	n.s.	n.s.	n.s.

Table 8.3. Mean values of a (0 °C intercept), k (slope of the temperature function), SR_{18} (soil respiration at 18 °C), Q_{10}, SR mean (mean values of soil respiration in the year 2005), SR vegetative (mean values of soil respiration in the vegetative period) and SR non vegetative (mean values of soil respiration in the non vegetative period). FACE effect is reported as average of not fertilized and fertilized soils. Nitrogen fertilization effect is reported as average of Control and FACE soils. Analysis of variance of FACE and N treatment and of poplar species is also reported. The not significant interactions were removed from the analysis. The values of p are reported: * p<0.05, ** p<0.01, *** p<0.001.

Inter-annual patterns of soil respiration

To avoid biased interpretation of interannual patterns of soil respiration related to temperature fluctuations, the temperature normalized respiration rates were used. As shown in tab. 8.4, all temperature function parameters were significantly different in the three years of measurement.

Considering only the Control plots, the 0 °C intercept of soil respiration was lowest in the year 2003, but in the same year the temperature response was maximum and the k and Q_{10} reached the highest values of the three years (Bonferroni post Hoc test, $p<0.001$). Years 2003 and 2005 showed instead similar values of respiration rates at 18 °C, while in 2004 the lowest values were observed (1.70 in 2004 with respect to an average of 2.38 in 2003 and 2005, Bonferroni post Hoc test, $p<0.001$). Regarding the effect of FACE treatment on temperature function parameters, it induced different responses depending on the year of measurement (tab. 8.4) both on respiration rates (a and SR18) and on k and Q_{10}. The FACE effect on *a* was maximum in 2003 and minimum in 2004 (+82% and + 2% respectively). The SR_{18} increase due to $[CO_2]$ enrichment reached the highest values in 2004, and in 2003 and 2004, the increase was similar in not fertilized and fertilized soil (fig. 8.7). In 2005 the increase in the plots previously exposed to elevated $[CO_2]$ was significant only in fertilized soils (fig. 8.7). Regarding the effect of FACE treatment on the response of soil respiration to the temperature increase, we observed the highest positive effect in 2004 and a negative effect in 2005 on both k and Q_{10}.

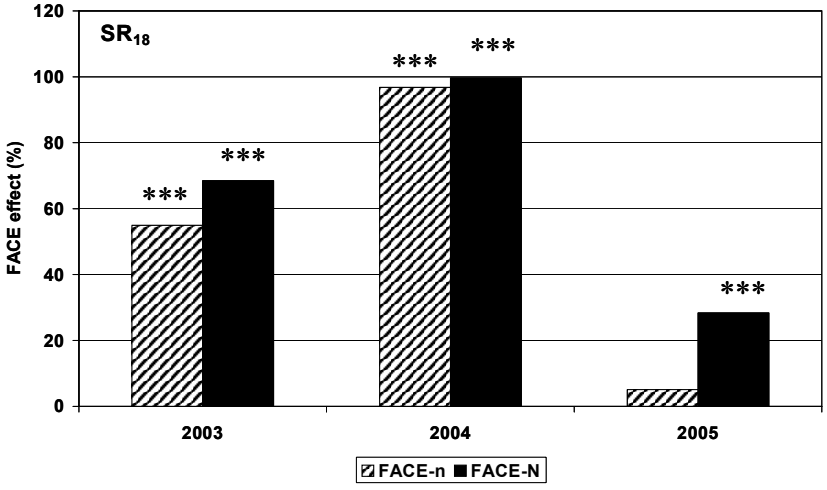

Figure 8.7. Elevated $[CO_2]$ effect of not fertilized (n) and fertilized (N) soils in 2003, 2004 and 2005 as average of the three clones. *** p<0.001.

The N fertilization did not affect soil respiration rates and Q_{10} in the three years of measurement. The poplar clones determined a significant difference in the three years only in the response of soil respiration to the temperature increase (k and Q_{10}), due to the positive temperature response of *P. nigra* in 2003, that was not repeated in the following years.

	a	k	SR_{18}	Q_{10}
	Analysis of Variance			
years	***	***	**	***
years * CO_2	*	***	*	***
years * nitrogen	n.s.	n.s.	n.s.	n.s.
years * species	n.s.	*	n.s.	**
years * CO_2 * nitrogen	*	*	n.s.	*

Table 8.4. Analysis of variance of the temporal variation in the three years of measurement and its interaction with FACE and fertilization treatment and the poplar clones.

By means of the multivariate statistical analysis we considered several possible determinants of soil CO_2 efflux for the years 2003-2004, as reported in fig. 8.8.

Mechanisms determining soil respiration rates were clearly different at 0 or at 18 °C. At low temperatures the soil CO_2 efflux was related mainly to the microbial activity (MR_{basal} and qCO_2), whereas at not limiting temperatures the plant activity (root and litter productivity) and the consequent release of water soluble C fractions were the main controlling factors of soil respiration. The soil microbial biomass and the mineral N content did not seem to be related to the soil respiration, but on the contrary were strictly correlated with the total organic C amount in POPFACE soils. The soil respiration response to the temperature increase (k and Q_{10}) showed a separate pattern, although was correlated with the litter fall and the WSC ($p<0.01$).

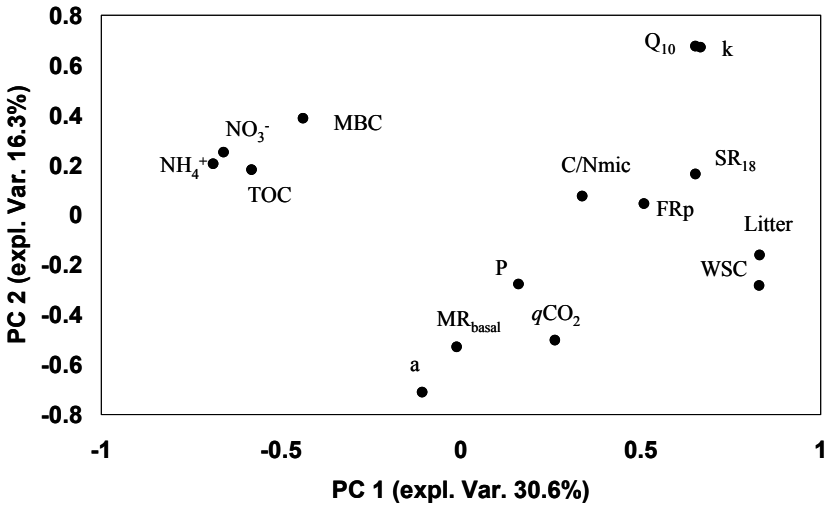

Figure 8.8. Principal component analysis for SR_{18} (temperature normalized respiration rates), a (0 °C intercept of soil respiration), k (slope of the temperature function), Q_{10}, Litter (litter fall production), WSC (water soluble carbon), FRp (fine root productivity), C/Nmic (C/N ratio of microbial biomass), P (extractable phosphorus), qCO_2 (metabolic quotient), MR_{basal} (basal microbial respiration), MBC (microbial biomass carbon), TOC (total organic carbon), NH_4 (soil ammonium content), NO_3 (soil nitrates content). Data referred to the years 2003-2004.

8.3 Discussion

Annual and inter-annual patterns of soil respiration

Several interrelated factors have been suggested to regulate seasonal patterns of soil respiration such as soil moisture, soil temperature, root biomass, and decomposer populations (Vose et al., 1997). Clearly, soil respiration rates were closely tied to the seasonal progression of soil temperature, as shown by the good fitting of the experimental data with the temperature functions (fig. 8.2, 8.4 and 8.6). The nearly threefold increase of soil respiration in the vegetative period was consistent with other studies that reported a four- or fivefold increase from the seasonal low in winter to the seasonal high in summer (Maier and Kress, 2000; Epron et al., 2001; King et al., 2004). Soil moisture was unlikely to be a significant factor of variation in the plantation, because of irrigation of the experimental site, which avoided the drought stress in summer, allowing soil respiration to increase exponentially with soil temperature.

Nevertheless, the increase of soil respiration with temperature might be coupled with other controlling factors. In temperate climate root growth activity may be positively correlated to the soil temperature, that it is believed to exert a dominant control over the autotrophic component of soil respiration (King et al., 2001, 2002). Moreover, root respiration is related to plant photosynthetic activity (Heilmeier et a., 1997) and the effect of temperature on root respiration is likely to be constrained by GPP (Janssens et al., 2001). These last authors concluded that on an annual timescale, and in the absence of drought stress, GPP more than temperatures can explain differences in autotrophic respiration among different sites and that higher rates of respiration in warmer climates can be sustained only by higher productivity and subsequent root activity and litter deposition.

Microbes can utilize a wide range of energy sources, nevertheless, easily available substances released by roots can stimulate microbial growth in the rhizosphere leading to an increased decomposition of soil organic matter (rhizosphere *priming effect*, Kuzyakov, 2002). Plant phenology was the most important variable in controlling the amount of rhizosphere-primed soil C loss in a greenhouse experiment using natural ^{13}C tracers (Cheng et al., 2003). Moreover, microbial metabolism depends also on new litter inputs (Schulze et al., 2000) and thus, indirectly, on site productivity.

Our results confirmed these findings: the temperature normalized respiration rates at 18 °C depended on root and litter productivity (fig. 8.8). The response was both autotrophic and

heterotrophic, since it was mediated by the amount of labile input to soil in the form of water soluble carbon fraction, rapidly metabolized by rhizomicrobial populations (see sect. 9). Moreover, higher microbial C/N ratio (indirect indicator of fungal biomass) was correlated to SR_{18}, confirming the findings of Kutsch et al. (2001). The authors in fact concluded, by field measurements and modelling, that photosynthesis and C partitioning within the plant, and the assimilate fluxes to the roots and further to the rhizosphere and the mycorrhizal fungi during active periods, are the driving forces of the respiration patterns of the rhizomicrobial system. On the other hand, soil respiration at lower temperatures depended mainly on microbial activity (fig. 8.8). This did not seem to be related to either root or litter production or the water soluble C fractions.

Considering the temperature normalized soil respiration rates at 18 °C in Control plots, and the response to the temperature increase, a significant difference between years was observed. Developing forests are supposed to present greater inter-annual variation than more established ones, due to the lower buffering capacity of heterotrophic communities, and an increase of soil respiration through years was expected to happen, as root system progressively colonized the soil volume (King et al., 2004). However, in 2004 the lowest respiration rates of the 2003-2005 period were observed, thus suggesting other controlling mechanisms on yearly fluctuations of soil CO_2 efflux, besides root system development. The amount of easily decomposable labile C seemed to be the key factor even in the inter-annual pattern of soil respiration. In fact, the sharp decrease of WSC observed in 2004 (-110% with respect to the previous year, see sect. 4) could explain, at least in part, the lower respiration rates measured in that year.

Mechanisms for increased soil respiration under elevated [CO_2]

Elevated [CO_2] increased the CO_2 efflux from soil. A positive effect of FACE treatment on sol respiration at 20 °C was already observed in the 1st cycle of the plantation (39% on average in not fertilized soils; King et al., 2004). In the 2nd cycle this effect persisted and even increased to 60% in 2003 and 95% in 2004 considering SR_{18}, indicating a much larger relative stimulation of soil respiration after the coppicing. A greater root biomass and litter fall was observed in the 2nd cycle (Lukac, unpublished data; De Angelis, unpublished data) with respect to the 1st cycle both in Control and FACE plots. At the same time, root biomass and turnover was enhanced by [CO_2] enrichment (Lukac et al., 2003), whereas the litter produced under elevated [CO_2] attained higher residual mass than control litter (Cotrufo et al., 2005). In this conditions a greater quantity of decomposable substrates was ac-

cumulating in FACE soil during years, thus sustaining higher respiration rates. Moreover, both roots productivity and litter fall were found to be significantly correlated with the WSC, which is considered the most immediate and easily degradable C source for micro-organisms (Wang et al., 2003).

Soil CO_2 efflux is the result of two distinct processes (see sect. 10): i) rhizosphere respiration or root-derived CO_2, ii) soil-derived CO_2, or microbial SOM decomposition.

The two processes may be linked through rhizosphere interactions, which may exert a stimulative (*priming effect*) or a suppressive influence on SOM decomposition (Cheng et al., 2003). Kuzyakov (2002) reviewed several environmental or plant-mediated mechanisms for rhizosphere priming effects. Root productivity and photosynthesis intensity are main factors controlling rhizodeposition and belowground C allocation, and therefore the CO_2 efflux from soil (Hogberg et al., 2001; Kuzyakov and Cheng, 2001).

Changes in the mass or activity of roots could directly increase soil CO_2 evolution by increased root respiration and, indirectly, by increased heterotrophic respiration in response to a larger rhizosphere (Vose et al., 1997). About 15% to 25% of belowground allocated C is exuded from the roots into the soil, inducing fast C turnover in the vicinity of the roots (Kuzyakov, 2002). Moreover, several results suggest that the degree of CO_2 enhancement of rhizosphere respiration is much higher than the enhancement of root biomass (Lekkerkerk et al., 1990; Hungate et al., 1997; Cheng and Johnson, 1998). Cheng (1999) explained these results through two mechanisms: i) roots from plants grown in elevated [CO_2] exuded more C and had higher turnover rates; ii) elevated [CO_2] enhanced rhizosphere microbial activities per unit of root growth.

The greater part of SR_{18} enhancement under elevated [CO_2] in the plantation was due to the rhizosphere inducing effect, comprehensive of root and rhizomicrobial respiration. In fact, when the FACE treatment was interrupted in 2005, the derived increase in SR_{18} was fourfold lower than that of the previous year. The lower increase of SR_{18} in FACE soil was presumably because of a lower flux of C fixed through photosynthesis, demonstrating the strict relationship between soil C losses and plant activity. On the other hand, root biomass was presumably still higher in FACE plots, as well as decomposing litter, thus sustaining the residual elevated [CO_2] effect. These processes were expected to be related to the soil nutrient *status*. Several theories hypothesized that in nutrient poor soil a stimulation of microbial activity could enhance SOM decomposition (see sect. 1) and a reduction of rhizosphere *priming effect* by N addition was observed in many studies (Cheng and Coleman, 1990; Liljeroth et al., 1994). However, an opposite effect could be due to the strong

competition between roots and microorganisms for mineral N where not availablethat can reduce the microbial utilization of organics released by roots (Merckx et al., 1985; Van Veen et al., 1989).

In the plantation, FACE effect was similar in not fertilized and fertilized soils when treatment was applied (2003-2004), and N fertilization did not induce any significant modification on soil CO_2 emissions. Our findings agreed to that of Vose et al. (1997), which postulated that the components of the belowground system responded in counteracting directions to N addition, with no measurable difference in soil CO_2 emissions. Nevertheless, in 2005, the "residual effect" of FACE treatment was significant only in fertilized soils, suggesting that in the absence of greater photosynthetic input, the N supply could favor microbial decomposition of plant residues.

Soil respiration-temperature relationship under elevated [CO_2]

The temperature response of soil respiration under elevated [CO_2] was significantly different in the three years of measurement. In 2003 the interactions between FACE and fertilization treatments were significant and the effect of elevated [CO_2] on Q_{10} was positive only in the absence of N limitation. In the following year, however, the positive FACE effect on Q_{10} was significant both in fertilized and not fertilized soils. The increase of Q_{10} under elevated [CO_2] might be induced by the direct effect of enhanced photosynthesis. Stable isotope studies indicated that during the growing season, recently fixed carbohydrates were used for root respiration within few days of C uptake (Ekblad and Hogberg, 2001). The greater effect of FACE treatment in the 2004 growing season seems to reflect this process: the effect of warmer climate and higher photosynthetic rates coupled under FACE in the vegetative period, therefore inducing a positive feedback on Q_{10}. Therefore we can confirm the main role of plant activity in determining soil CO_2 effluxes, enhanced in elevated [CO_2] because of increased activity in the vegetative season.

Nevertheless, these results disagree with those of the 1st cycle reported in King et al. (2004), when no elevated [CO_2] effects on Q_{10} were found. Estimates of Q_{10} derived from annual datasets incorporate seasonal changes in root biomass, litter inputs, microbial populations, and other seasonally fluctuating processes and conditions, and thus reflect community responses (Janssens and Pilegaard, 2003). Different responses through years might be a consequence of processes feedback, that can differ during long term studies and it is reasonable to suppose that plant – soil interactions can be strongly altered after a land use change, as in POPFACE from an agricultural field to a short rotation forest. Moreover, we

can suppose that the greater amount of labile C available under elevated [CO_2] in the 2nd cycle, could better sustain an increase of soil respiration at higher temperatures.

However, the negative FACE "residual effect" on Q_{10} observed in 2005 confirmed the main role of the direct flux of fixed C from plants to soil. The lack of elevated [CO_2] photosynthetic enhancement after the end of FACE treatment did not sustain higher respiration rates during plant growth, and a more pronounced elevated [CO_2] effect on microbial activity in the not vegetative period was found.

It has also to be kept in mind that autotrophic and heterotrophic components of soil respiration can exhibit different Q_{10} values (Buchmann, 2000) and that the competition for nutrients played an important role in the microbial utilization of C (sect. 6). We can also assume that, in 2005 growing season, the greater flux of rhizodeposition was no more supplied by enhanced photosynthetic rates. Moreover, the competition for nutrient is greater during the growing season. Although speculative, we can hypothesize that microbial decomposition could have been favoured in the not vegetative period when nutrient plant uptake was lower, whereas, during plant growth, an inhibition related to plants-microbes competition for nutrients did occur, leading to a decreased Q_{10}.

THE INFLUENCE OF TEMPERATURE AND LABILE C SUBSTRATES ON RHIZO-MICROBIAL RESPIRATION

9.1 Introduction

Soil respiration is the sum of the microbial and root respiration but there is no standard practice to include rhizospheric respiration in eterotrophic or in autotrophic respiration (Hanson et al., 2000). Rhizosphere CO_2 efflux must include not only direct root respiration, but also the CO_2 evolved by microbial utilization of exudates and the CO_2 derived by microbial decomposition of rhizosphere soil organic matter (Kuzyakov, 2002). Exudates and root residues are energy-rich; they enhance the underground C stock and are metabolized by soil microflora. These C sources, which are readily available to microorganisms, contribute to fast C turnover in the soil and to higher microbial activity in the rhizosphere when compared with root-free soil (Kuzyakov, 2002). Nevertheless, many authors hypothesized that only with an adequate mineral nutrient supply the increased availability of rhizodeposits to soil microorganisms under elevated [CO_2] may reduce microbial dependence on older SOM as a carbon source (Kuikman et al., 1990; Cardon et al., 2001).

Soil temperature and soil moisture, besides availability of labile substrates, are the most important factors controlling CO_2 fluxes in the short term (Raich and Schlesinger, 1992; Rustad et al., 2000). Within a range from –5 to 35 °C, the temperature dependence of soil respiration and other mineralisation processes can be described by a simple exponential function and by the parameter Q_{10} (Borken et al., 2002). Accurate prediction of climate effects on C cycles depends on a clear understanding of the effect of temperature on the microbially mediated release of CO_2 from soil organic matter (MacDonald et al., 1995).

Laboratory measurements of soil respiration by the incubation technique are normally used to analyze the microbiological functionality of soils and the effect of temperature on the microbial activity (Fang and Moncrieff, 2001; Wang et al., 2003). The main limitation of this method is related to the manipulation of the soil structure and a common problem is that respiration rate is initially high because of the disturbance by sample preparation and then gradually declines to a low level (Winkler et al., 1996). On the other hand, the understanding of the controlling factors of the actual soil CO_2 effluxes in natural and managed ecosystems is crucial to improve the existent scenarios of our future climate (Kirschbaum, 2000; Valentini et al., 2000). To study the microbial respiration without soil sieving and roots removing, we measured the temperature responses of CO_2 flux of intact soil cores, in laboratory, at steady state. Under these conditions the measured CO_2 flux could be used to investigate the soil biological processes (Pendall et al., 2004), with values that are compa-

rable with the field measurements and under the direct influence of root exudates.

The objectives of this study were: 1) to determine the parameters of the temperature functions of the microbial respiration (bulk soil *plus* rhizosphere microorganisms); 2) to identify some possible determinants of the observed variability of measured CO_2 fluxes; and 3) to determine the influence of the treatments on temperature functions parameter.

9.2 Results

After 24 hours from soil sampling, the root contribution to total respiration of soil cores ranged between 3 and 10% in February and between 2 and 10% in May (respectively Control n and FACE n, tab.1). In the two sampling dates the rates of the roots respiration were similar, and the increase of total respiration in May seemed to be due principally to the microbial component (fig.1). A positive effect of FACE treatment on root respiration was evident in fig.1 (+250% on average, $p<0.001$).

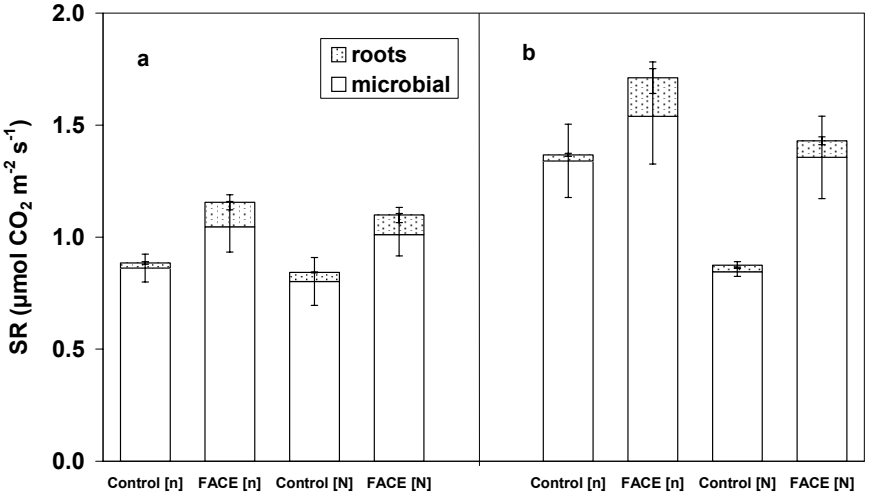

Figure 1. Estimated residual roots respiration and microbial respiration of soil cores after 24 hours from sampling. Temperature of measurement was 18 °C both for February (a) and May (b) sampling dates. [n] not fertilized, [N] fertilized subplots.

	February	May
Control - n	2.6%	1.9%
FACE - n	9.7%	9.6%
Control - N	4.9%	3.3%
FACE - N	7.6%	5.2%

Table 1. Residual root respiration after 24 h expressed as percentage of total respiration of the soil cores. The soil cores were collected in February and in May in Control and FACE plots, not fertilized [n] and fertilized [N]. The temperature of measurement was 18 °C.

Temperature functions of microbial respiration

The 0 °C intercept (α) of microbial CO_2 efflux was not affected by the season: in February and May the respiration rates at low temperature were similar (fig. 9.2 and tab. 9.2). The two treatments (elevated [CO_2] and fertilization) did not cause any significant effect on α, but the interaction between FACE and N treatments was significant (tab. 9.2): the largest effect of FACE treatment (+60%) was observed, in May, in fertilized subplots. The 0 °C intercept resulted positively correlated only with the microbial biomass size (MBC, tab. 9.4).

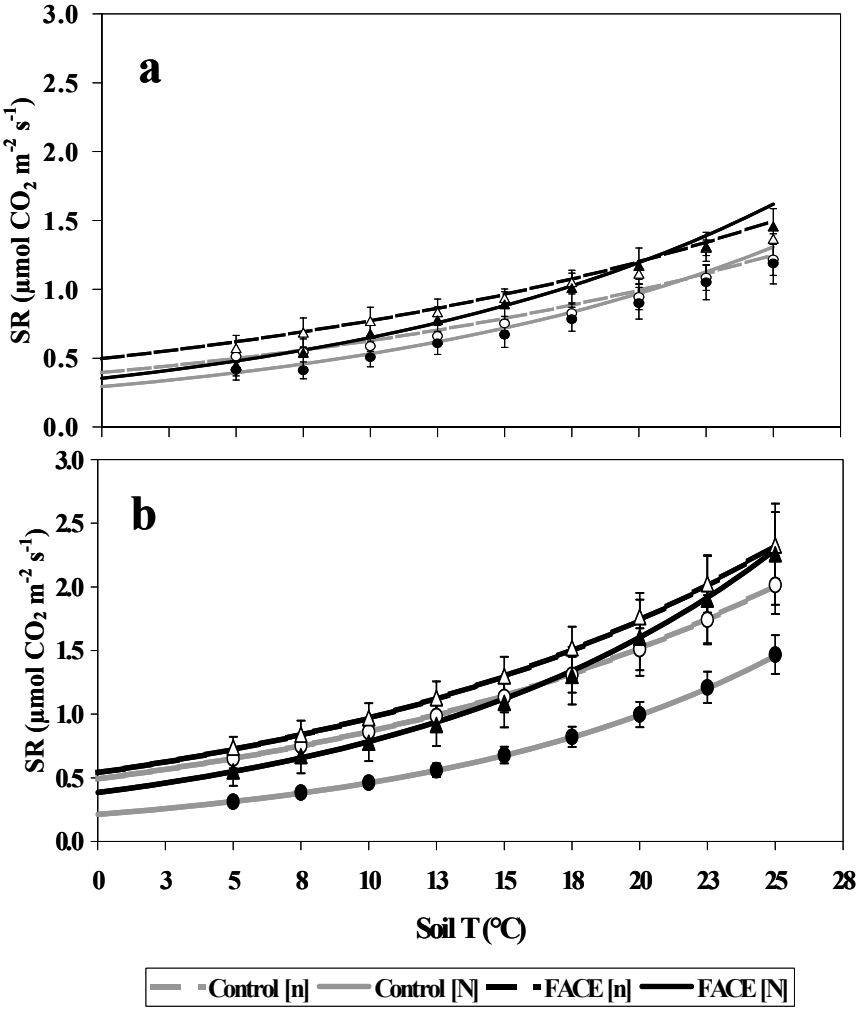

Figure 9.2. Temperature curves of microbial respiration in February (a) e in May (b) calculated from eq. 3. Data for Control [n], FACE [n], Control [N] and FACE [N] are reported. Mean values of experimental data are reported with standard error bars.

The microbial respiration at 18 °C was influenced by the season with a significant increase in May with respect to February (fig. 9.2 and tab. 9.2). The effect of CO_2 enrichment was evident in both sampling dates and, on average, a +30% significant increase was observed (tab. 9.2). The N fertilization did not cause any appreciable effect on microbial respiration at 18 °C. FACE and N treatments interacts significantly with the season. In fact, in fertilized subplots, the CO_2 enrichment induced the largest increase of microbial activity, and this effect was particularly evident in May (tab. 9.2). The CO_2 efflux at not limiting temperature was significantly correlated with the labile C content of the soils (WSC), as well as with the C and N content of the microbial cells (MBC and MBN) and the fine roots biomass (tab. 9.4).

The slope of the temperature function of microbial respiration (k) was significantly affected by the season: in May we observed, on average, a +25% increase with respect to February. This seasonal increase occurred both in FACE and in fertilized soils (tab. 9.2). FACE treatment did not produce any appreciable effect on k. On the contrary, N addition raised significantly the slope of the curve (fig. 9.2 and tab. 9.2). A significant negative correlation was observed between k and the microbial C/N ratio (tab. 9.4).

The slope of the curve and the Q_{10} were strictly correlated and gave similar information (tab. 9.4). A relevant increase of Q_{10} in May was detected, with a +13% on average, without interactions with treatments (tab. 9.2). Elevated [CO_2] did not affect this parameter neither in February nor May. In the fertilized subplots Q_{10} reached the highest values, although the effect of N fertilization was significant only at 0.07 probability value. The Q_{10} was negatively correlated with the C/N ratio of microbial biomass (tab. 9.4).

		α μmol CO_2 m^{-2} s^{-1}	k $°C^{-1}$	SR_{18} μmol CO_2 m^{-2} s^{-1}	Q_{10}
February	**Control - n**	0.40 *(0.04)*	0.05 *(0.01)*	0.86 *(0.06)*	1.60 *(0.10)*
	FACE - n	0.50 *(0.11)*	0.04 *(0.01)*	1.05 *(0.11)*	1.57 *(0.14)*
	Control - N	0.29 *(0.05)*	0.06 *(0.00)*	0.80 *(0.11)*	1.83 *(0.01)*
	FACE - N	0.35 *(0.06)*	0.06 *(0.01)*	1.01 *(0.09)*	1.86 *(0.015)*
Effects (%)					
FACE		+24%	-1%	+24%	0
Nitrogen		-28%	+34%	-5%	+16%
May	**Control - n**	0.49*(0.08)*	0.06 *(0.01)*	1.34 *(0.16)*	1.769 *(0.12)*
	FACE - n	0.54 *(0.09)*	0.06 *(0.00)*	1.54 *(0.21)*	1.796 *(0.04)*
	Control - N	0.23 *(0.01)*	0.08 *(0.00)*	0.85 *(0.02)*	2.162 *(0.08)*
	FACE - N	0.38 *(0.05)*	0.07 *(0.00)*	1.36 *(0.18)*	2.049 *(0.09)*
Effects (%)					
FACE		+31	-2	+32	-2
Nitrogen		-42	+29	-24	+18
Analysis of variance					
nitrogen		ns	0.034*	ns	ns
CO_2		ns	ns	0.005**	ns
season		ns	0.015*	0.001**	0.031*
CO_2 x nitrogen		0.023*	ns	0.003**	ns
season x CO_2 x nitrogen		ns	ns	0.033*	ns

Table 9.2. Values of the temperature functions parameters for February and May, s.e. are in parenthesis. For each date are reported the FACE and Fertilization effects in Control and FACE plots and in not fertilized (n) and fertilized (N) sub-plots. Analysis of variance of FACE and N treatment and of seasonal variability is also reported. The interactions without any significance were removed from the table. The values of p<0.1 are reported: * p<0.05, ** p<0.01, *** p<0.001.

Physical and biochemical parameters

Soil water content was unaffected by the season. The CO_2 enrichment determined a significant increase in the soil moisture, larger in winter. The N addition did not influence this parameter (tab. 9.2).

The roots in the soil cores were always less than 2 mm diameter, and therefore belong to the class of the fine roots. The fine roots biomass was similar in the two sampling dates and no seasonal effect was found. Elevated [CO_2] strongly influenced the fine roots biomass, with a +187% increase on average; the N fertilization did not induce any significant effect (tab. 9.3). Roots were significantly correlated with the water soluble C, the microbial biomass C and with the soil water content (tab. 9.4).

Considering the two forms of labile C, K_2SO_4 extractable C (ExC) was unaffected by the season, while water soluble C (WSC) increased significantly in May (+89% on average). Both fractions were significantly higher in elevated [CO_2] and, on average, we detected a +62% increase for the two C pools (tab. 9.3). No N fertilization effect on soil C pools was observed. WSC was significantly correlated with fine roots biomass and with the respiration rate at 18 °C (tab. 9.3).

Microbial biomass C (MBC) was unaffected either by the season or by the treatments (tab. 9.3). The correlation between MBC and the root biomass was significant (tab. 9.4). The nitrogen content of microbial cells (MBN) increased significantly in May, while no effects due to the treatments were detectable on it (tab. 9.3). As a consequence of the seasonal increase of microbial N, a relevant shift in the C/N ratio of microbial biomass was observed, with a threefold decrease in May (tab. 9.3). MBN was positively correlated with the water soluble C (tab. 9.4).

	WC	Roots	ExC	WSC	MBC	MBN	C/N
	% vol	g d.w. m^{-3}	µg C g^{-1}	µg C g^{-1}	µg C g^{-1}	µg N g^{-1}	µg C/ µg N
February							
Control - n	21 *(1)*	186 *(44)*	78 *(24)*	11 *(3)*	283*(53)*	19 *(2)*	19 *(5)*
FACE - n	24 *(2)*	728 *(135)*	76 *(22)*	21 *(3)*	442 *(80)*	27 *(2)*	19 *(5)*
Control - N	20 *(0.5)*	334 *(18)*	62 *(19)*	13 *(3)*	311 *(117)*	25 *(8)*	15 *(6)*
FACE - N	23 *(3)*	661 *(255)*	109 *(18)*	18 *(3)*	301 *(72)*	28 *(7)*	11 *(2)*
Effects (%)							
FACE	+15%	+167%	+31%	+60%	+25%	+24%	-8%
Nitrogen	-4%	+9%	+10%	-5%	-16%	+15%	-30%
May							
Control - n	20 *(2)*	220 *(56)*	60 *(24)*	24 *(4)*	373 *(35)*	66 *(17)*	6 *(1)*
FACE - n	22 *(3)*	727 *(398)*	86 *(18)*	34 *(7)*	341 *(72)*	68 *(14)*	5 *(1)*
Control - N	20 *(3)*	244 *(122)*	38 *(21)*	24 *(4)*	334 *(13)*	54 *(17)*	8 *(3)*
FACE - N	21 *(2)*	558 *(131)*	105 *(17)*	35 *(5)*	367 *(63)*	56 *(1)*	7 *(1)*
Effects (%)							
FACE	+8%	+176%	+94%	+43%	0	+4%	-13%
Nitrogen	0	-15%	-2%	+1%	-2%	-18%	+36%
Analysis of variance							
Nitrogen	ns	ns	ns	ns	ns	ns	ns
CO_2	0.011*	0.000***	0.025*	0.003**	ns	ns	ns
season	ns	ns	ns	0.000***	ns	0.000***	0.002**

Table 9.3. Values of the physical and biochemical parameters for February and May, s.e. are in parenthesis. For each date are reported the FACE and Fertilization effects in Control and FACE plots and in not fertilized (n) and fertilized (N) sub-plots. WC: soil Water Content; ExC: C extractable in K_2SO_4; WSC: Water Soluble C; MBC: Microbial Biomass C; MBN: Microbial Biomass N; C/N: ratio of C and N of Microbial Biomass. Analysis of variance of FACE and N treatment and of seasonal variability is also reported. The interactions without any significance were removed from the table. The values of p<0.1 are reported: * p<0.05, ** p<0.01, *** p<0.001.

	Q_{10}	k	α	SR18	WC	Roots	ExC	WSC	MBC	MBN
Q_{10}	1									
k	0.966***	1								
α	-0.649**	-0.568**	1							
SR18	ns	ns	0.754***	1						
WC	ns	ns	ns	ns	1					
Roots	ns	ns	ns	0.515*	0.425*	1				
ExC	ns	ns	ns	ns	ns	ns	1			
WSC	ns	ns	ns	0.523*	ns	0.413*	ns	1		
MBC	ns	ns	0.510*	0.524*	ns	0.442*	ns	ns	1	
MBN	ns	ns	ns	0.695***	ns	ns	ns	0.54**	ns	1
C/N	-0.441*	-0.435*	ns	ns	ns	ns	ns	ns	ns	-0.66***

Table 9.4. Correlation matrix of all parameters analyzed. Data collected in winter and spring were pooled together. Pearson correlation coefficient (r) are reported when significant: * p<0.05, ** p<0.01, *** p<0.001. WC: soil Water Content; ExC: C extractable in K_2SO_4; WSC: Water Soluble C; MBC: Microbial Biomass C; MBN: Microbial Biomass N; C/N: ratio of C and N of Microbial Biomass.

9.3 Discussion

Rhizosphere effect

The rhizosphere is the soil zone in which the soil microorganisms are influenced by the plant roots and is recognized as the zone of greatest microbial growth and activity due to the higher concentrations of carbon and other nutrients (Lynch and Whipps, 1990; Graystone and Campbell, 1996). The rhizodepositions have a profound quantitative and qualitative effect on rhizosphere microflora and the amount of available carbon has been assumed to be an important factor controlling microbial growth by several rhizosphere models (Darrah, 1991a,b; El-Shatnawi and Makhadmeh, 2001). With this experimental approach it was demonstrated that, in the rhizosphere complex, the presence of fine roots directly influenced the microbial population size and activity through the exudation of labile C. [CO_2] enrichment in the plantation increased root production (Lukac et al., 2003) and labile C pools (sect. 4). This experiment confirmed these results and established a strict relationship between roots and WSC, both significantly increased by the elevated [CO_2]. The large increment of fine root biomass caused by the FACE treatment, determined a significant increase in the labile C substrates with a positive effect on soil microbial activity. The C supply had also a positive effect on the microbial N, showing that in the presence of a labile C source, available soil N was rapidly immobilized. Microbial demand for available N is, in fact, generally greatest in soils with highest concentrations of simple C compounds (Magill and Aber, 2000; Vance and Chapin III, 2001). The elevated [CO_2] positive effect on soil moisture confirms results from other works (Hungate et al., 1997; Johnson et al., 2002). Moreover a strict relationship between Wc and root biomass was observed. Probably a cause-effect relationship between soil water content and root biomass occurred, but without relevant effects on soil soluble C and soil microbial respiration. A positive effect of nitrogen addition on root biomass is reported in literature (Rasse, 2002). In the plantation we found a not significant increase of roots biomass as a result of N fertilization, more pronounced in winter. Nevertheless this N effect did not seem to be related to a larger C exudation or microbial population size, and thus did not cause higher CO_2 efflux rates.

CO_2 efflux rates

Many authors observed a strong decline of root respiration within a few hours after excision (Chapin e Tryon, 1982; Bloom and Caldwell, 1988; Rakonczay et al., 1997). The CO_2 efflux measurements were carried out after 24 hours from the soil sampling. This elapsed time could cause the negligible contribution of roots to total respiration, sensibly lower

than the contribution reported in sect. 8. Nevertheless the residual root respiration was strongly affected by FACE treatment and the removal of this root residual respiration allowed a more careful approach with respect to the microbial response to elevated [CO_2].

The first order exponential function fit well the experimental data: R^2 values ranged between 0.88 and 0.99 for the two sampling dates and for the two treatments. The exponential regression is widely used in literature with good results and in absence of soil moisture limitation (the site is drip irrigated) it is suitable for our purposes (Raich and Potter, 1995; Boone et al., 1998; Davidson et al., 1998; Buchmann, 2000).

At very low temperature microbial respiration could be considered as maintenance respiration since microbial metabolic activities are reduced to minimum. Soil microbial activity and organic matter decomposition virtually ceases below about 5 °C (Brady and Weil, 1999). In fact the CO_2 efflux at 0 °C (α) was not related to roots contents or substrate availability. On the contrary the microbial population size was the main determinant of the CO_2 efflux rates at a limiting temperature. At 0 °C the microbial metabolism could not benefit actively of the largest pool of labile C available in FACE plots, and therefore the positive effect of elevated [CO_2] was not significant.

Under favourable temperature conditions substrate availability is considered to be the principal determinant to soil respiration, and WSC is the most active and immediate organic substrates for microorganisms (Wardle, 1992; Cheng et al., 1996; Wang et al., 2003). The labile carbon pool size varies widely in response to seasonal changes in representative plant material inputs (Gu et al., 2004). The significant increase of WSC in May well explains the positive response of microbial respiration in this month. Cheng et al. (1996) indicated that microbial respiration was not limited by available carbon when water soluble carbon concentrations were close to or higher than 0.1 mg C g^{-1} of soil. WSC values were sensibly lower with respect to this threshold, and this can explain how CO_2 efflux rates raised quickly in correspondence of an increase of labile C substrates. It is evident that in the very short period of our measurements only the fast decomposable C fractions were metabolized. As a consequence, we did not detect any relationship of the extractable C in K_2SO_4 (ExC) with the microbial respiration. In fact the ExC comprises more complex C fractions than WSC and microorganisms preferred the more easily decomposable C substrates.

The elevated [CO_2] stimulating effect on total soil respiration is widely reported in literature for this and other experimental sites (Janssens and Ceulemans, 2000; Zak et al., 2000; King et al., 2004). However, microbial responses are highly variable and ranged between a

4% decline to a not significant 72% increase beneath woody plants (Zak et al., 2000). Data collected in POPFACE experimental site since 2000 showed that microbial respiration over 10 days of incubation was unaffected by [CO_2] enrichment (sect. 7). The lack of significant results of elevated [CO_2] effect on microbial respiration can be due to soil manipulation and rhizosphere removing, since roots act as mediator for elevated [CO_2] effect on soil. With this experimental approach we were able to observe a direct influence of root-derived substrates on microbial activity, thus highlighting the positive effect of elevated [CO_2] on microbial respiration. In our soils microbial biomass and microbial respiration were strictly correlated, and the increase of labile C substrates raised significantly the microbial activity in FACE plots. Nevertheless in soil a co-limitation of C and N could occur and the mineral nutrient supply may severely limit microbial growth and activities (Allen and Schlesinger, 2004). MBN was not affected by treatments, but was significantly correlated with CO_2 efflux. Melillo et al. (1982) suggested that the microbial decomposition of leaf litter should increase with increasing N availability to microbes. Allen and Schlesinger (2004) found that N addition to forest floor increased respiration rates. Microbial mineralization of available C could reach indeed highest rates if N level into microbial cells is not limitant. The C/N ratio of microbial biomass reached very high values in February and the N limitation is a further possible cause, besides the availability of the labile C, of the lower respiration rates in this month.

The nitrogen treatment did not give significant results, but the CO_2 effect was the largest in fertilized sub-plots: as reported above, the N limitation of microbes could lower the microbial utilization of available C. In the short term of this experiment, at high temperature, the N availability under FACE induced a significant increase in the microbial metabolism and the largest CO_2 efflux rates.

Temperature response

Q_{10} values ranged between 1.6 and 2.2 and were comparable with those reported in literature for microbial respiration measured with roots exclusion experiments (Boone et al., 1998; Epron et al., 2001; Bääth and Wallander, 2003) and on sieved soil (Winkler et al., 1996; Fang et al., 2005). Field measurements on POPFACE soil produced Q_{10} values of 3.6 under elevated [CO_2] (average of fertilized and non fertilized sub-plots) and 1.55 in the Control plots (data not shown). Thus, these *in situ* measurements highlighted a strong effect of FACE treatment on soil sensitivity to temperature and a lack of response to the N fertilization. The difference with microbial Q_{10} is strong and we suppose that the field in-

crease of soil respiration under FACE treatment was due mainly to the roots activity. We can argue that during the vegetative season, when temperatures are higher, photosynthates are transported and metabolized by roots within few hours after initial assimilation. The root metabolism of C assimilated by the photosynthesis is in fact considered as a crucial controlling factor for total CO_2 efflux (Kuzyakov and Cheng, 2001).

In our laboratory experiment, elevated [CO_2] did not induce any relevant effect on Q_{10}. The microbial sensitivity to temperature was thus independent by the largest labile C pool in FACE plots. A debate is occurring in literature on the sensitivity of organic C decomposition to temperature increases (Powlson, 2005). The question concerns the different sensitivity to temperature of labile and resistant soil C to decomposition. This is a very short-term experiment and we detected only the decomposition of labile C fractions. Nevertheless we measured respiration from soils with large differences on fast turnover C pools between FACE and Control plots. The lack of FACE effects on Q_{10} could suggest that the microbial community sensitivity to temperature is not depending on C substrates composition (Fang et al., 2005). On the contrary, the main determinant of the sensitivity of soil microbial respiration to the temperature in our soils was the N addition. N availability allowed a more rapid response of microbial population to the temperature increase, which however was not explainable with a larger microbial population. The growth rate of microorganisms is controlled by the availability of nutrients, besides C, and when nitrogen is available *r*-strategist may grow quickly and consume mostly the fresh organic matter (Fontaine et al., 2003). Allen and Schlesinger (2004) reported also that N addition might also cause N-limited soil microbes to decompose organic matter more quickly. The C/N ratio of microbial biomass is commonly used to assume physiological differences inside microbial populations (i.e. fungal-bacterial ratio, Paul and Clark, 1989) and its correlation with Q_{10} can suggest a different utilization pattern of substrates by different microbial populations. The sharp differentiation of fungal genetic composition, and the lower number of fungal species due to N addition showed in sect. 6, might support the hypothesis of a dominance of microbial communities more rapid in consuming available substrates in fertilized soil.

SEPARATING ROOT CONTRIBUTION TO SOIL

RESPIRATION AND THE ROLE OF ROOTS ON SOIL CO_2

EFFLUX AND C STORAGE

10.1 Introduction

Soil respiration is a combination of the activity of roots (autotrophic respiration), rhizosphere and root free soil (eterotrophic respiration) (Bazin et al., 1990). To evaluate the implications of environmental change on soil carbon cycling and sequestration it is essential to understand the different contribution of each group. Nevertheless, when speaking of root contribution to soil respiration, it is important to define the role of rhizospheric microorganisms. "Root respiration" was defined by Wiant (1967) as "...all respiration derived from organic compounds originating in plants including the respiration of living root tissue, the respiration of symbiotic mycorrhizal fungi and associated microorganisms, and the decomposing organisms operating on root exudates and recent dead root tissues in the rhizosphere". Kuziakov and Cheng (2001) referred to the "root-derived CO_2", comprehensive of root and rhizomicrobial respiration of exudates and dead roots, as a whole that must be separated from the total CO_2 efflux in studies of soil C sequestration, since it is not part of soil C loss.

Three different methods can be used to separate total respiration in the different components (Hanson et al., 2000):

- ✓ component integration (Edwards and Harris, 1977): it involves the separation of the constituent soil components contributing to the CO_2 efflux (i.e. roots, sieved soil, litter) followed by measurement of the specific respiration rates;
- ✓ root exclusion: any procedure that indirectly estimates the root contribution by measuring respiration with and without the presence of roots as 1) roots removal (roots are removed, soil is placed back in reverse order of removal, and further root growth is prevented by barriers, Wiant et al., 1967); 2) trenching (existing roots are severed by trenching but not removed, and a barrier is installed to inhibit future root growth, Ewel et al., 1987); and 3) gap analysis (aboveground vegetation is removed from relatively large areas, Brumme, 1995);
- ✓ isotopic methods (as pulse labelling, repeated pulse labelling and continuous labelling with either radioactive ^{14}C or stable ^{13}C): they allow partitioning of soil respiration between root-derived and soil-derived CO_2.

These last isotopic methods have the advantage over other methods because they limit soil and root disturbance, but present high costs and complex analyses. However, Rochette et

al. (1999) found that the ^{13}C isotopic labelling and root exclusion methods produced similar values for root contribution, and concluded that both approaches were useful.
To separate the root contribution from the total soil respiration we carried out a field experiment by means of two techniques for root exclusion:

1) root removal and
2) root trenching.

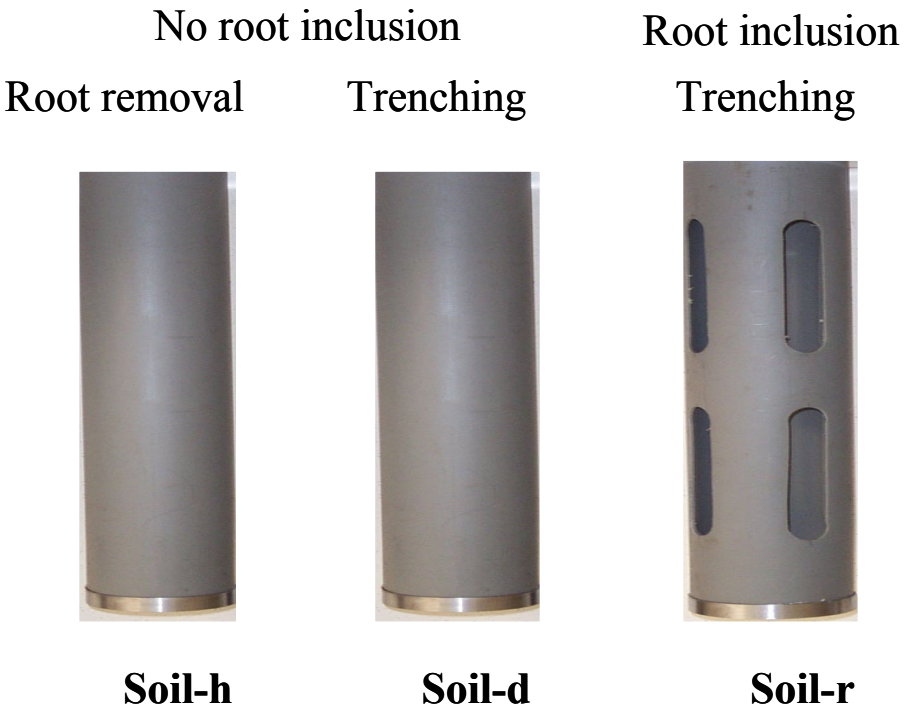

Figure 10.1. The three typologies of pvc tubes installed in each sector

The set up of the experimental design is described in detail in sect. 2. The resuming scheme is shown in fig. 10.1, where:

- ✓ Soil-h: roots removed and new roots inclusion prevented.
  → Measurement of eterotrophic respiration of root free soil (SRh);
- ✓ Soil-d: roots trenched and new roots inclusion prevented.
  → Measurement of eterotrophic respiration (bulk soil *plus* rhizosphere) in presence of dead roots material to decompose (SRd)

✓ Soil-r: roots trenched and new roots inclusion allowed.

→ Measurement of eterotrophic (bulk soil *plus* rhizosphere) plus autotrophic respiration with dead and living roots (SRr).

Starting from the respiration rates of the different soils, the following calculation of the root fraction can be made:

Root fraction = (SRr – SRd)/ SRr

The estimation of the relative contribution of the different components was carried out under future conditions of atmospheric CO_2 concentration and at two different level of fertilization.

At the end of the 18 months of CO_2 efflux measurements the Soil-r and Soil-h were used for further investigations about chemical and biochemical properties. The Total Organic C and some labile C fractions (ExC, WSC, MBC and labile C) were analysed in FACE and Control soils of *P. nigra* and *P. x euramericana*. The Community Level Physiological Profile of these soils was also determined.

Aims of this experiment were:

1) to separate the components of respiration and in particular to determine the root contribution to total soil CO_2 efflux;
2) to study the soil microbial activity without roots input during a long term field incubation;
3) to assess the role of roots on the soil CO_2 emissions and C storage, through the analysis of microbial functionality and activity with and without roots;
4) to estimate the role played by roots activity in determining the sensitivity of soil respiration to temperature.

10.2 Results

In the following sections we will refer to the soil of the different field experiments as “soil type”.

Root fraction contribution to soil respiration

In the vegetative season of year 2003, the Soil-r respiration was significantly different from the Soil-d respiration and reached twofold higher values (fig. 10.2 and tab. 10.1). The three species and the two treatments did not induce different respiration rates in both soil type. In the same period of the year 2004, values were on average lower for both soil types in comparison with 2003 (fig. 10.2).

The difference between the two soil types was significant and similar in amplitude to that of the previous year, but the variability of SRd values was much higher than for 2003. Soil respiration rates were similar for the three poplar clones in not fertilized and fertilized soils. The elevated [CO_2] increased significantly the respiration rates of both soil types, without interactions either with N availability or with clones (fig. 10.2 and tab. 10.1). The root fraction was calculated from the mean values of SRr and SRd respiration measurements carried out in the vegetative period between 21 may and 17 September 2003. Values for the respiration of the root fraction ranged between 32 and 62% of the total soil respiration, with an average of 48.5% (fig. 10.3). The lower values were observed for *P. x euramericana*, but were not significantly different. Neither elevated [CO_2], nor N fertilization induced significant variations on this fraction.

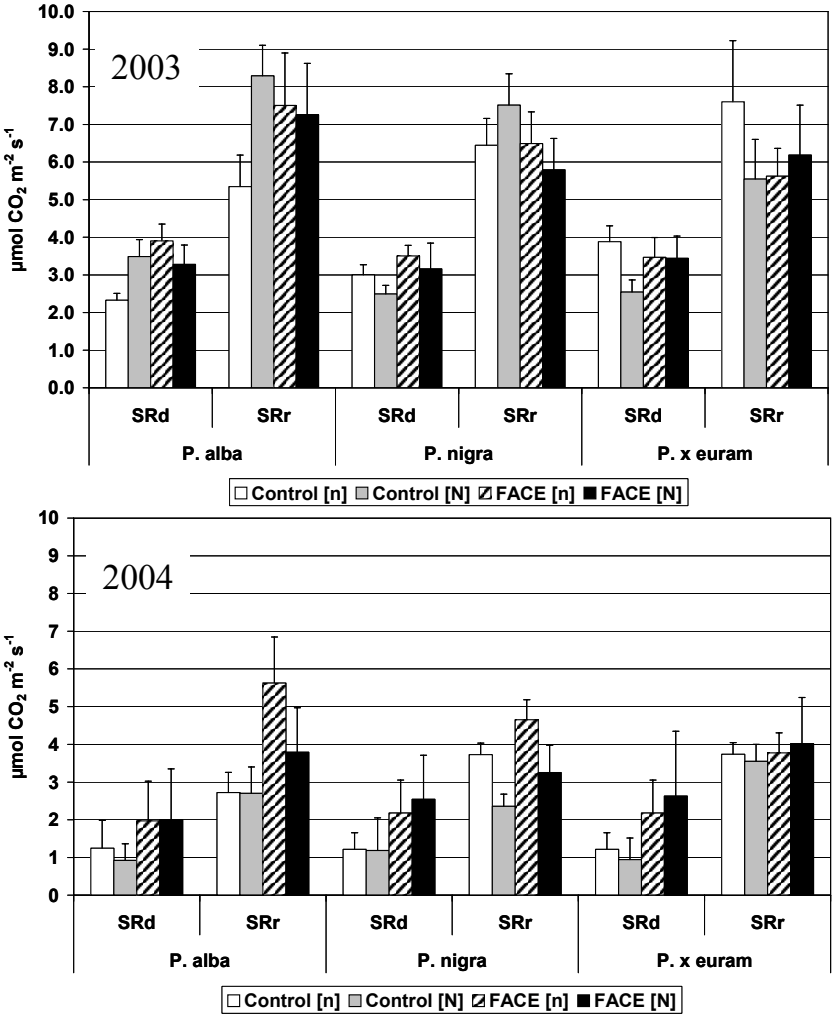

Figure 10.2. Soil respiration rates of Soil-r and Soil-d for the three poplar clones in the vegetative seasons of years 2003 and 2004. Measurements were made monthly between May and September in Control and FACE plots and not-fertilized and fertilized sub-plots.

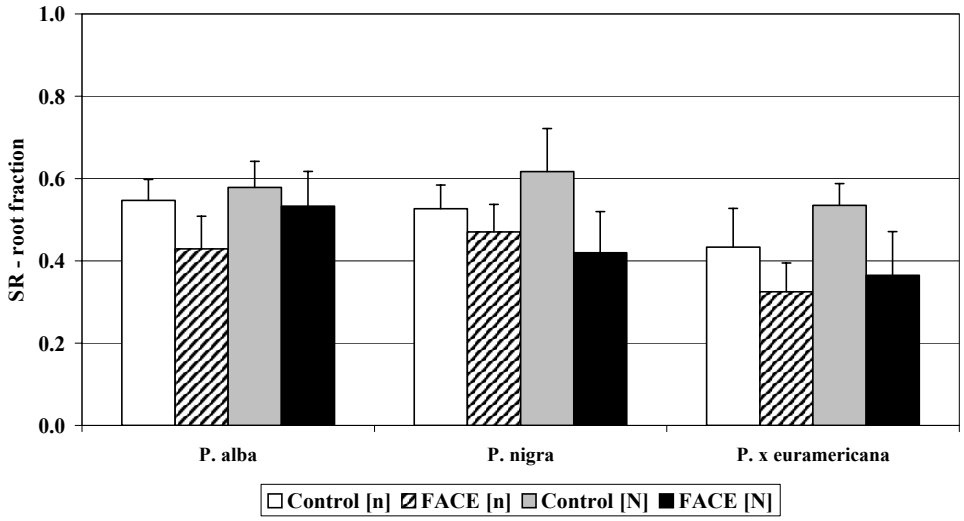

Figure 10.3. Root fraction of soil respiration measured in 2003 for *P. alba*, *P. nigra* and *P. x euramericana* in Control, FACE, fertilized and not fertilized soils.

	2003		2004		root fraction
	SRr	SRd	SRr	SRd	
CO_2	n.s.	n.s.	0.000	0.000	n.s.
Nitrogen	n.s.	n.s.	n.s.	n.s.	n.s.
Species	n.s.	n.s.	n.s.	n.s.	n.s.
Soil type	0.000		0.000		

Table 10.1. Analysis of variance of SRr and SRd for the years 2003 and 2004 and for the root fraction of soil respiration calculated for 2003. Values of $p<0.1$ are reported. The not significant interactions are excluded from analysis.

Soil microbial respiration and temperature functions

Soil-h:

Values of SRh were greater in 2003 than in 2004 (fig. 10.4). In the first year the elevated [CO_2] did not modify the CO_2 effluxes from this soil type, while in 2004 the increase of respiration was significant and particularly evident in FACE fertilized soils (tab. 10.2). The fertilization treatment induced a significant decrease of activity in the first year in *P. x euramericana*.

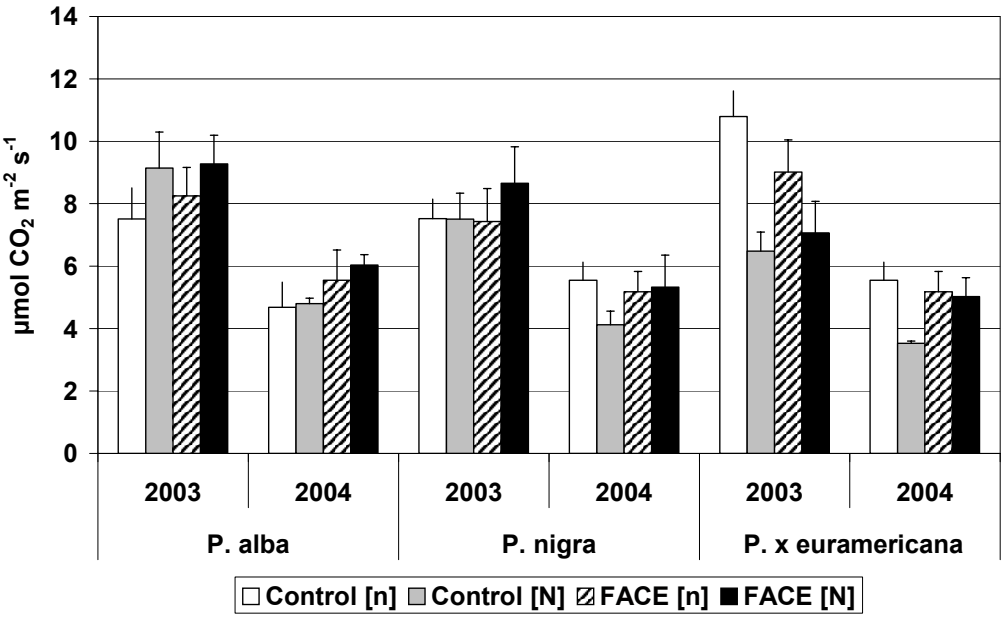

Figure 10.4. Soil respiration rates of Soil-h for the three poplar clones in the vegetative seasons of years 2003 and 2004. Measurements were made monthly between May and September in Control and FACE plots and not-fertilized and fertilized sub-plots.

	2003	2004
FACE	+1%	+14%
Nitrogen	-5%	-9%
	Analysis of variance	
CO_2	n.s.	0.03
nitrogen	n.s.	n.s.
species	n.s.	n.s.
Species x nitrogen	0.004	0.09

Table 10.2. Percentage effect of Soil-h microbial respiration in the vegetative season of 2003 and 2004, as average of the three species. Analysis of variance of SRh for the years 2003 and 2004 and is also reported. Values of $p<0.1$ are reported. The not significant interactions were excluded from analysis.

In the year 2004 the soil-h respiration maintained high rates, similar for the three poplar clones (fig. 10.5). The 0 °C intercept was significantly increased by the elevated [CO_2] treatment (+ 47% considering the average of fertilized and not fertilized soils), whereas the N availability and the interaction between FACE and fertilization treatments did not affected this parameter (tab. 10.3).

At not limiting temperature (18 °C) the SRh maintained higher rates under elevated [CO_2] with a significant increase of + 24% on average. The fertilization treatment and the poplar clones did not induce significant changes (tab. 10.3).

The slope of the temperature function of microbial respiration (k) was not affected either by poplar clones or by the two treatments. Also for Q_{10} no significant variations were detected (tab. 10.3). Q_{10} values were 2.9 on average.

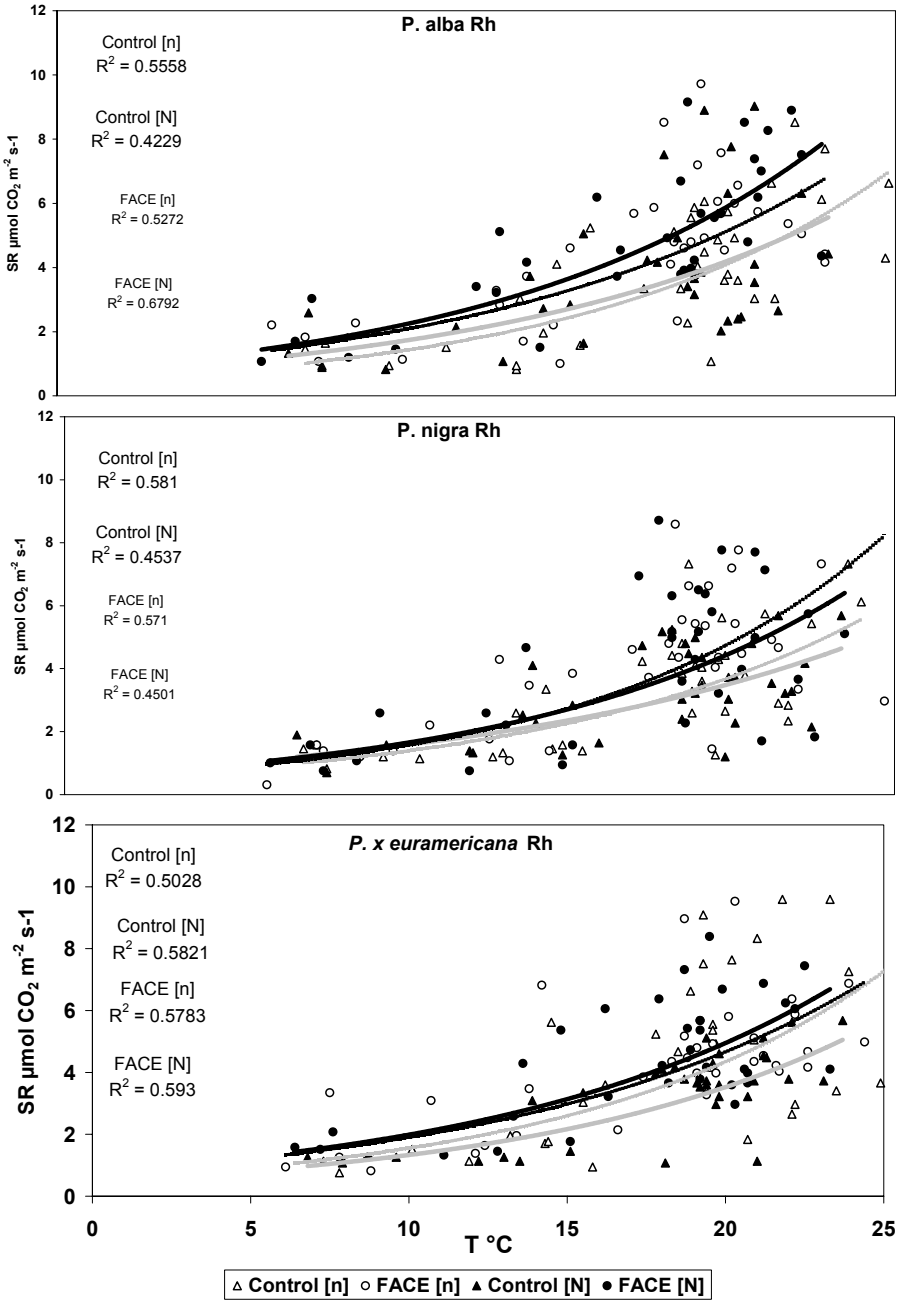

Figure 10.5. Temperature function of SRh for the year 2004. Data from *P. alba*, *P. nigra* and *P. x euramericana* in Control and FACE plots and in not fertilized (n) and fertilized (N) sub-plots are reported.

SRh	a	k	$SRh_{(18)}$	Q_{10}	$SRh_{(cum)}$
	µmol CO_2 m^{-2} s^{-1}	*1/ °C*	*µmol CO_2 m^{-2} s^{-1}*		*g C- CO_2 m^{-2} y^{-1}*
	P. alba				
Control [n]	0.46 ± 0.05	0.11 ± 0.00	3.32 ± 0.32	2.99 ± 0.10	1089 ± 92.6
FACE [n]	0.79 ± 0.29	0.10 ± 0.02	4.21 ± 0.49	2.89 ± 0.70	1244 ± 105.4
Control [N]	0.72 ± 0.16	0.09 ± 0.09	3.77 ± 0.97	2.49 ± 0.12	828 ± 83.9
FACE [N]	0.97 ± 0.33	0.10 ± 0.01	4.91 ± 0.52	2.64 ± 0.34	1380 ± 204.42
	P. nigra				
Control [n]	0.46 ± 0.05	0.11 ± 0.01	3.17 ± 0.58	2.92 ± 0.24	889 ± 148.7
FACE [n]	0.57 ± 0.17	0.11 ± 0.03	3.85 ± 0.25	3.31 ± 0.90	1109 ± 34
Control [N]	0.63 ± 0.17	0.09 ± 0.09	3.11 ± 0.66	2.49 ± 0.16	1305 ± 156.4
FACE [N]	0.66 ± 0.35	0.11 ± 0.02	3.94 ± 0.83	3.22 ± 0.64	1208 ± 97.2
	P. x euramericana				
Control [n]	0.44 ± 0.07	0.12 ± 0.02	3.86 ± 0.96	3.40 ± 0.62	1224 ± 226.5
FACE [n]	1.02 ± 0.57	0.09± 0.02	4.08 ± 0.74	2.65 ± 0.56	1193 ± 176
Control [N]	0.47 ± 0.03	0.10 ± 0.00	2.94 ± 0.32	2.76 ± 0.10	860 ± 56
FACE [N]	0.66 ± 0.35	0.11 ± 0.02	3.94 ± 0.83	3.22 ± 0.64	1067 ± 119
	Analysis of Variance				
CO_2	0.040	n.s.	0.018	n.s.	0.065
Nitrogen	n.s.	n.s.	n.s.	n.s.	n.s.
Species	n.s.	n.s.	n.s.	n.s.	n.s.

Table 10.3. Values of the temperature functions parameters and of cumulative respiration in Soil-h for the year 2004, ± standard errors. Data referred to *P. alba*, *P. nigra* and *P. x eruamericana* in Control (C) and FACE (F) plots and in not fertilized (n) and fertilized (N) sub-plots. Analysis of variance of FACE and N treatment and of clonal variability is also reported. The interactions without any significance were removed from the table. The values of p<0.1 are reported.

The Soil-h cumulative respiration was calculated for the year 2004 (fig. 10.6). Values ranged between 830 and 1380 g C-CO_2 m^{-2} and were significantly higher under elevated [CO_2] (+16% on average for the three clones). Values were similar for all poplar clones and were not influenced either by the fertilization treatment or by the interaction between the treatments.

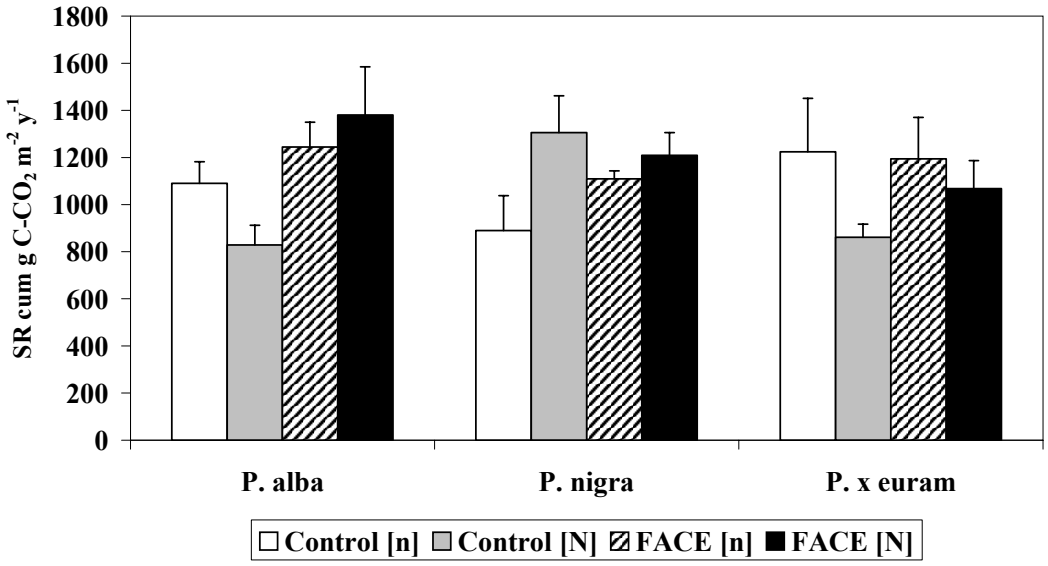

Figure 10.6. Soil-h cumulative respiration for the year 2004 calculated for *P. alba*, *P. nigra* and *P. x euramericana* in Control and FACE not fertilized (n) and fertilized (N) subplots.

At the end of the experiment, the C fractions and the Total Organic C analysed in the not fertilized soils of *P. nigra* and *P. x euramericana* did not show any significant effect due to the FACE treatment (tab. 10.4). The soil of the two clones had similar C contents. The Community Level Physiological Profile showed a significant difference between the two poplar species with higher values, on average, for *P. nigra*, whereas no significant effects due to elevated [CO_2] were observed (fig. 10.7).

Soil-h	TOC	ExC	WSC	MBC	Labile C	CLPP
	%	$\mu g\ g^{-1}$	$\mu g\ g^{-1}$	$\mu g\ g^{-1}$	$\mu g\ g^{-1}$	$\mu g\ CO_2\ g^{-1}\ h^{-1}$
P.nigra [C]	1.29 (0.2)	92.30 (18)	64.25 (40)	228.48 (44)	320.8 (40)	1.52 (0.06)
P.nigra [F]	1.10 (0.1)	95.39 (18)	76.75 (24)	243.37 (39)	338.8 (37)	1.61 (0.06)
P.x eur. [C]	1.21 (0.2)	79.10 (12)	66.14 (31)	115.07 (66)	194.2 (54)	1.53 (0.08)
P.x eur. [F]	1.03 (0.2)	79.03 (6)	43.11 (35)	269.41 (41)	348.4 (46)	1.42 (0.1)
	ANOVA					MANOVA
CO_2	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Species	n.s	n.s	n.s	n.s	n.s	0.000

Table 10.4. Soil-h chemical and biochemical parameters for *P. nigra* and *P. x euramericana* in not fertilized soils collected at the end of the experiment. Total Organic C (TOC), K_2SO_4 Extractable C (ExC), Microbial Biomass C (MBC), Water Soluble C (WSC) and the overall labile C (ExC + MBC) were reported with the Analysis of Variance. The Community Level Physiological Profile (CLPP) is reported with the Multivariate Analysis of Variance. Standard errors are in parenthesis. Values of $p<0.1$ are reported.

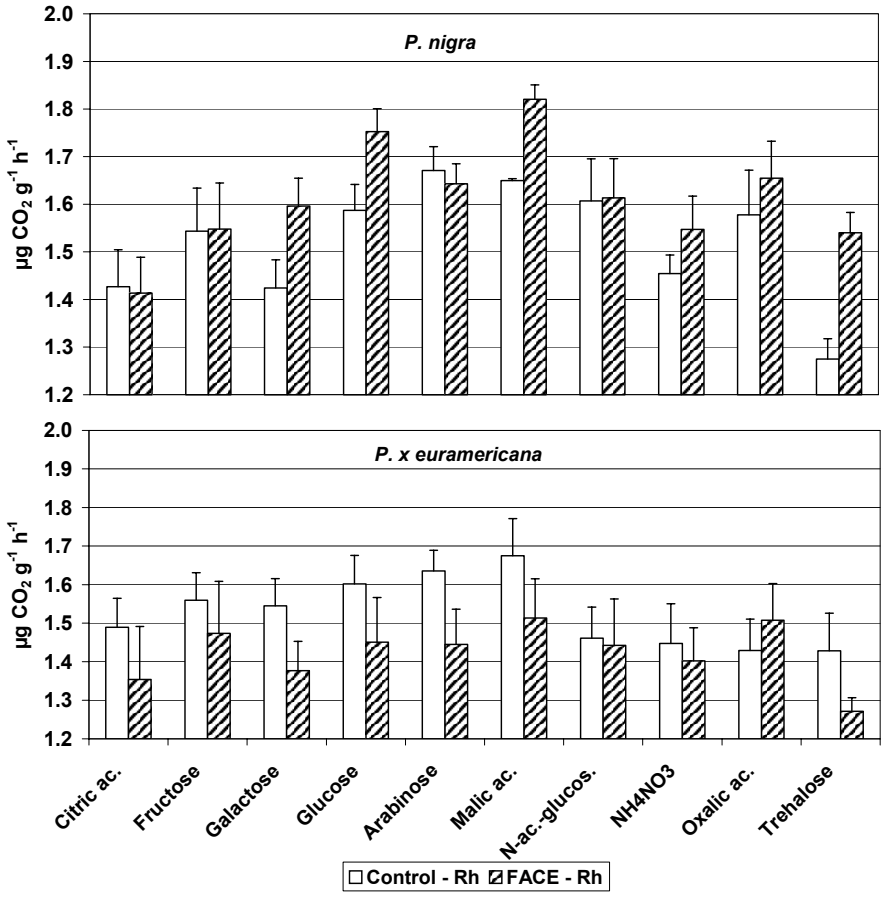

Figure 10.7. Community Level Physiological Profile for Soil-h. The microbial respiration rates of Control and FACE plots in *P. nigra* and *P. x euramericana* are reported for the different substrates added.

Soil-r:

Values of SRr in the year 2004 were similar for *P. alba*, *P. nigra* and *P. x euramericana* in fertilized and not fertilized soils (fig. 10.8). The 0 °C intercept was not affected by the two treatments and showed similar values for the three clones (tab. 10.5).

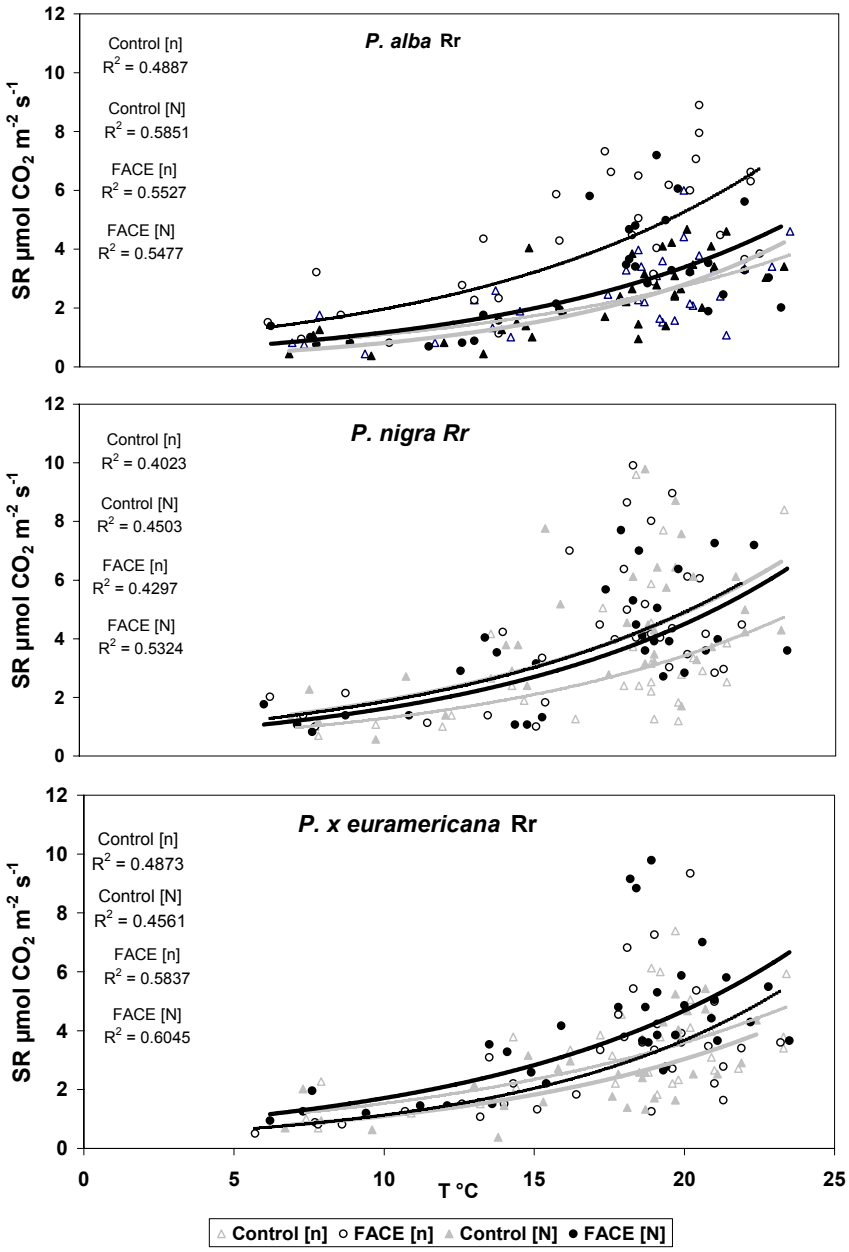

Figure 10.8. Temperature function of SRr for the year 2004. Data from *P. alba*, *P. nigra* and *P. x euramericana* in Control and FACE plots and in not fertilized (n) and fertilized (N) sub-plots are reported.

At not limiting temperature the increase under elevated [CO_2] was significant and particularly evident for *P. alba* and *P. nigra*, even if the clones did not induce significant differences (tab. 10.5). $SRr_{(18)}$ values and FACE effects were not influenced by the N availability.

SRr	a	k	$SRr_{(18)}$	Q_{10}	$SRr_{(cum)}$
	µmol CO_2 m^{-2} s^{-1}	*1/ °C*	*µmol CO_2 m^{-2} s^{-1}*		*g C- CO_2 m^{-2} y^{-1}*
	P. alba				
Control [n]	0.71 ± 0.41	0.09 ± 0.03	2.42 ± 0.29	2.53 ± 0.64	637 ± 34.2
FACE [n]	1.05 ± 0.53	0.09 ± 0.02	4.57 ± 0.82	2.66 ± 0.47	1112 ± 111.8
Control [N]	0.36 ± 0.14	0.11 ± 0.02	2.28 ± 0.32	3.17 ± 0.55	850 ± 303.9
FACE [N]	0.79 ± 0.23	0.09 ± 0.01	4.17 ± 0.73	2.59 ± 0.17	957 ± 14.8
	P. nigra				
Control [n]	0.56 ± 0.03	0.08 ± 0.02	2.80 ± 0.90	2.43 ± 0.52	786 ± 241.4
FACE [n]	0.75 ± 0.29	0.10 ± 0.01	4.36 ± 0.87	2.84 ± 0.27	1205 ± 162
Control [N]	0.79 ± 0.23	0.09 ± 0.01	4.17 ± 0.73	2.59 ± 0.17	807 ± 77.6
FACE [N]	0.64 ± 0.14	0.09 ± 0.00	3.57 ± 0.92	2.58 ± 0.07	1012 ± 189
	P. x euramericana				
Control [n]	0.99 ± 0.29	0.07 ± 0.01	3.19 ± 0.76	1.95 ± 0.15	926 ± 179.1
FACE [n]	0.34 ± 0.05	0.12 ± 0.01	2.96 ± 0.21	3.37 ± 0.25	841 ± 75.8
Control [N]	0.63 ± 0.17	0.08 ± 0.01	2.46 ± 0.57	2.19 ± 0.20	824 ± 194.8
FACE [N]	0.66 ± 0.09	0.10 ± 0.01	3.84 ± 0.04	2.71 ± 0.22	937 ± 169.1
	Analysis of Variance				
CO_2	n.s.	0.048	0.036	0.073	0.033
Nitrogen	n.s.	n.s.	n.s.	n.s.	n.s.
Species	n.s.	n.s.	n.s.	n.s.	n.s.

Table 10.5. Values of the temperature functions parameters and of cumulative respiration for Soil-r in the year 2004, ± standard errors. Data referred to *P. alba*, *P. nigra* and *P. x eruamericana* in Control (C) and FACE (F) plots and in non fertilized (n) and fertilized (N) sub-plots. Analysis of variance of FACE and N treatment and of clonal variability is also reported. The interaction without any significance were removed from the table. The values of p<0.1 are reported.

The slope of the temperature function was significantly higher under elevated [CO_2] in the three clones (mainly *P. nigra* and *P. x euramericana*) and in both fertilized and not fertilized subplots (tab. 10.5). This increase influenced also the Q_{10} with 2.48 and 2.79 values respectively for Control and FACE soils (tab. 10.5). The N addition and the clones did not influence the Q_{10}.

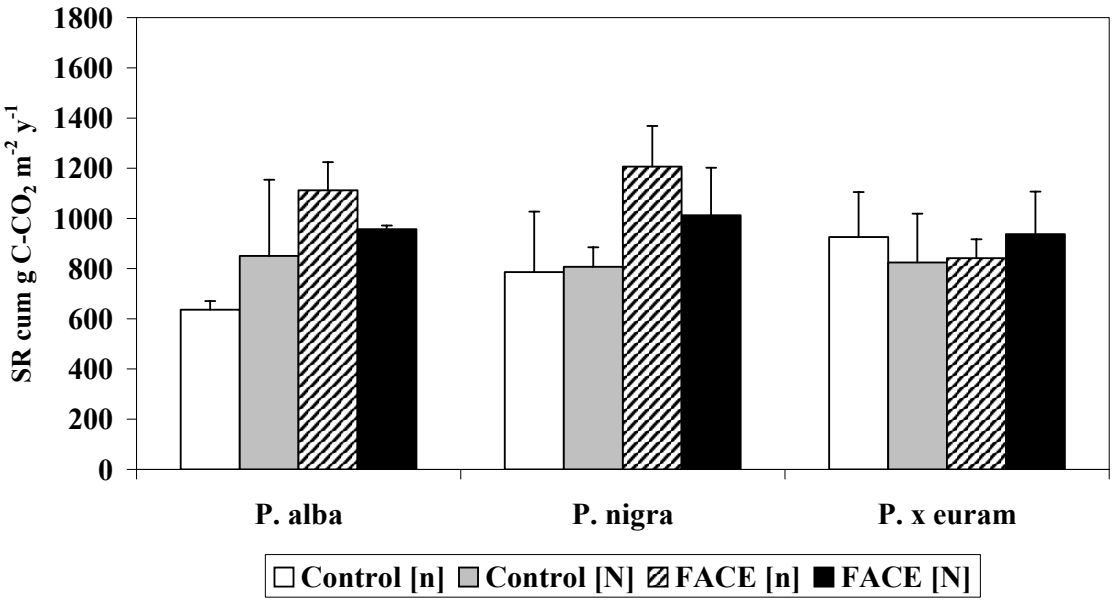

Figure 10.9. Soil-r cumulative respiration for the year 2004 calculated for *P. alba*, *P. nigra* and *P. x euramericana* in Control and FACE not fertilized (n) and fertilized (N) subplots.

The Soil-r cumulative respiration for the year 2004 showed values that ranged between 367 and 1206 g C-CO_2 m^{-2}. FACE treatment induced significantly higher rates with an average of + 26% with respect to Control, and the highest increase was observed on *P. alba* and *P. nigra* (fig. 10.9 and tab. 10.5). However, the fertilization treatment and the poplar clones did not induce significant variations.

At the end of the experiment, the C fractions and the Total Organic C analysed in not fertilized soils of *P. nigra* and *P. x euramericana* did not show any significant effect due to the FACE treatment (tab. 10.6). The soil of the two clones had similar C contents.

The root biomass was significantly higher in FACE soil in both species with an average of + 206% (tab. 10.6). The Community Level Physiological Profile showed significant differences between the two poplar species with higher values, on average, for *P. nigra* (fig. 10.10). The FACE treatment induced significantly higher substrate utilization rates with respect to control (tab. 10.6).

Soil-r	TOC	ExC	WSC	MBC	Labile C	Roots	CLPP
	%	µg g^{-1}	µg g^{-1}	µg g^{-1}	µg g^{-1}	g m^{-2}	µg CO_2 g^{-1} h^{-1}
P.nigra [C]	1.83 (0.16)	98.08 (14.0)	94.56 (25.9)	457.71 (78.05)	555.79 (85.53)	2.87 (2.13)	1.709 (0.06)
P.nigra [F]	1.57 (0.21)	98.09 (13.1)	83.43 (33.5)	227.57 (36.39)	325.65 (25.34)	15.96 (5.98)	1.788 (0.06)
P.x eur [C]	1.40 (0.08)	97.80 (3.94)	80.98 (34.3)	260.59 (31.76)	358.39 (28.85)	7.16 (3.97)	1.571 (0.07)
P.x eur [F]	1.52 (0.06)	90.46 (9.58)	49.62 (25.5)	416.87 (25.33)	507.33 (34.49)	14.79 (11.65)	1.597 (0.07)
	ANOVA						MANOVA
CO_2	n.s.	n.s.	n.s.	n.s.	n.s.	0.0679	0.0259
Species	n.s	n.s	n.s	n.s	n.s	n.s.	0.000

Table 10.6. Soil-r chemical and biochemical parameters for *P. nigra* and *P. x euramericana* in not fertilized soils collected at the end of the experiment. Total Organic C (TOC), K_2SO_4 Extractable C (ExC), Microbial Biomass C (MBC), Water Soluble C (WSC) and the over-all labile C (ExC + MBC) are reported with the Analysis of Variance. The Community Level Physiological Profile (CLPP) is reported with the Multivariate Analysis of Variance. Standard errors are in parenthesis. Values of $p<0.1$ are reported.

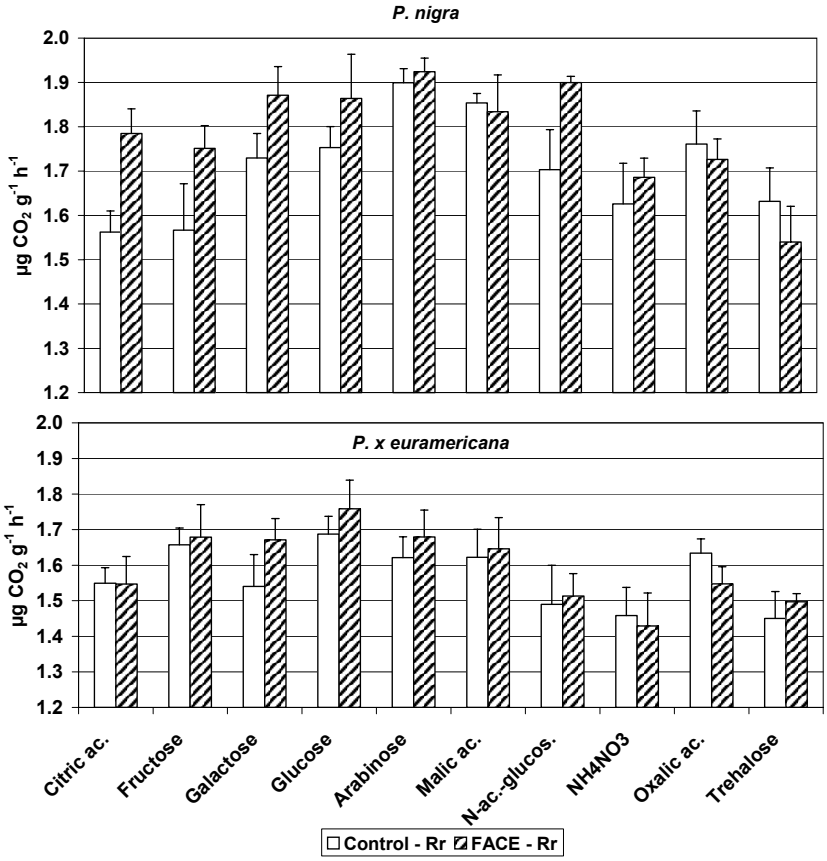

Figure 10.10. Community Level Physiological Profile for Soil-r. The microbial respiration rates of Control and FACE plots in *P. nigra* and *P. x euramericana* were reported for the different substrates added.

Considering the difference between the two soil types, Soil-h and Soil-r, the CO_2 efflux from soil at 18 °C and the cumulative respiration for the year 2004 were significantly higher in Soil-h (tab. 10.7). Regardless the treatments, there was a +11% and a + 23% increase of respiration rates in soil with roots removal ($SRh_{(18)}$ and SRh_{cum} respectively) with respect to the soil with living roots.

On the contrary, the microbial biomass C and the labile C, as well as the Total Organic C, were significantly lower in Soil-h with respect to the Soil-r (tab. 10.7). A decrease of 36, 31 and 26% respectively was observed as an average for the two clones, *P. nigra* and *P. x euramericana* (tab. 10.7). Similarly, the Community Level Physiological Profile was significantly lower in soil-h (tab. 10.7). The differences between the soil types were independent of elevated [CO_2] and no interactions were found between neither elevated [CO_2] nor poplar clones and the variability due to the soil type (tab. 10.7).

A significant inverse relationship between the cumulative respiration of Soil-h and Soil-r and the Total Organic C content of these two soil types was found, considering Control and FACE not fertilized soils of *P. nigra* and *P. x euramericana* (fig. 10.11).

	Soil-h	Soil-r	*p value*
Fluxes			
a *(µmol CO_2 m^{-2} s^{-1})*	0.65	0.69	n.s.
k *(1/ °C)*	0.10	0.09	n.s.
SR_{18} *(µmol CO_2 m^{-2} s^{-1})*	3.76	3.40	0.0797
Q_{10}	2.91	2.64	n.s.
SR_{cum} *(g C- CO_2 m^{-2} y^{-1})*	1116.81	908.09	0.0018
CLPP *(µg CO_2 g^{-1} h^{-1})*	1.524	1.663	0.000
Chemical and Biochemical properties			
TOC *(%)*	1.16	1.57	0.0018
ExC *(µg g^{-1})*	86.46	96.11	n.s.
WSC *(µg g^{-1})*	62.56	77.15	n.s.
MBC *(µg g^{-1})*	214.08	340.69	0.0104
Labile C *(µg g^{-1})*	300.54	436.79	0.0062

Table 10.7. Mean values of the CO_2 effluxes for 2004 and the chemical and biochemical properties of Sol-h and Soil-r, reported as the average of Control and FACE plots of *P. nigra* and *P. x euramericana*. p values<0.1 were reported.

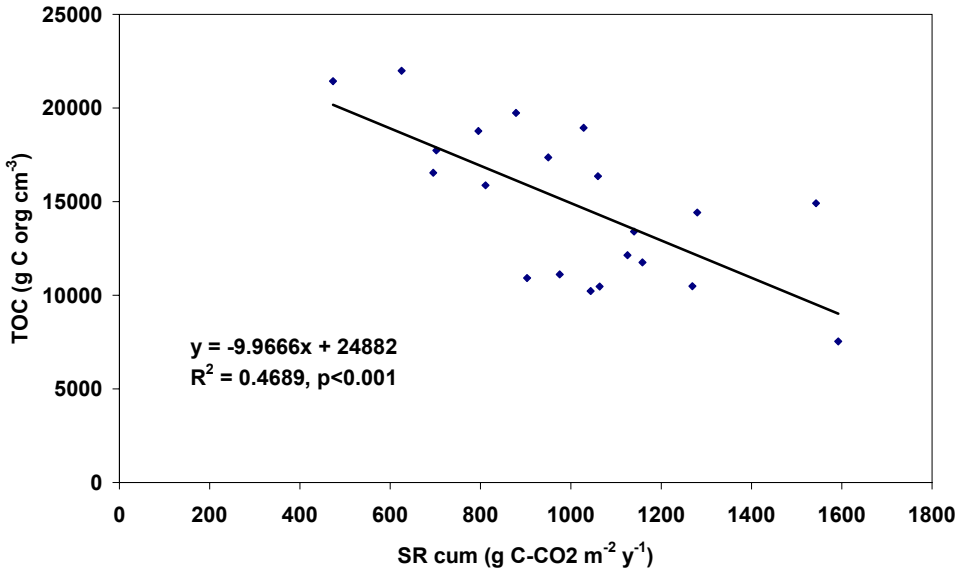

Figure 10.11. Linear regression between the cumulative respiration of the year 2004 and the TOC content measured at the end of the experiment in Soil-h and Soil-r. The p value is reported in the figure.

10.3 Discussion

Before starting the discussion, it is important to draw attention to three main limitations related to the experimental approach:

1) Observing the two considered periods (2003 and 2004 vegetative seasons) in the first year the respiration rates were much higher, also compared with field soil respiration measured with collars (sect. 8), while in the second growing season a strong decrease of CO_2 effluxes from all soils was evident (fig. 10.2). The insertion of the tubes and the consequent root trenching caused in fact a strong impact on the soil, inducing higher respiration rates soon after the disturbance and a following decline. Several authors reported strong disturbance of soil CO_2 effluxes, suggesting a period to allow re-equilibration to steady state conditions (Hanson et al., 2000).
2) Soil-h showed the higher respiration rates since the beginning of the experiment, and in 2004 it maintained CO_2 emissions around 5 µmol CO_2 m^{-2} s^{-1} against 3.8 µmol CO_2 m^{-2} s^{-1} of Soil-r. Two possible processes could be occurred at the same time: 1) the soil manipulation effected to remove roots, in addition to mixing and oxygenate the soil, could have broken up aggregates, thus exposing organo-mineral surfaces otherwise not available to microbes (Post and Know, 2000). Microorganisms found a more favourable environment to SOM degradation, and could therefore increase the metabolic activity. 2) The soil placed back in the tubes, even if compacted, presented a modified structure. The CO_2 transport relies on a diffusion mechanism in soil and a turbulent transport in the atmosphere (Healy et al., 1996). It is possible that changes in the soil structure could favour the CO_2 diffusion in soil, also altering the soil moisture, thus increasing the CO_2 efflux of Soil-h.
3) At the end of the autumn 2003 the Soil-d had problems with the drainage and water stagnated in tubes, with consequent anaerobic conditions, until the following summer 2004. The plantation soil was classified as loam or silt loam (Hoosbeck et al., 2004) and it is possible that the insertion of the tubes compacted the clay rich soil not allowing a correct drainage. In Soil-r the opening inside the tubes and the inclusion of living roots, and thus the absorption of water, prevented water accumulation; while in the third type, Soil-h, the manipulation and the partial sieving of the

soil to remove roots allowed better draining conditions. In Soil-d the drainage of water was less efficient and with abundant rainfall the water stagnated in the tubes.

Separating the root contribution to soil respiration

One of the biggest concern with the trenching approach to separate the root contribution to soil respiration is the influence of residual decomposing roots left in the trenched plots and their contribution to soil respiration (Hanson et al., 2000). To avoid this problem, Bowden et al. (1993) and Ewel et al. (1987) assumed a low contribution after 9 or more months from the trenching. We prevented this problem by comparing the respiration of soil with trenched roots (SRd) with that of soil with trenched roots plus new living roots that were allowed to enter in the tubes (SRr). In this way, calculating the difference in the respiration rates between the two soil type (SRr-SRd), the contribution of dead roots decomposition to soil respiration was supposed to be excluded. Nevertheless, the presence of living roots might have influenced the decomposition of dead material, and it is possible that a bias due to roots/microbes interactions could have been occurred, mainly in FACE soil.

The anaerobic conditions strongly influenced the soil respiration rates and the decomposition activity of microbial communities in Soil-d, and the comparison between SRr and SRd was much more approximate in 2004 than in 2003. The root fraction was therefore calculated using the average CO_2 efflux between May and September 2003, that is a period of great root production and plant activity (see also Lukac et al., 2003) and when the inhibition of soil respiration due to anaerobic conditions of soil did not occurr yet.

Regardless the treatments, the contribution of root and living root derived CO_2 to total soil CO_2 efflux assessed around the 48% (fig. 10.3). Data reported in literature showed wide range of variation, depending on different methods of measurements and on the large variation between different biomes. With root exclusion experiments, the root contribution to total soil CO_2 efflux ranged from 30 to 93% (Nakane et al., 1983; Bowden et al., 1993; Laudelot and Thierron, 1996; Ryan et al., 1997; Xu et al., 2001; Li et al., 2004). Using C tracer techniques, it has been shown that root derived CO_2 can contribute from 19% to 80% to the total CO_2 efflux from planted soils (Warembourg and Paul, 1977; Martin and Merckx, 1992). In any case Hanson et al. (2000) showed that from the analysis of 50 studies, the modal root contribution to soil respiration lied in the range from 40 to 50%, and our values of root fraction confirmed this result.

The increase of root biomass under elevated [CO_2] in Soil-r, although highly variable, was consistent with the results of Lukac et al. (2003) that showed a significant increase of root

biomass under elevated [CO_2] on 0-20 and 0-40 cm soil depth in the plantation. Moreover, in many works a higher root activity in FACE soils is reported (Janssens et al., 1998; Pritchard et al., 2001), and the production and longevity is also expected to increase (Rasse, 2002). From these remarks, a greater root contribution to soil respiration under elevated [CO_2] was expected. The lack of effects of [CO_2] enrichment on the root fraction indicated however that the soil respiration increase reported in sect. 8, and confirmed by SRr, was a consequence of the greater activity of both auto- and heterotrophic components. The contribution of the two components to the increase of soil CO_2 efflux was in fact similar. Although Zak et al. (2000) reported very different responses of microbial respiration to elevated [CO_2], an enhancement of rhizospheric respiration was found (sect. 9) and from decomposing roots in Soil-d derived a further greater CO_2 efflux. Anyhow, we cannot exclude that the rhizosphere interactions between root and microorganisms (i.e. competition for nutrients) could lead to contrasting feedbacks. In Soil-d microbes could benefit of C released by dead roots, while in Soil-r further competition for nutrients between microbes and the new roots occurred, thus limiting the increase of respiration rates under elevated [CO_2] (Reid and Goss, 1982; Sparling et al., 1982).

Soil microbial activity

Since the beginning of the incubation, the microbial respiration in Soil-h decreased significantly from 6.3 to 4.2 after one year. A decline in soil respiration rates was expected to occur as a mechanism commonly observed with long incubation time due to the depletion of C (Fang et al., 2005).

The microbial respiration increase under elevated [CO_2] observed in 2004 was in agreement with the laboratory temperature response of rhizomicrobial respiration discussed in sect. 9. In that work it was found a significant relationship between α and the size of microbial population and it was also demonstrated that, at not limiting temperatures, the labile C supply was the main driving force of microbial respiration (sect. 7). We know that the living fraction of organic C (MBC) was increased by elevated [CO_2], as well as the labile C fractions (see sect. 4). It is also worth to remember that, in Soil-h, the new C input in the 18 months of incubation came exclusively from litter and macrofaunal activity.

It is therefore possible to hypothesize the following: 1) The rates of early stage microbial litter degradation were accelerated under elevated [CO_2] and could prime the decay of native SOM, increasing the microbial soil CO_2 efflux as suggested by Cotrufo et al. (2005). Nevertheless, this effect was supposed to be a consequence of a rhizosphere activity en-

hancement (Lin et al., 2001) and was not expected to act in the absence of roots. 2) The hypothesized competition between microbes and plants for N in FACE soils (Calfapietra et al., 2003) did not occur in Soil-h, since roots were removed. The consequent availability of N allowed microbes to utilize C if present, without immobilizing it. 3) The higher amount of labile C in FACE soils could feed microorganisms in the following 18 months. 4) Respiration was enhanced by the release of C from microbial cells. Microbial biomass turnover rates have been reported to vary around 1-2 years (Schnurer et al., 1985; Jenkinson and Ladd, 1981; Paul and Vorney, 1984; Smith et al., 1985, 1986). In FACE soils the living fraction of C was greater and it is presumable that the released C during microbial turnover could fuelled the new microbial cells. This last hypothesis is supported by the lack of elevated [CO_2] effects on microbial respiration in 2003, while the increase was significant only in 2004, in particular in fertilized soils. The microbial biomass is considered to be the labile pool of nutrients and C, and its turnover, to a certain extent, determines N, P and S availability (Smith et al., 1993). Robertson et al. (1986) found that a significant amount of mineralized C and N was correlated with a concurrent decrease in microbial biomass during a 12 week incubation. The absence of roots competition for nutrient therefore could have allowed a greater microbial mineralization supported in FACE soils by a larger pool of labile C during a period of time equal to that of microbial turnover. Therefore, this process it is not supposed to occur in normal field conditions in the plantation, where plant nutrient uptake and further inputs might alter microbial C metabolism, as seen for shorter term incubations (sect. 5, 6 and 7).

The cumulated respiration in 2004 remarks these results. The positive effect of elevated [CO_2] was not a seasonal effect, linked to plant activity, but the microbial respiration was influenced by the C and N substrates present in the soil during the year 2004. The input from litter can be more recalcitrant under FACE, but the review of Norby et al. (2001) showed that changes of litter quality induced by elevated [CO_2] did not result in a significant reduction of decay rates. Thus we can suppose that the higher C availability in FACE (already present in soil as labile C), combined with the lack of competition for N with plants, allowed microbes to respire and to metabolise more C under elevated [CO_2]. A depletion of C was expected in general (Fang et al., 2005) and there was not a greater litter production in FACE plots (Cotrufo et al., 2005). It is reasonable to suppose that the higher C losses under elevated [CO_2] resulted in similar C content between the Control and the FACE soils at the end of the experiment. The same interpretation could be made for Soil-d. The FACE soils had greater C availability, in the form of labile C (sect. 4) and dead roots

(Lukac et al., 2003). The significant positive effect of elevated [CO_2] on SRd in the summer 2004 suggested that microbes could metabolise this C surplus during the incubation.
The fertilization did not influence in any case the microbial respiration, neither in the respiration rates nor in the slope of the temperature function for SRh. We should keep in mind that the N fertilization effect could be only residual, since no N additions were made after the insertion of tubes. Therefore the only difference between not fertilized and fertilized sub-plots was on the initial different N concentration of the soil, that probably was depleted after a few time.

The role of roots on soil CO_2 efflux and C storage

Roots can affect soil microbial activities either by exuding C-rich organic substances easily available for microorganisms and by altering the soil physical and chemical environment, consequently controlling also the soil-derived CO_2 efflux. This can lead to either acceleration or retardation of Soil Organic Matter (SOM) decomposition (Kuzyakov and Cheng, 2001).
At the end of the experiment, the organic C was lower in Soil-h in comparison with Soil-r because of the C mineralization activity during the incubation, as demonstrated by the relationship between the Soil-h and Soil-r TOC and cumulative respiration (fig. 10). The effect of living roots on SOM decomposition was reported as inhibitory of microbial minmeralization activity due to the competition between roots and microflora for substrates (Reid and Goss, 1982; Sparling et al., 1982). Some models predicted a microbial immobilization of plant derived C, with reducing rates of C mineralization in the presence of living roots: Bottner et al. (1991) and Körschens and Müller (1994) found in fact an accumulation of SOM in cultivated compared to the bare soil. Nevertheless, the structural disturbance occurred in Soil-h could have favoured the strong decrease of C, therefore overweighting the effect of living roots. Moreover, evidences of a positive impact of roots on microbial substrates utilization potential were found. The microbial Community Level Physiological Profile in the plantation was in fact significantly influenced by the presence of roots. The addition of each C and N substrates increased the microbial activity in Soil-r with respect to Soil-h (tab. 10.6). It is reasonable to suppose that, where more new C was available as in Soil-r, microbes were prompt to utilize the added C substrates for their metabolism, whereas, where labile C input was low as in Soil-h, the added C was immobilized with lower respiration rates.

Moreover, the ability of microbes to utilise the added substrates increased significantly under elevated [CO_2] only in the presence of living roots (tab. 10.5). These results suggested that the activity of microbes was strictly dependent on the roots activity, also in the bulk soil. Microbes could be more active in consuming the available C where more roots and rhizodeposition were present. We can deduce that microorganisms living in planted soils were more active and prompt to utilize substrates. The added C was mainly in the form of rhizodeposition (carboxylic acids and carbohydrates) and a major ability, or practice, in their consume in soil with roots was expected. Several authors reported a stimulatory effect of living roots due to the *priming effect* of exudates (Helal and Sauerbeck, 1986, Kuzyakov et al., 2001). The presence of roots leads to higher C utilization rates when this was available in the form of easily degradable substrates, as roots exudates. In any case, was not evident a mechanism as the *priming effect*, and the consequent break down of stable organic matter, but it seemed more likely that only added C were respired: the difference between respiration rates of soil without added substrates and those of soil with added substrates did not exceed the added C.

It is possible therefore that the easily decomposable C supplied by roots allowed microbes to utilize it without breaking down the stable organic matter. As a consequence, the depletion of organic C was prevented, or balanced by the new inputs.

In Soil-r the increase of soil respiration with temperature was significantly influenced by elevated [CO_2] (tab. 10.4), as well as the plant photosynthetic activity was (Wittig et al., 2005) that, through the root respiration and the C supply to microbes by rhizodeposition, might have induced higher soil respiration rates during the vegetative period. Results from Högberg et al. (2001) and Yuste et al. (2004) indicated that the flux of photosynthates has a big impact on soil respiration, that were influenced either by seasonal patterns of plant activity or by variations in soil temperature. Moreover, the microbial respiration response to the temperature increase was not affected by elevated [CO_2] in soil without roots (tab. 10.2). As found for rhizomicrobial respiration (see sect. 9), the slope of the temperature function and thus the Q_{10} was independent of the elevated [CO_2] (tab. 10.2). The higher labile C availability found in FACE soil (sect. 4) did not affect the microbial response to the temperature increase, confirming the finding that the microbial Q_{10} was not dependent on the amount of labile C in soils.

Thus, we can conclude that the increase of Q_{10} in Soil-r under elevated [CO_2] was not related to an enhanced microbial SOM decomposition with higher temperature, but to a direct flux of C through roots derived from plant photosynthetic activity.

6. CONCLUSIONS

Soil fertility

The high soil N content indicated a good fertility of the experimental site, although a strong N-NH_4^+ depletion did occur at the end of the 1st cycle. The nitrification was in fact the prevalent mineralization process in the plantation, and N-NO_3^- was the only mineral N form to be affected by experimental treatments.

The total nitrogen in soil decreased under elevated [CO_2] and the consequent plants-microbes competition for nitrogen favoured microbial N immobilization. The decrease of nitrification and mineralization rates under elevated [CO_2] resulted in fact in a lower N-NO_3^- availability in soil. Conversely, fertilizer applications increased availability of N-NO_3^- only. The added N-NH_4^+ was in part converted in nitrates because of a very fast nitrification activity, and in part immobilized in soil, in forms less accessible to plant uptake, as suggested by the increase of total N. Although the negative effect of [CO_2] enrichment on total N soil content acted similarly in fertilized and not fertilized soil, the decrease of nitrates under elevated [CO_2] was partly compensated by the positive interaction with the fertilization treatment. A more intense microbial mineralization activity induced in fact a N-NO_3^- increase in FACE fertilized soil, more evident in *P. nigra.*

Phosphorus availability in soil was under biotic control of acid-phosphatase activity, and was not limiting in the plantation, being present in relatively high amounts. In the 2nd cycle a negative trend was observed, with a progressive decrease more pronounced under elevated [CO_2] due to a greater phosphorus requirement from plants and microorganisms. The 2002 P addition determined an increase of this nutrient, also because of an enhancement of acid-phosphatase activity in fertilized soil, due to a *priming effect* mechanism. Nevertheless, the fertilization treatment had a negative impact on phosphorus availability under elevated [CO_2]. The [CO_2] enrichment-induced P decrease was in fact higher in nutrient rich soil, because of the lower enzymatic activity, combined with the greater P demand.

As seen for phosphorus, the increase of several extracellular enzymes indicated a greater soil nutrient requirement under elevated [CO_2]. This resulted in a general enhance-

ment of soil biological activity in order to maintain an adequate supply of nitrogen, sulphur and phosphorus in soil. The N addition lowered arylsulphatase and did not induce modification in chitinase activity; whereas the *priming effect* on acid-phosphatase had a short-term impact induced by the 2002 phosphorus fertilization. Although soil nutrient *status* played a main role in determining nutrient acquisition activity, the increase of extracellular enzymes under elevated [CO_2] was similar in fertilized and not fertilized soil. The greater enzymatic synthesis was indeed related to a general enhancement of microbial processes mediated by plants activity, as confirmed by the different responses of poplar clones.

Soil cation exchange capacity (CEC) increased during the 2nd rotation cycle, because of the positive impact of the plantation on soil organic matter. Under elevated [CO_2] the effect was more pronounced, and was sustained by the higher C/N ratio of labile soil organic matter, the greater root and fungal biomass, and the greater rhizosphere activity. N addition, as well as treatments interaction, did not induce any modification on CEC, confirming the main role of the amount and quality of C input, in its turn not affected by fertilization treatment.

Soil C pools

C input from plants to soil favoured the organic C storage in the plantation: in the three years of the 2nd cycle, after a first period of unbalance, a 23% increase of total organic C was in fact detected.

Elevated [CO_2] resulted in a more pronounced increase of all labile C fractions; however the recalcitrant C fraction and the total organic C were unaffected by the treatment. The lack of significant changes was indeed related to the difficulty of measuring small changes against the background C-content of the soil in the relative short term of the experiment, and did not exclude longer-term modifications.

The C/N ratio of labile fractions increased due to [CO_2] enrichment, indicating a lower quality of easily decomposable compounds, thus confirming results obtained for litter quality in other studies.

N addition decreased the availability of labile C fractions, and induced a temporary decrease of total organic C. At the end of the 2nd cycle, however, soil organic C content was similar in fertilized and not fertilized soil, suggesting a rapid recovery of C in soil. Organic

matter quality was unaffected by N addition, that influenced only the living fraction of soil C.

[CO_2] enrichment-induced modifications on labile and stable organic matter quantity and quality were similar in fertilized and not fertilized soil, and no interactions between the treatments were found on soil C pools.

Changes in microbial communities

The mineral N availability and the quantity and quality (C/N ratio) of labile soil organic matter, were the driving variables of microbial physiological *status* in the plantation. The N-NH_4^+ depletion induced a nutritional umbalance that caused microbial loss and altered microbial maintenance energy requirement. At the same time, the seasonal pattern of microbial processes observed in the plantation, was strongly related to changes in substrates quality (C/N ratio) and the consequente modifications of microbial community structure. At the beginning of the vegetative season an enhancement of microbial nutrient acquisition activity was observed; whereas, at the end of the growing season the high N concentration in microbial cells and in labile organic matter sustained higher microbial metabolic rates.

The extra C made available for microbes in elevated [CO_2] induced an increase in microbial biomass C sustained by a less energetically expensive metabolism, reflecting more a cell enlargement process than a real microbial proliferation. The relationship between microbial and soluble C suggested in fact that at least part of plants C input was immobilized and used for microbial growth, instead that respired and released back in the atmosphere. Elevated [CO_2] induced a preferential stimulation of fungi because of the increase in quantity but decrease in quality of plants input, which favoured a more efficient microbial population in converting substrate into biomass. This modification prevailed at the beginning of the vegetative season, when a lower quality of organic compounds and a further decrease of specific respiration rates were observed.

Fertilization treatment did not induce changes in microbial population size, but influenced the fungal community composition and lowered the fungal species richness. Moreover the microbial N immobilization was favoured only in June, during the fertilizer application, therefore lowering the microbial biomass C/N ratio and sustaining in the short-term fast growing microbial communities and higher metabolic rates.

Although the treatments interaction did not modify either microbial biomass C or microbial C/N ratio, the soil nutrient status and substrates quality influenced the impact of

elevated [CO_2] on the microbial structural and functional diversity. The interaction between [CO_2] enrichment and N addition selected different fungal communities without lowering the species richness, and the utilization of added C compounds was higher in FACE fertilized soil, where the combined C and N availability allowed higher metabolic rates, particularly in *P. nigra*.

Soil C losses and storage

As general mean, the autotrophic and the heterotrophic components of soil respiration contributed similarly to soil CO_2 efflux, and the root-derived CO_2 was on average the 48% of the total. Soluble C supply, by means of root and litter production, played a main role in determining soil respiration rates in the plantation, as well as yearly fluctuations.

The large increase of soil C losses under elevated [CO_2] was sustained during years, and after the coppicing a much larger relative stimulation of soil respiration did occur due to the greater availability of roots and decomposable substrates.

The increase of soil CO_2 emissions from both auto- and heterotrophic components was related to the enhancement of plants photosynthetic activity, to the increase in below-ground productivity, and to the consequent greater flux of fixed C from plants to soil. The consequent increase of rhizospheric activity, comprehensive of roots and rhizo-microbial respiration, caused higher soil CO_2 effluxes in FACE soil.

N addition did not influence soil respiration rates, and the increase under elevated [CO_2] did not depend on the nutritional *status* of the soil and was evident in both fertilized and not fertilized soils. Indeed, N was the limiting factor in microbial C utilization rates and the heterotrophic positive response to [CO_2] enrichment in the rhizosphere was in general favoured by a greater N concentration in microbial cells.

The influence of root activity had also a positive impact on soil Q_{10}: warm temperatures and higher photosynthetic rates, coupled in the vegetative period, induced a positive feedback on soil respiration under elevated [CO_2], independently of the soil nutrient *status*. The increase of soil respiration with temperature was indeed strongly dependent on living roots, whereas the Q_{10} of the heterotrophic component did not change in response to [CO_2] enrichment. N availability was instead the main determinant of heterotrophic Q_{10} increase, because of fast growing microbial communities, favoured in fertilized soil.

Greater root biomass and labile C compounds availability induced higher heterotrophic respiration rates only in the rhizosphere, as demonstrated by the lack of [CO_2] enrichment effect on microbial potential respiration in the absence of a direct root influence. A lower quality of labile C pools and the consequent changes in microbial communities selected a more efficient microbial biomass, dominated by fungal populations and characterized by a slower metabolism.

The N addition determined a seasonal increase in microbial decomposition activity due to a fertilizer addition priming effect, and to related changes in microbial N concentration, that however had a short-term impact on C storage. Moreover, the interaction between elevated [CO_2] and fertilization did not induce any modification on microbial SOM decomposition activity as a result of similar substrate quality and microbial N.

From these evidences we conclude that the elevated [CO_2] enhancement of autotrophic and heterotrophic respiration was more than balanced by the greater C input from plants to soil, and that the increase of labile C fractions was the net result of these processes, suggesting a positive trend of C storage in FACE soil in the longer-term.

12. REFERENCES

Agnelli A., Ascher J., Corti G., Ceccherini M.T., Nannipieri P., Pietramellara G., 2004. Distribution of microbial communities in a forest soil profile investigated by microbial biomass, soil respiration and DGGE of total and extracellular DNA. Soil Biology & Biochemistry 36, 859-868.

Allen A.S., Andrews J.A., Finzi A.C., Matamala R., Richter D.D., Schlesinger W.H., 2000. Effects of Free Air CO2 Enrichment (FACE) on belowground processes in a Pinus Taeda forest. Ecological Applications 10, 437-448.

Allen A.S., Schlesinger W.H., 2004. Nutrients limitations to soil microbial biomass and activity in loblolly pine forests. Soil Biology and Biochemistry 36, 581-589.

Amundson R., Stern L., Baisden T., Wang Y., 1998. The isotopic composition of soil and soil-respired CO_2. Geoderma 82, 83-114.

Anderson, J.M., Ingram, J.S.I., 1993. Colorimetric determination of ammonium. In: J.M. & Ingram, J.S.I. (Eds), Tropical Soil Biology and Fertility, a handbook of methods. Second Edition. Anderson,. CAB International, Wallingford, U.K, pp.73-74.

Anderson, T.H., 2003. Microbial eco-physiological indicators to assess soil quality. Agriculture, Ecosystems and Environment 98, 285-293.

Anderson, T.H., Domsch, K.H., 1989. Ratios of microbial biomass carbon to total organic-C in arable soils. Soil Biology and Biochemistry 21, 471-479.

Anderson, T.H., Domsch, K.H., 1990. Application of eco-physiological quotients (qCO_2 and qD) on microbial biomasses from soils of different cropping histories. Soil Biology and Biochemistry 22, 251-255.

Andrews J. A., Schlesinger W. H., 2001. Soil CO_2 dynamics, acidification, and chemical weathering in a temperate forest with experimental CO_2 enrichment. Global Biogeochemical Cycles 15, 149-162.

Aon, M.A., Cabello, M.N., Sarena, D.E., Colaneri, A.C., Franco, M.G., Burgos, J.L., Cortassa S., 2001. I. Spatio-temporal patterns of soil microbial and enzymatic activities in an agricultural soil. Applied Soil Ecology 18, 239-254.

Arnebrant K., Baath E., Soderstrom B., Nohrsted H. O., 1996. Soil microbial activity in eleven Swedish coniferous forests in relation to site fertility and nitrogen fertilization. Scandinavian Journal of Forest Research 11, 1-6.

Bääth E and Wallander H 2003 Soil and rhizosphere microorganisms have the same Q_{10} for respiration in a model system. Glob. Change Biol. 9, 1788-1791.

Badalucco, L., Grego, S., Dell'Orco, S., Nannipieri, P., 1992. Effect of liming on some chemical, biochemical and micro-biological properties of acid soil under spruce (*Picea abies* L.). Biology and Fertility of Soils 14, 76-83.

Badiane, N.N.Y., Chotte, J.L., Pate, E., Masse, D., Rouland, C., 2001. Use of soil enzyme activities to monitor soil quality in natural and improved fallows in semi-arid tropical regions. Applied soil ecology 18, 229-238.

Bandick, A.K., Dick, R.P., 1999. Field management effects on soil enzyme activities. Soil Biology & Biochemistry 31, 1471-1479.

Baral A., Guha G.S., 2004. Trees for carbon sequestration or fossil fuel substitution: the issue of cost vs. Carbon bnefit. Biomass and Bioenergy 27, 41-55.

Barnard R., Barthes L., Le Roux X., Harmens H., Raschis A., Soussana J. F., Winkler B., Leadley P. W., 2004. Atmospheric CO_2 elevation has little effect on nitrifying and denitrifying enzyme activity in four European grasslands. Global Change Biology 10, 488-497.

Barnard R., Barthes L., Le Roux X., Leadley P. W., 2004. Dynamics of nitrifying activities, denitrifying activities and nitrogen in grassland mesocosms as altered by elevated CO_2. New Phytologist 162, 365-376.

BassiriRad H., Griffin K. L., Reynolds J. F. and Strain B. R.. 1997. Changes in root NH_4 and NO_3 absorption rates of loblolly and ponderosa pine in response to CO_2 enrichment. Plant and Soil 190: 1–9.

Batjes NH., 1996. The total C and N in soils of the world. European Journal of Soil Science 47, 151– 63.

Bazin M.J., Markham P., Scott E.M., Lynch J.M., 1990. Population dynamics and rhizosphere interactions. In: The Rhizosphere, ed. J.M.Lynch, pp. 99-127. John Wiley, New York.

Beauchamp E. G., Reynolds W. D., Brasche-Villeneuve D., Kirby K., 1986. Nitrogen mineralization kinetics with different soil pretreatments and cropping histories. Soil Science Society American Journal 50, 1478-1483.

Berntson G.M., Bazzaz F.A., 1996. Belowground positive and negative feedbacks on CO_2 growth enhancement. Plant and Soil 187, 119-131.

Berntson, G. M., Bazzaz F. A., 1998. Regenerating temperate forest mesocosms in elevatedCO2:Belowground growth and nitrogen cycling. *Oecologia* 113, 115-125.

Blevins R. L., Thomas G. W., Smith M. S., Frye W. W., Cornelius P. L., 1983. Changes in soil properties after 10 years of continuous non-tilled and conventionally-tilled corn. Soil Tillage and Research 3, 135-136.

Bloom A J and Caldwell R M 1988 Root excision decreases nutrient absorption and gas fluxes. Plant physiol. 87, 794-796.

Boone R D, Nadelhoffer K J, Canary J D and Kaye J P 1998 Roots exert a strong influence on the temperature sensitivity of soil respiration. Nature 396, 570-572.

Borken W, Xu Y, Davidson E A and Beese F 2002 Site and temporal variation of soil respiration in European beech, Norway spruce, and Scots pine forests. Glob. Change Biol 8, 1205-1216.

Bottner P., Cortez J., Sallih Z., 1991. Effect of living roots on carbon and nitrogen of the soil microbial biomass. In: Atkinson D. (Ed.). British Ecological Society Special Publication 10. Blakwell Scientific Publications, Oxford, pp. 201-210.

Bowden R.D., Nadelhoffer K.J., Boone R.D., Melillo J.M., Garrison J.B., 1993. Contributions of above-ground litter, belowground litter, and root respiration to total soil respiration in a temperate mixed hardwood forest. Canadian Journal of Forest Research 23, 1402-1407.

Brady N.C., Wail R.R., 1999. The nature and properties of soils, 12th edn. Prentice-Hall, New Jersey.

Bremner J. M., Mulvaney C. S., 1982. Nitrogen-Total. In: Page A. L., Miller R. H., Keeney D. R. (Eds.). Methods of soil analysis Part 2. Chemical and microbiological properties. American Society of Agronomy, Inc., Soil Science Society of America, Inc., Madison, Wisconsin, USA. pp. 595-624.

Bronick C. J., Lal R., 2005. Soil structure and management: a review. Geoderma 124, 3-22.

Brookes, P.C., 1995. The use of microbial parameters in monitoring soil pollution by heavy metals. Biol. Fert. Soils 19, 269-279.

Brown S., 1996. Management of forests for mitigation of greenhouse gas emissions. In Climatic Change 1995. Impacts, Adaptations and Mitigation of Climate Change: Scientific-technical Analyses (eds. J. T. Houghton, G. J. Jenkins, and J. J. Ephraums). Cambridge University Press, Cambridge, pp. 773–797.

Brumme R., 1995. Mechanisms of carbon and nutrient release and retention in beech forest gaps. Plant and Soil 168/169, 593-600.
Buchmann N., 2000. Biotic and abiotic factors controlling soil respiration rates in *Picea abies* stands. Soil Biol. Biochem. 32, 1625-1635.
Burford J.R., Bremner J.M., 1975. Relationships between the denitrification capacities of soils and total, water-soluble and readily decomposable soil organic matter. Soil Biology and Biochemistry, 7: 389-394.
Caesar-TonThat T. C., 2002. Soil binding properties of mucilage produced by a basidiomycete fungus in a model system. Mycological Research 106, 930-937.
Calfapietra C., Gielen B., Sabatti M., De Angelis P., Scarascia Mugnozza G., Ceulemans R., 2001. Growth performance of *Populus* exposed to "Free Air Carbon dioxide Enrichment" during the first growing season in the POPFACE experiment. Ann. For. Sci. 58, 819-828.
Calfapietra C., Tulva I., Eenslu E., Perez M., De Angelis P., Scarascia Mugnozza G., Kull O., 2005. Canopy profiles of photosynthetic parameters under elevated CO2 and N fertilization in a poplar plantation. Environmental pollution, in press
Calfapietra, C., Gielen, B., Galema, A.N.J., Lukac, M., De Angelis, P., Moscatelli, M.C., Ceulemans, R., Scarascia-Mugnozza, G., 2003. Free-air CO_2 enrichment (FACE) enhances biomass production in a short-rotation poplar plantation (POPFACE). Tree Physiology 23, 805-814.
Campbell C.D., Chapman S.J., Cameron C.M., Davidson M.S., Potts J.M., 2003. A rapid microtiter plate method to measure carbon dioxide evolved from carbon substrates amendments so as to determine the Physiological Profiles of Soil Microbial Communities by using the whole soil. Applied and environmental microbiology, 3593-3599.
Canadell J.G., Pitelka L.F., Ingram J.S.I., 1996. The effects of elevated [CO_2] on plant-soil carbon below-ground: a summary and synthesis. Plant and Soil 187, 391-400.
Cardon Z.G., Hungate B.A., Cambardella C.A., Chapin III F.S., Field C.B., Holland E.A. Mooney H.A., 2001. Contrasting effect of elevated CO_2 on old and new soil carbon pools. Soil Biology and Biochemistry 33, 365-373.
Cardon, Z.G., 1996. Influence of rhizodepositions under elevated CO_2 on plant nutrition and soil organic matter. Plant and Soil 187, 277-288.
Carnol M., Hogenboom L., Jach M. E., Remacle J., Ceulemans R., 2002. Elevated atmospheric CO_2 in open top chambers increases net nitrification and potential denitrification. Global Change Biology 8, 590-598.
Cataldo, D.A., Haroon, M., Schrader, L.E., Young, V., 1975. Rapid colorimetric determination of nitrate in plant tissue by nitration of salycilic acid. Communications in Soil Science and Plant Analysis 6, 71-80**.**
Cerling T. E., 1984. The stable isotopic composition of modern soil carbonate and its relationship to climate. Earth and Planetary science letters 71, 229-240.
Ceulemans R., Janssens I.A., Jach M.E., 1999. Effects of CO_2 enrichment on Trees and Forests: Lessons to be Learned in View of Future Ecosystem Studies. Annals of Botany 84, 577-590.
Chadwick O. A., Derry L. A., Vitousek P. M., Hedin L. O., 1999. Changing sources of nutrients during four million years of ecosystem development. Nature 397, 491-497.
Chapin F S and Tryon P R 1982 Phosphate absorption and root respiration of different plant growth forms from northern Alaska. Holoarctic Ecol 5, 164-171.
Cheng W., Zhang Q., Coleman D. C., Carroll C. R., Hoffman C. A., 1996. Is available carbon limiting microbial respiration in the rhizosphere? Soil Biol. Biochem. 28, 1283-1288.

Cheng W., Johnson D.W., 1998. Elevated CO_2, rhizosphere processes, and soil organic matter decomposition. Plant Soil 202, 167-174.
Cheng W., Johnson D.W., Fu S., 2003. Rhizosphere effects on decomposition: controls of plant species, phenology, and fertilization. Soil Science Society of America Journal 67, 1418-1427.
Cheng W., Zhang Q., Coleman D. C., Carroll C. R., Hoffman C. A., 1996. Is available carbon limiting microbial respiration in the rhizosphere? Soil Biology and Biochemistry 28, 1283-1288.
Cheng, W.X., 1999. Rhizosphere feedbacks in elevated CO_2. Tree physiology 19, 313-320.
Christ M. J., David M. B., 1996. Temperature and moisture effects on the production of dissolved organic carbon in a spodosol. Soil Biology and Biochemistry 28, 1191-1199.
Ciais P., Reichstein M., Viovy N., Granier A., Oge´e J., Allard V., Aubinet M., Buchmann N., Bernhofer C., Carrara A., Chevallier F., De Noblet N., Friend A. D., Friedlingstein P., Grunwald T., Heinesch B., Keronen P., Knohl A., Krinner G., Loustau D., Manca G., Matteucci G., Miglietta F., Ourcival J. M., Papale D., Pilegaard K., Rambal S., Seufert G., Soussana J. F., Sanz M. J., Schulze E. D., Vesala T., Valentini R., 2005. Europe-wide reduction in primary productivity caused by the heat and drought in 2003. Nature 437, 529-533.
Coleman, D.C., Crossley, D.A. jr, 1996. Fundamentals of Soil Ecology. Academic Press Limited, London.
Cotrufo M. F., De Angelis P., Polle A., 2005. Leaf litter production and decomposition in a poplar short-rotation coppice exposed to free air CO_2 enrichment (POPFACE). Global Change Biology 11, 1-12.
Cotrufo M. F., Ineson P., Scott A., 1998. Elevated CO_2 reduces the nitrogen concentration of plant tissues. Global Change Biology 4, 43-54.
Couteaux M. M., Mousseau M., Celerier M. L., Bottner P., 1991. Increased atmospheric CO_2 and litter quality : decomposition of sweet chestnutleaf litter with animal food webs of different complexities. Oikos 61, 54-65.
Cropper W P and Gholz H L 1991 *In situ* needle and fine root respiration in mature slash pine (*Pinus elliottii*) trees. Can. J. Forest Res. 21, 1589-1595.
Dahlgren R. A., McAvoy D. C., Driscoll C. T., 1990. Acidification and recovery of a spodosol Bs horizon from acidic depositions. Environmental Science and Technology 24, 531-537.
Darrah P. R., 1991a. Models of the rhizosphere. I: Microbial population dynamics around a root releasing soluble and insoluble carbon. Plant Soil 133,187-199.
Darrah P. R., 1991b. Models of the rhizosphere. II: A quasi threedimensional simulation of the microbial population dynamics around a root releasing soluble exudates. Plant Soil 133,187-199.
Davidson E. A., Belk E., Boone R. D., 1998. Soil water content and temperature as independent or confounded factors controlling soil respiration in a temperate mixed hardwood forest. Glob. Change Biol. 4, 217-227.
Davidson E. A., Savage K., Bolstad P., Clark D. A., Curtis P. S., Ellsworth D. S., Hanson P. J., Law P. E., Luo Y., Pregitzer K. S., Randolph J. C., Zak D., 2002. Belowground carbon allocation in forests estimates from litterfall and IRGA-based soil respiration measurements. Agric. For. Meteorol. 113, 39-51.
De Angelis, P., Chigwerewe, K.S., Scarascia Mugnozza, G., 2000. Litter quality and decomposition in a CO_2-enriched forest ecosystem. Plant and Soil 224 (2), 31-41.
De Lucia E.H., Hamilton J.G., Naidu S.L., Thomas R.B., Andrews J.A., Finzi A., Lavine M., Matamala R., Mohan J.E., Hendrey G.R., Schlesinger W.H., 1999. Net primary

production of a forest ecosystem with experimental CO2 enrichment. Science 284, 1177-1179.
De Lucia, E.H., Callaway, R.M., Thomas, E.M., Schlesinger, W.H., 1997. Mechanisms of phosphorus acquisition for ponderosa pine seedlings under high CO2 and temperature. Annals of Botany 79, 111-120.
Debosz K., Rasmussen P. H., Pedersen A. R., 1999. Temporal variations in microbial biomass C and cellulolytic enzyme activity in arable soils: effects of organic matter input. Applied Soil Ecology 13, 209-218.
Debosz K., Vognsen L., Labouriau R., 2002. Carbohydrates in hot water extracts of soil aggregates as influenced by long-term management. Commun. Soil Science Plant Anal. 33, 623-634.
Degens B.P., 1999. Catabolic response profiles differ between microorganisms grown in soils. Soil Biol. Biochem. 31, 475-477.
Del Galdo I., De Angelis P., Cotrufo M. F., 2004. Land-use change and increasing atmospheric CO_2 concentration: how they act on soil organic matter pools? Presentation at the International FACE workshop "Short- and long-term effects of elevated atmospheric CO_2 on managed ecosystems", Ascona (Svizzera) 20-25 Marzo 2004.
Demetz M., Insam H., 1999. Phosphorus availability in a forest soil determined with a arespiratory assay compared to chemical methods. Geoderma 89, 259-271.
Dewar R.C., Cannell M.G.R., 1992. Carbon sequestration in the trees, products and soils of forest plantations: an analysis using UK examples. Tree Physiology 11, 49–71.
Diaz S.A., Grime J.P., Harris J, McPherson E., 1993. Evidence of a feedback machanism limiting plant response to elevated carbon dioxide. Nature 364, 616-617.
Dick W. A., Tabatabai M. A., 1993. Significance and potential use of soil enzymes. In: F. Blaine (Eds) Soil Microbial Ecology. Marcel Dekker. NY.
Dilly O., Munch J.C., 1998. Ratios between estimates of microbial biomass content and microbial activity in soils. Biol. Fert. Soils 27, 374-379.
Dimoyiannis D. G., Tsadilas C. D., Valmis S., 1998. Factors affecting aggregate instability of Greek agricultural soils. Commun. Soil Science Plant Anal. 29, 1239-1251.
Dixon R. K., Brown S., Houghton R. A., Solomon A. M., Trexler M. C., Wisniewski J., 1994. Carbon pools and flux of global forest ecosystem. Science 263, 185-190.
Dommergues, Y., 1960. La notion de coefficient de minéralisation du carbone dans le sols. L'Agronomie tropicale XV n. 1, 54-60.
Drake E. H., Motto H. L., 1982. An analysis of the effect of clay and organic matter content on the cation exchange capacity of New Jersey soils. Soil Science 133, 281-288.
Dumontet S., Mazzatura A., Casucci C., Perucci P., 2001. Effectiveness of microbial indexes in discriminating interactive effects of tillage and crop rotations in a Vertic Ustorthens. Biology and Fertility of soils 34, 411-416.
Ebersberger, D., Niklaus, P., Kandeler, E., 2003. Long term CO_2 enrichment stimulates N-mineralisation and enzyme activities in calcareous grassland. Soil Biology & Biochemistry 35, 965-972.
Edwards N.T., Harris W.F., 1977. Carbon cycling in a mixed deciduous forest floor. Ecology 58,431-437.
Edwards N.T., Norby R.J., 1999. Below-ground respiratory response of sugar maple and red maple saplings to atmospheric CO_2 enrichment and elevated air temperature. Plant and Soil 206, 85-97.
Ekblad A., Hodberg P., 2001. Natural abundance of ^{13}C in CO_2 respired from forest soils reveals speed of link between tree photosynthesis and root respiration. Oecologia 127, 305-308.

Eliasson P. E., McMurtrie R. E., Pepper D. A., Stromgren M., Linder S., Agren G. I., 2005. The response of heterotrophic CO2 flux to soil warming. Global Change Biology 11, 167-181.

Elsgaard, L., Andersen, G.H., Eriksen, J., 2002. Measurement of arylsulphatase activity in agricultural soils using a simplified assay. Soil Biology & Biochemistry 34,79-82.

El-Shatnawi M K J and Makhadmeh I M 2001 Ecophysiology of the Plant-Rhizosphere System. J. Agron. Crop Sc. 187, 1-9.

Epron D., Le Dantec V., Dufrene E., Granier A., 2001. Seasonal dynamics of soil carbon dioxide efflux and simulated rhizosphere respiration in a beech forest. Tree physiol. 21, 145-152.

Eswaran H, Van den Berg E, Reich P, Kimble J., 1995. Global soil carbon resources. In: Lal R, Kimble JM, Levine E, Stewart BA, editors. Soils and global change, pp. 27–43. Boca Raton: CRC/Lewis.

Ewel K.C., Cropper W.P. Jr, Gholz H.L., 1987. Soil CO_2 evolution in Florida slash pine plantations. II. Importance of root respiration. Canadian Journal of Forest Research. 17, 330-333.

Fang C., Moncrieff J. B., 2001. The dependence of soil CO_2 efflux on temperature. Soil Biol. Biochem. 33, 155-165.

Fang C., Smith P., Moncrieff J.B., Smith J.U., 2005. Similar response of labile and resistant soil organic matter pools to changes in temperature. Nature 433, 57-59.

FAO, 1995. Forest products 1991-1995. Yearbook, FAO Forestry Series n°30. FAO statistic series n°137. Roma, Italy: FAO, 57 pp.

FAO, 2001. Soil carbon sequestration for improved land management. World Soil Resources Reports n°96. Rome, Italy.

Felske A., Engelen B., Nübel U., Backhaus H., 1996. Direct ribosomal isolation from soil to extract bacterial rRNA for community analysis. Applied Environonmental Microbiology 62, 4162–4167.

Field C B, Chapin III F S, Matson P A and Mooney H A 1992 Responses of terrestrial ecosystems to the changing atmosphere: a resource-based approach. Annu. Rev. Ecol. Syst. 23, 201-235.

Finzi A. C., Allen A. S., DeLucia E. H., Ellsworth D. S., Schlesinger W. H., 2001. Forest litter production, chemistry, and decomposition following two years of free-air CO_2 enrichment. Ecology 82, 470-484.

Flanagan P. W., Van Cleve K., 1983. Nutrient cycling in relation to decomposition and organic matter quality in taiga ecosystems. Canadian Journal of Forest Research 13, 795-817.

Fokin A. D., Radzhabova P. A., 1996. Availability of phosphates in soils as a function of the state and transformation of organic matter. Eurasian Soil Science 29, 1216-1221.

Folland C.K. and Karl T.R., 2001. Observed climate variability and change. In: Climate change 2001: The Scientific Basis. Pp. 99-181. Intergovernmental Panel on Climate Change, Cambridge Univ. Press, Cambridge, U.K.

Fontaine S, Mariotti A and Abbadie L 2003 The priming effect of organic matter: a question of microbial competition? Soil Biol. Biochem. 35, 837-843.

Foster N. W., Beauchamp E. G., Corke C. T., 1980. Microbial activity in a *Pinus banksiana* Lamb. forest floor amended with nitrogen and carbon. Canadian Journal of Soil Science 60, 199-209.

Franzluebbers A. J., 1999. Potential C and N mineralization and microbial biomass from intact and increasingly disturbed soils of varying texture. Soil Biology and Biochemistry 31, 1083-1090.

French D.D., 1988. Some effects of changing soil chemistry on decomposition of plant litters and cellulose on a Scottish moor. Oecologia 75, 608-618.
Gebauer R.L.E., Strain B.R., Reynolds J.F., 1998. The effect of elevated CO2 and N availability on tissue concentrations and whole plant pools of carbon-based secondary compounds in loblolly pine (Pinus Taeda). Oecologia 113, 29-36.
Ghani A., Dexter M., Perrott K. W., 2003. Hot-water extractable carbon in soils: a sensitive measurement for determining impacts of fertilization, grazing and cultivation. Soil Biology and Biochemistry 35, 1231-1243.
Giardina C. P., Ryan M. G., Binkley D., Fownes J. H., 2003. Primary production and carbon allocation in relation to nutrient supply in a tropical experiment forest. Global Change Biology 9, 1438-1450.
Giardina C. P., Ryan M. G., 2000. Evidence that decomposition rates of organic carbon in mineral soil do not vary with temperature. Nature 404, 858-861.
Gielen B., Calfapietra C., Lukac M., Wittig V. E., De Angelis P., Janssens I. A., Moscatelli M. C., Grego S., Cotrufo M. F., Godbold D.L., Hoosbeck M. R., Long S. P., Miglietta F., Polle A., Bernacchi C. J., Davey P. A., Ceulemans R., Scarascia Mugnozza G., 2005. Net carbon storage in a poplar plantation (POPFACE) after three years of free-air CO_2 enrichment. Tree Physiology 25, 1399-1408.
Gielen B., Ceulemans R., 2001. The likely impact of rising atmospheric CO_2 on natural and managed *Populus*: a literature review. Environmental Pollution 115, 335-358.
Gillman G. P., 1979. A proposed method for the measurement of exchange properties of highly weathered soils. Australian Journal of Soil Research. 17, 129-139.
Gomes N.C.M., Fagbola O., Costa R., Rumjanek N.G., Buchner A., Mendona-Hagler L., Smalla C., 2003. Dynamics of fungal communities in bulk and Maize rhizosphere soil in the Tropics. Applied and Environmental Microbiology 69, 3758-3766.
Gorissen A., Cotrufo M. F., 1999. Elevated carbon dioxide effects on nitrogen dynamics in grasses, with emphasis on rhizosphere processes. Soil Science Society of America Journal 63, 1695-1702.
Gorissen A., Van Ginkel J. H., Keurentjes J. J. B., Van Veen J. A., 1995. Grass root decomposition is retarded when grass has been grown under elevated CO_2. Soil Biology and Biochemistry 27, 117-120.
Graham R.L., Wright L.L., Turhollow A.F., 1992. The potential for short-rotation woody crops to reduce US CO2 emissions. Climatic Change 22, 223–38.
Grayston S. J., Campbell C. D., 1996. Functional biodiversity of microbial communities in the rhizosphere of Hybrid larch (*Larix eurolepis*) and Sitka spruce (*Picea sitchensis*). Tree Physiol. 16, 1031-1038.
Graystone S.J., Campbell C.D., Lutze J.L., Gifford R.M., 1998. Impact of elevated CO2 on the metabolic diversity of microbial communities in N-limited grass swards. Plant and Soil 203, 289-300.
Griffiths B.S., Ritz K., Ebblewhite N., Paterson E., Killham K., 1998. Ryegrass rhizosphere microbial community structure under elevated carbon dioxide concentrations, with observation of wheat rhizosphere. Soil Biol. Biochem. 30, 315-321.
Gu L, Post W M and King A W 2004 Fast labile carbon turnover obscures sensitivity of heterotrophic respiration from soil to temperature: A model analysis. Global Biogeochem. Cycles 18, GB1022, doi:10.1029/2003GB002119
Gu L, Shugart H H, Fuentes J D, Black T A and Shewchuck S R 1999 Micrometeorology, biophysical exchanges and NEE decomposition in a two story boreal forest : Development and test of an integrated model. Agric. For. Meteorol. 94, 123-148.

Guo L.B., Gifford R.M., 2002. Soil carbon stocks and land use change: a meta analysis. Global Change Biology 8, 345-360.
Gupta, R.K. and Rao, D.L.N. 1994. Potential of wastelands for sequestering carbon by reforestation. *Curr. Sci*. 66: 378–380.
Hagedorn F., Spinnler D., Bundt M., Blaser P., Siegwolf R., 2003. The input and fate of new C in two forest soils under elevated CO_2. Global Change Biology 9, 862-872.
Hagedorn F., Maurer S., Egli P., Blaser P., Bucher J.B., Siegwolf R., 2001. Carbon sequestration in forest soils: effects of soil type, atmospheric CO_2 enrichment and N deposition. European Journal of Soil Science 52, 619-628.
Hall D.O., Mynick H.E., Williams R.H., 1991. Cooling the greenhouse with bioenergy. Nature 353, 11–20.
Hamilton J. G., Thomas R. B., DeLucia E. H., 2001. Direct and indirect effects of elevated CO_2 on leaf respiration in a forest ecosystem. Plant Cell Environment 24, 975-982.
Hamilton J.G., De Lucia E.H., Naidu K.G.S.L., Finzi A.C., Schlesinger W.H., 2002. Forest carbon balance under elevated CO2. Oecologia 131, 250-260.
Hanson P.J., Edwards N.T., Garten C.T., Andrews J.A., 2000. Separating root and soil microbial contributions to soil respiration: A review of methods and obsrevations. Biogeochemistry 48, 115-146.
Harley P. C., Thomas R. B., Reynolds J. F., Strain B. R., 1992. Modelling of photosynthesis of cotton grown in elevated CO2. Plant, Cell and Environment 15, 271-282.
Harte J., Kinzig A.P., 1993. Mutualism and competition between plants and decomposers: implications for nutrient allocation in ecosystems. American Naturalist 141, 829-846.
Hartley, S.E., Jones, C.G., Couper, G.C., Jones H.T., 2000. Biosynthesis of plant phenolic compounds in elevated atmospheric CO_2 . Global Change Biology 6, 497-506.
Hasenkamp K.P., 1992. Global reforestation to solve the problem of CO2: or mankind will be burning the wrong tree until it finds the right one. In: Yearbook of renewable energies, vol. I. Bochum, Germany: Ponte Press.
Haynes R. J., Naidu R., 1998. Influence of time, fertilizer and manure applications on soil organic matter content and soil physical conditions: a review. Nutrient cycling Agroecosystem 51, 123-137.
Haynes R.J. 1986. The decomposition process: mineralization, immobilization, humus formation and degradation. In: Mineral nitrogen in the plant-soil system. Physiological Ecology, A series of monographs, text and treatises (Kozlowski T.T. Ed.),pp 52-109. Academic Press, Inc.
Healy R. W., Striegl R. G., Russell T. F., Hutchinson G. L., Livingston G. P., 1996. Numerical evaluation of static-chamber measurement of soil-atmosphere gas exchange: identification of physical processes. Soil Science Society of America Journal 60, 740-747.
Heath J., Ayres E., Possell M., Bardgett R. D., Black H. I. J., Grant H., Ineson P., Kerstiens G., 2005. Rising atmospheric CO_2 reduces sequestration of root-derived soil carbon. Science 309, 1711-1713.
Heilmeier H., Erhard M., Schulze E. D., 1997. Biomass allocation and water use under arid conditions. In: Plant Resource Allocation (Bazza F. A., Grace J., eds.), pp. 93-112. Academic Press, San Diego, CA.
Helal H. M., Sauerbeck D., 1986. Effect of plant roots on carbon metabolism of soil microbial biomass. Zeitschrift für Pflanzenernährung und Bodenkunde 149, 181-188.
Henriksen T. M., Breland T. A., 1999. Nitrogen availability affects on carbon mineralization, fungal and bacterial growth, and enzyme activities during decomposition of wheat straw in soil. Soil Biology and Biochemistry 31, 1121-1134.

Himes, F.L. 1998. Nitrogen, sulfur and phosphorus and the sequestering of carbon. In: *Soil Processes and the Carbon Cycle*, pp. 315–319, Lal, R., Kimble, J.M., Follett, R.F., and Stewart, B.A., Eds., CRC, Boca Raton, FL.

Hodge A., Paterson E. Greystone S. Campbell C., Ord B.G., Killham K., 1998. Characterisation and microbial utilisation of exudate material from the rhizosphere of *Lolium Perenne* grown under CO2 enrichment. Soil Biology and Biochemistry 28, 47-54.

Hodge A., Robinson D., Fitter A., 2000. Are microorganisms more effective than plants at competing for nitrogen? Trends in plant science 5, 304-308.

Hogberg P., Nordgren A., Buchmann N., Taylor A.F.S., Ekblad A., Hogberg M.N., Nyberg G., Ottoson-Lofvenius M., Read D.J., 2001. Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature 411, 789-792.

Holland E. A., Coleman D. C., 1987. Litter placement effects on microbial and organic matter dynamics in an agroecosystem. Ecology 68, 425-433.

Holland H. D., Lazar B., McCaffrey M., 1986. Evolution of the atmosphere and oceans. Nature 320, 27-33.

Hoosbeek M R, Lukac M, van Dam D, Godbold D L, Velthorst E J, Biondi F A, Peressotti A, Cotrufo M F, De Angelis P and Scarascia Mugnozza G 2004 More new carbon in the mineral soil of a poplar plantation under Free Air Carbon Enrichment (POPFACE): Cause of increased priming effect? Global Biogeochem. Cycles 18, GB1040, doi:10.1029/2003GB002127.

Houghton R. A., 1996. Converting terrestrial ecosystems from sources to sinks of carbon. Ambio 25 , 267–272.

Houghton R. A., 2003. The contemporary carbon cycle. In: Treatise on Geochemistry. ISBN, pp. 473-513.

Houghton R.A., Ackler J.L., Lawrence K.T., 1999. The U.S. carbon budget: contributions from land use change. Science 285, 574-578.

Houghton, R. A., Joos F., and Asner G. P., 2004. The effects of land use and management on the global carbon cycle. In: Gutman, G., editor, Land Change Science: Observing, Monitoring, and Understanding Tranjectories of Change on the Earth's Surface, *Springer*, E-book ISBN: 1-4020-2562-9.

Hu S., Firestone M.K., Chapin III F.S., 1999. Soil microbial feedbacks to atmospheric CO_2 enrichment. Trends in ecology and evolution 14, 433-437.

Hu S., Chapin III, Firestone M.K., Field C.B., Chiariello N.R., 2001. Nitrogen limitation of microbial decomposition in a grassland under elevated CO2. Nature 409, 188-191.

Hu S., van Bruggen A. H. C., 1997. Microbial dynamics associated with multiphasic decomposition of ^{14}C-labeled cellulose in soil. Microbial Ecology 33, 134-143.

Hu S., Zhang W., 2004. Impact of global change on biological processes in soil: Implications for agroecosystem management. Journal of crop improvement 12, 289-314.

Hungate B. A., Chapin F. S. III, Zhong H., Holland E. A., Field C. B., 1997. Stimulation of grassland nitrogen cycling under carbon dioxide enrichment. Oecologia 109,149-153.

Hungate B. A., Dijkstra P., Johnson D. W., Hinkle C. R., Drake B. G., 1999. Elevated CO_2 increases nitrogen fixation and decreases soil nitrogenmineralization in Florida scrub oak. Global Change Biology 5, 781-489.

Hungate B. A., Dukes J. S., Shaw M. R., Luo Y., Field C. B., 2003. Nitrogen and Climate Change. Science 302, 1512-1513.

Hungate B. A., Jackson R. B., Field C. B., Chapin III F. S., 1996. Detecting changes in soil carbon in CO_2 enrichment experiments. Plant and Soil 187, 135-145.

Hungate B.A., Chapin III F.S., 1995. Terrestrial ecosystem response to elevated CO_2: effects on microbial N transformations across gradients of nutrients availability. GCTE Focus 1 Workshop. Lake Tahoe, California, USA.

Hungate B.A., Jaeger C.H., Gamara G., Chapin III F.S., Field C.B., 2000. Soil microbiota in two annual grasslands: responses to elevated atmospehric CO_2. Oecologia, 124:589-598.

Hungate B.A., Lund C., Pearson H.L., Chapin III F.S., 1997. Elevated CO_2 and nutrient addition alter soil C cycling and N trace gas fluxes with early season wet-up in a California annual grassland. Biogeochemistry 37, 89-109.

Ineichen K., Wiemken V., Wiemken A., 1995. Shoots, roots and ectomycorrhizal formation of pine seedlings at elevated atmospheric carbon dioxide. Plant Cell Environ. 18, 703-707.

Ineson P., Cotrufo M.F., Bol R. et al., 1996. Quantification of soil C inputs under elevated CO_2 : C_3 plants in a C_4 soil. Plant and Soil 187, 345-350.

Insam H., Baath E., Berreck M., Frostegard A., Gerzabek M.H., Kraft A., Schinner F., Schweiger P., Tschuggnall G., 1999. Responses of the soil microbiota to elevated CO2 in an artificial tropical ecosystem. Journal of microbiological methods 36, 45-54.

Insam H., Goberna M., 2004. Community level physiological profiles (Biolog substrate use tests) of environmental samples. In: Akkermans ADL, van Elsas JD, DeBruijn FJ (eds): Molecular Microbial Ecology Manual, Second Edition, Kluwer Academic Publishers, 5.3.2: 1-8.

Intergovernmental Panel on Climate Change, 2000. Land Use, Land Use Change and Forestry. Special Report, Cambridge Univ. Press, Cambridge, U.K.

Intergovernmental Panel on Climate Change, 2001. Climate change: The Scientific Basis. Cambridge Univ. Press, Cambridge, U.K.

Irvine J., Law B. E., Kurpius M. R., 2005. Coupling of canopy gas exchange with root and rhizosphere respiration in a semi-arid forest. Biogeochemistry 73, 271-282.

Janssens I. A., Ceulemans R., 2000. The response of soil CO_2-efflux under trees grown in elevated atmospheric CO_2: a literature review. Phyton-Annales Rei Botanicae 40, 97-101.

Janssens, I.A., Crookshanks, M., Taylor, G., Ceulemans, R., 1998. Elevated atmospheric CO_2 increases fine root production, respiration, rhizosphere respiration and soil CO_2 efflux in Scots pine seedlings. Global Change Biology 4, 871-878.

Janssens I. A., Lankreijer H., Matteucci G., Kowalski A. S., Buchmann N., Epron D., Pilegaard K., Kutsch W., Longdoz B., Grunwald T., Montagnani L., Dore S., Rebmann C., Moors E. J., Grelle A., Rannik U., Morgenstern K., Oltchev S., Clement R., Gudmundsson J., Minerbi S., Berbigier P., Ibrom A., Moncrieff J., Aubinet M., Bernhofer C., Jensen O., Vesala T., Granier A., Schulze E. D., Lindroth A., Dolman A. J., Jarvis P. G., Ceulemans R., Valentini R., 2001. Productivity overshadows temperature in determining soil and ecosystem respiration across European forests. Global Change Biology 7, 269-278.

Janssens I. A., Pilegaard K., 2003. Large seasonal changes in Q_{10} of soil respiration in a beech forest. Global Change Biology 9, 911-918.

Jastrow J. D., Miller R. M., Owensby C. E., 2000. Long-term effects of elevated atmospheric CO_2 on below-ground biomass and transformations to soil organic matter in grassland. Plant and Soil 224, 85-97.

Jenkinson D.S., Ladd J.N., 1981. Microbial biomass in soil: measurement and turnover. In: Paul E.A., Ladd J.N. (Eds.) Soil Biochemistry, vol.5. Dekker, New York, pp. 415-471.

Jobbàggy E. G. and Jackson R. B. (2000) The vertical distribution of soil organic carbon and its relation to climate and vegetation. Ecol. Appl. 10, 423–436.

Joergensen R G and Brookes P C 1990 Ninhydrin-reactive nitrogen measurements of microbial biomass in 0.5 M K2SO4 soil extracts. Soil Biol. Biochem. 22, 1023-1027.

Joergensen R. G., Anderson T. H., Wolters V., 1995. Carbon and nitrogen relationships in the microbial biomass of soils in beech (*Fagus sylvatica* L.) forests. Biology and Fertility of Soils 19, 141-147.

Johansson M., 2001. Composition of the saprotrophic fungi in *Calluna* heathland soil and the influence of ammonium nitrate application. Water, Air and Soil Pollution: Focus 1, 231-239.

Johnson J. D., Tognetti R. and Paris P., 2002. Water relations and gas exchange in poplar and willow under water stress and elevated atmospheric CO_2. Physiol. Plantarum 115, 93-100.

Jonasson S., Vestergarrd P., Jensen M., Michelsen A., 1996. Effects of carbohydrate amendments on nutrient partitioning, plant and microbial performance of grassland-shrub ecosystem. Oikos 75, 220-226.

Jones C. A., 1984. Estimation of an active fraction of soil nitrogen. Commun. Soil Science Plant Anal. 15, 23-32.

Jongern M., Jones M. B., Hebeisen T., Blum H., Hendrey G., 1995. The effects of elevated CO_2 concentrations on the root growth of *Lolium perenne* and *Trifolium repens* grown in a FACE system. Global Change Biology 1, 361-371.

Kelting D.L., Burger J.A., Edwards G.S., 1998. Estimating root respiration, microbial respiration in the rhizosphere, and root-free soil respiration in forest soils. . Soil Biology and Biochemistry. 30, 961-968.

Keyser P. T., Kirk K., Zeikus J. G., 1978. Ligninolytic enzyme system of *Phanerochaete chrysosporium*: synthesized in the absence of lignin in response to nitrogen starvation. Journal of bacteriology 135, 790-797.

Killham K., 1994. Soil Ecology, Cambridge University Press.

King J.S., Hanson P.J., Bernhardt E., De Angelis P., Norby R.J., Pregitzer K.S., 2004. A multiyear synthesis of soil respiration responses to elevated atmospheric CO_2 from four forest FACE experiments. Global Change Biology 10, 1027-1042.

King, J.S., Kurt, S., Pregitzer, S., Zak, D.R., Kubiske, M.E., Ashby, J.A., Holmes, W.E., 2001. Chemistry and decomposition of litter from Populus tremuloides Michaux grown at elevated atmospheric CO_2 and varying N availability. Global Change Biology 7,65-74.

King J. S., Albaugh T. J., Allen T. H. L., 2002. Below-ground carbon input to soil is controlled by nutrient availability and fine root dynamics in loblolly pine. New Phytologist 154, 389-398.

Kinney K.K., Lindroth R.L., 1997. Responses of three deciduous tree species to atmospheric CO_2 and N supply. Plant and Soil 224, 75-84.

Kirschbaum M.U.F., 1994. The sensitivity of C_3 photosynthesis to increasing CO_2 concentration. A theoretical analysis of its dependence on temperature and background CO_2 concentration. Plant, Cell Environment. 17, 747-754.

Kirschbaum M.U.F., 1995. The temperature dependence of soil organic matter decomposition, and the effect of global warming on soil organic carbon storage. Soil Biology and Biochemistry 27, 753-760.

Kirschbaum M.U.F., 2000. Will changes in soil organic carbon act as a positive or negative feedack on global warming?. Biogeochemistry. 48, 21-51.

Klamer M., Roberts M.S., Levine L.H., Drake B.G., Garland J.L., 2002. Influence of elevated CO_2 on the fungal community in a coastal Scrub Oak forest soil investigated with Terminal-Restriction Fragment Length Polymorphism Analysis. Applied and environmental microbiology 68, 4370-4376.

Knorr W., Prentice I. C., House J. I., Holland E. A., 2005. Long-term sensitivity of soil carbon turnover to warming. Nature 433, 298-301.
Kohlmaier G. H., Weber M., and Houghton R. A., 1998. Carbon Dioxide Mitigation in Forestry and Wood Industry. Springer, Berlin.
Körschen M., Müller A., 1994. Nachaltige Bodennutzung, gemessen am Ertrag sowie an C- und N- Bilanzen. Archiv für Acker-, Pflanzenbau und Bodenkunde 38, 373-381.
Kowalchuk G., 1999. Fungal community analysis using denaturing gradient gel elettrophoresis (DGGE). In: Akkermans A.D.L., van Elsas J.D., De Bruijn F.G. (Eds.), Molecular Microbial Ecology Manual, vol. 3.4.6. Kluwer Academic Publisher, Dordrecht, pp. 1-16.
Kubiske M.E., Pregitzer K.S., Zak D.R. et al., 1998. Growth and C allocation of Populus Tremuloides genotypes in response to atmospheric CO2 and soil N availability. New Phytologist 140, 251-260.
Kuikman P J, Lekkerkerk L J A and van Veen J A 1990 Carbon dynamics of a soil planted with wheat under elevated CO_2 concentration. In: Advances in Soil Organic Matter Research: The impact on Agriculture and the Environment. Ed. W S Wilson. pp 177-196. Academic Press, San Diego, USA.
Kutsch W. L., Staack A., Wotzel J., Middelhoff U., Kappen L., 2001. Field measurements of root respiration and total soil respiration in an alder forest. New Phytologist 150, 157-168.
Kuzyakov Y., 2002. Separating microbial respiration of exudates from root respiration in non-sterile soils: a comparison of four methods. Soil Biol. Biochem. 34, 1621-1631.
Kuzyakov Y., 2002. Review: Factors affecting rhizosphere priming effects. Journal of Plant Nutrition ans Soil Science 165, 382-396.
Kuzyakov Y., Cheng W., 2001. Photosynthesis controls of rhizosphere respiration and organic matter decomposition. Soil Biology and Biochemistry 33, 1915-1925.
Kuzyakov, Y., Friedel, J.K., Stahr, K., 2000. Review of mechanisms and quantification of priming effects. Soil Biol & Biochem 32, 1485-1498.
Lal R., 2003. Global potential of soil carbon sequestration to mitigate the greenhouse effect. Critical Reviews in Plant Sciences 22(2), 151-184.
Lal R., Kimble J.M., 1997. Conservation tillage for carbon sequestration. Nutrient Cycling in Agroecosystems 49, 243-253.
Lal, R., Kimble, J.M., 2000. Pedogenic carbonates and the global carbon cycle. In: Lal, R., Kimble, J.M., Eswaran, H., and Stewart, B.A., (Eds.), Global Climate Change and Pedogenic Carbonates, pp. 1–13. CRC/Lewis Publishers, Boca Raton, FL.
Lal, R. 2001. Potential of desertification control to sequester carbon and mitigate the greenhouse effect. *Climate Change* 15: 35–72.
Larson, J.L., Zak, D.R., Sinsabaugh, R.L., 2002. Extracellular enzyme activity beneath temperate trees growing under elevated carbon dioxide and ozone. Soil Science Society American Journal 66,1848-1856 .
Laudelot H., Thierron V., 1996. Contribution of root respiration to total CO_2 efflux from the soil of a deciduous forest. Canadian Journal of Forest Research 26, 1142-1148.
Lekkerkerk L.J.A., van De Geijn S.C., van Veen J., 1990. Effects of elevated atmospheric CO2 levels on the carbn economy of a soil planted with wheat. In: Bouwman A.F., ed. Soils and the greenhouse effect. New York, USA: John Wiley & Sons, 423-429.
Lewis J. D., Strain B. R., 1996. The role of mycorrhizal in the response of *Pinus taeda* seedlings to elevated CO_2. New phytologist 133, 431-443.
Li Y., Xu M., Sun O.J., Cui W., 2004. Effects of root and litter exclusion on soil CO_2 efflux and microbial biomass in wet tropical forests. Soil Biology and Biochemistry 36, 2111-2114.

Liberloo M., Gielen B., Calfapietra C., Veys C., Pigliacelli R., Scarascia Mugnozza G., Ceulemans R., Growth of a poplar short rotation coppice under elevated atmospheric CO2 concentrations (EUROFACE) depends on fertilization and species. Annals of Forest Science 299-307.

Liljeroth E., Kuikman P., Van Veen J. A., 1994. Carbon translocation to the rhizosphere of maize and wheat and influence on the turnover of native soil organic matter at different soil nitrogen levels. Plant and Soil 161, 233-240.

Lin G., Rygiewicz P. T., Ehleringer J. R., Johnson M. G., Tingey D. T., 2001. Time-dependent responses of soil CO_2 efflux components to elevated atmospheric [CO_2] and temperature in experimental forest mesocosms. Plant and Soil 229, 259-270.

Lu S., Mattson K.G., Zaerr J.B., Marshall J.D., 1998. Root respiration of Douglas-Fir seedlings: effect of N concentration. Soil Biology and Biochemistry 30, 331-336.

Lukac M., Calfapietra C., Godbold D.L., 2003. Root production and turnover in , pulus grown under elevated CO_2 using a free air enrichment system POPFACE. Glob. Change Biol. 9, 838-848.

Luo Y., Wu L., Andrews J.A., White L., Matamala R., Schafer K.V.R., Schlesinger W.H., 2001. Elevated CO_2 differentiates ecosystem carbon processes: deconvolution analysis of Duke forest FACE data. *Ecological monographs* 71(3), 357-376.

Lynch J. M., Whipps J. M., 1990. Substrate flow in the rhizosphere. Plant and Soil 129, 1-10.

MacDonald N. W., Zak D. R., Pregitzer K. S., 1995. Temperature effects on kinetics of microbial respiration and net nitrogen and sulfur mineralization. Soil Sci. Soc. Am. J. 59, 233-240.

Maier C. A., Kress L. W., 2000. Soil CO_2 evolution and root respiration in 11 year-old loblolly pine (*Pinus taeda*) plantations as affected by moisture and nutrient availability. Canadian Journal of Forest Research 30, 347-359.

Magill A. H., Aber J. D., 2000. Variation in soil net mineralization rates with dissolved organic carbon additions. Soil Biology and Biochemistry 32, 597-601.

Marland G., nders R.J., Boden T.A., Johnston C., Brenkert A., 1999. Global, regional and national CO_2 emission estimates from fossil fuel burning, cement production and gas flaring, 1751-1996. Report NDP-030, Carbon Dioxide Information Analysis Center, Oakridge National Laboratory, Oakridge, TN, USA.

Martin J.K., Merckx R., 1992. The partitioning of photosynthetically fixed carbon within the rhizosphere of mature wheat. Soil Biology and Biochemistry 24, 1147-1156.

Martin-Olmedo P. Rees R.M., Grace J., 2002. The influence of plants grown under elevated CO2 and N fertilization on soil nitrogen dynamics. Global Change Biology 8, 643-657.

Mary B., Recous S., Darwis D., Robin D., 1996. Interactions between decompositions of plant residues and nitrogen cycling in soil. Plant and Soil 181, 71-82.

Mayr, C., Miller, M., Insam, H., 1999. Elevated CO_2 alter community-level physiological profiles and enzyme activities in alpine grassland. Journal of Microbiological Methods 36, 35-43.

McAndrew D. W., Malhi S. S., 1992. Long-term N fertilization of a solonetzic soil : effects on chemical and biological properties. Soil Biology and Biochemistry 24, 619-623.

McGill W. B., Cannon K. R., Robertson J. A., Cook F. D., 1986. Dynamics of soil microbial biomass and water-soluble organic C in Breton L after 50 years of cropping to two rotations. Canadian Journal of Soil Science 66, 1-19.

Melillo J.M., Aber J.D., Muratore J.F., 1982. Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. Ecology 63, 621-626.

Merckx R., Den Hartog A., Van Veen J. A., 1985. Turnover of root-derived material and related microbial biomass formation in soils of different texture. Soil Biology and Biochemistry 17, 565-569.

Miglietta F, Peressotti A, Vaccari F P, Zaldei A, De Angelis P and Sarascia Mugnozza G 2001 Free-air CO_2 enrichment (FACE) of a poplar plantation: the POPFACE fumigation system. New Phytol. 150, 465-476.

Miller S.L., 1995. Functional diversity in fungi. Canadian Journal of Botany 73, 50-57.

Miller, M., Palojarvi, A., Rangger, A., Reeslev, M., Kjioller, A., 1998. The use of fluorogenic substrates to measure fungal presence and activity in soil. Applied and Environmental Microbiology 64, 613-617.

Monreal C. M., Bergstrom D. W., 2000. Soil enzymatic factors expressing the influence of land use, tillage system and texture on soil biochemical quality. Canadian Journal of Soil Science 80, 419-428.+

Moorhead, D.L., Linkins, A.E., 1997. Elevated CO2 alters belowground exoenzyme activities in tussock tundra. Plant and Soil 189, 321-329.

Morgan J. A., Knight W. G., Dudley L. M., Hunt H. W., 1994. Enhanced root system C-sink activity, water relations and aspects of nutrient acquiaition in mycorrhizal *Bouteloua graciliis* subjected to [CO_2] enrichment. Plant and Soil 165, 55-65.

Moscatelli M.C., Lagomarsino A., De Angelis P., Grego S., 2005. Seasonality of soil biological properties in a poplar plantation growing under elevated atmospheric CO_2. Applied Soil Ecology 30, 162-173.

Moscatelli, M.C., Fonck, M., De Angelis, P., Larbi, H., Macuz, A., Rambelli, A., Grego, S., 2001. Mediterranean natural forest living at elevated carbon dioxide: soil biological properties and plant biomass growth. Soil use and management 17, 195-202.

Muller T., Hoper H., 2004. Soil organic matter turnover as a function of the soil clay content: consequences for model applications. Soil Biology and Biochemistry 36, 877-888.

Murwira H. K., Kirchmann H., Swift M. U., 1990. The effect of moisture on the decomposition rate of cattle manure. Plant and Soil 122, 197-199.

Myrold D. D., Patson P. A., Peterson D. L., 1989. Relationships between soil microbial properties and aboveground stand characteristics of conifer forests in Oregon. Biogeochemistry 8, 265-281.

Nabuurs G.J., Dolman A.V., Verkaik E., Kuikman P.J., van Diepen C.A., Whitmore A., Daamen W., Oenema O., Kabat P., Mohren G.M.J., 2000. Article 3.3 and 3.4 of the Kyoto Protocol – consequences for industrialised countries' commitment, the monitoring needs and possible side effect. Environmental Science Policy 3 (2/3) 123-134. Economic Impacts of Climate Change on the Global Forest Sector. In: Economics of Carbon Sequestration in Forestry. S.R. Sedjo (ed.), Lewis Publishers, Boca Raton.

Nakane K., Yamamoto M., Tsubota H., 1983. Estimation of root respiration rate in a mature forest ecosystem. Jpn. J. Ecol. 33, 397-408.

Nannipieri P., Ascher J., Ceccherini M.T., Landi L., Pietramellara G., Renella G., 2003. Microbial iversity and soil functions. European Journal of Soil Science 54, 655-670.

Nannipieri, P., 1994. The potential use of soil enzymes as indicators of productivity, sustainability and pollution. In: Pankhurst C.E., Doube B.M., Gupta V.V.S.R., Grace P.R. (Editors). Soil biota: management and sustainable farming systems. CSIRO Australia, pp. 238-244.

Niklaus P.A., Alphey J., Ebersberger D., Kamplicher C., Kandeler E. Tscherko D., 2003. Six years of in situ CO_2 enrichment evoke changes in soil structure and soil biota of nutrient-poor grassland. Global change Biology 9, 585-600.

Niklaus P.A., Körner C., 1996. Responses of soil microbiota of a late successional alpine grassland to long term CO_2 enrichment. Plant and Soil 184, 219-229.
Nohrstedt H.O., Arnebrant K., Baath E., Soderstrom B., 1989. Changes in carbon content, respiration rate, ATP content, and microbial biomass in nitrogen-fertilized pine forest soils in Sweden. Canadian Journal of Forest Research 19, 323-328.
Norby R.J., Cotrufo M.F., Ineson P., O'Neill E.G., Canadell J.G., 2001. Elevated CO_2, litter chemistry and decomposition: a synthesis. Oecologia 127, 153-165.
Norton J. M., Firestone M. K., 1996. N dynamics in the rhizosphere of *Pinus ponderosa* seedlings. Soil Biology and Biochemistry 28, 351-362.
Oades J. M., Gillman G. P., Uehara G., Hue N. V., van Noordwijk M., Robertson G. P., Wada K., 1989. Interactions of soil organic matter and variable-charge clays. In: Coleman D. C., Oades J. M., Uehara G. (Eds.), Dynamics of Soil Organic Matter in Tropical Ecosystems. University of Hawaii Press, Honolulu, HI, pp. 69-95.
Oh N. H., Richter D. D., 2004. Soil acidification induced by elevated atmospheric CO_2. Global Change Biology 10, 1936-1946.
Okano S., Sato K., Inoue K., 1991. Turnover rate of soil biomass nitrogen in root may layer of pasture. Soil Science and Plant Nutrition 33, 373-386.
Olsson P., Linder S., Giesler R., Hogberg P., 2005. Fertilization of boreal forest reduces both autotrophic and heterotrophic soil respiration. Global Change Biology 11, 1745-1753.
Oorts K., Vanlauwe B., Cofie O. O., Sanginga N., Merckx R., 2000. Charge characteristics of soil organic matter fractions in a Ferric Lixisol under some multipurpose trees. Agroforestry Systems 48, 169-188.
Oorts K., Vanlauwe B., Merckx R., 2003. Cation exchange capacities of soil organic matter fractions in a Ferric Lixisol with different organic matter inputs. Agriculture Ecosystems and Environment 100, 161-171.
Parham, J.A. and Deng, S.P., 2000. Detection, quantification and characterization of b-glucosaminidase activity in soil. Soil Biology & Biochemistry 32, 1183-1190.
Paterson, E., Hall, J.M., Rattray, E.A.S., Griffiths, B.S., Ritz, K., Killham, K., 1997. Effect of elevated CO_2 on rhizosphere carbon flow and soil microbial processes. Global Change Biology 3, 363-377.
Paul E.A., Clark F.E., 1989. Soil Microbiology and Biochemistry, Academic Press, San Diego, CA.
Pendall E., Bridgham S., Hanson P. J., Hungate B., Kicklighter D. W., Johnson D. W., Law B. E., Luo Y., Megonigal J. P., Olsrud M., Ryan M. G., Wan S., 2004. Below-ground process responses to elevated CO_2 and temperature: a discussion of observations, measurements methods, and models. New Phytologist 162, 311-322.
Pennanen T., Caul S., Daniell T.J., Griffiths B.S., Ritz K., Wheatley R.E., 2004. Community-level responses of metabolically-active soil microorganisms to the quantity and quality of substrate inputs. Soil Biology & Biochemistry 36, 841-848.
Phillips, R.L., Zak, D.R., Holmes, W.E., White, D.C., 2002. Microbial community composition and function beneath temperate trees exposed to elevated atmospheric carbon dioxided and ozone. Oecologia 131, 236-244.
Pinzari, F., Trinchera, A., Benedetti, A., Sequi, P., 1999. Use of biochemical indices in the mediterranean environment: comparison among soils under different forest vegetation. Journal of Microbiological Methods 36, 21-28.
Post W.M., Know K.C., 2000. Soil carbon seuestration and land-use change: processes and potential. Global Change Biology 6, 317-327.
Powlson D., 2005. Will soil amplify climate change? Nature 433, 204-205.

Pregitzer K.S., Zak D.R., Curtis P.S., Kubiske M.E., Teeri J.A., Vogel C.S., 1995. Atmospheric CO_2, soil nitrogen and turnover of fine roots. New Phytologist 129, 579-585.

Prentice I.C., 2001. The carbon cycle and the atmospheric carbon dioxide. In: Climate change 2001: The Scientific Basis. Pp. 204-237. Intergovernmental Panel on Climate Change, Cambridge Univ. Press, Cambridge, U.K.

Prior S. A., Rogers H. H., Runion G. B., Torbert H. A., Reicosky D. C., 1997. Carbon dioxide-enriched agroecosystems: influence of tillage on short-term soil carbon dioxide efflux. Journal of Environmental Quality 26, 244-252.

Pritchard S G, Rogers H H, Davis M A, van Santen E, Prior S A and Schlesinger W H 2001 The influence of elevated atmospheric CO2 on fine root dynamics in an intact temperate forest. Glob. Change Biol. 7, 829-837.

Raich J. W., Nadelhoffer K. J., 1989. Belowground carbon allocation in forest ecosystems: global trends. Ecology 70, 1346-1354.

Raich J. W. and Potter C. S., 1995. Global patterns of carbon dioxide emissions from soils. Global Biogeochem. Cycles 9, 23-36.

Raich J.W., Schlesinger W.H., 1992. The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate. Tellus 44, 81–89.

Raich J.W., Tufekcioglu A., 2000. Vegetation and soil respiration: correlations and controls. Biogeochemistry 48, 71-90.

Rakonczay Z, Seiler J R and Samuelson L J 1997 A new method for the *in situ* measurement of fine root gas exchange of forest trees. Environmental and Experimental Botany 38, 243-249.

Ramaswamy V., 2001. Radiative forcing of climate change. In: Climate change 2001: The Scientific Basis. Pp. 349-416. Intergovernmental Panel on Climate Change, Cambridge Univ. Press, Cambridge, U.K.

Rasse D.P., 2002. Nitrogen deposition and atmospheric CO2 interactions on fine root dynamics in temperate forests: a theoretical model analysis. Global Change Biology 8, 486-503.

Raun W. R., Johnson G. V., Phillips S. B., Westerman R. L., 1998. Effect of long-term N fertilization on soil organic C and total N in continuous wheat under conventional tillage in Oklahoma. Soil and Tillage Research 47, 323-330.

Reid J. B., Goss M. J., 1982. Suppression of decomposition of ^{14}C carbon isotope-labelled plant roots in the presence of living roots of maize and perennial ryegrass. Journal of Soil Science 33, 387-395.

Reuss J. O., Johnson D. W., 1986. Acid Deposition and the Acidification of Soils and Water. Springler-Verlag, New York.

Rice C. W., Moorman T. B., Beare M., 1996. Role of microbial biomass carbon and nitrogen in soil quality. Methods for Assessing Soil Quality. In: Doran J., Jones A. J., (Eds.), SSSA Special Publication Number 49. Soil Science Society America Inc., Madison, WI, pp. 203-215.

Richards B.N., 1987. The Microbiology of Terrestrial Ecosystems, Longman/Wiley, New York.

Riffaldi R., Saviozzi A., Levi-Minzi R., 1996. Carbon mineralization kinetics as influenced by soil properties. Biology and Fertility of Soils 22, 293-298.

Rillig, M. C., Field C. B., and Allen M. F., 1999a. Soil biota responses to long-term atmospheric CO_2 enrichment in two California annual grasslands. Oecologia 119, 572-577.

Rillig, M. C., Field C. B., and Allen M. F., 1999b. Fungal root colonization responses in natural grasslands after long-term exposure to elevated atmospheric CO_2. Global Change Biology 5, 577-585.

Rochette P. Flanagan L.B., Gregorich E.G., 1999. Separating soil respiration into plant and soil components using natural abundance of ^{13}C. Soil Science Society of America Journal 63, 1207-1213.

Ross D. J., Tate K. R., Newton P. C. D., 1995. Elevated CO_2 and temperature effects on soil carbon and nitrogen cycling in ryegrass/white clover turfs of an Endoaquept soil. Plant ans Soil 176, 37-49.

Rothstein D. E., Zak D. R., Pregitzer K. S. And Curtis P. S. 2000. Kinetics of nitrogen uptake by *Populus tremuloides* in relation to atmospheric CO_2 and soil nitrogen availability. *Tree Physiology* 20, 265–270.

Rouhier H., Read D.J., 1998. Plant and fungal responses to elevated atmospheric carbon dioxide in mycorrhizal seedlings of *Pinus Sylvestris*. Environ. Exp. Bot. 40, 237-246.

Rustad L E, Huntington T. G., Boone R. D., 2000. Controls of soil respiration: Implications for climate change. Biogeochemistry 48, 1-6.

Rutherford P. M., Jume N. G., 1992. Effect of glucose amendment on microbial biomass, spelling fertilizer ^{15}N recovery and distribution in a barley-soil system. Biology and Fertility of Soils 12, 228-232.

Ryan M.G., Binkley D., Fownes J. H., Giardina C. P., Senock R. S., 2004. An experimental test of the causes of forest growth decline with stand age. Ecological Monographs 74, 393-414.

Ryan M.G., Lavigne M.G., Gower S.T., 1997. Annual carbon cost of autotrophic respiration in boreal forest ecosystems in relation to species and climate. Journal of Geophysical Research 102 (D24), 28871-28883.

Ryan M. G., Law B. E., 2005. Interpreting, measuring, and modeling soil respiration. Biogeochemistry 73, 3-27.

Sadowsky, M. J. and Schortemeyer, M., 1997. Soil microbial responses to increased concentrations of atmospheric CO_2. Global Change Biology 3, 217-224.

Salamanca E. F., Raubuch M., Joergensen R. G., 2002. Relationships between soil microbial indices in secondary tropical forest soils. Applied Soil Ecology 21, 211-219.

Salisbury F.B., Ross C.W., 1991. Plant physiology 4th ed. Wadsworth, Belmont.

Sallih Z., Bottner P., 1998. Effect of wheat (*Triticum aestivum*) roots on mineralization rates of soil organic matter. Biology and fertility of soils 7, 67-70.

Sarathchandra S.U., Ghani A., Yeates G.W., Burch G., Cox N.R., 2001. Effects of nitrogen and phosphate fertilizers on microbial and nematode diversity in pasture soils. Soil Biol. Biochem 33, 953-964.

Saugier B., Roy J., and Mooney H. A., 2001. Estimations of global terrestrial productivity: converging toward a single number? In: J. Roy, B. Saugier, and H. A. Mooney (eds.) Terrestrial Global Productivity. Academic Press, San Diego, California, pp. 543–557.

Saviozzi A., Levi-Minzi R., Riffaldi R., 1993. Mineralization parameters from organic materials added to soil as a function of their chemical composition. Biores. Technol. 45-131-135.

Scarascia Mugnozza G, De Angelis P, Sabatti M, Calfapietra C, Ceulemans R, Peressotti A and Miglietta F 2000 A FACE experiment on short rotation, intensive poplar plantation: objective and experimental set up of POPFACE. In: Terrestrial ecosystems research in Europe: success, challenges and policy. Eds. M A Sutton, J M Moreno, W H van der Putten, S Struwe. pp. 136-140. European Communities, Luxemburg, EUR 19375.

Schimel D. S., 1995. Terrestrial biogeochemical cycles: global estimates with remote sensing. Remote Sens. Environ. 51, 49-56.

Schlamadinger B., Garland G., 1996. The role of forest and bioenergy strategies in the global carbon cycle. Biomass and Bioenergy 10, 275-300.

Schlesinger W.H., Andrews J.A., 2000. Soil respiration and the global carbon cycle. Biogeochemistry 48, 7-20.

Schortmeyer, M., Dijkstra, P., Johnson, D.W., Drake, B.G., 2000. Effects of elevated atmospheric CO_2 concentration on C and N pools and rhizosphere processes in a Florida scrub oak community. Global Change Biology 6, 383-391.

Schulten H. R., Leinweber P., 2000. New insights into organic-mineral particles: composition, properties and models of molecular structure. Biology and Fertility of Soils 30, 399-432.

Schulze E. D., Hogberg P., van Oene H., 2000. Interactions between the carbon- and nitrogen cycle and the role of biodiversity: a synopsis of a study along a north-south transect through Europe. In: Carbo and Nitrogen Cycling in European Forests Ecosystems (Schulze E. D. ed), Ecological studies 142, pp. 468-491. Springer, Berlin.

Shepherd T. G., Saggar S., Newman R. H., Ross C. W., Dando J. L., 2001. Tillage-induced changes to soil structure and organic carbon fractions in New Zealand soils. Australian Journal of Soil Research 39, 465-489.

Sinsabaugh, R.L., Moorhead, D.L., 1994. Resource allocation to extracellular enzyme production: a model for nitrogen and phosphorus control of litter decomposition. Soil biology and Biochemistry 26, 1305-1311.

Sinsabaugh, R.L., Moorhead, D.L., Linkins, A.E., 1994. The enzymic basis of plant litter decomposition: emergence of an ecological process. Applied Soil Ecology 1, 97-111.

Six J. Carpentier A., van Kessel C., Merckx R., Harris D., Horwath W. H., Luscher A., 2001. Impact of elevated CO_2 on soil organic matter dynamics as related to changes in aggregate turnover and residue quality. Plant and Soil 234, 27-36.

Six J., Elliott E. T., Paustian K., 2000. Soil structure and soil organic matter : II. A normalized stability index and the effect of mineralogy. Soil Science Society of America Journal 64, 1042-1049.

Smith S.E. 1980. Mycorrhizas of autotrophic higher plants. Biol. Rev. Cambridge Philos. Soc. 55: 475-510.

Smith J. L., McNeal B. L., Cheng H. H., Campbell G. S., 1986. Calculation of microbial maintenance rates and net nitrogen mineralization in soil at staedy-state. Soil Science Society of America Journal 50, 332-338.

Smith J. L., Papendick R. I., Bezdicek D. F., Lynch J. M., 1993. Soil Organic matter dynamics and crop residue management. In: Metting F. B. Jr. (ed). Soil microbial ecology pp. 65-94. Marcel Dekker, NY.

Smith, P., Powlson, D.S., Glendining, M.J., and Smith, J.U. 1997. Potential for carbon sequestration in European soils: preliminary estimates for five scenarios using results from long-term experiments. *Global Change Biol.* 3**:** 67–79.

Smolander A., Kurka A., Kitunen V., Malkonen E., 1994. Microbial biomass C and N, and respiratory activity in soil of repeatedly limed and N-and P-fertilized Norway spruce stands. Soil Biology and Biochemistry 26, 957-962.

Sollins P., Homann P., Caldwell B. A., 1996. Stabilization and destabilization of soil organic matter: mechanisms and controls. Geoderma 74, 65-105.

Sparling G. P., Cheshire M. V., Mundie C. M., 1982. Effect of barley plants on the decomposition of ^{14}C-labelled soil organic matter. Journal of Soil Science 33, 89-100.

Springer, U., Klee, J., 1954. Prüfung der Leistungsfähigkeit von einigen wichtigeren Verfahren zur Bestimmung des Kohlenstoffs mittels Chromschwefelsäure sowie Vorschlag einer neuen Schnellmethode. Z. Pflanzenernährung. Bodenk. 64, 1.

Stanford G., Smith S. J., 1972. Nitrogen mineralization potentials of soils. Soil Science Society of America Proc. 36, 465-472.
Stitt M., 1991. Rising CO_2 levels and their potential significance for carbon flow in photosynthetic cells. Plant, cell and Environment 14, 741-762.
Subke J.A., Reichstein M., Tenhunen J.D., 2003. Explaining temporal variation in soil CO2 efflux in a mature spruce forest in Southern Germany. Soil Biology and Biochemistry 35, 1467-1483.
Syvertsen J. P., Graham J. H., 1999. Phosphorus supply and arbuscular mycorrhizas increase growth and net gas exchange responses of two *Citrus* spp. grown at elevated CO_2. Plant and Soil 208, 209-219.
Tabatabai M. A., Bremner J. M., 1970. Arylsulphatase activity of soils. Soil Science Society of America Proceedings 34, 225-229.
Tate III R.L., 1995. Soil Microbiology, Wiley, New York.
Tate K.R., Ross D.J., 1997. Elevated CO_2 and moisture effects on soil carbon storage and cycling in temperate grasslands. Global Change Biology 3, 225-235.
Thirukkumaran C. M., Parkinson D., 2000. Microbial respiration, biomass, metabolic quotient and litter decomposition in a lodgepole pine forest floor amended with nitrogen and phosphorus fertilizers. Soil Biology and Biochemistry 32, 59-66.
Thomas R. B., Whitehead D., Reid J. B., Cook F. J., Adams J. A., Lakie A. C., 1999. Growth, loss, and vertical distribution of Pinus radiata fine roots growing at ambient and elevated CO_2 concentration. Global Change Biology 5, 107-121.
Tian G., Kang B. T., Brussaard L., 1992. Biological effects of plant residues with contrasting chemical compositions under humid tropical conditions-decomposition and nutrient release. Soil Biology and Biochemistry 24, 1051-1060.
Tingey D.T., Phillips D.L., Johnson M.G., 2000. Elevated CO2 and conifer roots: effects on growth, life span and turnover: New Phytologist 147, 169-187.
Treseder K. K., 2004. A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO_2 in field studies. New phytologist 164, 347-355.
Trueman R. J., Gonzalez-Meler A., 2005. Accelerated belowground C cycling in a managed agriforest acosystem exposed to elevated carbon dioxide concentrations. Global Change Biology 11, 1-14.
Tuchman, N.C., Wetzel, R.G., Rier, S.T., Kirk, A.W., Teeri, J.A, 2002. Elevated atmospheric CO2 lowers leaf litter nutritional quality for stream ecosystem food webs. Global Change Biology 8, 163-170.
Turner B. L., Hopkins D. W., Haygarth P. M., Ostle N., 2002. β-Glucosidase activity in pasture soils. Applied Soil Ecology 20, 157-162.
Vainio E.J., Hantula J., 2000. Direct analysis of wood-inhabiting fungi using denaturing gradient gel electrophoresis of amplified ribosomal DNA. Mycol Res 104, 927-936.
Valentini R, Matteucci G, Dolman A J, Schulze E D, Rebmann C, Moors E J, Granier A, Gross P, Jensen N O, Pilegaard K, Lindroth A, Grelle A, Bernhofer C H, Grunwald T, Aubinet M, Ceulemans R, Kowalski A S, Vesala T, Rannik Ü, Berbiger P, Loustau D, Gudmundsson J, Thorgeirsson H, Ibrom A, Morgenstern K, Clement R, Moncrieff J, Montagnani L, Minerbi S and Jarvis P G 2000 Respiration as the main determinant of European forests carbon balance. *Nature* 404, 861-865.
Van Dijk H., 1971. Colloid chemical properties of humic matter. In: McLaren A. D., Skujins J. (Eds.), Soil Biochemistry, vol. 2. Marcel Dekker, NY, pp. 16-35.
Van Ginkel J. H., Gorissen A., van Veen J. A., 1996. Long-term decomposition of grass roots (*Lolium perenne* L.) as affected by elevated atmospheric CO_2. Journal of Environmentl Quality 25, 1122-1128.

Van Ginkel J. H., Gorissen A., Polci D., 2000. Elevated atmospehric carbon dioxide concentration: effects of increased carbon input in a *Lolium perenne* soil on microorganisms and decomposition. Soil Biology and Biochemistry 32, 449-456.

Van Groenigen, K., Harris, D., Horwath, W.R., Hartwig, U.A., Van Kessel, C., 2002. Linking sequestration of ^{13}C and ^{15}N in aggregates in a pasture soil following 8 years of elevated atmospheric CO_2. Global Change Biology 8, 1094-1108.

Van Hees P. A. W., Jones D. L., Finlay R., Godbold D. L., Lundstrom U. S., 2005. The carbon we do not see – the impact of low molecular weight compounds on carbon dynamics and respiration in forest soils: a review. Soil Biology and Biochemistry 37, 1-13.

Van Veen J. A., Ladd J. N., Amato M., 1985. Turnover of carbon and nitrogen through the microbial biomass in a sndy loam and a clay soil incubated with (U-^{14}C)glucose and $(^{15}NH_4)_2SO_4$ under different moisture regimes. Soil Biology and Biochemistry 17, 747-756.

Van Veen J.A., Liljeroth E., Lekkerkerk L.J.A., van de Geijn S.C., 1991. Carbon fluxes in plant-soil systems at elevated atmospheric CO_2 levels. Ecol. Appl. 1, 175-181.

Vance E. D., Chapin F. S. III, 2001. Substrate limitations to microbial activity in taiga forest floors. Soil Biology and Biochemistry 33, 173-188.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biology & Biochemistry 19, 703-707.

Vanlauwe B., Nwoke O. C., Sanginga N., Merckx R., 1996. Impact of residue quality on the C and N mineralization of leaf and root residues of three agroforestry species. Plant and Soil 183, 221-231.

Vepsalainen, M., Kukkonen, S., Vestberg, M., Sirvio, H., Niemi, R.M., 2001. Application of soil enzyme activity test kit in a field experiment. Soil Biology & Biochemistry 33, 1665-1672.

Vepsalainen, M., Kukkonen, S., Vestberg, M., Sirvio, H., Niemi, R.M., 2001. Application of soil enzyme activity test kit in a field experiment. Soil Biology & Biochemistry 33, 1665-1672.

Verstraete W. 1981. Nitrification. In: Terrestrial nitrogen cycles: processes ecosystem strategies and management impacts. (F.E.Clark and T. Rosswall eds.) pp. 303-314. Ecological Bulletin, Stockholm.

Vesterdal L. Ritter E., Gundersen P., 2002. Change in soil organic carbon following afforestation of former arable land. Forest Ecolgy and Management 169, 137-147.

Vose J. M., Elliott K. J., Johnson D. W., Tingey D. T., Johnson M. G., 1997. Soil respiration response to three years of elevated CO_2 and N fertilization in ponderosa pine (*Pinus ponderosa* Doug. ex Laws.). Plant and Soil 190, 19-28.

Wallace A., 1994. Soil acidification from use of too much fertilizer. Commuhn. Soil Science Plant Anal. 25, 87-92.

Wang, W.J., Dalal, R.C., Moody, P.W., Smith, C.J., 2003. Relationships of soil respiration to microbial biomass, substrate availability and clay contentSoil Biology & Biochemistry 35, 273-284.

Wardle D. A., 1992. A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soil. Biological Review 67, 321-358.

Wardle, D.A. and Ghani, A., 1995. A critique of the microbial metabolic quotient (qCO_2) as a bioindicator of disturbance and ecosystem development. Soil Biology & Biochemistry 27 n.12, 1601-1610.

Warembourg F.R., Paul E.A., 1973. The use of $^{14}CO_2$ canopy techniques for measuring carbon transfer through the plant-soil system. Plant and Soil 38, 331-345.

Wiant H.V., 1967. Contribution of roots to forest soil respiration". Advanced in Plant Science. 18,163-167.

Wiemken V., Ineichen K., Boller T., 2001. Development of ectomycorrhizas in model beech-spruce ecosystems on siliceous and calcareous soil: a 4-year experiment with atmospheric CO_2 enrichment and nitrogen fertilization. Plant and Soil 234, 99-108.

Wiemken, V., E. Laczko, K. Ineichen, and T. Boller., 2001. Effects of elevated carbon dioxide and nitrogen fertilization on mycorrhizal fine roots and the soil microbial community in beech-spruce ecosystems on siliceous and calcareous soil. *Microbial Ecology* 42, 126-135.

Winkler J P, Cherry R S and Schlesinger W H 1996 The Q_{10} relationship of microbial respiration in a temperate forest soil. Soil Biol. Biochem. 8, 1067-1072.

Wittig V. E., Bernacchi C. J., Zhu X. G., Calfapietra C., Ceulemans R., De Angelis P., Gielen B., Miglietta F., Morgan P. B., Long S. P., 2005. Gross primary production is stimulated for three *Populus* species grown under free-air CO_2 enrichment from planting through canopy closure. Global Change Biology 11, 1-13.

Woomer P.L., Martin A., Albrecht A., Resk D.V.S., Scharpenseal H.W., 1994. The importance and management of soil organic matter in the tropics. In: P.L. Woomer and M.J. Swift (eds.) "The Biological Management of Tropical Soil Fertility", J. Wiley & Sons, Chichester, U.K. 47-80.

Xu M., De Biase T., Qi Y., Goldstein A., Liu Z., 2001. Ecosystem respiration in a young ponderosa pine plantation in the Sierra Nevada Mountains, California. Tree Physiology 21, 309-318.

Yuste J. C., Janssens I. A., Carrara A., Ceulemans R., 2004. Annual Q_{10} of soil respiration reflects plant phonological patterns as well as temperature sensitivity. Global Change Biology 10, 161-169.

Zak D.R., Holmes W.E. Finzi A.C., Norby R.J., Schlesinger W.H., 2003. Soil nitrogen sycling under elevated CO_2: a synthesis of forest FACE experiments. Ecological applications 13(6), 1508-1514.

Zak D.R., Pregitzer, K.S., Curtis P.S., Teeri J.A., Fogel R., Randlett D.L., 1993. Elevated atmospheric CO_2 and feedback between carbon and nitrogen cycles. Plant and Soil 151, 103-116.

Zak D.R., Ringelberg D.B., Pregitzer K.S., Randlett D.L., White D.C., Curtis P.S., 1996. Soil microbial communities beneath *Populus grandidentata* grown under elevated atmospheric CO_2. Ecol. Appl. 6, 257-262.

Zak D.R., Tilman D., Parmenter R.R., Rice C.W., Fisher F.M., Vose J., Milchunas D., Martin C.W., 1994. Plant production and soil microorganisms in late-successional ecosystems: a continental scale study. Ecology 75, 2333-2347.

Zak D.R., Pregitzer, K.S., King, J.S., Holmes, W.E., 2000. Elevated atmospheric CO_2, fine roots and the response of soil microorganism: a review and hypotesis. New Phytologist 147, 201-222.

Zsuffa L., Giordano E., Pryor L.D., Stettler R.F., 1996. Trends in poplar culture: some global and regional perspectives. In: Stettler R.F., Bradshaw Jr H.D., Heilman P.E., Hinckley T.M. (Eds.), Biology of *Populus* and its implications for Management and Conservation (Part II, Chapter 19. NRC Research Press, National Research Council of Canada, Ottawa, pp. 515-539.

RINGRAZIAMENTI

Il lavoro di ricerca è stato svolto grazie al contributo del progetto CE EUROFACE (EVR1-CT-2002-40027) e MIUR Centro di Eccellenza "Foreste & Clima". Si ringrazia il coordinatore prof. Giuseppe Scarascia Mugnozza.

Un ringraziamento particolare alla dott.ssa M. Cristina Moscatelli per il fondamentale contributo scientifico dato all'attività di ricerca, all'elaborazione dei dati e alla stesura dell'elaborato, nonchè per la sua disponibilità, per i suggerimenti e per la fiducia data in questi anni.

Si ringrazia il laboratorio di Biochimica del suolo (DABAC), il laboratorio di Ecofisiologia (DISAFRI), tutti i dottorandi e gli assegnisti per il supporto tecnico e scientifico e per i preziosi consigli di questi anni. In particolare si vuole ringraziare la dott. Sara Marinari per i sempre utili suggerimenti, tutti i tesisti che hanno contributo allo sviluppo di questa ricerca ed il Sig. Tullio Oro per l'assistenza tecnica e la disponibilità.

Si ringrazia il laboratorio di microbiologia dell'Università di Innsbruck per gli insegnamenti ed il supporto tecnico e scientifico ed il prof. Insam per la disponibilità e la pazienza.

Un profondo ringraziamento va ai tutori di questa tesi:

al prof. Stefano Grego, guida e sostegno sin dall'inizio di questo lavoro, per le indicazioni e per i fondamentali consigli, per la sua continua disponibilità e per la supervisione del lavoro svolto;

al prof. Paolo De Angelis per il suo instancabile lavoro come coordinatore del corso di dottorato e come tutore di questa tesi, per lo spirito critico sempre costruttivo, per l'accuratezza delle indicazioni e dei suggerimenti che sono stati indispensabili per la crescita di questa ricerca.

Un ultimo ringraziamento, non per importanza, va alla mia famiglia per la pazienza, il sostegno, la sopportazione e l'aiuto instancabile e gratuito di questi anni.