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Effects of atmospheric CO enrichment on root processes and mycorrhizal functioning in short rotation intensive poplar plantation

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Effects of Atmospheric CO_2 Enrichment on Root Processes and Mycorrhizal Functioning in Short Rotation Intensive Poplar Plantation

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Abstract

As a result of rising atmospheric CO_2 concentrations, numerous studies have been aimed at determining and clarifying the response of photosynthesising plants to this phenomenon. Despite the significant role of trees and forests in the carbon cycle, most of the CO_2 research carried out so far has been focused on studying single plants grown in controlled environments for relatively short periods. This study intends to bridge this gap in knowledge and it is a part of European Free Air Carbon Enrichment Experiment on Poplar Plantations (POPFACE). Specifically, the objective of this research is to characterise the effects of elevated CO_2 on root biomass production and associated mycorrhizal activity and functioning of the following three *Populus* species: *P. alba*, *P. nigra* and *P. euramericana* (I-214). The trees were grown in a 1 x 1m spaced plantation under ambient (350ppm) and elevated (550ppm) atmospheric CO_2 conditions provided by a FACE system. After three growing seasons, it was shown that elevated CO_2 increases below-ground allocation of biomass in all three species examined. This enhancement of root production was approximately twofold when compared to the increase of aboveground biomass induced by FACE, when standing root biomass was increased by 47% to 76%. Similarly, fine root biomass present in the soil increased by 35% to 84% as a result of elevated CO_2. FACE treatment resulted in 55% faster fine root turnover in *P. alba* and a 27% increase in turnover of roots of *P. nigra* and *P. euramericana*. *P. alba* and *P. nigra* invested more root biomass into deeper soil horizon under elevated CO_2. Response of the mycorrhizal community to elevated CO_2 was more varied, the rate of infection increased only in *P. alba* for both ECM and AM. The roots of *P. nigra* showed greater infection only by AM and the colonisation of the root system of *P. euramericana* was not affected FACE treatment. The results suggest that elevated atmospheric CO_2 conditions induce greater belowground biomass investment, which could lead to accumulation of assimilated C in the soil profile. This might have further implications for C sequestration and must be taken into account when considering long-term C storage in the soil.

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List of Abbreviations

AFLP	Amplified Fragment Length Polymorphism
CVCP	Committee of Vice-Chancellors and Principals of UK universities
DNA	Deoxyribonucleic acid
ECM	Ecto-mycorrhiza
EU	European Union
FACE	Free Air Carbon Enrichment
IPCC	Intergovernmental Panel for Climate Change
IRGA	Infra-red gas analyser
OTC	Open top chamber
PCR	Polymerase chain reaction
POPFACE	European Free Air Carbon Enrichment Experiment on Poplar Plantations
RAPD	Randomly Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
SOM	Soil organic matter
TNC	Total non-structural carbohydrates
VAM	Vesicular-arbuscular mycorrhiza

1 Introduction

With the concentration of atmospheric CO_2 increasing at a rate of 1% or more per year (Scarascia-Mugnozza *et al.*, 2001), there exists a consensus and a growing concern that environmental conditions are being modified globally (IPCC, 1996). Following the establishment of the first long-term observation of CO_2 concentration on Mauna Loa, Hawaii (Keeling *et al.*, 1996) it became apparent, that the composition of the atmosphere is being changed at an accelerating rate. The impact of these changes on global climate and hence on the functioning of ecosystems is being progressively studied (Jain *et al.*, 2000). However, apart from indirect effects, the increase in CO_2 concentration has a direct consequence on photosynthesising plants (Bazzaz, 1990). Through increased rate of photosynthesis (Curtis & Wang, 1998) elevated atmospheric CO_2 introduces changes into the carbon cycle. This could affect the productivity of ecosystems, both natural and man maintained, and also alter their ability to absorb CO_2 from the atmosphere.

In view of these circumstances, it became apparent that if green plants are to be used to mitigate the effects of ever increasing consumption of fossil fuels and of past and present land-use changes, more and detailed information is necessary about the response of whole ecosystems to elevated levels of atmospheric CO_2 (Norby, 1994). This work presents the results of one such study; the effects of elevated CO_2 on below-ground processes in an agro-forestry *Populus* plantation were examined for a period of three years.

1.1 Background of the study

Much of the scientific knowledge about responses of plants to elevated CO_2 gathered so far relates to non-woody herbs and agricultural crops (Ceulemans & Mousseau, 1994). This is understandable given their fast growth and development and, in the case of crops, their importance in

food production. However, if the potential role in carbon sequestration is being considered, tree dominated ecosystems must be taken into account. It has been estimated that about 85% of carbon present in plants and around 35% of all C present in soils can be found in forests (Kirschbaum & Fischlin, 1996). This fact may nominate tree ecosystems as the primary resource that could be exploited in the effort to mitigate future increases in the concentration of atmospheric CO_2.

To establish the responses of trees to elevated CO_2 and to assess their role in C sequestration a host of studies has been carried out during the 1990s (Jarvis, 1998). However, most of the information about the effects of elevated CO_2 on woody species has been obtained from studies of seedlings or very young plants (Norby *et al.*, 1999). Given the difficulties arising from the large size of most trees and their long life cycle, coupled with constraints on resources available, these studies resulted in some invaluable insights and provided initial information about the response of trees to elevated CO_2. Mooney & Chapin (1994), among others, called for an expansion of these studies to include physiological responses on the whole-tree scale and for an investigation of the interactions among individual plants in a tree community.

Appropriate research methodologies were developed and the technology of CO_2 enrichment improved, both tightly connected to the scale at which the processes have been studied (Scarascia-Mugnozza *et al.*, 2001). Besides large open-top chambers enclosing portions of natural ecosystems and mature stands growing in the vicinity of CO_2 springs, another technique allows for investigation of ecosystem's response to elevated CO_2 - Free Air Carbon Enrichment (FACE) (Hendrey *et al.*, 1993).

Based on the aforementioned rationale, a fast growing agro-forestry system with atmospheric CO_2 enrichment provided by a FACE has been employed to study responses to elevated CO_2 at (an) ecosystem's level. Codenamed POPFACE, the effect of elevated CO_2 on a variety of

important processes, such as C assimilation, plant respiration, stomatal activity and respiration, biomass production, plant architecture and below-ground processes in a *Populus* plantation have been studied for a period of three growing seasons. Although all mentioned processes have been studied according to the same experimental design and the results will be evaluated collectively, this thesis concerns only one of the areas studied: below-ground processes.

1.2 *Aims of the study*

This work aims to investigate whether and how elevated CO_2 affects below-ground processes in a *Populus* plantation. It has been shown that in a *Populus* hybrid more than 50% of assimilated C has been transferred below-ground (Horwath *et al.*, 1994). Given this extend of C transfer to the soil, it is of importance to assess the effect of elevated CO_2 on root production and associated soil processes. Moreover, since *Populus* trees form mycorrhizal symbioses with both vesicular-arbuscular and ectomycorrhizas, an additional below-ground sink for assimilated C and its response to elevated CO_2 needs to be investigated in order to examine the C sequestering potential of a *Populus* plantation. Because of these facts, two main hypotheses were tested in this research:

- Elevated CO_2 does affect root production of *Populus* trees grown in a plantation.
- Mycorrhizal symbionts do respond to elevated CO_2 with increased colonisation of *Populus* roots.

In order to test the aforementioned hypotheses and to explain the effect of elevated CO_2 on C cycling through the soil, the following sub-questions were investigated:

- Does elevated CO_2 increase fine root turnover of *Populus* trees?

- Does elevated CO_2 change spatial allocation of root biomass of *Populus* trees?
- Does the species composition of mycorrhizas change under elevated CO_2?

1.3 *Structure of the study*

The structure of this thesis is as follows:

Chapter 2 offers an overview of the circumstances that led to the selection of a *Populus* plantation as the ecosystem of choice to be studied under elevated CO_2 conditions. Current state of knowledge in the field of root response to elevated CO_2 is reviewed, together with the methods applied in root investigation. Available knowledge about the effect of elevated CO_2 on mycorrhizas is presented, together with the brief summary of existing information about C cycling and storage in the soil in connection with elevated CO_2.

Chapter 3 discusses the techniques of atmospheric CO_2 enrichment available at present and explains why FACE technology has been chosen for the POPFACE experiment. The experimental design of POPFACE is described in detail, together with basic characteristics of the set-up and performance of CO_2 enrichment system.

Chapter 4 deals with the effects of elevated CO_2 on root production of *Populus* species utilised in POPFACE. Standing biomass of coarse and fine roots was studied during all three growing seasons. The influence of elevated CO_2 on spatial allocation of roots is assessed and discussed.

Chapter 5 concerns fine root dynamics and how they are influenced by elevated CO_2. The ingrowth core method of investigation of fine root production is explained alongside minirhizotron observation of fine root growth. Root turnover rates are calculated for both CO_2 treatments and examined.

Chapter 6 gives details of the root identification method chosen to provide evidence of origin of roots extracted from the soil in POPFACE. RAPD combined with PCR was utilised to identify *Populus* roots according to their different banding patters.

Chapter 7 assesses the effect of elevated CO_2 on mycorrhizal colonisation and functioning. The rate of colonisation of *Populus* fine roots both by vesicular-arbuscular and by ectomycorrhizas is examined. An attempt to capture the influence of elevated CO_2 on the composition of vesicular-arbuscular mycorrhizas is described and analysed.

Chapter 8 combines the results of examinations of the effects of elevated CO_2 on root production, turnover and mycorrhizal colonisation and puts them into the context of C cycling and storage in the soil. The amount of C transferred below-ground under both CO_2 treatments is calculated and discussed.

Finally, Chapter 9 summarises the results of this study, outlines its limitations and suggests directions for future research in this area.

2 Framework of the study

It has been shown that the impeding global climate change will have an effect on the functioning of ecosystems and might have profound and long-lasting effect on the biosphere. Due to their role in providing living habitat and nutrition to the human population, assessment of nature and magnitude of the response of terrestrial ecosystems to this rapid change of environmental conditions is of utmost importance. Moreover, living ecosystems do not only passively undergo climatic alteration, but they are also driving factors influencing the course of climatic changes (Dixon *et al.*, 1994). A considerable amount of effort and resources has been invested into investigating what climate change will mean for human society and how to avert the worst-case scenarios. In the light of these circumstances, this chapter justifies the value of this study and discusses the present state of knowledge in the field under investigation.

2.1 *The politics of elevated CO_2*

Although some scientists and policymakers still have reservations, it is an accepted fact that human activity is the main driving force behind increasing levels of CO_2 and associated global change (Shine & Forster, 1999). In an attempt to cap the emissions of CO_2, under the terms of the Kyoto Protocol[1], EU countries committed themselves to reduce their CO_2 emissions to 92% of the baseline - represented by the amount of emissions in 1992 - by the end of the first commitment period (2008-2012). Because the Kyoto Protocol allows for calculation of net CO_2 emissions as a balance between total CO_2 emitted and the C sequestration capacity, it is worth for

[1] Originally drafted in Kyoto (Japan) in December 1997 and later modified and signed in Bonn (Germany) in July 2001 and in Marrakech (Morocco) in November 2001. The full wording of the protocol can be viewed at http://unfccc.int/resource/docs/convkp/kpeng.html

each country to explore and develop its C sinks. According to the wording of the protocol, not only natural forest ecosystems, but also agro-forestry plantations are considered to be C sinks.

Due to overproduction of agricultural produce inside its borders, the EU has decided to set aside around 5 million ha of agricultural land. Biomass forestry is one of many potential uses envisaged for this land released from agriculture. Apart from already existing natural sinks, of all options examined by Smith *et al.* (2001), bioenergy crops show the greatest potential for C sequestration. Smith *et al.* (1997) calculated, that the long-term conversion into biomass tree crops and natural woodlands of 20% of present arable land in the EU would increase soil carbon stocks by about 5%. If this is so, this could be one of means worth consideration in order to mitigate C emissions.

2.2 *The choice of species*

Extensive research conducted in Europe, and elsewhere, has shown that poplar and willow are the most reliable species for use as biomass and energy crops (Makeschin & Makeschin, 1999). This is due to their fast growth that allows for great biomass production, which results in sizeable C sequestration capacity. Very important in this consideration, apart from harvestable biomass, is the proportion of C allocated below ground. It is likely that some of this C, after its conversion to soil organic matter, will remain locked in the soil for a prolonged period of time. It has been demonstrated, that in forest and agricultural soils C can accumulate at a mean rate of 0.3 t ha^{-1} y^{-1}, rising to a maximum of about 3 t ha^{-1} y^{-1} (Post & Kwon, 2000). In addition to the direct C sequestration potential of a fast-growing plantation, after publication of some alarming reports about the effects of global change in recent years, there has been an increased interest in renewable energy sources.

Since one of the main objectives of the POPFACE experiment was to study the effect of elevated CO_2 on tree dominated ecosystem, tree

species which could provide conditions typical of forest ecosystem in a relatively short period of time was needed. For this reason, together with wider ecological, social and economical considerations, a poplar plantation was selected as a medium of experimentation. *Populus* trees, growing in an agro-forestry system, provide an opportunity to study processes and mechanisms determining biomass production and allocation at the ecosystem level. They are extremely fast growing, quickly providing a tall, closed canopy, have high genetic reproducibility and a short life-span (Stetter *et al.*, 1988).

2.3 *Below-ground effects of elevated CO_2*

The need to assess the role of ecosystems in the C cycle and how that role will be affected by or will affect global change gave rise to many experiments over a wide range of scales (Norby *et al.*, 1999). It has been estimated that forests and forest soils stock around 90% of total C present in the terrestrial ecosystems (Armentano & Ralston, 1980), signifying the fact that the response of forests to global change might have important consequences for the C cycle in the biosphere. Studies conducted at the whole tree and community scale have indicated that elevated CO_2 results in a marked increase of primary production, often accompanied by greater biomass allocation below-ground (IPCC, 2000). Naturally, global change does not constitute only increasing levels of atmospheric CO_2, it encompasses other aspects such as long-term increases of mean temperature, nutrient supply, tropospheric ozone levels, UV radiation and changes in global climate patterns. However, the collective impact of all these environmental factors is difficult, if not impossible, to simulate in experimental conditions. Due to this fact, the POPFACE experiment investigates only the influence of elevated CO_2 on growth, functioning and C balance of a fast growing poplar plantation. More specifically, this study examines the effects elevated CO_2 has on below-ground processes and functioning of this fast-growing agro-forestry system.

Mooney *et al.* (1999) have classified the most important physiological plant processes influenced by elevated CO_2 into the following five categories: effects on stomatal conductance and water-use efficiency, photosynthesis and respiration, plant structure and phenology, plant nutrient concentration and C allocation and growth. There exists a consensus, that due to their size, longevity and complexity, the effect of elevated CO_2 on trees cannot be considered along the same guidelines as the effect on herbaceous plants (Wullschleger *et al.*, 1997; Janssens *et al.*, 2000). Therefore, for the purposes of reference and comparison, mainly the responses of woody species to elevated CO_2 are discussed in this work.

2.3.1 Root production

It is estimated that 50% or more of total plant biomass in terrestrial ecosystems is allocated below-ground (Fogel, 1990). In a large scale experiment using 1- and 2-year old *Populus* trees, Horwath *et al.* (1994) and Scarascia-Mugnozza *et al.* (1999) found that between 20 and 60% of assimilated C were transferred into the soil through the production of roots. Through increased production of photosynthates, elevated CO_2 is known to increase the amount of biomass allocated below-ground (Norby, 1994; Curtis & Wang, 1998). Because trees are the dominant plants in most ecosystems they occupy, tree roots are an important source of C for the complex web of organisms inhabiting the soil (Zak *et al.*, 1993; Klironomos *et al.*, 1997). Any change in the quantity and quality of organic matter entering the soil resulting from increasing levels of CO_2 is therefore likely to result in a change of an ecosystems' C (Jastrow *et al.*, 2000) and nutrient cycles (Johnson, 1999).

Most of the studies investigating the effect of elevated CO_2 on root production in trees report their results only as a static observation. A single measurement of root biomass allocation is available in most cases, due to the fact that a complete measurement of root biomass invariably requires destructive harvest (Norby *et al.*, 1999). Although it has been

noted by some authors that this static measure of below-ground allocation is of limited importance (Norby, 1994), because of scarcity of time-series data it is worth mentioning the results of some observations.

2.3.1.1 *Standing root biomass*

In general, tree species - both angiosperms and gymnosperms - increase the amount of root biomass present in the soil when grown under elevated CO_2. O'Neill (1994) found that *Liriodendron tulipifera* increased root biomass at both +150 and +300 ppm CO_2 enrichment. Likewise, greater root production under elevated CO_2 was reported for *Betula papyrifera* (Godbold & Berntson, 1997), *Pinus strobus* (Godbold *et al.*, 1997), *Quercus alba* (O'Neill, 1994) and *Populus tremuloides* (Pregitzer *et al.*, 1995). However, this response is not universal, no effect of CO_2 on root biomass was found in *Betula papyrifera* (Berntson & Bazzaz, 1998) and *Pinus sylvestris* (Markkola *et al.*, 1996), suggesting that the effect of elevated CO_2 might be mediated by other environmental factors.

Some studies consider root:shoot ratio as a measure of root allocation response to elevated CO_2. There was no change in root:shoot ratio in *Liriodendron tulipifera* (Norby *et al.*, 1992), *Betula pendula* (Rey & Jarvis, 1997) or *Fraxinus excelsior*, *Quercus petraea* and *Pinus sylvestris* (Crookshanks *et al.*, 1998). Curtis & Wang (1998) in their meta-analysis of more than 500 studies provided statistical evidence that no change of root:shoot biomass partitioning occurs as a result of elevated CO_2. However, it is still a matter of discussion whether a static estimate of root:shoot ratio confers valuable information - a ratio obtained only from a single measurement may mask important responses to elevated CO_2 that are confounded in developmental changes of C allocation (Norby *et al.*, 1999).

2.3.1.2 *Fine roots and root turnover*

Due to the continuous growth and dieback of fine roots, standing root biomass is a very poor indicator of total below-ground allocation (Kubiske & Godbold, 2001). Norby (1994) advocated that because of this functional difference, the response of root systems to elevated CO_2 should be studied separately for coarse woody roots and for fine roots. The system of ephemeral fine roots has often been found to be the most responsive to elevated CO_2 among all plant parts. Fine root density (mass of roots per unit ground area) has been found to increase in *Pinus sylvestris* (Janssens *et al.*, 1998), *Populus grandidentata* (Zak *et al.*, 1993), *Quercus alba* (Norby *et al.*, 1995a), to name just a few studies reporting positive effect of elevated CO_2 on fine root biomass. Although it is unlikely that increased biomass of fine roots will have effect on whole plant biomass (Norby *et al.*, 1999), increased cycling of C through greater fine root mass and faster turnover could have a long-lasting effect on the C cycle in the soil. Increased rate of root turnover has been reported in elevated CO_2 conditions (Fitter *et al.*, 1997; Pregitzer *et al.*, 2000). Despite these efforts, not enough information about the direct impact of elevated CO_2 on root turnover is available. Furthermore, the amount of assimilates transferred below-ground through fine roots can be affected by changes in the rate of root respiration (Matamala & Schlesinger, 2000; Nijs *et al.*, 2000) and exudation (Hodge & Millard, 1998) under elevated CO_2.

It is known, that the response of tree root systems to elevated CO_2 is mediated by interactions with other factors (Pregitzer *et al.*, 2000). Soil nutrient status, especially N availability, was found to interact strongly with elevated CO_2 to determine root response (Curtis *et al.*, 1994; Zak *et al.*, 2000; Kubiske & Godbold, 2001). High N content of the soil, combined with elevated CO_2, usually results in proportionally more biomass allocated to roots than to shoots. However, it is not clear whether this effect lasts over a long period of time, when the soil becomes fully exploited due to higher fine root density. It has been speculated, that

elevated CO_2 reduces the rate of decomposition of plant organic matter due to a lower N:C ratio (Cotrufo *et al.*, 1998), thus resulting in N deficiency in the long run (Norby *et al.*, 2001). Other factors of climate change, such as temperature (Atkin *et al.*, 2000) and water availability (Leuschner *et al.*, 2001) have been found to affect fine root production and turnover, potentially influencing the response of roots to elevated CO_2.

2.3.2 Mycorrhizal functioning

As in most other plants, tree roots often have symbiotic associations with mycorrhizal fungi. Trees allocate between 10% and 30% of their photoassimilates to their mycorrhizal symbionts (Markkola *et al.*, 1996). Photosynthesising plant hosts exchange C in the form of carbohydrates with mycorrhizal symbionts. This C is invested into the symbiosis in exchange for the provision of nutrients (Hawkins *et al.*, 2000; Smith *et al.*, 2001), water (Hampp *et al.*, 1999) and pathogen (Miller, 1993), heavy metal pollutant (Godbold *et al.*, 1997) and drought resistance (Auge, 2001) by the fungal partner. It has been hypothesised that trees growing in elevated CO_2 allocate more C to mycorrhizas, resulting in improved water and nutrient acquisition by the hosts (Luxmoore, 1981) and in greater proliferation of mycorrhizal fungal structures in the soil (Rillig *et al.*, 1999). Since elevated CO_2 has the largest positive effect on production in high N conditions (Ceulemans & Mousseau, 1994), increased mycorrhizal proliferation could be of great importance in maintaining enhanced tree growth rates in soils with low N availability (Hodge, 1996). Increased mycorrhizal biomass provides further C sink for additional photosynthate assimilated in elevated CO_2 conditions, reducing downregulation pressure on the rate of photosynthesis (Bazzaz, 1990). The literature describing the effect of elevated CO_2 on mycorrhizas is inconclusive, mainly due to failure to separate the effect of CO_2 on plant growth from any specific effect on fungal symbionts (Norby & Jackson, 2000). There are several reviews published recently evaluating the response of mycorrhizas to

elevated CO_2, notably those of Staddon & Fitter (1998), Treseder & Allen (2000) and Kubiske & Godbold (2001), concluding that elevated CO_2 does not affect all mycorrhizas equally and that other factors such as nutrient availability and plant community structure have an effect on root colonisation under elevated CO_2.

2.3.2.1 *VAM under elevated CO_2*

Percentage root colonisation is the most commonly used variable in examining the effect elevated CO_2 has on mycorrhizal symbiosis. Frequently, vesicular-arbuscular mycorrhizas (VAM) are reported not to respond to elevated CO_2 with a higher rate of root colonisation, especially in high N conditions (Klironomos *et al.*, 1997; Rillig & Allen, 1998). Equally, increases of VAM infection caused by elevated CO_2 have been reported (Lovelock *et al.*, 1996; Rillig *et al.*, 2001) and even a decrease of root colonisation by VAM was observed under elevated CO_2 conditions (Klironomos *et al.*, 1996).

It is important to distinguish between various fungal structures found within a plant root, because of their functional significance. Although the structure-function relationship is not yet fully understood (Klironomos, 1995), morphologic features can be used as indicators of mycorrhizal functioning. Therefore, different response by hyphae, vesicles and arbuscules to CO_2 enrichment might indicate a functional shift in the plant-fungus association. In a study carried out with *Artemisia tridentata*, it has been found that in elevated CO_2 and low nutrient conditions there were higher frequencies of hyphae and arbuscules, while in high nutrient availability a dominance of vesicles and spores was observed (Klironomos *et al.*, 1996).

2.3.2.2 *ECM under elevated CO_2*

The response of ECM to elevated CO_2 has been widely studied and was found to be dependent on specific plant-fungal associations. In the

majority of cases, a positive effect of elevated CO_2 on ECM colonisation of root tips was observed. For example, ECM colonisation increased in *Quercus alba* (Norby *et al.*, 1987), *Betula papyrifera* (Godbold *et al.*, 1997), *Pinus palustris* (Runion *et al.*, 1997) and in *Pinus ponderosa* (DeLucia *et al.*, 1997) under elevated CO_2. However, no effect was found in *Pinus taeda* (Lewis *et al.*, 1994) or in *Pinus sylvestris* (Peres-Soba *et al.*, 1995).

With reference to tree hosts of ECM, Kubiske & Godbold (2001) noted that, in the studies comparing the response of ECM on a number of tree species a difference between the effect on broadleaves and on conifers can be seen. It seems that the positive effect of elevated CO_2 on ECM colonisation is greater in deciduous broadleaves, signifying the importance of specific plant-fungus relationships.

2.3.2.3 *Change in mycorrhizal species composition*

Mycorrhizal groups vary in the direction and magnitude of their response to elevated CO_2 resulting in changes in the mycorrhizal community structure (Cairney & Meharg, 1999). Because of their identifiable morphology, shifts in the species composition of ECM are more frequently reported in the literature, although observations of VAM community structure exist.

The composition of ECM colonising roots of *Quercus alba* (O'Neill *et al.*, 1987), *Betula papyrifera* (Godbold *et al.*, 1997), *Betula pendula* (Rey & Jarvis, 1997) and *Pinus sylvestris* (Kasurinen *et al.*, 1999) changed under elevated CO_2. In contrast, Runion *et al.* (1997) reported no change in ECM species composition on *Pinus palustris*.

Klironomos *et al.* (1998) report that hyphal lengths of *Acaulospora denticulata* and *Scutellospora caulospora* increased under elevated CO_2 and those of two *Glomus* species used in the study did not. However, the fungi were grown in separate cultures and did not compete for resources.

Since mycorrhizal fungi differ in their ability to acquire nutrients (Jakobsen, 1995), any shift in mycorrhizal community structure is likely to result in altered nutrient status of the host plant. In reverse, the functioning of mycorrhizas depends on the supply of carbohydrates by the host plant. Due to increased photosynthesis under elevated CO_2 more assimilates are supplied to the root systems (Norby, 1994). It has been suggested (Godbold *et al.*, 1997; Rey & Jarvis, 1997), that greater amounts of available carbohydrates are favouring late-successional mycorrhizas, which might have higher demands for C supplied by the host. It has not been established whether this process takes place, but it could be one of the mechanisms causing changes in mycorrhizal community structure.

2.3.2.4 *Synergic effect on root biomass and mycorrhizas*

It has been shown that percentage root colonisation is a poor estimate of mycorrhizal response to elevated CO_2 (Hodge, 1996). It is important to consider this measure of mycorrhizal activity in conjunction with the response of the plant host's root system. Since most studies report an increase in fine root biomass (Curtis & Wang, 1998), even no change of percentage colonisation implies an increase of mycorrhizal biomass present in the soil.

In addition, extra-radical hyphal networks have been found to differ in their response to elevated CO_2 from intra-radical structures. Although the colonisation of VAM inside the root of *Prunella vulgaris* remained constant, the length of external VAM hyphae under elevated CO_2 was up to 5 times greater (Sanders *et al.*, 1998). Since the extra-radical network is the functional structure where acquisition of nutrients and water by the fungus takes place, its response to elevated CO_2 might play an important role in determining the character of mycorrhizal associations in a CO_2 rich world.

2.3.3 C cycle in the soil

It is reasonable to assume, that rhizodeposition increases as a result of greater biomass production stimulated by elevated CO_2. Hence, enhanced delivery of organic matter into the soil could influence soil microbial communities and subsequently alter decomposition rates, nutrient availability and soil C storage (Curtis *et al.*, 1994). The biomass of soil microorganisms is estimated to contain 1-3% of C held in a terrestrial ecosystem (Wardle, 1992). It is therefore unlikely that any change of microbial biomass mediated by elevated CO_2 will have a significant direct impact on the amount of C stored in the soil. However, since soil microbes, including mycorrhizal fungi, alter the quality and quantity of C that is allocated below ground (Rygiewicz & Andersen, 1994), the flow of carbohydrates through microbial biomass is a key factor in determining the fate of C in the soil.

The flux of CO_2 from the soil can be utilised to characterise integrated response of plant root systems and soil microorganisms to elevated CO_2. Zak *et al.* (2000) in their review of published studies conclude that elevated CO_2 results in more rapid CO_2 efflux from the soil, indicating a greater soil C cycle in terms of size. However, very few of the reported increases are statistically significant. Greater rates of soil C efflux could result from increased root biomass, root respiration, microbial respiration or a combination of all the factors. To date, the balance between increased input of C into the soil and more rapid soil CO_2 efflux has not been fully investigated and its effect on C storage in the soil is not known (Norby & Jackson, 2000).

In addition to the quantitative changes of organic matter entering the soil profile, qualitative alterations in plants growing in elevated CO_2 can affect C cycling in the soil. These originate from changes in plant, fungal and microbial species composition and shifts in biomass partitioning, resulting in a bigger proportion of more slowly decomposing

roots (Ceulemans *et al.*, 1999). One of the main chemical changes observed in plant tissues grown in elevated CO_2 is accumulation of total non-structural carbohydrates (TNC) (Schappi & Corner, 1997; Wurth *et al.*, 1998). Since these are readily decomposable substances, decomposition rates of plant parts should be enhanced in the initial stages, but it is unlikely that the decomposition rates will be affected after this initial stage (Berntson & Bazzaz, 1996). It is also accepted, that plants grown in elevated CO_2 have higher tissue C/N ratios by 14% on average (Cotrufo *et al.*, 1998). Large differences in the changed C/N ratio mediated by elevated CO_2 were observed between plant species. Reduced N concentration is expected to result in a decrease of the rate of decomposition of organic matter in the soil (Verburg *et al.*, 1998). However, this issue has not been clarified yet, as decreased (Boerner & Rebbeck, 1995; Cotrufo & Ineson, 1995), unchanged (O'Neill & Norby, 1996) and increased (Couteaux *et al.*, 1991) decomposition rates were reported for organic matter originating from elevated CO_2 conditions when compared to ambient. This inconsistency might be related to large interspecific variation of the effect elevated CO_2 has on C/N ratio (Cotrufo *et al.*, 1998).

A major factor affecting the amount of C stored in the soil under elevated CO_2 conditions is likely to be the amount of C transferred from the plant roots to mycorrhizal symbionts. C derived from mycorrhizal tissue can account for a significantly sized pool within ecosystems (Rillig & Allen, 1999), mainly because a substantial proportion of C transferred to mycorrhizas is turned into chitin and glomalin, both long-living substances (Gooday, 1994). The turnover rate of mycorrhizal fungal hyphae is largely unknown, together with any effect elevated CO_2 might have on it (Staddon & Fitter, 1998; Treseder & Allen, 2000). However, it was hypothesised that since elevated CO_2 can increase fine root turnover, it could subsequently increase the turnover of fungal networks dependent on C supply from fine roots (Pregitzer *et al.*, 1995).

It has been established, that increased input of organic matter into the soil can affect nutrient availability. However, there exist contrasting hypotheses about the direction in which it will be affected by elevated CO_2. Zak *et al.* (1993) argued that increased deposition of organic matter into the soil would support larger microbial biomass and thus increase mineralisation and nutrient availability. Conversely, Diaz *et al.* (1993) suggested that greater microbial biomass would lock up nutrients and cause nutrient deficiency in the soil. Klironomos *et al.* (1996) have shown that the effect of elevated CO_2 on the functioning of the soil microbial community is closely related to nutrient status of the soil. Under elevated CO_2 and in low nutrient conditions they observed a more mutualistic C flow system with predominance of mycorrhizas, while high nutrient availability resulted in an opportunistic saprotroph/pathogen system of C flow in the soil.

2.4 *C sequestration*

The concentration of atmospheric CO_2 has reached values that have not occurred during the recent geological past (Thomas, 2001). Climate models predict that it will continue to rise and could exceed 500 ppm during the second half of this century (Amthor, 1995). It has been suggested that one of the possible means of removing excess CO_2 from the atmosphere is locking up C in the soils of terrestrial ecosystems. According to van Kessel *et al.* (2000), this can be achieved only if the following two conditions will be satisfied in a CO_2 rich world. (i) A sustained increase in the rate of photosynthesis will be accompanied by increased allocation of organic matter into the soil. (ii) Soil C mineralisation will lag behind increased C input. At present, the influence of elevated CO_2 on soil C storage is estimated through ecosystem CO_2 exchange (soil CO_2 efflux), C isotope tracking and direct C stock measurement.

Several studies have shown that the surface soil CO_2 efflux increases under elevated CO_2 under *Pinus radiata* (Thomas *et al.*, 1996) and *Pinus ponderosa* (Vose *et al.*, 1997). However, Niklaus *et al.* (2000) in their analysis of methods warn, that CO_2 efflux measurement severely overestimates the amount of C sequestered in the soil. Although methodologically difficult, stable C isotope measurements present a more accurate alternative. Hungate *et al.* (1997) observed a large increase of C allocation to labile below-ground C pools in rye grassland grown under elevated CO_2. A similar effect was observed in cotton (Leavitt *et al.*, 1994) and in *Betula pendula* (Ineson *et al.*, 1996). Since direct C stock observations are insensitive to increases in the soil C storage in the range expected (Niklaus *et al.*, 2000) and because of the inherent heterogeneity of the soil and variability introduced by sampling (Hungate *et al.*, 1997), direct estimate of the effect of elevated CO_2 on soil C storage remains a formidable task.

Following an increase in the atmospheric CO_2 concentration, an increase in mean atmospheric temperature (Houghton *et al.*, 1990) is expected to enhance the rate of decomposition of organic matter. However, the rate of decomposition could also decrease due to increased C:N ratio (Diaz *et al.*, 1993), altered degree of soil weathering (van Kessel *et al.*, 2000) or due to a shift in species composition mediated by elevated CO_2 (Heijmans *et al.*, 2001).

Added all together, these factors influencing the retention of C in the soil embody a complex web of interactions which make a generalised estimate of soil C sequestration under elevated CO_2 very difficult to make. It is likely that the amount of C locked up in the soil will be dependent on local conditions and because both input and output processes are dependent on a variety of feedback mechanisms, the ultimate result of elevated CO_2 on C sequestration in the soil can only be determined from long term experiments (van Kessel *et al.*, 2000).

3 Carbon dioxide enrichment

The ability to predict the likely influences of atmospheric change on natural ecosystems is one of the major challenges of ecology at present. Particularly, the increase in the concentration of CO_2 - a gas necessary for photosynthesis - constitutes a factor likely to affect the functioning of ecosystems which include green plants. Attempts to predict future vegetation responses to elevated CO_2 have resulted in a diverse range of experimental designs and facilities created to test the reaction of anything, from a single photosynthesising cell to a whole ecosystem, to a CO_2 level much higher than today. As well as scale, researchers varied the length of exposure of plants to elevated CO_2 from only a few days fumigation to assess immediate physiological response to continuous measurements on plants grown under elevated CO_2 conditions for several growing seasons (Ashenden & McLeod, 1993). This chapter explains various techniques used to simulate future atmospheric levels of CO_2; describes the experimental set-up of POPFACE and, in brief, outlines the performance of the fumigation system utilised for this research.

3.1 Review of available CO_2 enrichment technology

The concentration of carbon dioxide in the atmosphere is thought to have been rising due to human activity in the past two centuries. The level of CO_2 in the pre-industrial period, estimated at $\approx$ 280ppm, has increased to the present value of $\approx$ 360 ppm (Watson *et al.*, 1990) and is predicted to double by the middle of this century. As soon as the scientific community realised that this phenomenon might have profound consequences for ecosystems, the effect of elevated CO_2 came under close scrutiny. As is always the case with emerging fields of research, the first to be examined were the simplest of ecosystems: those based on agricultural crops (Lemon, 1983). Numerous studies were carried out on agricultural plants, followed by examination of wild herbaceous species and communities,

such literature is reviewed by, among others, Bazzaz (1990). However, it is estimated that trees account for up to 70% of terrestrial carbon fixation (Melillo *et al.*, 1993) and therefore might play an important role in any attempt at carbon sequestration. Subsequently, studies aimed at improving our understanding of possible effect of elevated CO_2 on tree species emerged (Ceulemans and Mousseau, 1994; Norby *et al.*, 1999), even though, in comparison to crop species, the response of trees to CO_2 enrichment is slightly less well understood. This might be due to a large buffering effect of the woody storage compartments (Janssens *et al.*, 2000) or due to the obvious complications arising from the size of the ecosystems studied and from simulating the development of natural canopy in time. As already mentioned, numerous techniques have been devised to study the effects elevated CO_2 has on plants, the most important of which are briefly explained in the following four sections. Since the subject of this research is *Populus* plantation, emphasis has been put on studies dealing with woody species.

3.1.1 Greenhouses and closed chambers

At the beginning of the CO_2 work, greenhouses and closed chambers have been the main methodology of choice given their simplicity and, in some cases, existing infrastructure. This method involves growing plants in pots in greenhouses with altered atmospheric conditions to simulate ambient and future levels of CO_2 (Enoch and Kimball, 1986, Korner and Arnone, 1992). Given the space constrictions, studies of tree species have been limited to examining seedlings of very young trees (Saxe *et al.*, 1998). With the invention of phytotron techniques, tree species have also been examined in chambers where all environmental conditions were controlled (Ceulemans and Mousseau, 1994). A phytotron allows for exposure of examined plants to a wide combination of factors, CO_2 concentration being only one of them (Bazzaz, 1990). A number of variations of the closed chamber technique have been

used, for example installation of controlled plastic chambers inside a greenhouse (Radoglou & Jarvis, 1990) or field application of chambers with environmental variables other than CO_2 concentration kept at ambient levels (Overdieck, 1993).

With some level of flexibility, a 'branch bag' method can be considered a modification of closed chamber technique. This method involves enclosing selected branches of usually mature tree in plastic bags and exposing them to elevated CO_2 concentrations (Barton and Jarvis, 1999; Rayment and Jarvis, 1999; Hogg *et al.*, 2000) and assumes the validity of branch autonomy theory (Sprugel *et al.*, 1991).

Common to all these closed-space facilities are some limiting factors hindering their usage for tree research, notably limited space and inadequate light conditions.

3.1.2 Open top chambers (OTC)

At the moment, OTC are the most widely used facilities for studies involving CO_2 enrichment. As the name suggests, this technique entails enclosing part of an ecosystem in an open chamber and using the 'half closed' space thus created to expose plants to varying environmental factors. The conditions inside an OTC differ only slightly from those on the outside, although some effects on temperature, humidity and wind speed cannot be excluded. The OTC technique originally developed for pollution studies (Olczyk *et al.*, 1980; Nystrom *et al.*, 1982; Wang *et al.*, 1986) has later been used for examining a variety of plant communities (Allen *et al.*, 1992; Ashenden *et al.*, 1992; Owensby *et al.*, 1993). Although this method has been extensively developed, it still suffers from artefacts inherent in its basic design and a comparison with conditions on the outside or construction of control chambers remains necessary. It has to be mentioned that, similarly to the closed chamber technique, application of OTC imposes a considerable size constraint on the trees being studied. This point is stressed by Norby *et al.* (1999), who in their review present a

protocol of replicated experiments in which whole trees were exposed to elevated CO_2 under field conditions. Out of 31 studies listed in the review, only 4 have been carried out on trees older that 5 years with the majority of experiments involving seedlings or clonal cuttings.

3.1.3 Natural CO_2 springs

The possibility of using natural CO_2 springs to study long-term effects of elevated CO_2 on plants has been noted only recently (Miglietta *et al.*, 1993). These springs existed for decades or centuries and offer a unique opportunity to study acclimation of natural ecosystem to elevated CO_2 conditions, albeit in a rather uncontrolled manner (Newton *et al.*, 1996). Experimental use of these sites has highlighted their variability and their spread across the world (*CO_2 springs and their use in biological research workshop*, San Miniato, 1993). Naturally occurring CO_2 vents have been used to study perennial grasslands (Cook *et al.*, 1998), Mediterranean macchia (Tognetti *et al.*, 1998; Stylinski *et al.*, 2000) or carbon and nitrogen pools in the soil (Ross *et al.*, 2000).

Drawbacks that have to be taken into account when studying plants exposed to elevated CO_2 by a natural vent include lack of proper control plots and great spatial and temporal variability of CO_2 enrichment.

3.1.4 Free air CO_2 enrichment (FACE)

To exclude all environmental disturbances between the plants treated with elevated CO_2 and control plants and to expose entire ecosystems, the FACE technique has been developed (Harper *et al.*, 1973; McLeod *et al.*, 1985; Hendrey & Kimball, 1994; Hendrey *et al.*, 1993). Originally designed for agricultural crops, the system had been applied to a wide range of ecosystems. Table 3.1 lists some of experiments utilising FACE technology of CO_2 enrichment which are carried out at present.

Table 3.1 FACE experiments implemented at present.

Location	Ecosystem	CO_2 level	Plot Diam.
Braunschweig (D)	agronomic crops in rotation	550 ppm	20 m
Bulls (NZ)	grazed pasture grassland	475 ppm	12 m
Cedar Creek (USA)	C3 and C4 grasses (BioCON)	550 ppm	20 m
Clermont-Ferrand (F)	temperate grassland (MEGARICH)	600 ppm	1-2 m
Cumbria (UK)	bog (BERI)	560 ppm	1 m
Dublin (IRL)	temperate grassland (MEGARICH)	600 ppm	1-2 m
Durham (USA)	Loblolly pine forest (FACTS-I)	Amb+200 ppm	30 m
Eschikon (CH)	grassland	600 ppm	18 m
Giessen (D)	potatoes (CHIP) and grassland	560 ppm	8 m
Godollo (H)	perennial grassland (MEGARICH)	600 ppm	1-2 m
Jasper Ridge (USA)	grassland	2 x amb	2 m
Kopparasmyren (S)	bog (BERI)	560 ppm	1 m
Les Chaux-des-Breuleux (CH)	bog (BERI)	560 ppm	1 m
Maricopa (USA)	agronomic crops	Amb+200 ppm	23-25 m
Mekrijarvi (FI)	bog (BERI)	560 ppm	1 m
Munich (D)	temperate grassland (MEGARICH)	600 ppm	1-2 m
Nevada desert (USA)	Mojave desert scrub community (NDFF)	550 ppm	23 m
Oak Ridge (USA)	sweetgum plantation	Amb+200 ppm	25 m
Rapolano (I)	temperate grassland (MEGARICH)	600 ppm	1-2 m
Rapolano (I)	potatoes (CHIP), agronomic crops	560,600 ppm	22 m, 30 m
Rhinelander (USA)	Aspen forest (FACTS-II)	Amb+200 ppm	30 m
Shizukuishi (JPN)	rice and paddy fields	Amb+200 ppm	12 m
Sky Oaks (USA)	chaparral	550 ppm	16 m
Viterbo (I)	poplar plantation (POPFACE)	550 ppm	20 m
Wageningen (NL)	bog (BERI)	560 ppm	1 m
Yabulu (AU)	tropical savanna + planted eucalyptus and acacia seedlings	450 ppm, 500 ppm	15 m

Source: http://cdiac.esd.ornl.gov/programs/FACE/whereisface.html

FACE experiments are almost unanimously considered to provide the best opportunity to expose parts of plant communities to conditions of elevated atmospheric CO_2. In its original design, a FACE system consists of sets of horizontally or vertically positioned blowers releasing either ambient or CO_2 enriched air into the atmosphere. The blowers are usually arranged in some sort of circular shape enclosing the experimental area into which air mixed with CO_2 is blown. This allows for observation of the

reaction of the plant community to elevated CO_2 with minimal artefacts due to the operation of CO_2 enrichment equipment. However, the use of blowers requires large air feeding pipes to be installed and to circulate large volumes of air significant infrastructure must be installed. This also implies construction of control plots where everything, apart from CO_2 enrichment, is operated as in the FACE (Miglietta *et al.*, 2001a).

To further improve the technique, a method releasing pure CO_2 into the air has been devised (Okada *et al.*, 2001). This design might offer some advantages over the conventional system, notably dispensing with the need for large infrastructure and with construction of ambient air releasing pipes in the control plots. This system is explained in greater detail in the following section, as it represents the CO_2 enrichment method applied for this research.

3.2 *POPFACE*

The research presented in this thesis forms a part of 'A European Free Air Carbon Dioxide Enrichment Experiment on Poplar plantations' project. The main objective of this collaborative work is to investigate the response of a number of *Populus* species to the expected increase in atmospheric CO_2 concentrations. For this purpose, project participants designed and built a large scale Free Air Carbon Enrichment experiment which was operated from June 1999 until November 2001. The following sections explain the technology behind CO_2 fumigation and the field performance of the POPFACE system.

3.2.1 Experimental set-up

The plantation and FACE facility is located in central Italy, near the city of Tuscania, province of Viterbo. The site is at 42°37'04''N , 11°80'87''E and is 150 m above the sea level. *P.euramericana* (I-214) cuttings were planted during the spring of 1999 over 9 ha of former wheat field at a commercial spacing of 2 m x 1 m. Within this plantation, 6 experimental

plots were equally spaced in order to avoid enrichment pollution of 'control' plots with air blown in from 'FACE' plots (Figure 3.1). The whole of the 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. The amount of water was sufficient to avoid water stress even during the hottest days of a growing season.

Before planting, the whole of the field was ploughed to a depth of approximately 50 cm and tilled. The soil is derived from volcanic deposits and characterised by large clay content. This fact, together with extensive fertilisation by previous users, resulted in soil rich in N and other nutrients. The C content of the soil was uniform all over the field, at around 1%. Similarly, other characteristics of the soil, such as water holding capacity, porosity and bulk density, were similar in all location on the selected field. The only exception was stone content, significantly higher in and around plot number 5, due to the somewhat elevated position of this part of the field.

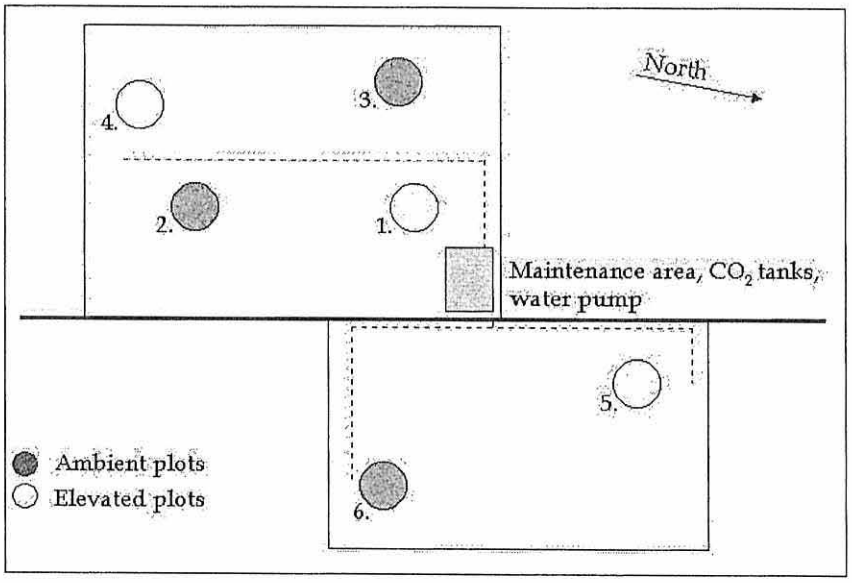


Figure 3.1 Layout of POPFACE plantation

In each of the experimental plots cuttings of the following tree species were planted at the same time as the rest of the plantation: *Populus alba* (clone 2AS-11), *Populus nigra* (clone Jean Pourtet) and *Populus euramericana* (*Populus deltoides* x *Populus nigra*, clone I-214). Table 3.2 lists some general characteristics and differences between the three species used. The plots were 20 m x 20 m in size and the trees were planted at 1 m x1 m spacing 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. Figure 3.2 illustrates the planting scheme for plots 1 and 2. In the remaining two pairs of plots, i.e. 3 - 4 and 5 - 6, the planting scheme was rotated to avoid possible bias due to uneven growth of different species.

Table 3.2 General characteristics of utilised *Populus* species (* - indicative for Central Italy).

Species	*P. alba* L.	*P. nigra* L.	*P. x euramericana* Dode (Guinier)
Genotype	2AS11	Jean Pourtet	I-214
Sex	Male	Male	Female
Origin	Italy (seed)	France (seed)	Italy (cuttings)
Rooting capacity	medium	very good	very good
Branching habit	medium	very high	low
Apical control	good	good	very good
Bud-burst*	end of March	end of March	end of March
Bud set*	end of October	beginning of October	middle of September

Within each plot, a circle 20 metres in diameter determined experimental trees, enclosing 314 individuals on which the measurements were carried out. Trees located on the border of each section, either next to trees of a different species or on the perimeter of the plot, were excluded from the measurements.

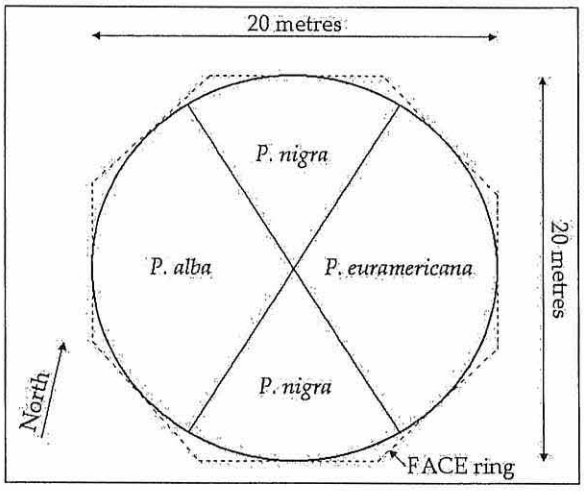


Figure 3.2 Layout of experimental plot

Three plots (2, 3 and 6) were without any FACE infrastructure and were used as ambient CO_2 controls. The remaining three plots were equipped with octagonal-shaped FACE rings providing atmospheric CO_2 enrichment. Thus, this experimental set-up provided a block design consisting of three replicates per treatment. The data, from both below- and aboveground measurements, were tested for block effect with experimental plots blocked as follows: 1 and 2, 3 and 4, 5 and 6. However, no significant block effect was found and bias introduced due to positioning of the blocks was not considered further in the analysis. The statistical tests performed on this basis offer a good chance to consider the CO_2 treatment effect. Prior to performing ANOVA analysis, all measurements reported in this work were averaged for each *Populus* species and for each plot, resulting in a single value for each variable per plot per species. All errors reported in this work were calculated from the three averaged values for each treatment. Data were checked for normality (Kolmogorov-Smirnov test) and for equality (Levene's test) and two-way repeated measures ANOVA analysis was performed (CO_2 treatment X *Populus* species; df=215), where time was used as split-plot factor. Thus, CO_2 treatment represented 2 factors (df=1), *Populus* species 3 (df=2) and 9 repeated measurements were carried out for each replicate (df=8). It has to be mentioned, that this approach, although presenting sound basis for

testing the effects of elevated CO_2, obscures possible interactions between plot and CO_2 treatment.

Each of the FACE plots enclosing an area of circa 350 m^2. The size of the ring was designed to provide an internal area with equal distribution of CO_2 with a diameter of 16 metres. Each ring had eight telescopic masts supporting layers of horizontally positioned polyethylene pipes (diameter 25 mm) releasing pure CO_2. The pipes were suspended at various heights to facilitate even vertical distribution of CO_2 within the canopy and the masts allowed for easy and rapid vertical movement of the pipes to account for fast growth of the poplars. At the beginning of the experiment, immediately after planting, an initial single layer of pipes was located at approximately 50 cm above ground. Later into the experiment second and third layer of pipes were added, reaching a maximum height of 12 m. In each layer, a side of the octagon consisted of two paired pipes carrying 350 and 500 jets respectively (Figure 3.3).

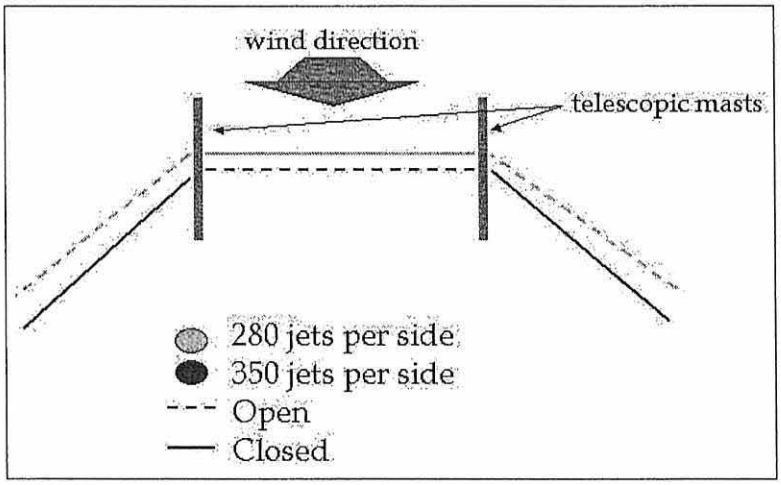


Figure 3.3 Operational principle of a FACE ring

The jets were of a mean diameter of 0.3 mm, drilled manually for the initial season, using laser technology for the subsequent two seasons to improve performance and accuracy of CO_2 release. The directional control of CO_2 fumigation was provided by 16 on/off solenoid valves and an automated pressure regulator controlling the amount of released CO_2. The

system was run by a programmable microprocessor unit based in the centre of each FACE ring. An algorithm used by this unit adjusted the amount of released CO_2 every second, according to the measurements of wind speed, wind direction (30 sec average) and CO_2 concentration. Detailed description and analysis of the design of POPFACE fumigation system is given by Miglietta *et al.* (2001b).

3.2.2 Performance of CO_2 enrichment system

The CO_2 enrichment system became operational on 29th June 1999 and remained in operation throughout the first growing season until 5th December 1999. At the onset of fumigation only 1 layer of gas delivery pipes was installed in each FACE ring. Over this period the three replicated elevated CO_2 systems have been operational for 148 days, or 93% of the time. Major interruptions were caused only by system maintenance and occasional delays in liquid CO_2 refilling. The daily duration of fumigation decreased from an initial 16 hours per day in June down to 9 hours at the end of the season. The performance of the FACE system was evaluated by recording of 1-minute average CO_2 concentration by an IRGA[2] located at the centre of each FACE plot. There is some consensus in the scientific community that the performance of a FACE can be judged as successful if 1-min average of CO_2 concentration measured every second is within ±20% variation off a target for more than 80% of the time (Miglietta *et al.*, 2001b). In the first growing season this target was reached for all three FACE replicates with the seasonal means at 545, 544.8 and 541.7 ppm for plots 1, 4 and 5 respectively – representing a 91% rate of CO_2 concentration being within ±20% of 550ppm target. Figure 3.4 shows daily mean CO_2 concentrations (full line) and mean daily wind speed (dotted line) measured at the centre of the plot throughout 1999 growing season for plot 5.

[2] IRGA – Infra-red Gas Analyser, here model PPSystem, SBA-1, Hitchin, UK

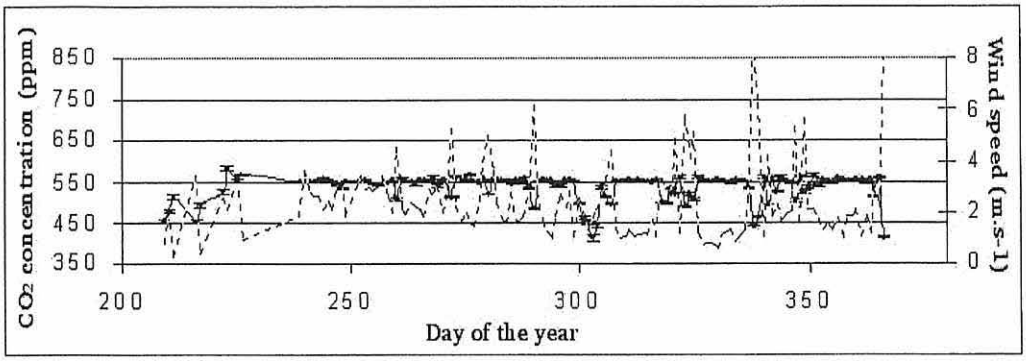


Figure 3.4 Daily mean CO2 concentration and wind speed in 1999

The major exceptions from overall satisfactory performance were days with very high wind conditions, during which the daily mean CO_2 concentrations dropped significantly. This can be explained by the fact, that an automatic cut-off of CO_2 supply was imposed for wind in excess of 10 $m.s^{-1}$, as gas flow through the pressure regulator became insufficient. However, it should be noted that the wind climate of Tuscania had a positive effect on the system performance as the air-CO_2 mixing was enhanced by windy conditions. On average, the wind rotated from north-east in the morning to south-west in the afternoon with a slight dominance of wind from the south-westerly direction. This fact must be taken into consideration, because the plants on the south-west side of the FACE plots were thus exposed to marginally higher concentrations of CO_2 than the plants growing on the north-east side.

During the second growing season, the fumigation was maintained for 198 consecutive days, from 14th April 2000 to 31st October 2000. CO_2 enrichment was achieved for 94% of this time, despite unexpected and increasing internal corrosion of the CO_2 tank in the second half of the season. This, together with the number of days with high wind conditions during which a target of 500 ppm instead of 550 ppm was maintained is shown in Table 3.3.

Table 3.3 Days of fumigation during second year

Month	Days with no fumigation	Days at 500 instead of 550 ppm target	Days with full enrichment
April	3	-	11
May	-	-	31
June	3	-	27
July	1	-	30
August	1	3	26
September	1	6	23
October	-	2	28
TOTAL	**11(5.5%)**	**11 (5.5%)**	**176 (89%)**

During the second growing season a consistent degradation of system performance was observed, a likely consequence of the increasing height of the canopy when in august 2000 a second layer of pipes had to be installed. At the beginning of the season the CO_2 concentration was within ±20% of the 550 ppm target for 92% of the time, a satisfactory value which decreased to 80-82% at the end of the season (Table 3.4).

Table 3.4 Fraction of successful fumigation in the second year

Month	Fraction of time	Standard deviation
April	0.92	0.068
May	0.91	0.093
June	0.85	0.110
July	0.88	0.066
August	0.84	0.086
September	0.80	0.095
October	0.82	0.065
Seasonal	**0.86**	-

Overall, successful fumigation was achieved for 86% of the time in 2000, representing seasonal averages of 544.2, 541.8 and 544.8 ppm for plots 1, 4 and 5 respectively.

The third and final year of CO_2 enrichment was marked by some modifications of the fumigation system to account for increasing height of the poplars and for 'honami' effect caused by plants waving during windy conditions. Once the initial problems were solved, the performance of the modified system markedly improved as figure 3.5 illustrates for the April to August period.

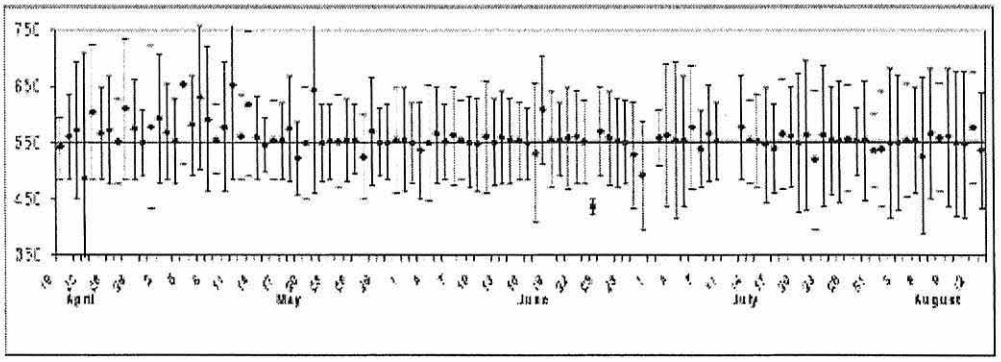


Figure 3.5 Average CO_2 concentrations during 20 01

Overall performance of the fumigation system was satisfactory even during the third growing season. At the end of the experiment, with *Populus* plants reaching 10 m in height, 5 layers of horizontal pipes were necessary to provide sufficient amount of CO_2.

4 Root biomass

There is growing consensus among scientific community that environmental conditions are being modified globally (IPCC, 1996). The worldwide increase in the atmospheric concentration of CO_2 constitutes part of the evidence of this global change, increasing at annual rate of 1% or more (Scarascia-Mugnozza *et al.*, 2001). This change might have profound effects on terrestrial ecosystems and it is an imposing task to unravel the complexity of ecosystems' responses. Numerous studies have attempted to shed light on this issue with varying degree of success. One of the reasons for difficulties encountered might be the fact that the key components of ecosystem response may reside out of sight - the below-ground system of roots, soil and associated organisms (Norby and Jackson, 2000). Despite complications inherent to investigating below-ground processes, evidence accumulated so far suggests a possible link between elevated atmospheric CO_2 and increased deposition of organic matter into the soil profile (Rogers *et al.*, 1994). This chapter briefly outlines the current state of knowledge in this area, describes the methods utilised for measuring root production at POPFACE and discusses the findings in view of the present state of knowledge.

4.1 Elevated CO_2 and root production

Root production has been suggested to constitute about half of the carbon annually cycled in forests (Vogt *et al.*, 1998) and 33% of the global net primary production (Jackson *et al.*, 1996). Hence, if green plants are to play a significant role in carbon sequestration, an accurate estimate of the effects future levels of CO_2 might have on below-ground processes is needed. It is argued, that additional C fixed under a CO_2-enriched atmosphere increases total plant biomass production, including that of roots (Curtis and Wang, 1998). However, this increase of C fixation due to stimulation of photosynthesis might be short-lived owing to a reduction in

photosynthetic capacity (Ceulemans and Mousseau, 1994). In these circumstances, it is of importance to conduct long-term CO_2 exposure experiments which allow acclimation processes to run their course. Different mechanisms have been invoked to explain the CO_2 acclimation processes, particularly changes in sink strength and nitrogen or water limitation (Scarascia-Mugnozza *et al.*, 2001). This is especially true for rhizosphere processes, where a change mediated by elevated CO_2 might take a longer time to demonstrate itself.

A number of reviews focused on below-ground plant response to elevated CO_2 have been published in the past, notably those of Norby *et al.* (1994); Rogers *et al.* (1994); Berntson and Bazzaz (1996a) and Curtis and Wang (1998). In general, the presented conclusion is that the roots often achieve the biggest and the most consistent growth enhancement among all plant parts (Pritchard and Rogers, 2000). However, understanding of the root response to elevated CO_2 might be limited because most data available come from experiments carried out with juvenile plants or come from only a single sampling period (Rogers *et al.*, 1999).

Root responses to CO_2 have been studied in a wide diversity of plant species, from agronomic and horticultural species to forest species and even natural communities. However, the majority of CO_2 studies containing root data have been conducted using crop plants (Rogers *et al.*, 1994). The most frequently measured response of root systems to elevated CO_2 has been dry weight, with virtually all studies reporting an increase of below-ground dry weight under elevated CO_2. Whether this increase is sustainable at ecosystem level and in the longer term remains a question worth of further investigation (Norby and Jackson, 2000). This is particularly so, when other environmental variables which have been found to have significant interacting effects with elevated CO_2 (e.g. soil moisture, nutrient availability and competition) are taken into account (Pregitzer *et al.*, 1995).

Similarly, the effect that elevated CO_2 might have on photosynthesised C partitioning within plants received ample attention. Results of these investigations do not allow for a straightforward conclusion, as the influence of elevated CO_2 on root:shoot (R:S) ratio produces variable responses. As Rogers *et al.* (1994) report in their review, a similar proportion of studies describe both an increase and a decrease of R:S ratio as a result of elevated CO_2 treatment. The ratio was suggested to increase more in agricultural crops, particularly in root and tuber ones, while forest species and trees show no alteration in R:S partitioning of biomass. This hypothesis is supported by the meta-analysis of Curtis and Wang (1998) which shows no significant increase of R:S ratio in tree species as a result of elevated CO_2 alone.

The availability of nutrients for plant growth is largely influenced by roots, since for most plants roots are the main organ for nutrient acquisition, particularly that of N. Given that the uptake of N is dependent upon the availability of C to fine roots (Oaks, 1993) and, in reverse, C assimilation in leaves is dependent on the supply of N from roots (Poorter *et al.*, 1995), effects of CO_2 on roots will affect whole plant nutrition. As already mentioned, elevated CO_2 usually increases the size of plants resulting in an increased need for the uptake of nutrients. Plants grown in poor or ambient nutrient conditions show greater biomass with lower nutrient concentration (Norby *et al.*, 1986), but if plants are supplied with additional nutrients, elevated CO_2 does not have an effect on nutrient concentration in tissues (Israel *et al.*, 1990) or on root mass ratio (Sigurdson *et al.*, 2001).

All these changes could have a profound effect on below-ground processes. Increased input and a change in quality of root derived organic C in the soil leads to many intriguing problems: a change in mass and turnover of soil biota (Lussenhop *et al.*, 1998), availability of N and other nutrients (Mikan *et al.*, 2000) and the relative contribution of leaf and root

derived C into the soil C pool (Gorissen *et al.*, 2000). These questions will have to be answered if we are to consider the ultimate fate of C in the soil and the only solid foundation offering a chance to understand them is an accurate and reliable measure of the amount C input into the soil.

4.2 *Measurement of root biomass*

4.2.1 Auger coring

Since the object of measurement – roots – is not easily accessible to the researcher, most techniques utilised are either disruptive or totally destructive to the root system studied or to the surrounding environment (Taylor *et al.*, 1991). The sampling design almost invariably presents a compromise between maximising the amount of samples due to the spatial variability of root systems and minimising the disturbance of the studied system while taking into account high labour requirements of the work carried out. One of the most common methods used to assess standing root biomass present in a volume of soil is auger coring. The basic auger corer described by Schuurman and Goadewaagen (1971) has undergone many modifications but invariably consists of a metallic cylindrical tube for extraction of soil cores and a T shaped handle facilitating soil penetration and removal. The core must be large enough to obtain a reasonable sample volume. In general, a core 5-8 cm in diameter is the most commonly used. This method is suitable for a wide range of soil conditions, although excessively stony soils might present a difficulty. All soils must be sampled to a minimum depth of 30 cm or to the bottom of the plough layer if one is present in the soil (Oliveira *et al.*, 2000). In general, this method for measuring standing root biomass is well developed and reliable.

4.2.2 Sampling design at POPFACE

4.2.2.1 Timetable of measurements

Standing root biomass was measured by auger sampling throughout the duration of the experiment. The first measurement was carried out in November 1999, subsequent sampling sessions took place in March, July and November 2000 and in March, May, July, September and November 2001. At all times the sampling strategy remained the same with the sampling alternately carried out in northern and southern halves of experimental plots[3]. All lab measurement described below were conducted identically for all samples of roots from each sampling session.

4.2.2.2 In situ *root sampling*

An auger corer 8 cm in diameter was utilised with root samples being taken from 2 soil horizons separately: surface layer 0 – 20 cm in depth and a bottom layer 20 – 40 cm in depth. Three samples per species per plot were taken from the vicinity of randomly selected tree at 30 cm, 50 cm and 70 cm distance from the stem to allow for measurement of spatial distribution of roots[4]. Care was taken not to sample root system of one tree more than once. Extracted soil cores were soaked in water overnight to allow for easy extraction of roots from the soil. Subsequently, cores were placed on a 2 mm mesh and the soil was washed off with running water. Roots were then collected from the surface of the mesh, divided into two diameter classes of fine (<2 mm) and coarse (>2 mm) roots and placed into labelled paper bags.

[3] See Figure 3.2 for details of plot design.

[4] For more information about POPFACE sampling and statistical design see section 3.2.1.

4.2.2.3 *Lab based measurements*

After transfer to the lab, all samples of roots were dried for 5 days at 60 ^{0}C and weighed. All samples were then kept for later analyses, such as ash content at 500 ^{0}C and DNA identification. All resulting data were tested for statistical significance of differences between treatments using repeated measures ANOVA in SigmaStat for Windows.

4.3 *Results*

The following sections present and describe the results of measurements carried out during the experiment. The data covering root production are divided into four subsections, each representing a separate root category or characteristic that can be utilised when constructing a larger picture depicting below-ground production.

4.3.1 Total standing root biomass

Exposure of all three *Populus* species to elevated atmospheric CO_2 resulted in bigger trees with greater root systems, which is in accordance with generally accepted hypothesis. Figure 4.1 shows standing root biomass of *Populus alba* throughout the duration of the experiment. A steady build-up of root biomass of *Populus alba* is evident for both FACE and control. Apart from the measurement in November 2000, trees grown under elevated CO_2 consistently display greater root biomass. Repeated measurement ANOVA has shown statistically significant difference between the treatments.

Similar results were obtained for the remaining two species utilised in the experiment: *Populus nigra* and *Populus euramericana*, data for total root biomass are shown in Figures 4.2 and 4.3 respectively. For both species, trees grown under FACE treatment maintain greater root biomass at any point in time and the difference between the treatments is statistically significant.

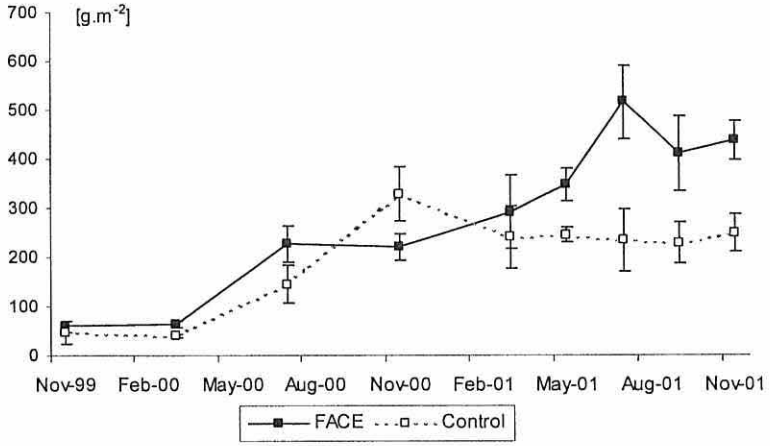


Figure 4.1 Total root biomass of *Populus alba* (±st.error; $P = 0.046$)

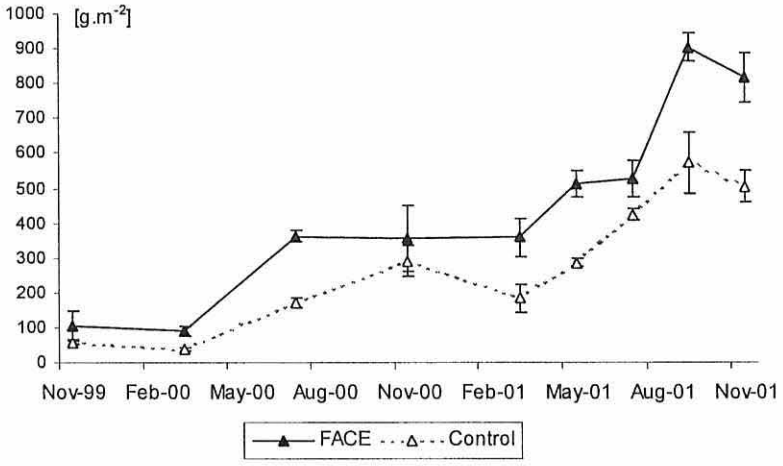


Figure 4.2 Total root biomass of *Populus nigra* (±st.error; $P = 0.002$)

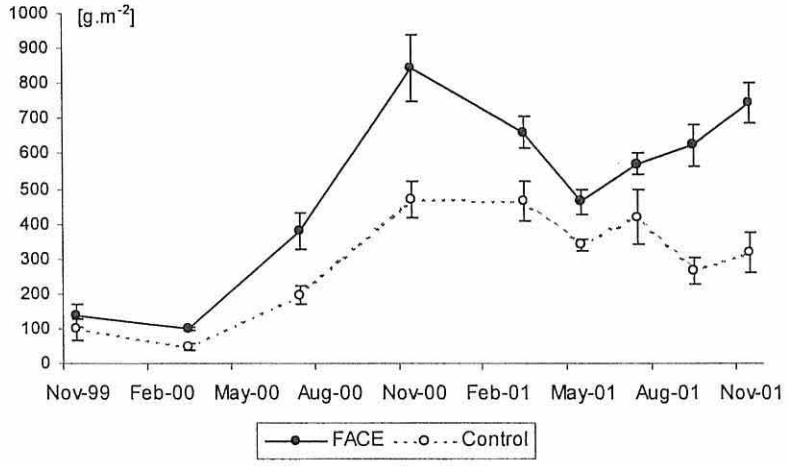


Figure 4.3 Total root biomass of *Populus euramericana* (± st.error; $P = 0.002$)

Elevated CO_2 has the same effect on all three species - bigger root systems are produced and maintained. However, the *Populus* species utilised in this research do not respond to FACE treatment with the same magnitude of increase in root production. *Populus alba* is the species with the smallest increase of standing root biomass induced by FACE: +47% (±26% st.error). *Populus nigra* and *Populus euramericana* responded to FACE treatment with +76% (±24% st.error) and +71% (±21% st.error) increase respectively. All data report live root biomass, because only negligible amounts of root necromass were detected in the soil. This is probably due to very fast rate of decomposition of dead root biomass in this ecosystem.

4.3.2 Fine root biomass

Fine roots represent physiologically the most important part of a root system, therefore it is of importance to present the data describing standing fine root biomass even though fine root production and turnover in the POPFACE plantation are presented in more detail in Chapter 5. In general, fine root biomass follows the same trends and shows a similar response to FACE as total root biomass, of which it is part. Figures 4.4, 4.5 and 4.6 show fine root biomass for *Populus alba, Populus nigra* and *Populus euramericana* respectively.

Similarly to total root biomass, the species with the smallest increase in fine root biomass is *Populus alba*, for which FACE treatment increased the amount of fine roots present in the top 40 cm of the soil by +35% (±24% st.error). *Populus nigra* and *Populus euramericana* responded to elevated CO_2 with +84% (±22% st.error) and +53% (±22% st.error) enhancement of fine root biomass. Increases due to FACE are statistically significant for *Populus nigra* (P=0.004) and for *Populus euramericana* (P=0.014), whereas for *Populus alba* (P=0.205) the enhancement due to elevated CO_2 is not significant.

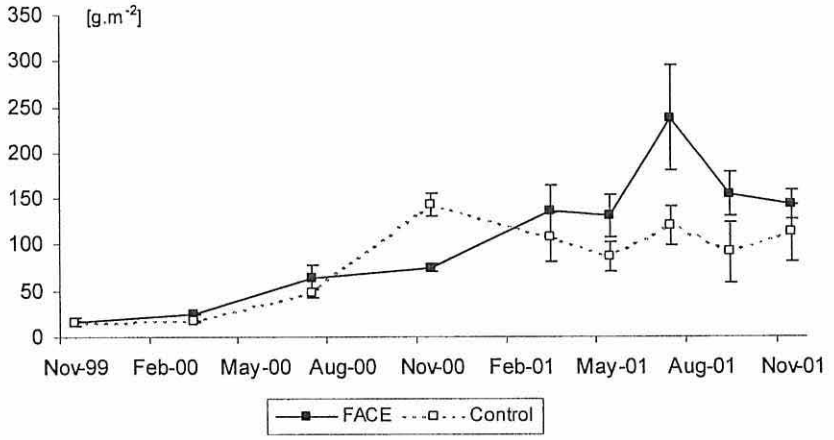


Figure 4.4 Fine root biomass of *Populus alba* (±st.error; $P = 0.205$)

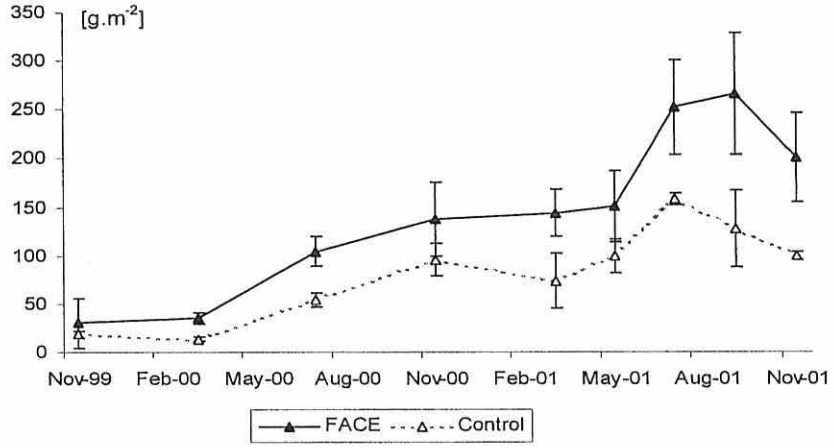


Figure 4.5 Fine root biomass of *Populus nigra* (±st.error; $P = 0.004$)

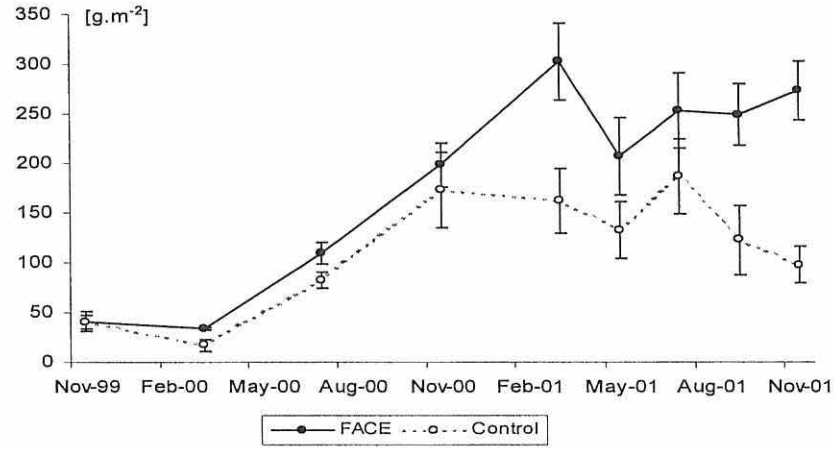


Figure 4.6 Fine root biomass of *Populus euramericana* (± st.error; $P = 0.014$)

The proportion of fine roots in total root biomass is not greatly altered by FACE (Table 4.1). This ratio changed significantly as a result of elevated CO_2 treatment only for *Populus alba*, where FACE treatment caused a slight decrease in the proportion of fine roots. In general, fine roots constitute between 35 and 40% of total root biomass for all three species. In addition to this, the percentage remains stable over time with the same amount of below-ground biomass allocated to fine roots throughout the whole duration of the experiment, as figure 4.7 shows for *Populus nigra*.

Table 4.1 Proportion of fine roots in total root biomass

	Populus alba		*Populus nigra*		*Populus euramericana*	
	FACE	Control	FACE	Control	FACE	Control
Average	37%	41%	34%	32%	36%	39%
St. error	3.8	3.6	4.3	3.9	4.6	2.9
P-value	0.030		0.289		0.398	

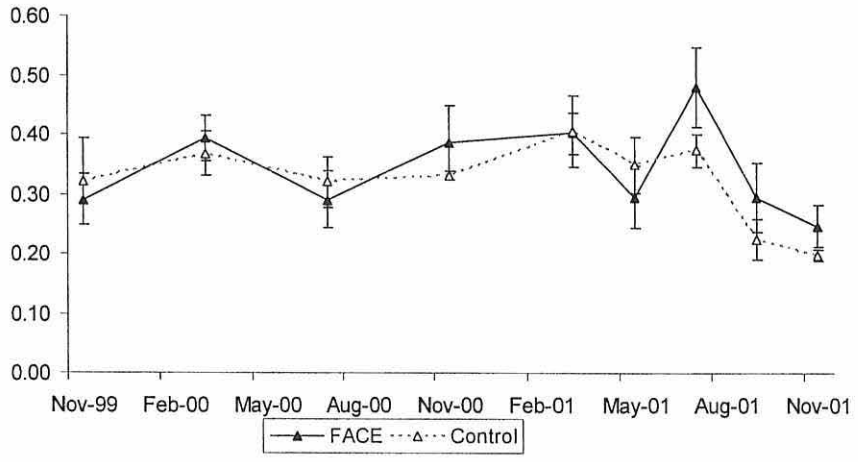


Figure 4.7 Proportion of fine roots in total root biomass of *Populus nigra*

4.3.3 Root biomass as a function of distance from the stem

Data show, that soil profile more distant from a stem was progressively colonised, as the trees grew bigger. Figures 4.8 (FACE) and 4.9 (control) depict the amount of roots of *Populus euramericana* present in the soil at three distances from the stem: 30 cm, 50 cm and 70 cm on a diagonal line drawn across a square drawn between four neighbouring trees. Data are shown as a percentage difference between the measured value and the average value over all distances (100%=average). Therefore, three horizontal lines at 100% would signify uniform colonisation of available space the soil and, conversely, lines distant from 100% display wide variation in the amount of roots present in the soil at various distances from the stem.

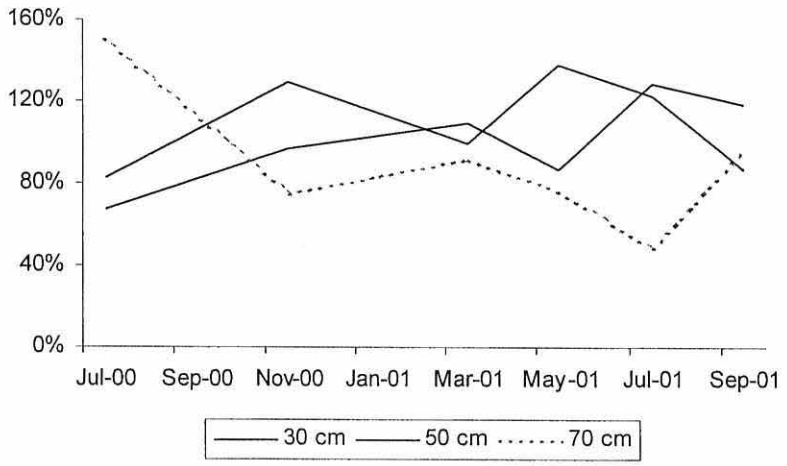


Figure 4.8 Proportion of root biomass according to distance from the stem for *Populus euramericana* - FACE treatment

It can be seen from the figures, that at the end of the last growing season of the experiment all the values are converging towards the average, confirming the assumption that the soil profile is becoming fully colonised by the roots. It is clear especially from figure 4.9 that at the beginning of the observation the soil is practically devoid of roots, hence all the values are close to 100%. As the soil becomes colonised, close to the stem at first, the relative proportion of roots at 30 cm from the stem rises to

170%. At the end to the observation, when soil is fully colonised, even at the furthest measurement point the proportionate values converge around 100% - which represents the average. Similar results were obtained for *Populus alba* and *Populus nigra,* although no statistically significant difference in the pace of soil colonisation was detected between FACE and control.

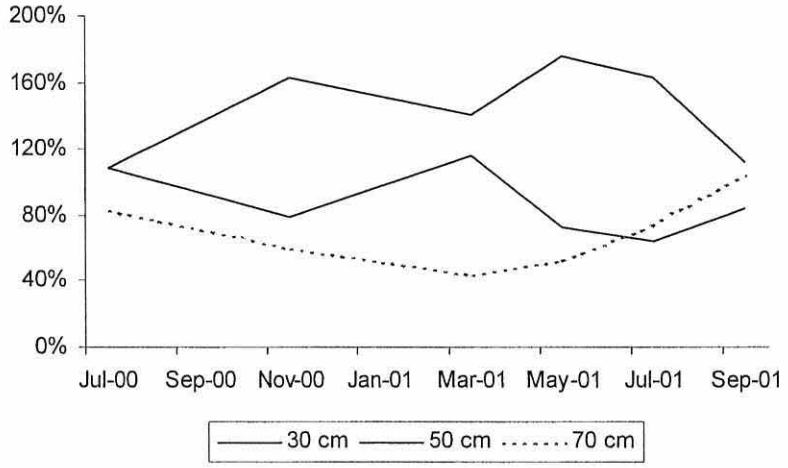


Figure 4.9 Proportion of root biomass according to distance from the stem for *Populus euramericana* – Control

4.3.4 Root biomass depth allocation

Both *Populus alba* and *Populus nigra* invested a significantly greater proportion of their root biomass into the deeper soil horizon under the FACE treatment. Average root biomass present in the top 20 cm of soil expressed as a percentage of total root biomass measured is shown in Table 4.2.

Table 4.2 Proportion of root biomass in top 20 cm

	Populus alba		*Populus nigra*		*Populus euramericana*	
	FACE	Control	FACE	Control	FACE	Control
Average	62%	78%	63%	76%	60%	62%
St. error	2.6%	2.9%	5.2%	5.5%	7.0%	7.9%
P-value	<0.001		0.001		0.778	

Populus euramericana did not respond to FACE treatment by changing the depth allocation of root biomass, suggesting that this type of response might be species specific. Both *Populus alba* and *Populus nigra* increased rooting depth as a result of FACE treatment from the beginning of the experiment, as illustrated in figures 4.10 and 4.11.

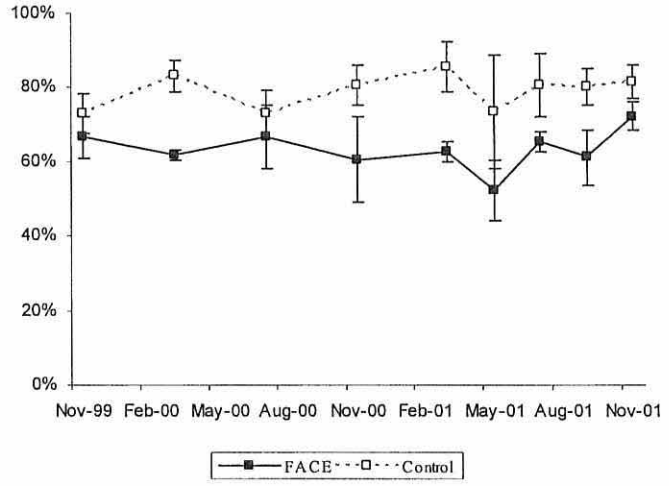


Figure 4.10 Percentage of root biomass in top 20 cm - *Populus alba*

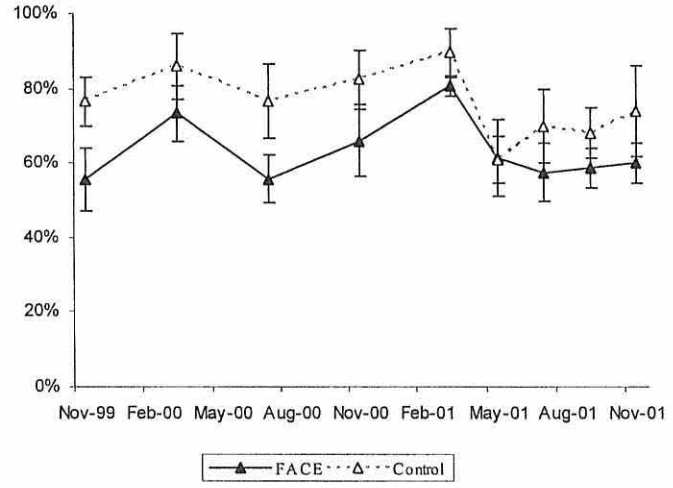


Figure 4.11 Percentage of root biomass in top 20 cm - *Populus nigra*

4.4 *Discussion*

Tingey *et al.* (2000) in their review present a range from -21% to +225% (median +52%) as a response of root production of coniferous trees to elevated CO_2. Similarly, broadleaves are reported to respond with an

increase of below-ground production (Kinney & Lindroth, 1997). In this research, differences in below-ground production in response to FACE treatment were observed.

Comparing the root production between species (Table 4.3) demonstrates that *Populus nigra* and *Populus euramericana* outproduce *Populus alba* with respect to below-ground biomass.

Table 4.3 Comparison of root biomass between species (values in g.m^{-2}), max and mean after three years growth

	Populus alba		*Populus nigra*		*Populus euramericana*	
	FACE	Control	FACE	Control	FACE	Control
Maximum	515	328	902	571	843	469
Average	266	188	401	252	471	287

Data for all 3 years of the experiment show average total root biomass of *Populus nigra* to be 34% and that of *Populus euramericana* 52% greater than root biomass of *Populus alba* in ambient conditions. This difference further increases under FACE treatment, to 50% and 76% respectively. This might be explained by the poorer rooting capacity of *Populus alba* in comparison to other two species (see Table 3.2) or by different rooting strategies employed by these species. A similar picture can be drawn above-ground, both biomass production and the magnitude of the response to elevated CO_2 show similar trends (Calfapietra, 2002). *Populus nigra* and *Populus euramericana* produce greater above-ground biomass than *Populus alba*. However, this difference is of lesser magnitude than that below-ground.

The abovementioned measurements could provide some insight into R:S partitioning of assimilated carbon. Above-ground response to FACE of *Populus alba* and *Populus euramericana* is approximately half the

size of below-ground response (45% and 49% respectively), while the same ratio for *Populus nigra* is 75% (Calfapietra, 2002). Based on this evidence, it might be safe to conclude that the R:S ratio of selected species responds to elevated CO_2 in different fashion.

Table 4.4 Relative enhancement of biomass production by FACE (Above - D^2H [cm^3], Below - total root biomass [gm^{-2}])

	Populus alba		*Populus nigra*		*Populus euramericana*	
	Above	Below	Above	Below	Above	Below
Average	23%	50%	57%	75%	39%	78%

At present, the general consensus is that effects on biomass partitioning between roots and shoots are dependent on the interaction between elevated CO_2 and N availability (Eamus & Jarvis, 1989; Kubiske & Godbold, 2001). Given that the *Populus* species in this research were all grown in identical environmental conditions, a third characteristic might have a role in R:S partitioning - the response to elevated CO_2 could be species specific (Kinney & Lindroth, 1997). An important factor worthy consideration may also be the relative sink strength of the root systems (Arp, 1991). Different rooting strategies that sustain adequate nutrient uptake and water supply under elevated CO_2 (Rogers *et al.*, 1992) might offer an explanation as to why R:S ratios differ among the species utilised.

Fine roots are of particular physiological importance for water and nutrient uptake (Bosac *et al.*, 1995) and at the same time represent a major sink of carbon as in most ecosystems a significant portion of carbon fixed through photosynthesis is allocated to production and maintenance of fine roots (Pregitzer *et al.*, 1995). Given these facts, it is likely that plants will respond to elevated CO_2 and to associated above-ground physiological changes with an alteration of biomass or with a change in the functioning of fine roots. The results of this research indicate that both alterations are

taking place, but are species specific. Data for *Populus nigra* and *Populus euramericana* seem to support the general assumption that elevated CO_2 produces bigger plants with greater root systems. If the proportion of fine roots over total root biomass remains stable regardless of the size of the plant, it could be hypothesised that there is little functional change in the role the fine roots play. Greater nutritional needs of bigger plants growing under elevated CO_2 are satisfied by greater number of roots foraging the soil. On the other hand, *Populus alba* - the species with the smallest root system - responded to FACE treatment with a small but significant decrease of the proportion of fine roots. This fact might point to a functional change in fine roots leading to an increase in acquisition of water and nutrients.

As Farrar and Jones (2000) in their analysis of carbon allocation into roots point out, it is of great importance to try to distinguish between short-term response - which might just be bigger plants - and long-term response occurring after the plants begin to deplete soil resources. As a result of stratified coring, it was possible to evaluate whether all the soil available has been fully colonised by the roots. Due to the relatively high planting density in the experimental plots (10,000 stems per ha) and fast growth of *Populus* trees, full colonisation of the soil was facilitated by the end of the 3rd growing season. Based on the results, it can be argued that towards the end of the experiment the trees were experiencing effects usually associated with an established forest ecosystem, where root competition or nutrient depletion affects root production.

To evaluate the effect of elevated CO_2 on root distribution in vertical direction, each sample of root biomass has been divided into two strata: 0 -20 cm and 20 - 40 cm in depth. It has been suggested that approximately 50 - 80% of roots in forests can be found in the top 30 cm of soil (Jackson *et al.*, 1996). Given that the POPFACE plantation has been

drip irrigated from the surface, it might be safe to assume that similar or greater proportion of roots can be accessed by sampling in the top 40 cm.

There does not exist a consensus in the literature about whether plants respond to elevated CO_2 by vertical shifts in the distribution of root biomass. Arnone *et al.* (2000) report an upward shift in root length density in native grassland grown for several successive seasons under FACE treatment. Similarly, elevated CO_2 was reported to stimulate root growth near the surface in grass growing on a limestone soil (Fitter *et al.*, 1997). On the other hand, *Pinus radiata* growing under elevated CO_2 produces relatively more fine roots deeper in the soil profile. Thomas *et al.* (1999) and Day *et al.* (1996) have shown a greater proliferation of fine roots both near the surface and at greater depth in an oak-palmeto scrub ecosystem subjected to elevated atmospheric CO_2. One of the possible explanations as to why some species produce more roots in deeper soil horizons could be an increase of competition for resources due to bigger and more extensive root systems present under elevated CO_2. However, the fact that *Populus alba* produced smaller root biomass than *Populus euramericana*, yet invested significantly more biomass into a deeper soil horizon seems to contradict this suggestion. Moreover, as figures 7.10 and 7.11 show, the difference in depth allocation of roots in *Populus alba* and *Populus nigra* was evident since the onset of the experiment, but the competition due to full colonisation of the surface of soil emerged only at the end of the last growing season.

More observations later in the development of this plantation would be needed if the question of functional response of root systems to FACE is to be answered reliably. According to Fitter *et al.* (1996), the effect of elevated CO_2 on spatio-temporal pattern of root growth is system dependent, affecting root distribution in some ecosystems and root turnover in others.

4.5 *Conclusions*

Elevated CO_2 is positively affecting the amount of root biomass and the enhancement of root production is statistically significant in all three *Populus* species utilised in this research. Similarly, fine root production is increased under elevated CO_2 while the proportion of fine roots in total root biomass does not change. Two of the species, *Populus alba* and *Populus nigra* significantly altered the depth allocation of root biomass as a result of exposure to FACE treatment.

These findings must be taken into account when considering the fate of assimilated carbon. Both the amount of C transferred underground and its depth allocation might have profound effects on its fate within the soil. Moreover, when considering the impact of elevated CO_2 on an ecosystem or when assessing long-term plant response to this phenomenon, its effects on intra- and inter-specific competition must be taken into account.

5 Root dynamics

A sizeable body of literature suggests that increasing levels of atmospheric CO_2 enhance net primary production of terrestrial plant ecosystems (Ceulemans *et al.*, 1999). Higher production of biomass could lead to accumulation of C in plant tissues or in the soil - thus offering a possible explanation for the fate of C which remains unaccounted for in the global carbon cycle models (Schimel *et al.*, 1995; Keeling *et al.*, 1996). Elevated CO_2 seems to increase the total input of organic material into the soil not only by enhanced root production (Berntson & Bazzaz, 1996b; Godbold *et al.*, 1997) but also by increasing root turnover rates (Fitter *et al.*, 1997). Fine root growth and dieback play an important role in these processes (Matamala & Schlesinger, 2000) and assimilated C allocation into growth and maintenance of fine roots might constitute a significant proportion of C transferred below-ground. Although studies of biomass allocation in terrestrial ecosystems have often emphasized the role of long-lived and therefore larger and more accessible parts (Kodrik, 1998) it could be that investigating the smallest components with high rates of turnover might give us a better insight into the functioning of an ecosystem. In this chapter, the influence of elevated CO_2 on fine root production and turnover is investigated and the data presented detail the amount of organic matter transferred into the soil under the two different CO_2 treatments.

5.1 Response of root dynamics to elevated CO_2

Root turnover constitutes a crucial component of ecosystem carbon cycling (Aber *et al.*, 1985) and its response to elevated CO_2 might be of great importance when considering the ecosystems' potential for C sequestration. As mentioned in section 4.1, root systems are often reported, in terms if size, as the biggest beneficiaries of elevated atmospheric CO_2 among plant parts. This, in connection with the fact that

fine root growth and maintenance costs in trees may represent as much as 67-70% of total net primary production (Fogel, 1985), makes the investigation of the effects elevated CO_2 might have on fine roots especially worthy.

For example, yellow-poplar saplings grown under elevated CO_2 showed an increased rate of photosynthesis which was accompanied by increased fine root production with a consequent increase in the C flux through the root system (Norby *et al.*, 1992). Similarly, this is also the case for many studies of trees growing under elevated CO_2 where higher root production, and subsequently turnover, increases carbon losses from the root systems (Janssens *et al.*, 1998; Cheng, 1999). There is no general relationship between the amount of photosynthate produced and the amount of fine roots, mainly because factors such as nutrient and water supply come into play (Schulze *et al.*, 1996). Hence plants growing under elevated CO_2 might simply invest more biomass to fine roots in order to satisfy their increased need for nutrients and water (Matamala & Schlesinger, 2000).

Numerous studies report a strong link between fine root response to elevated CO_2 and the availability of nutrients in the soil, usually signified as availability of N. Pregitzer *et al.* (1995) suggest that the availability of N in soil is a major factor regulating fine root growth of *Populus eugenei* under different CO_2 treatments. High soil N availability combined with elevated CO_2 translates into larger fine root systems, while in low N elevated CO_2 does not increase fine root production in *Populus tremuloides* (Zak *et al.*, 2000). Moreover, increased fine root growth and turnover can have a positive feedback effect on soil C and N dynamics, producing greater N availability (Zak *et al.*, 1993). The indications, however, are not clear - soil N mineralisation decreased in soil under *Castanea sativa* seedlings grown under elevated CO_2 (Rouhier *et al.*, 1994).

It seems that the mechanisms controlling fine root response to elevated CO_2 are rather complicated and will require further analysis. However, evidence collected thus far suggests that additional sugars produced by photosynthesis under elevated CO_2 are usually transferred below-ground and utilised for increased fine root production and turnover.

5.2 *Fine root measurement*

Several methods have been used to estimate fine root biomass, production and turnover (Nadelhoffer & Reich, 1992). It is likely that the most frequently used method is sequential coring, although this method has been reported to underestimate fine-root production (Hendrick & Pregitzer, 1992) due to its inability to estimate root growth and death between sampling dates. Other methods include use of ingrowth cores (Messier & Pluttonen, 1993) and minirhizotrons (Majdi, 1996). All three techniques were applied in combination in this research to estimate fine root production and turnover. Sequential coring has been explained in detail in Chapter 4, more space in this section is therefore devoted to the latter two methods.

5.2.1 Ingrowth cores

Ingrowth coring is one of the most common methods used to measure root production (Oliveira *et al.*, 2000). Ingrowth cores are usually used to give an independent estimate of root production when determining root turnover and changes in standing root biomass by sequential coring methods. In general, a volume of soil is extracted from the soil profile, sieved free of roots and then re-packed into the original space in the profile separated from surrounding soil by a nylon mesh. Fine root production is then estimated as the amount of roots penetrating into the ingrowth core per specified duration of time (Steen, 1984). However, ingrowth cores have disadvantages, namely that they may alter the

physical and chemical characteristics of the soil used to fill them (Makkonen and Helmisaari 1998) and also that severing roots during installation may increase root production (Oliveira *et al.*, 2000). Because of these drawbacks the ingrowth core method is especially suitable for comparison of fine-root production between sites or treatments (Messier and Pluttonen 1993) rather than straightforward measurement of root production. Most commonly, the cores are installed well before the onset of measurement to allow for root growth to occur (Vogt *et al.*, 1998) and are left in the soil for prolonged time periods. Rytter and Rytter (1998) have shown that fine root turnover in a fast-growing willow plantation to be 4.8 to 8.8 year^{-1}. Thus the roots of fast growing systems are renewed every 2 months on average, suggesting that for long incubation periods (0.5 - 1 year) high level of root turnover might be expected in ingrowth cores. Expecting a similarly high fine root turnover in POPFACE plantation, a modification of ingrowth core method was introduced.

5.2.1.1 *Evaluation of a modified ingrowth coring method*

A Short Rotation Coppice Plantation at Henfaes Farm, University of Wales, Bangor was utilised to evaluate the modification of ingrowth coring which was later used at the POPFACE site. In this investigation 3 plots planted with *P. interamericana* (clone Beaupre) and 3 plots with *P. euramericana* (clone Ghoy) were used[5]. In two separate experiments ingrowth cores were inserted into the soil profile either immediately after

[5] A Short Rotation Coppice Plantation No.691 (Forestry Commission Research Division, UK), with *Populus* clones was utilised for this experiment. The site is located at Henfaes Farm, University of Wales, Bangor (53 °14'N, 4°01'W, altitude 15 m). The plantation was established in March 1996 and coppiced in March 1997 and again in February 2000. In this investigation three plots planted with *P. interamericana* (clone Beaupre) and three plots with *P. euramericana* (clone Ghoy) were used. All plots are 10 trees x 10 trees spaced alternately 1.5 m and 0.75 m between rows, and 0.9m in the rows. Within each plot a set of five randomly placed replicates of each type of ingrowth core was established. These were always placed in the row between two trees to minimise the influence of distance from a stump on root production .[6] For detailed planting design and layout of the plantation see section 3.2.1

a fresh hole was dug in the soil or after a 'settling-down' period during which a PVC spacer tube was used to keep a pre-dug hole open. Both experiments showed that, contrary to Oliveira *et al.* (2000), root injury caused by installation of ingrowth core does not cause an increase in root growth.

In the first trial, two series of ingrowth cores were left in the soil for 13 weeks. One series was inserted into freshly dug holes, the other into holes which were pre-dug and kept open for 8 weeks by spacer-tubes before the insertion of an ingrowth core. A third series of ingrowth cores was left in the soil for 21 weeks as a control. As figure 5.1 shows, in the ingrowth cores that used spacer-tubes, after 13 weeks the fine root biomass of both 'Beaupre' and 'Ghoy' was similar to the biomass determined in the 21 week old conventional ingrowth cores. This can be explained by fine roots reaching full establishment in the ingrowth core. However, in cores using a spacer-tube, root production greatly exceeds that of the freshly cored ingrowth-cores for both clones during the latter 13 weeks of the experiment. More detailed explanation of this experiment can be found in Lukac & Godbold (2001).

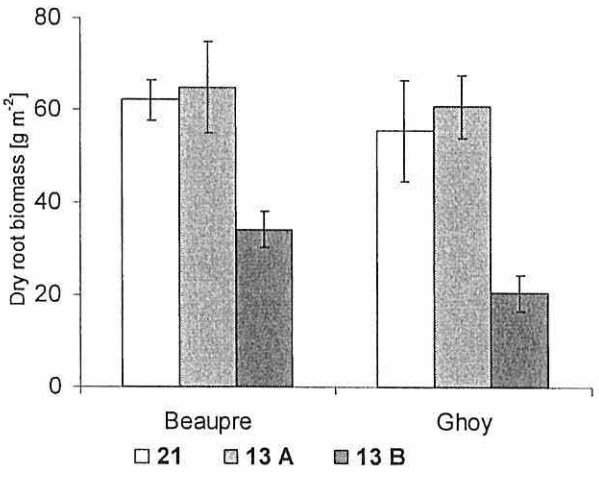


Figure 5.1 Comparison of ingrowth cores with and without spacer-tubes (± st. error)

Key: 21- cores left in the soil for 21 weeks, 13A - 13 weeks with pre-installed spacer tubes, 13B - 13 weeks without spacer- tubes

The second trial evaluating the modification of ingrowth coring method was aimed at establishing the time length of the delay in fine root growth caused by installation of ingrowth cores. Three plots planted with *P.euramericana* (clone Ghoy) at the abovementioned location were utilised. Two series of ingrowth cores were installed at the beginning of June 2001, one series into freshly dug holes, the other into holes already kept open for three months with the use of spacer-tubes. Cores were then extracted at monthly intervals until November 2001.

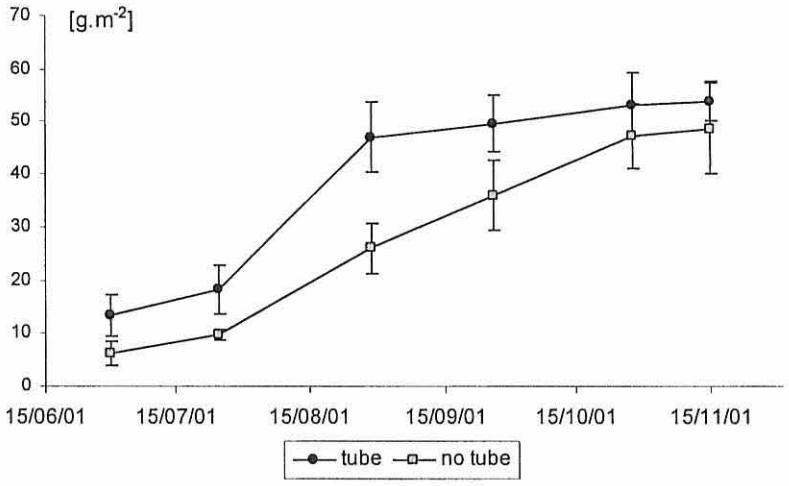


Figure 5.2 Ingrowth of fine roots into cores with a nd without spacer-tubes (± st. error)

As figure 5.2 suggests, for the environmental conditions at Henfaes Farm, Bangor, fresh installation of ingrowth core delays fine root production in *P. euramericana* (Ghoy) by about 1.5 to 2 months when compared to a core inserted into a pre-dug hole. In other words, the ingrowth cores inserted into freshly dug holes take about 2 months longer to fill with fine roots and after this period the difference between the two types of cores is not significant. However, if a continuous measure of fine root production without delays is required, the modified technique appears to be better suited. Due to these circumstances, the spacer-tube method of ingrowth coring was utilised in POPFACE.

5.2.1.2 *Ingrowth core set-up utilised in POPFACE*

Series of randomly spaced holes were pre-dug in the soil profile before planting of all species utilised in the POPFACE experiment[6]. There were 20 holes per species in each of the experimental plots, every hole was 4cm in diameter and 40 cm deep. Each set of holes was divided into four series of five holes, three of them (15 holes in total) were inserted with 4 cm PVC spacer-tubes. The tubes were closed at both ends with expanding foam filler to minimise soil moisture and temperature changes in the vicinity of a tube. All tubes were labelled and inserted into the soil flush with the surface. The remaining 5 holes per species per plot were inserted with a series of ingrowth cores in order to measure fine root production from the onset of the experiment.

In order to fill an ingrowth core, first an allotment of local soil from the vicinity of each plot was sieved free of roots. Then a 2 mm mesh was inserted into the pre-dug hole and filled with the sieved soil. Care was taken to compact the soil to the original bulk density, as this might be one of the key factors affecting ingrowth of roots into the core (Steingrobe *et al.*, 2000). Average bulk density of undisturbed soil was obtained and an amount of soil to be compacted into each ingrowth core was calculated in order to achieve appropriate compaction.

To facilitate the measurement of root production over a given time period, at the beginning of the period a series of 5 spacer-tubes were extracted from the soil and 5 ingrowth cores were inserted into freed holes and left in the soil profile for the duration of the chosen period of time. At the end of the period, ingrowth cores were extracted and the holes again inserted with PVC spacer-tubes to keep them open for later use. The series of holes were utilised for fine root production measurement in strict rotation to allow maximum time possible for recovery of the roots severed during ingrowth core extraction. On average, spacer-tubes were left in the soil for a period of 13-15 months.

After extraction from the soil, roots in the ingrowth cores were washed out *in situ* using a 2 mm sieve. All roots thinner than 2 mm were collected, placed in paper bags, transported into laboratory and dried at 60 ^{0}C until constant weight.

5.2.2 Minirhizotrons

Root measurement methods which require extraction of roots from the soil profile are unable to measure fine root production, death and disappearance simultaneously (Majdi, 1996). Rhizotrons overcome this shortcoming and allow for a detailed study of root dynamics at the interface between a transparent viewing medium and soil (Smith *et al.*, 2001). The use of rhizotrons, and the more recent increased use of minirhizotrons, provided some useful insights into phenology and productivity of below-ground plant organs (Pregitzer *et al.*, 1993). The minirhizotron, which consists of a transparent acrylic tube inserted into the soil profile, permits continuous observation of the same roots in an undisturbed volume of soil thus allowing for an estimate of root growth together with root senescence and decomposition. However, it is questionable how representative this method of root measurement is when compared to bulk soil methods. For this reason, use of minirhizotrons in combination with soil coring is advisable if an accurate measure of root biomass production is needed (Rytter, 1999).

5.2.2.1 *Minirhizotron set-up in POPFACE*

The aim of the Minirhizotron observation was to assess the effect of elevated CO_2 on phenology of root growth, after termination of above-ground growth during the second year of the experiment. Minirhizotron installation was carried out in March 2000 in experimental plots 1-4 only. A series of 5 minirhizotrons per *Populus* species was inserted into the soil profile in each of these plots. A 65cm long acrylic tube 3 cm in diameter was divided into 5-cm sections covering 55 cm of the length of the tube.

The remaining 10 cm at one end was painted black and then white to minimise light penetration and to maximise albedo of the tube surface. The tube was then inserted into the soil at 45^0 angle up to the painted end of the tube, thus covering the top 40 cm of the soil profile. The top end was closed with an empty film container to prevent light and rainfall entering the inside of the minirhizotron. An endoscope fitted with a battery powered light source was used for root observation. The number of roots observed in each 5 cm section of the minirhizotron was noted during each inspection spanning from 29th September to 23rd November 2000.

5.3 *Results*

Measurement of fine root production with the use of ingrowth cores was carried out throughout all the duration of POPFACE experiment, while observation of roots with minirhizotrons took place only during the autumn of the second growing season. Results for these two measurements are therefore presented separately.

5.3.1 Fine root production in ingrowth cores

Elevated CO_2 treatment resulted in larger fine root production in all three *Populus* species utilised in this research. The amount of fine roots produced under FACE conditions was higher than that in the control from the beginning of the experiment and this trend remained unchanged until the last observation at the end of the third growing season. During the first two seasons of POPFACE, ingrowth cores were left in the soil for half of the season (±4.5 months) at a time. In order to obtain a more accurate estimate, this period was halved in the third growing season as a result of very fast fine root turnover observed in the POPFACE experiment.

Figures 5.3, 5.4 and 5.5 show fine root production for all species utilised in POPFACE, as measured with ingrowth cores in the top 40 cm

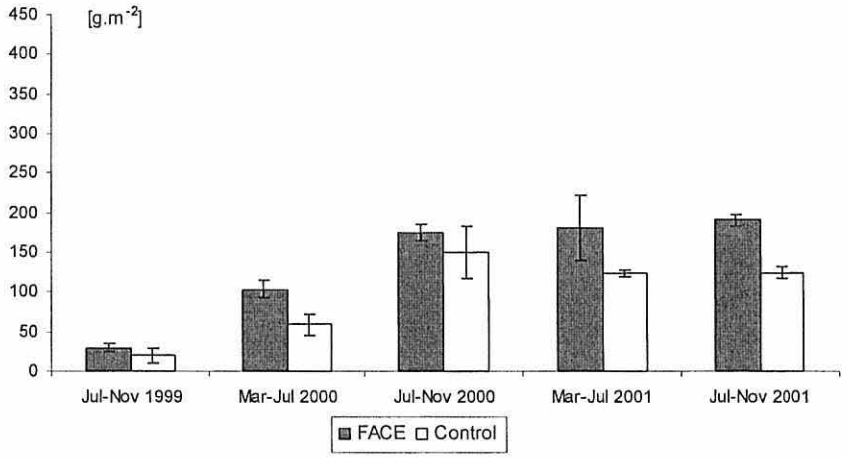


Figure 5.3 Fine root production of *Populus alba* (±st.error)

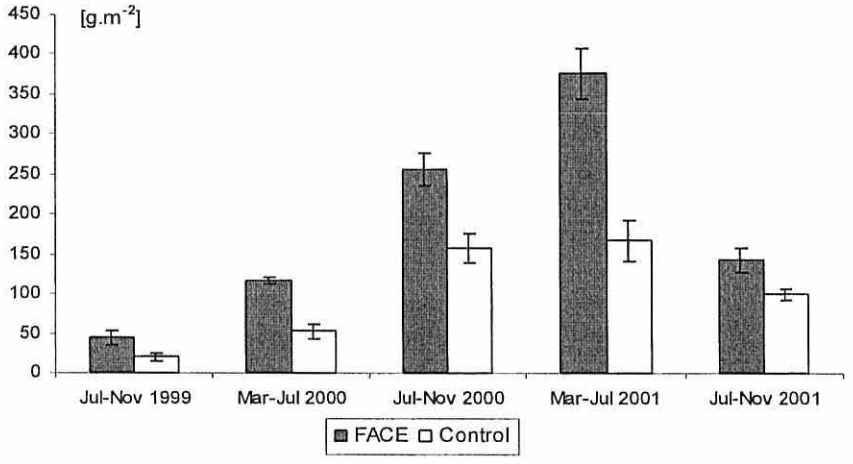


Figure 5.4 Fine root production of *Populus nigra* (±st.error)

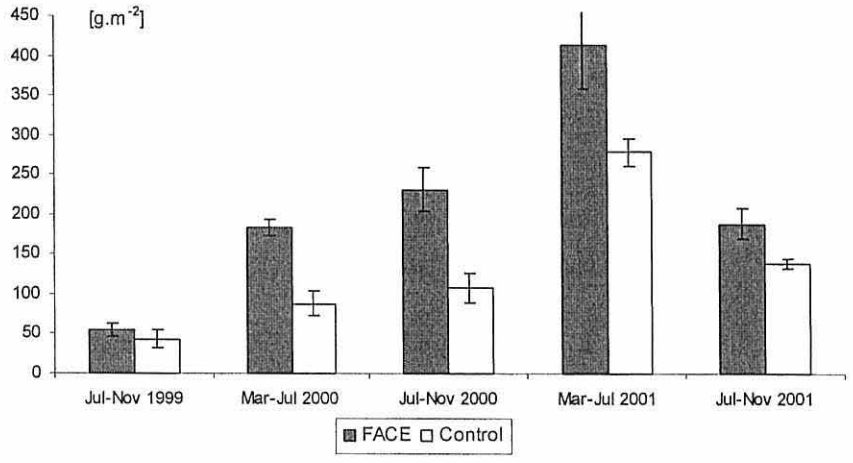


Figure 5.5 Fine root production of *Populus euramericana* (±st.error)

of the soil. The difference between FACE treatment and control is statistically significant at P=0.026 for *Populus alba*, P=0.022 for *Populus nigra* and P=0.032 for *Populus euramericana*. Relative enhancement of fine root production caused by elevated CO_2 is illustrated in table 5.1, which also details the amounts of fine roots produced in ingrowth cores in each growing season.

Table 5.1 Annual mean fine root production ($gm^{-2}y^{-1}$, ± st. error)

	Populus alba			*Populus nigra*			*P. euramericana*		
Season	FACE	Control	Effect	FACE	Control	Effect	FACE	Control	Effect
1	29 (±5)	20 (±9)	+50%	44 (±9)	20 (±4)	+124%	54 (±8)	43 (±11)	+25%
2	278 (±16)	209 (±45)	+33%	372 (±22)	210 (±10)	+77%	414 (±17)	196 (±22)	+111%
3	371 (±46)	247 (±5)	+50%	517 (±46)	266 (±20)	+94%	601 (±57)	417 (±13)	+44%
Total	**678 (±56)**	**475 (±53)**	**+42%**	**934 (±71)**	**496 (±16)**	**+88%**	**1069 (±40)**	**656 (±32)**	**+63%**

It is clear from Table 5.1, that *Populus alba* was the species which produced the least fine root biomass. In addition to this, *Populus alba* has also shown the smallest response to elevated CO_2, which resulted in an even bigger difference among the species when fine root production under FACE treatment is assessed. However, a statistically significant difference (p=0.029) between species in fine root production over the period of all three growing seasons was found only between *Populus alba* and *Populus euramericana* in FACE treatment.

5.3.2 Minirhizotron observation of root growth

Measurements were taken every 10 to 12 days at the end of the second growing season, starting on 29th September until 23rd November. The number of roots observed per 5 cm section of a minirhizotron tube is presented in figures 5.6 and 5.7. The data are divided into two depth categories; top soil layer 0-20 cm and bottom layer 20-40 cm in depth to assess depth allocation of fine roots under different CO_2 treatments.

As figure 5.6 suggests, as a result of FACE treatment all *Populus* species produced more fine roots in the top soil layer. The difference is statistically significant ($P<0.001$) for all species. Moreover, the measurement from the lower soil horizon, representing depth of 20 to 40 cm (figure 5.7), indicates even more clearly the effect elevated CO_2 has on fine root production.

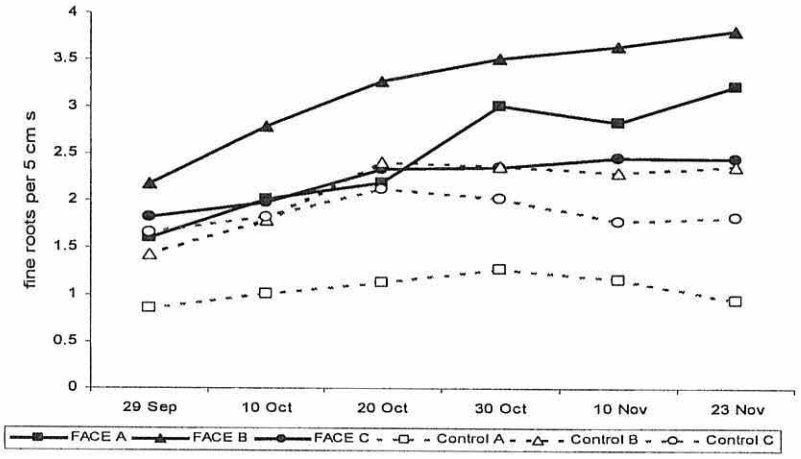


Figure 5.6 Number of fine roots per 5 cm section of minirhizotron, 0-20cm depth (A=*P. alba*, B=*P. nigra*, C=*P. euramericana*)

The minirhizotron measurements do show some influence of FACE on root phenology, especially in the top horizon. The curves displayed in figure 5.6 show trends of a slowly decreasing rate of new fine root formation under FACE similar for all species up until the end of the growing season.

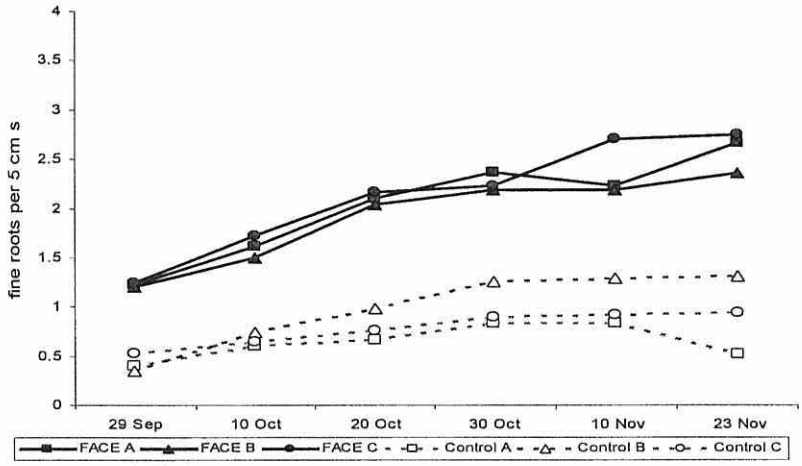


Figure 5.7 Number of fine roots per 5 cm section of minirhizotron, 20-40cm depth (A=*P. alba*, B=*P. nigra*, C=*P. euramericana*)

In contrast, data collected from control plots demonstrate the termination of root production and onset of root decomposition in the period between 20th October to 30th October for *Populus nigra* and *Populus euramericana* and around 30th October for *Populus alba*. This suggests that elevated CO_2 prolongs fine root production by at least a month in all *Populus* species examined.

5.3.3 Fine root turnover

Root turnover was determined according to the model originally proposed by Dahlman & Kucera (1965), who identified root turnover as annual below-ground production divided by maximum below-ground standing crop. Only fine root turnover was considered in this study; measures of fine root production were obtained by ingrowth coring and maximum standing fine root biomass was determined from sequential cores. Table 5.2 details the rate of fine root turnover for all species utilised. It is clear, that elevated CO_2 increases not only the amount of fine roots produced, but also the rate of root turnover in all three species. This increase is the biggest for *Populus alba*, which speeded up the turnover of its roots by 43% under FACE. *Populus nigra* and *Populus euramericana* show

similar increase of about 25 to 27% in the rate of fine root turnover as a result of elevated CO_2.

Table 5.2 Fine root production [g.m^{-2}], maximum crop [g.m $^{-2}$], and turnover [y^{-1}]

Growing season		*Populus alba*		*Populus nigra*		*P. euramericana*	
		FACE	Control	FACE	Control	FACE	Control
1	Production	29	19	44	19	54	43
	Max Crop	16	15	30	18	40	41
	Turnover	**1.7**	**1.2**	**1.4**	**1.0**	**1.3**	**1.0**
2	Production	278	209	372	210	414	196
	Max Crop	74	142	137	95	198	172
	Turnover	**3.7**	**1.4**	**2.7**	**2.1**	**2.0**	**1.1**
3	Production	371	247	517	266	601	417
	Max Crop	237	120	265	158	302	186
	Turnover	**1.6**	**2.1**	**2.0**	**1.8**	**2.0**	**2.2**
Mean turnover		**2.3**	**1.6**	**2.0**	**1.6**	**1.8**	**1.4**

5.4 *Discussion*

The results of this study conform to the generally accepted hypothesis that elevated CO_2 increases the amount of root biomass. *Populus* species utilised in this research showed responses of different magnitude when exposed to FACE when fine root production is considered. *Populus alba* showed the smallest investment in the fine root system, both under elevated and ambient CO_2 conditions. The remaining two species also differed in their response to FACE, suggesting that the response might be species specific and vary even among closely related species.

The increase of fine root production caused by FACE does not diminish in time, all species show roughly the same size of enhancement

in all three years of the experiment. This suggests that either acclimation to elevated CO_2 takes more than 3 years in this *Populus* plantation or that the enhancing effect of elevated CO_2 does not weaken in time. In addition, the results show that N availability does not influence the response of fine root production in this ecosystem to elevated CO_2. No N treatment was applied throughout the course of the experiment and despite decreasing availability of N (P. De Angelis, pers. com.) the response of *Populus* trees to FACE did not change. This fact challenges the findings of Pregitzer *et al.* (2000), who found that elevated CO_2 increased fine root biomass of *Populus tremuloides* by 52% in high N, but had no significant effect in low N.

Similarly to root production, fine root turnover was increased by FACE in all species. *Populus alba* responded with 43% increase of root turnover, while *Populus nigra* and *Populus euramericana* both showed a 25-27% increase in the rate of root turnover. Li *et al.* (1998) report increased root turnover in *Betula papyrifera* grown under elevated CO_2; similarly Kubiske *et al.* (1998) found that elevated CO_2 increases root turnover in *Populus tremuloides.* However, this effect of elevated CO_2 is not uniform, Pritchard *et al.* (2001) established that root turnover did not change in artificially constructed communities of five early successional forest species. In some species, the increase in fine root turnover is likely to be a function of the magnitude of root production increases (Berntson & Bazzaz, 1996b), in others a function of decreases in life span of individual roots (Kubiske *et al.*, 1998). The results of this study support the second hypothesis; *Populus alba* showed the biggest increase of fine root turnover (+43%) in response to elevated CO_2, yet the smallest increase of standing fine root biomass (+35%).

It has to be mentioned, that during and after the summer of the third growing season, a significant drop in fine root production was observed under both CO_2 treatments in *Populus nigra* and *Populus*

euramericana. Figure 5.8 shows fine root production in *Populus alba*, detailed for all measurement periods. It can be seen that since the beginning of the second growing season the amount of fine root produced remains stable, especially when taking into account that the Jul-Nov 2000 measurement should be split into Jul-Sep and Sep-Nov values for better comparison with 2001 measurements[7].

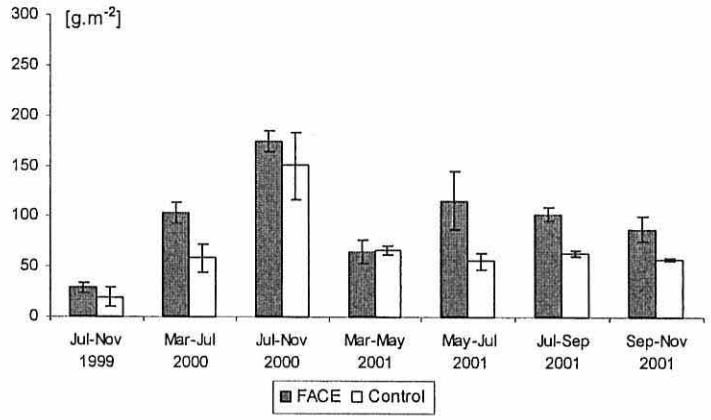


Figure 5.8 Fine root production - *Populus alba* (± st. error)

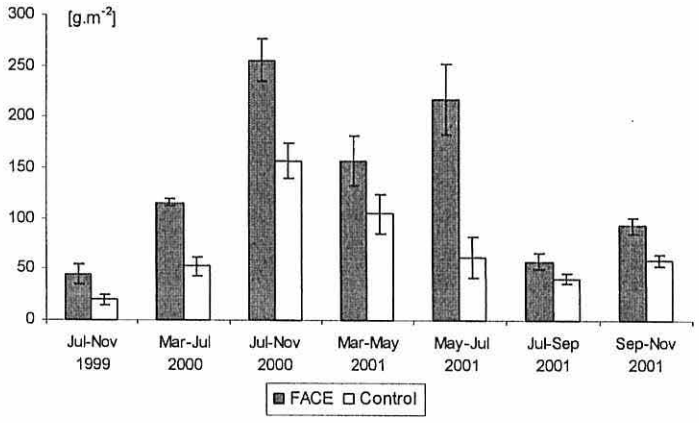


Figure 5.9 Fine root production - *Populus nigra* (± st. error)

Measurement for both *Populus nigra* (Figure 5.9) and *Populus euramericana* revealed instead a sudden decrease of fine root production after July 2001. It is not clear what caused this reduction, possible explanations include species specific responses to a summer drought or

[7] Figures 5.3, 5.4 and 5.5 show cumulated fine root production for half growing seasons.

full colonisation of available space by the root systems of *Populus nigra* and *Populus euramericana*. However, despite this decrease in fine root production, a positive effect of elevated CO_2, albeit smaller, was still observed in all species.

These changes have direct implications for the amount of assimilated C transferred below-ground. Rasse *et al.* (2001) estimated with a photosynthate allocation model, that fine root turnover is the single most important source of C to temperate forest soils. Where calculating the additional amount of C transferred to soil as a result of elevated CO_2, it is important to consider the amount of roots present in the soil together with the rate of root turnover. For example, in the second growing season the largest increase in fine root turnover resulting from elevated CO_2 treatment, that of *Populus alba* (160%), overshadows that of *Populus nigra* (28%) and *Populus euramericana* (90%). However, if average standing fine root biomass is taken into account, *Populus alba* (93%) shows the smallest increase of biomass invested in fine roots over the growing season when compared to *Populus nigra* (107%) and *Populus euramericana* (130%).

5.5 *Conclusions*

The results of this study show significant increase of fine root production in all three *Populus* species as a result of FACE. The difference in magnitude of this response appears to be species specific, although was not found to be statistically significant. Alongside fine root production, root turnover expressed as a ratio of root production and maximum standing biomass, was increased by elevated CO_2. Similarly to fine root production, the change in the rate of turnover is different for each species utilised, resulting in diverse amounts of organic matter transferred below-ground.

6 Polymerase chain reaction (PCR) identification of *Populus* roots

Correct identification of roots extracted from the soil has always been an important issue when conducting an analysis of below ground production with resolution down to species level. This is especially so in highly diverse natural ecosystems where root systems often overlap and distinguishing roots just by morphological characteristics might be difficult. However, recent advances in molecular techniques and tools, in particular those related to DNA analysis, enable researchers to distinguish tissue samples between two species or even between different populations of the same species. The following section depicts one such technique and describes its utilisation for distinguishing roots of *Populus* trees used in the POPFACE experiment.

6.1 DNA fingerprinting

Traditionally the identification and classification of organisms was based on the comparisons and homologies of phenotypic features. This methodology gave rise to the present taxonomy of species, although it carries the risk of different organisms being considered identical simply because a common environment led to development of similar morphological characteristics (Avise, 1994). Alternative approaches for inter- and intra- specific classification have been reported, most commonly based on geographical origin, karyotype and isozyme analysis (Kaser and Steiner, 1983). After the discovery of the genetic base of all organic traits it became possible to distinguish organisms by analysing the template common to all individuals belonging to one species - DNA.

A number of techniques were developed to carry out analysis of DNA present in all eucaryotic cells. These techniques can be applied to DNA originating both from the nucleus and organelles because the basic

composition of DNA remains the same no matter where it is found. The advent of these easy-to-use methods resulted in many studies analysing the evolutionary relationships and investigating the differences between species, clones, cultivars etc.

Polymerase chain reaction (PCR) enables multiple copies of a DNA molecule to be generated by enzymatic amplification of a target sequence of nucleotides (Saiki *et al.*, 1988). Microgram quantities of DNA fragments can be generated from as little as a single template DNA molecule which is repeatedly copied, leading to an exponential increase in DNA concentration (Boiteux *et al.*, 1999). This method received substantial attention and was repeatedly modified to suit particular experimental designs. Most notably, restriction fragment length polymorphism -RFLP (Gawel *et al.*, 1992), amplified fragment length polymorphism -AFLP (Vos *et al.*, 1995) and random amplified polymorphic DNA -RAPD (Millan *et al.*, 1996) analyses used in conjunction with PCR have yielded encouraging results.

On the one hand, both RFLP and AFLP are considered labour intensive methods and some knowledge of the constitution of the genome under study is required for successful application of the techniques (Rajaseger *et al.*, 1997). A part of the genome considered to be of influence with regard to the analysed characteristic is multiplied and further analysed. On the other hand, RAPD offers a straightforward alternative when quick identification of a sample is required and a reference DNA is available. Arbitrary chosen random primers are applied during amplification of the DNA template, yielding DNA fragments of identical lengths for samples representing the same genome. Alternatively, when comparing samples from closely related organisms, this method has the potential to reveal a large amount of variation with good coverage of the entire genome (Melchinger *et al.*, 1994).

In this work, the RAPD method was applied to identify the roots of all three *Populus* species utilised in order to validate the measurements of root production and turnover. Due to the close spacing of the sections containing trees of the different species, it was necessary to assess whether roots of different species advanced into an area planted with another species. A RAPD 'signature' of each species was developed from leaf DNA and this was later used to compare the banding patterns of DNA extracted form root samples.

6.2 *Methods*

Initially, a number of leaf samples from each of the *Populus* species was obtained during the first year of POPFACE experiment. One sampling session with three trees sampled was considered sufficient, since all three species utilised are propagated vegetatively and therefore all trees of one species have identical genomes. Subsequently, throughout the duration of the POPFACE experiment, 30 samples per species of root tissue from randomly selected root samples utilised for root biomass measurement were taken and used for DNA extraction.

6.2.1 DNA extraction from plant tissue

The DNA extraction procedure followed the protocol first described by Doyle & Doyle (1987) with some modifications for poplar leaves (Bradshaw & Stettler, 1993). Plant tissue (0.5 - 1.5 g) was ground to a fine powder with a pre-chilled mortar and pestle in the presence of liquid nitrogen. A 0.1 g aliquot of the powdered, frozen plant tissue was placed into 1.5 ml Eppendorf tubes containing 500 µl of hot (60 °C) DNA isolation buffer (100 mM Tris pH 8.0, 20 mM EDTA pH 8.0, 2 % w/v CTAB and 0.2 % β-mercaptoethanol - Sigma). A further 500 µl of hot DNA isolation buffer was added to each Eppendorf tube, the tubes were incubated in a water bath at 60 °C for 1 hour with periodic mixing. After the incubation phase, the tubes were centrifuged at 10,000 g for 2 minutes

to pellet out the cell debris. Avoiding the pellet, 500 µl of the supernatant was transferred into fresh tubes. Under a fume hood 500 µl 24:1 chloroform:isoamyl alcohol (Sigma) was added to each tube and mixed with the supernatant by gentle inversion. The tubes were then centrifuged at 10,000 rpm for 1 minute to separate the aqueous and solvent phases. Avoiding the phase interface, the upper aqueous layer was pipetted into new Eppendorf tubes and the chloroform:isoamyl alcohol step was repeated. A 300 µl volume of the upper aqueous layer was pipetted into new tubes and 150 µl of 5 M NaCl and 225 µl cold isopropanol (- 20 °C) was added (Sigma). The contents of the tubes were mixed by gentle inversion and chilled at - 20 °C for 30 minutes. After chilling, the tubes were centrifuged at 14,000 g for 20 minutes to pellet out the nucleic acids. The supernatant was poured away and the pellet was washed in 1 ml DNA wash buffer (80 % v/v ethanol, 10 mM ammonium acetate - Sigma) for 30 minutes with periodic swirling. The tubes were centrifuged at 14,000 g for a further 2 minutes and the wash buffer was carefully poured away The DNA pellet was allowed to dry for 3 - 4 minutes and then re-suspended in 50 - 100 µl (depending on the size of the pellet) DNA re-suspension buffer containing RNase (10 mM Tris pH 8.0, 1 mM EDTA pH 8.0 - Sigma, with 10 µg/ml RNase - Sigma Cat. N° R6513). The tubes were incubated at 37 °C in a water bath for 30 minutes with periodic swirling. After 30 minutes the DNA sample was diluted with two volumes of de-ionised water. Ammonium acetate (5 M) was added to a final concentration of 1 M and mixed by gentle inversion. The suspended DNA was precipitated out by adding 2.5 volumes of cold (- 20 °C) ethanol. The DNA was then pelleted out of suspension by centrifugation for 15 minutes at 14,000 rpm. The pellet was then re-suspended in DNA re-suspension buffer without RNase and stored at - 20°C.

6.2.2 RAPD amplification by PCR

Extracted samples of leaf DNA were used to construct a marker specific for each of the *Populus* species later to be utilised for identification of roots. Previous experience with identification of RAPD markers for commercially used poplar species has shown that several of the readily available random 10-base primers give rise to useful fragment polymorphism (A. Armstrong, pers. com.) The following primers were tested in this study: A3, A4, B07, B08, B09, B10, B15, C01, C02, C03 and C04 (Operon Technologies)[8]. DNA samples were agitated gently to ensure thorough mixing of the DNA in the re-suspension buffer. A 1 μl sample of the template DNA was pipetted into a 0.5 ml Eppendorf tube containing a PCR reaction mixture consisting of 25 μl RedTaq polymerase (Sigma Cat. N° R2523), 23 μl de-ionised water and 1 μl of 10-base primer. The reaction mixture was overlaid with 50 μl of mineral oil (Sigma) and the PCR reaction was run on a thermal cycler (Hybaid, Omnigene). The optimal thermocycle consisted of 35 cycles denaturation at 94 °C for 60 seconds, annealing at 50 °C for 45 seconds and extension at 72 °C for 60 seconds, followed by a single extension step at 72 °C for 10 minutes. The PCR products were separated out by molecular weight on a 1.3 % agarose gel and visualised by ethidium bromide staining. A digital photograph of each gel visualised on a UV-light table (TFX-20M, Life Tech) was taken with a Kodak DC120 camera.

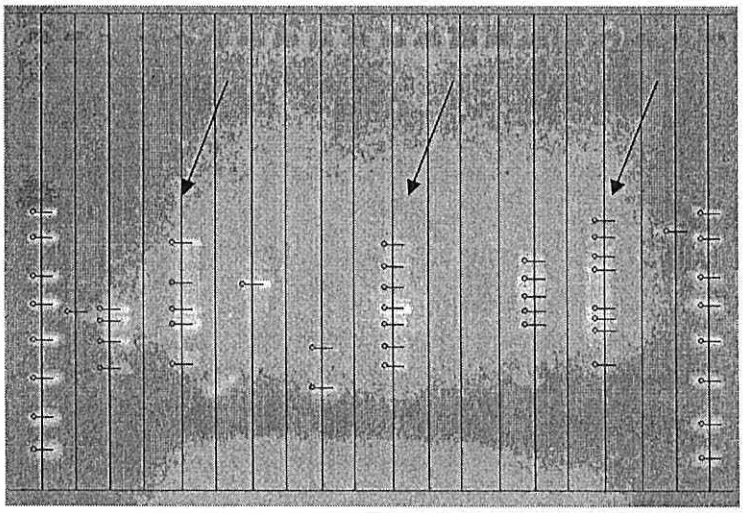
DNA extracted from randomly selected root samples at later stages of the experiment was amplified and separated according to the same

[8] A03 - AGTCAGCCAC, A04 - AATCGGGCTG, B07 - GGTGACCCAG, B08 - GTCCACACGG, B09 - TGGGGGACTC, B10 - GTAGACCCGT, B15 - GGAGGGTGTT, C01 - TTCCAGCCAG, C02 - GTGAGGCGTC, C03 - GGGGGTCTTT, C04 - CCGCATCTAC.

protocol. Resulting band patterns were analysed by Kodak 1D 3.0.0 software and compared with those obtained from leaf DNA.

6.3 *Results*

Initially, a major part of the work consisted of identifying the primer which would reveal the differences in banding patterns between the species. Since in this study just three species of *Populus* were considered, only a small number of primers had to be tested. DNA amplified with the use of selected primers was useful in creating a RAPD identification key for the *Populus* plants. The patterns for the three species were distinguishable both visually (Figure 6.1) and quantitatively (Table 6.1). Figure 6.1 depicts the banding patterns obtained with primers A3, A4, B9, B10, C1 and C2. Primers A4 and B10 gave rise to different patterns for each species, primer B10 was chosen for later identification of root DNA.



L A3 A4 B9 B10 C1 C2 A3 A4 B9 B10 C1 C2 A3 A4 B9 B10 C1 C2 L
Populus alba *Populus nigra* *Populus euramericana*

Figure 6.1 Banding patters from leaf DNA. Lanes from left to right: ladder (L); *P. alba* primers A3, A4, B9, B10, C1, C2; *P.nigra* primers A3, A4, B9, B10, C1, C2; *P.euramericana* primers A3, A4, B9, B10, C1, C2; ladder (L). Empty lanes are due to amplification failure.

PCR products consisted of 5, 7 and 8 banded patterns for *Populus alba*, *Populus nigra* and *Populus euramericana* respectively. Table 6.1 details the lengths of fragments obtained with primer B10.

Table 6.1 Lengths of DNA fragments obtained with primer B10, values have been rounded to the nearest 10.

	Populus alba	*Populus nigra*	*Populus euramericana*
1	370	370	370
2		460	
3			570
4	600	600	
5			650
6	720	720	720
7		900	
8	950		
9			1100
10		1150	
11			1270
12		1420	
13	1450		
14			1520
15			1850

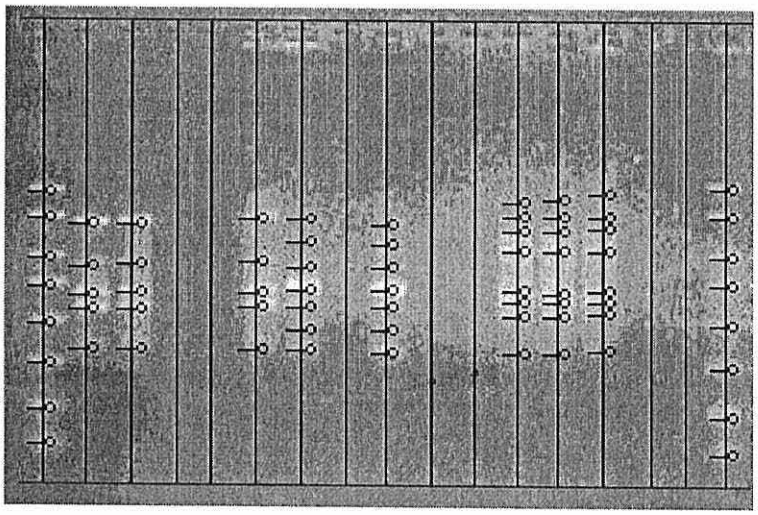


Figure 6.2 Banding patterns for root DNA. Lanes from left to right: ladder (L), *P. alba* (5 lanes), *P. nigra* (5 lanes), *P.euramericana* (5 lanes); ladder (L). Empty lanes are due to amplification failure.

Running PCR with the same primer and the DNA extracted from roots gave rise to the same banding patterns for each species. Root DNA was successfully amplified in approximately 30% of the runs on the first

attempt, non-successful amplifications were repeated with DNA extracted from the same sample of ground-up roots. In all, 30 samples of root DNA were analysed for each species. Figure 6.2 shows the separation of bands according to molecular weights for root samples extracted during the third year of POPFACE.

Out of a total of 90, only on three occasions has DNA of a species different from the one planted in the section been detected. DNA from two species of poplar in one sample of roots was found only in one instance, in a sample originating from the section planted with *Populus euramericana*. The two contaminated samples from *Populus alba* contained DNA originating from an unidentified species other than the *Populus* species utilised in POPFACE. Table 6.2 summarises the results of DNA analysis from roots.

Table 6.2 Incidence of DNA from another species of *Populus* planted in a different section of the experimental plot and from other unidentified species

	P. alba	*P. nigra*	*P. euramericana*
Other species of *Populus*	0	0	1
Unidentified species	2	0	0
Total samples analysed	**30**	**30**	**30**

6.4 *Discussion*

RAPD method for identification of plant roots allows for reliable detection of origin of organic matter present in the soil. The most common visual methods rely on recognition of morphological and anatomical features (Moore *et al.*, 1977) which is often hampered by the similarity of the studied material. There are two pre-requisites for the viability of RAPD method. Firstly, extractable DNA must be present in the sample analysed. Secondly, a branding pattern or 'signature' must be obtained

from DNA originating from a more easily identifiable part of the same plant – usually an above-ground structure.

Welsh and McClelland (1990) have found that between three and twenty products, i.e. DNA pieces, predominate from most eucaryotic organisms. The resulting variability of the number of bands and of the length of fragments is deemed sufficient for distinguishing even closely related organisms (Melchinger *et al.*, 1994). RAPD's greatest advantage is the use of random arbitrary chosen primers without previous knowledge of the genetical makeup of the plant studied. Nevertheless, it is questionable if the banding patterns produced from a species in one geographical area can be used elsewhere. Intraspecific variation might prevent this or, in reverse, a very similar or identical pattern might be obtained from a different species resulting in a false positive identification.

A similar technique for identification of plant roots is described by Bobowski *et al.* (1999) – RFLP of the PCR amplified *rbcL* gene. The technique is also reliant on constructing a 'key' from DNA obtained from above-ground plant tissue. However, since the amplified gene sequence originates from a chloroplast, the low success rate (3%) of amplification was attributed to a high level of contaminants and the low number of proto-chloroplasts in the roots. Brunner *et al.* (2001) in their RFLP identification of common alpine tree roots increased the success rate of DNA amplification by modifying the extraction protocol of Chang *et al.* (1993). Full identification down to species level was achieved for all 30 tree species, although simultaneous use of four restriction enzymes was necessary to achieve this. Much higher resolution between plants is obtainable by application of the AFLP method. Both Arens *et al.* (1998) and Winfield *et al.* (1998) applied this method to study genetic diversity of *Populus nigra* and *Populus nigra* subsp. *betulifolia* respectively. The results are reliable and reproducible, however a two-step PCR is required for this technique.

If a quick identification of plant material is desired, RAPD seems to be the best method to achieve this goal. However, it is important to mention the drawbacks, which might impede its successful application. The problems of DNA extraction are well known (Staub *et al.*, 1996) and become even more challenging in woody tissues and roots. It is essential to standardise the method of DNA extraction and amplification as differing amounts and concentrations of solutions and temperatures can have a large effect on resulting banding patterns (Welsh & McClelland, 1990). In addition, it is uncertain how the presence of pathogenic or symbiotic organism in the root tissue influences the results of RAPD analysis. All species in an area must be sufficiently surveyed to allow for reliable positive identification.

6.5 *Conclusions*

It is evident that correct identification of plant roots poses a considerable challenge. RAPD technique of analysing DNA from below-ground plant tissue can provide reliable genus and species identification. A 'signature' banding pattern must be obtained for each species to be identified prior to detection of below-ground samples.

An application of this method to the three *Populus* species utilised in POPFACE indicates that none or minimal growth of roots occurred between the species. Hence, it is safe to conclude that the data of root production and turnover for each section of the experimental plots corresponded only to the species of poplar planted in that section.

7 Mycorrhizal colonisation of *Populus* roots

The response of green plants to rising concentrations of CO_2 is likely to be linked with their nutritional status and the availability of water. Since most plants, poplars included, form mycorrhizal symbioses which enable them to increase their water and nutrient acquisition, it is of importance to consider the potential effect elevated atmospheric CO_2 could have on this relationship. Mycorrhizal fungi are mostly dependent on the plant host for their C uptake. This fact, coupled with increased below-ground allocation of C, is likely to result in a change in the amount and cycling of mycorrhizal organic matter in the soil. The following chapter investigates the effect FACE treatment had on mycorrhizal colonisation of *Populus* roots and attempts to assess the composition of the mycorrhizal community.

7.1 Mycorrhizas and elevated CO_2

Mycorrhizal fungi symbiotically colonise more than 80% of plant roots and are found in nearly every habitat in the world (Smith & Read, 1997). Since the mycobionts are dependent on the host as their source of C (Allen, 1991), increased supply of C by means of increased photosynthesis and root allocation can be beneficial for the fungi (Rillig *et al.*, 1999). This causality is likely to affect all types of mycorrhizas in a similar fashion, although it is not proven that experimental results obtained from one can be applied to another (Fitter *et al.*, 2000). This study concerns investigating the following two types of mycorrhizas: vesicular-arbuscular (VAM) and ectomycorrhizas (ECM).

On the one hand, fungal species involved in VAM symbioses are members of a single order (*Glomales*), all of which are obligate symbionts. The fungi form structures inside the root of a host plant through which the exchange of water, nutrients and C takes place. On the other hand, ECM fungi form a diverse range of species originating from various orders.

Many different species can colonise a single plant (Allen *et al.*, 1995a) utilising external structures formed on root tips to perform their symbiotic role. What these two types of symbionts have in common is the function they carry out in relation to the host plant.

Both receive photosynthate from the green plant and in return provide increased capacity for uptake of water and nutrients from the soil and protection against fungal root pathogens (Smith & Read, 1997). Due to this, mycorrhizal fungi form an integral part of the C and nutrient dynamics of an ecosystem. Studies have shown that a significant proportion of net primary production is invested in maintaining mycorrhizas (Finlay & Soderstrom, 1992) and the presence of the symbiosis increases the growth of mycorrhiza-dependent plants (Jakobsen *et al.*, 1994). It is hypothesized that the symbiotic fungi will be greatly affected by an increasing atmospheric CO_2 concentration (Allen *et al.*, 1995b), since elevated CO_2 increases C allocation below-ground (Rogers *et al.*, 1994). Any such effect is likely to be only an indirect one: the fungus exists in an environment rich in CO_2, both inside the root and in the soil (Fitter *et al.*, 2000). A positive feedback loop can be envisaged in elevated CO_2 conditions, plants transfer more assimilated C below ground, consequently mycorrhizal fungi can grow more and exploit a greater volume of soil allowing them to supply more water and nutrients to the host plant.

Any such effects have so far been investigated mostly in 'model systems' consisting of a single plant species and a single or several fungal symbionts (Rillig *et al.*, 1999) which do not have the potential to reveal the response of the whole ecosystem. The effects of elevated CO_2 on mycorrhizas have been reviewed by O'Neill (1994), Diaz (1996) and Staddon & Fitter (1998) indicating that the percentage of roots with mycorrhizas can increase significantly and that this effect seems to be greater for ECM associations. However, as root biomass tends to rise, total

mycorrhizal biomass might do so as well (Treseder & Allen, 2000). Klironomos *et al.* (1997) found that although the percentage colonisation of roots of *Populus tremuloides* was not affected by elevated CO_2, the extra-radical hyphal network was altered both quantitatively and qualitatively.

Different mycorrhizal groups vary in their response to elevated CO_2, resulting in potential shifts in the mycorrhizal community (Cairney & Meharg, 1999). These shift have been documented both for VAM (Klironomos *et al.*, 1998) and for ECM (Godbold *et al.*, 1997). While the response of the ECM community is somewhat easier to measure, due to their morphological characteristics, the assessment of VAM community structure poses a considerable challenge, partly because of the inadequacies of our understanding of fungal taxonomy (Fitter *et al.*, 2000) and partly because of a lack of success in cultivating these fungi axenically (Rosendahl & Taylor, 1997).

Various genetic markers have been used in attempts to identify VAM in soil and plant roots. Specific isozyme markers have been used to detect know species of VAM (Bonde *et al.*, 1993). However, a large number of spores or other material is needed for a successful application of this method. PCR with fungal-specific primers offers a viable alternative when only a small amount of fungal DNA is available. The technique has been used, in various modifications, to study genetic variation of VAM (Wyss & Bonfante, 1993; Clapp *et al.*, 1995).

In this study, the effect of elevated CO_2 on the rate of colonisation of *Populus* roots by VAM and ECM was studied. In addition to this, the composition of the fungal community was assessed by analysis of fungal DNA by specific primers.

7.2 *Methods*

7.2.1 Percentage colonisation by VAM fungi

Two samples of fine roots were collected from the depth of 10 to 20 cm at randomly chosen locations within the sampling range of each section within each plot. The samples were collected during the following sampling sessions: July and November 2000 and March, May, July, September and November 2001. Samples were immediately submerged in 70% alcohol, transferred into the laboratory and stored at 4 ^{0}C. Prior to analysis the roots were carefully washed free of soil, chopped into 2 cm pieces and stained. Clearing and staining was carried out according to the technique described by Brundrett (1994). The tissues were cleared in 2.5 % KOH at 90 ^{o}C for 30 minutes, rinsed three times in tap water, bleached in alkaline hydrogen peroxide solution (a 1:10 ratio of 20 % NH_4OH and 3 % H_2O_2 respectively) for 10 - 30 minutes at 90 ^{o}C, rinsed in tap water again 3 times and acidified in ample 1 % HCl solution at 90 ^{o}C for 1 hour. Once the roots have been acidified the tissue was stained with 0.05 % trypan blue for 20 minutes at 90 ^{o}C and then de-stained and stored in acidic glycerol.

Root colonisation by VAM was then estimated according to a modified version of a protocol described by McGonigle *et al.* (1990). From each sample, four randomly selected 2 cm root sections were mounted on slides and viewed under 200X magnification. An eyepiece grid was overlaid over a central point of each section of root and the presence or absence of VAM colonisation in the form of hyphae, vesicles and arbuscules was noted under 100 intersections of the grid. The grid was then moved over a neighbouring section of the root and another 100 intersections were inspected, resulting in a total of 800 intersections inspected per sample. Ratio between the total number of intersections and those containing fungal structures was used as an estimate of percentage root colonisation. The data have been averaged for each experimental plot,

according to the overall statistical design[9]. For each CO_2 treatment a repeated measures ANOVA was carried out taking into account *Populus* species and percentage colonisation by hyphae, vesicles and arbuscules. Percentage values were tested log transformed and ANOVA was carried out using both percentages and log transformed values, yielding the same results.

7.2.2 Percentage colonisation by ECM fungi

Three fine root samples per *Populus* species per plot were collected in May and September 2001 according to the same scheme as described previously. After collection the roots were carefully shaken free of soil particles and placed into sealed vials containing moist cotton and kept at 4 ^{0}C until analysis. The roots were then viewed under binocular dissecting microscope (ZEISS Stemi SV11, 20 - 60X) and inspected for ECM colonisation. On average, 250 root tips were examined per sample. Repeated measures ANOVA was employed to assess the treatment effect in a similar fashion to VAM colonisation.

7.2.3 Species composition of the mycorrhizal community

The potential influence elevated CO_2 might have on the assemblage of mycorrhizas colonising *Populus* roots was examined with the help of two-step PCR with fungal-specific primers. DNA from mycorrhizal roots was extracted according to the protocol described in section 6.2.1. A 1 µl sample of the template DNA was pipetted into a 0.5 ml Eppendorf tube containing 25 µl of Ready Mix RedTaq (Sigma Cat. N^o R2523), 22 µl de-ionised water and 1 µl (2 µl in total) of each of the following oligonucleotide primers: VANS1 and VANS22 (Operon Technologies, VhBio Ltd.). The reaction mixture was overlaid with 50 µl of mineral oil (Sigma Cat. N^o M5904) and the PCR reaction was run on a thermal cycler

[9] See section 3.2.1

(Hybaid, Omnigene) according to the following cycle: 35 cycles denaturation at 94 °C for 60 seconds, annealing at 50 °C for 45 seconds and extension at 72 °C for 60 seconds, followed by a single extension step at 72 °C for 10 minutes. The abovementioned primers amplify a 720 bp length of fungal-ribosomal DNA from the 18S gene region (Simon *et al.*, 1993). The PCR product from this reaction is diluted 100 times and used as the DNA template in the second PCR reaction (PCR step-two), which utilises the family-specific primers VAGLO[10] (family *Glomaceae*), VAGIGA (family *Gigasporaceae*) and VAACAU (*Acaulosporaceae*), in conjunction with primer VANS1 (Sharrock *et al.*, 2000). If members of the above fungal families are present in the root tissue under analysis a single PCR product is generated, approximately 150 - 200 bp in length (Simon *et al.*, 1993). In total, 20 randomly selected root samples known to be mycorrhizal and extracted in September and November 2001 were analysed per *Populus* species per treatment.

A total of 36 (6 per species per treatment) samples of ECM hyphae were taken from mycorrhizal root tips with the help of extra fine forceps, fungal DNA was extracted and amplified with the use of selected random 10bp primers[11] (Operon Technologies, VhBio Ltd.) to determine whether the ECM mantles colonising *Populus* root tips belong to the same species. In addition, DNA from fruiting bodies found on the soil surface was extracted and analysed according to the same protocol. Resulting banding patterns were then compared with those obtained from root colonising ECM.

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[10] The sequences of family-specific primers utilised were as follows: VAGLO - CAAGGGAATCGGTTGCCCGAT, VAGIGA - TCACCAAGGGAAACCCGAAGG, VAACAU - TGATTCACCAATGGGAAACCCC.

[11] The following primers were used: B03 - CATCCCCCTG, B04 - GGACTGGAGT, B05 - GTAGACCCGT, B06 - TGCTCTGCCC, B07 - GGTGACCCAG, B08 - GTCCACACGG, B09 - TGGGGGACTC.

7.3 *Results*

7.3.1 Mycorrhizal colonisation

Evidence of mycorrhizal symbiosis was found under both CO_2 treatments and on the roots of all three *Populus* species. The first colonisation by VAM fungi was detected in March and by ECM in September during the second growing season.

7.3.1.1 VAM colonisation

After initial delay, probably caused by the absence of suitable mycorrhizal spores due to agricultural use of the site or after initial high N content of the soil affecting the viability of symbiosis (Klironomos *et al.*, 1997), *Populus* roots were rapidly colonised by VAM species. The hyphal density inside roots already reached high values in July 2000 and remained virtually the same until the end of the experiment (Figures 7.1, 7.2 and 7.3).

A significant effect of elevated CO_2 on hyphal colonisation of roots was found in *Populus alba* (P=0.032) and *Populus nigra* (P =0.002). Although in *Populus euramericana* an increase in hyphal colonisation was shown, the difference between the treatments was not significant (P=0.125). A comparison between different *Populus* species revealed that in ambient CO_2 conditions there is no significant difference in percentage hyphal colonisation of roots. However, under elevated CO_2 the differences in the amount of hyphae present in the roots of different *Populus* species were found to be statistically significant. This appears to be caused by the difference in the response of mycorrhizal symbioses of each species to elevated CO_2.

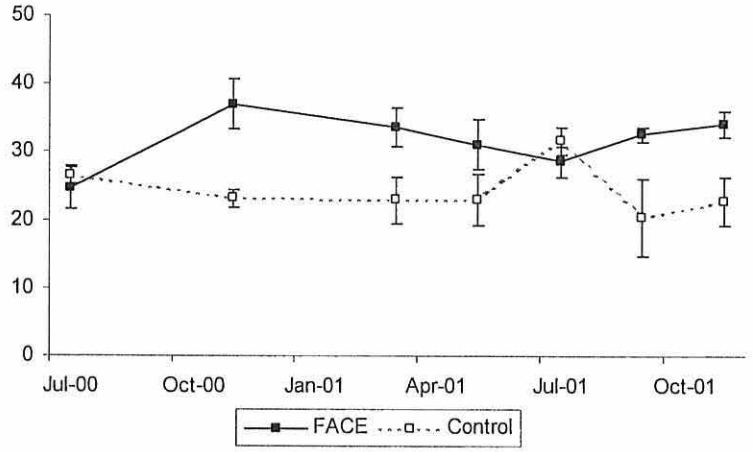


Figure 7.1 Percentage of roots colonised by hyphae - *Populus alba* (± st. error)

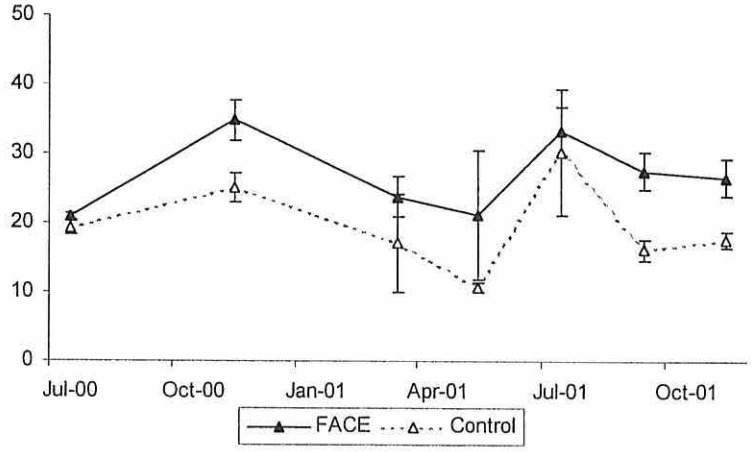


Figure 7.2 Percentage of roots colonised by hyphae - *Populus nigra* (± st. error)

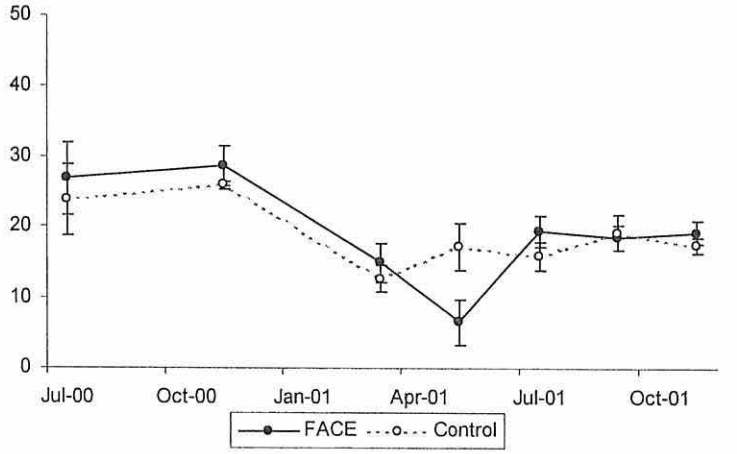


Figure 7.3 Percentage of roots colonised by hyphae - *P. euramericana* (± st. error)

The remaining two VAM structures examined, vesicles and arbuscules did not respond to FACE. There was no variation in occurrence of arbuscules between *Populus* species or CO_2 treatments (Table 7.1), root colonisation by vesicles was significantly higher in *Populus euramericana* that in the other two species utilised both under FACE and control. Increased growth of vesicles in the roots of *Populus euramericana* took place only during the third growing season and showed similar trends both in elevated and ambient CO_2 (Figure 7.4, Figure 7.5 and 7.6).

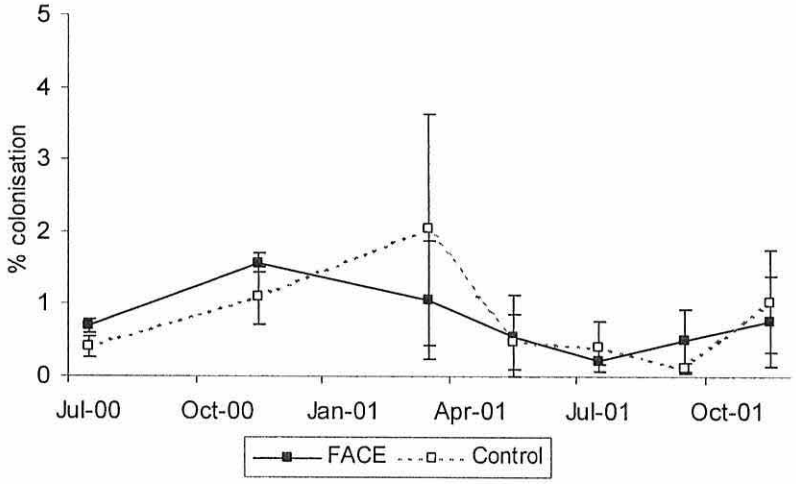


Figure 7.4 Percentage of roots colonised by vesicles - *Populus alba* (± st. error)

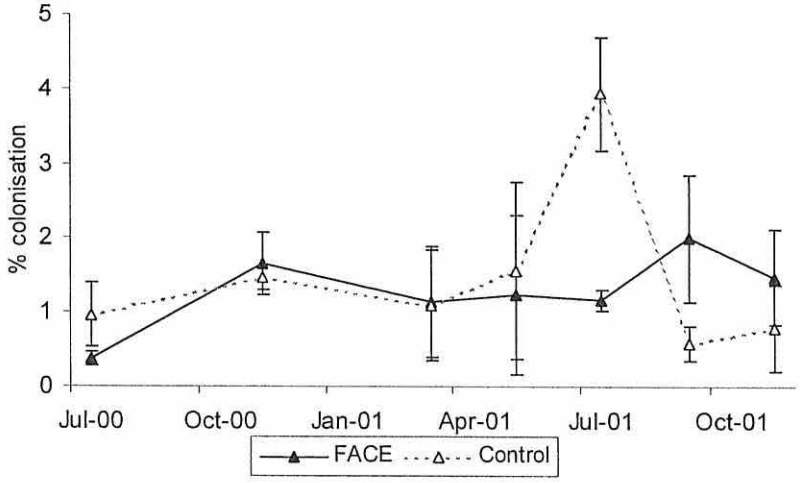


Figure 7.5 Percentage of roots colonised by vesicles - *Populus nigra* (± st. error)

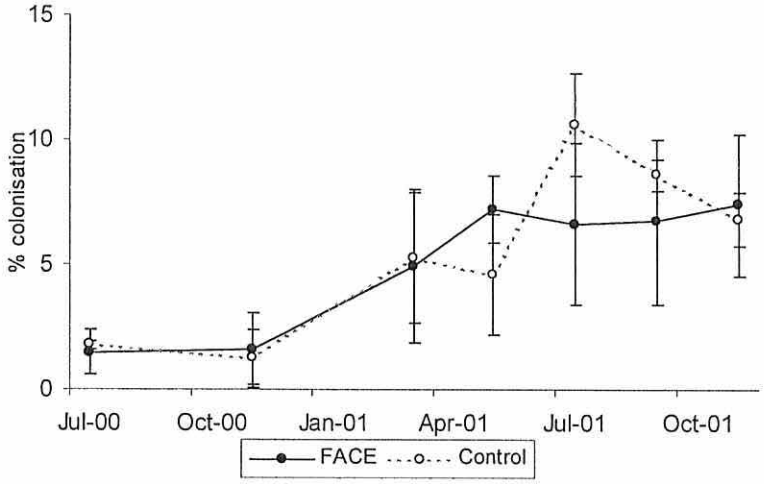


Figure 7.6 Percentage of roots colonised by vesicles- *Populus euramericana* (± st. error)

Table 7.1 Average percentage of root colonisation by VAM (% , ± st. error).

	Populus alba		*Populus nigra*		*Populus euramericana*	
	FACE	Control	FACE	Control	FACE	Control
Hyphae	31.7 (±2.6)	24.4 (±3.0)	26.8 (±3.5)	19.5 (±3.2)	19.2 (±2.8)	18.8 (±2.4)
Vesicles	0.8 (±0.4)	0.8 (±0.7)	1.3 (±0.6)	1.5 (±0.9)	5.1 (±2.0)	5.6 (±1.4)
Arbuscules	0.6 (±0.3)	0.4 (±0.1)	0.6 (±0.3)	0.4 (±0.3)	0.3 (±0.1)	0.5 (±0.3)
Negative	66.8 (±2.8)	73.7 (±2.8)	71.2 (±3.8)	79.7 (±2.3)	76.1 (±3.7)	75.9 (±2.6)

7.3.1.2 ECM colonisation

One distinct ectomycorrhizal morphotype was found to colonise the root tips of all *Populus* species. The morphotype was characterised by white mantle fully enveloping each colonised root tip. ECM fungus was common on roots of *Populus alba* and *Populus nigra* (Figure 7.7 and 7.8), the colonisation of *Populus euramericana* was only patchy and did not reach

large values (Figure 7.9). The variation in colonisation between the clones was statistically significant in all comparisons but *Populus alba* vs *Populus nigra* under ambient CO_2.

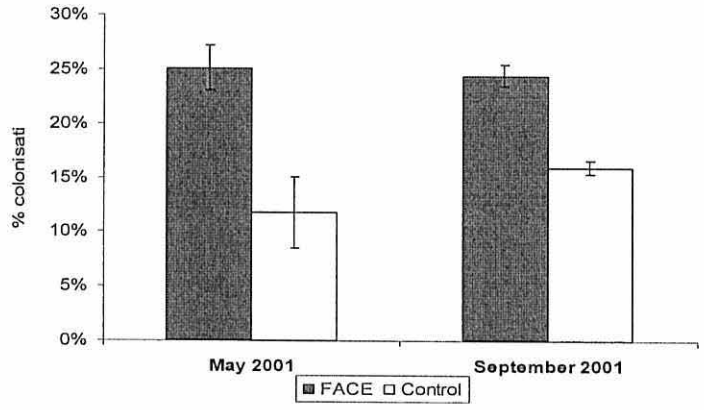


Figure 7.7 ECM colonisation of roots, *Populus alba* (± st. error)

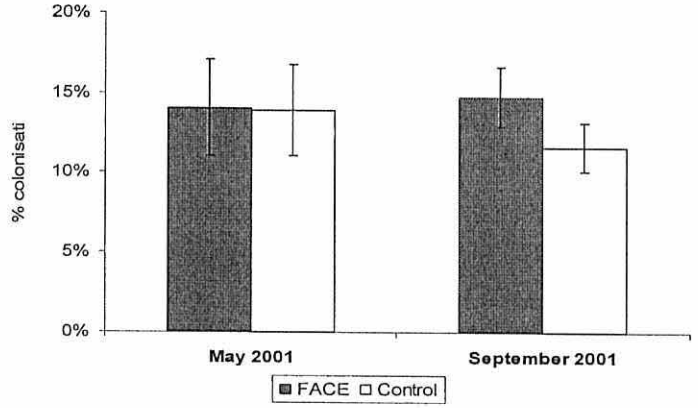


Figure 7.8 ECM colonisation of roots, *Populus nigra* (± st. error)

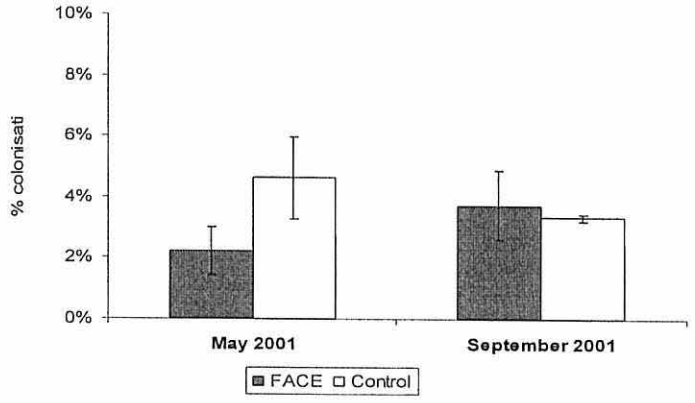


Figure 7.9 ECM colonisation of roots, *Populus euramericana* (± st. error)

Elevated CO_2 significantly increased ECM colonisation of roots only in *Populus alba* (P=0.003), while in the remaining two species the FACE treatment did not have a significant effect (P=0.853 and 0.285 for *Populus nigra* and *Populus euramericana* respectively).

7.3.2 Mycorrhizal species composition

7.3.2.1 Vesicular-arbuscular mycorrhizas

The effect of FACE treatment on VAM community composition was assessed by analysing fungal DNA from fungal structures present in fine roots of all three *Populus* species. Usage of three family-specific primers revealed that no fungi from the *Gigasporaceae* and *Acaulosporaceae* families were colonising roots in POPFACE experiment[12]. Species of mycorrhizal fungi belonging to *Glomaceae* appear to colonise more roots under elevated CO_2 in *Populus alba* only, in *Populus nigra* and *Populus euramericana* there being no change due to FACE treatment (Table 7.2).

Table 7.2 Number of samples found to contain *Glomaceae* fungus (out of a total of 20)

	Populus alba		*Populus nigra*		*Populus*	
	FACE	Control	FACE	Control	FACE	Control
Glomaceae	15	12	8	7	6	7
Gigasporaceae	0	0	0	0	0	0
Acaulosporaceae	0	0	0	0	0	0

However, it is important to bear in mind that the results have to be interpreted with caution due to difficulties with the amplification of

[12] In order to verify that no substance found in *Populus* roots is interfering with fungal DNA amplification, a template DNA extracted fro m roots of a common grass know to contain species from both the *Gigasporaceae* and *Acaulosporaceae* families was mixed with DNA extracted from *Populus* roots. Amplification of fungal DNA was successful in both cases, confirming that mycorrhizal fungi from these families were not present in POPFACE.

fungal DNA on a number of occasions during this analysis. This fact, together with low resolution of the analysis (only 3 VAM families) leaves space for a further, more detailed, study.

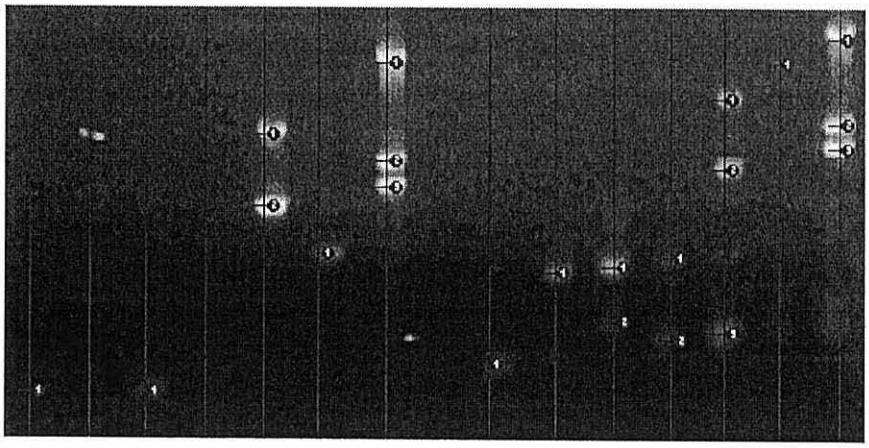
7.3.2.2 *Ectomycorrhizas*

Examination of DNA extracted from the ECM hyphae confirmed the initial observation, that only one ECM species colonised roots of all three *Populus* species under both FACE and control treatments. After initial assessment, primer B09 was found to give the best banding pattern for this mycorrhizal fungus (Figure 7.10), resulting in three distinct bands. All samples of ECM hyphae analysed resulted in the same pattern, verifying that *Populus* trees in POPFACE were colonised only by one species of ECM.

Figure 7.10 Banding patterns from ECM fungus. Lanes from left to right: *P. alba* sample from plot 1, 4, 5 (fail) , 2, 3 (fail), 6 (fail), *P. nigra* sample from plot 1, 4 (fail), 5 and 2.

Throughout the second and third growing season, but especially in September 2000, fruiting bodies were found growing on the soil surface both under elevated and ambient CO_2 treatments. According to their morphological characteristics, the fruiting bodies were identified as species belonging to the *Hebeloma* and *Pluteus* genera and as *Laccaria*

laccata. Genomic DNA extracted from each type of fruiting body was compared to that obtained from ectomycorrhizas colonising the roots. Figure 7.11 shows the banding patterns resulting from application of 10 bp primers, with the best result obtained from application of primer B09. A positive identification of *Laccaria laccata* as the ectomycorrhizal fungus colonising the roots of the *Populus* trees was achieved: the banding pattern of DNA extracted from the fruiting body of the fungus (lane 7) matches the one produced from DNA extracted from hyphae colonising a root tip (lane 14).



primer: B03 B04 B05 B06 B07 B08 B09 B03 B04 B05 B06 B07 B08 B09

DNA source: fruiting body hyphal mantle

Figure 7.11 RAPD identification of ectomycorrizas

7.4 *Discussion*

A significant response of mycorrhizal colonisation to FACE treatment was found to be host-species specific. The mycorrhizal response of both VAM and ECM to elevated CO_2 has been studied on a variety of types of plants: trees (O'Neill *et al.*, 1991), shrubs (Klironomos *et al.*, 1996), crops (Runion *et al.*, 1994) and grasses (Rillig *et al.*, 1999). In general, increased level of atmospheric CO_2 was found to have no effect or to an increase the intensity of mycorrhizal symbiosis (Staddon & Fitter, 1998; Tingey *et al.*, 1995). This effect is not universal and seems to be mediated by several factors, namely by the plant host species (Rillig *et al.*, 1999) and

the availability of N in the soil (Wallenda & Kottke, 1998). Klironomos *et al.* (1997) have shown that the growth of mycorrhizal hyphae was stimulated under combined high CO_2 and low N availability and the opposite trend prevailed under high N.

The response of VAM percentage colonisation to elevated CO_2 was found to be varied among the *Populus* species. In both *Populus alba* and *Populus nigra* hyphal presence inside fine roots increased (by +29% and +36% respectively), but little effect was observed in *Populus euramericana* (+2%). Since in this research all three *Populus* species were grown in identical soil conditions, it is safe to assume that the difference of mycorrhizal response to FACE treatment was due to inter-specific variation in poplars. In addition, it is likely that the fine roots of all three *Populus* species were colonised by the same assemblage of VAM fungi due to close proximity of the *Populus* trees within experimental plots and because of extensive soil tillage and homogenisation prior to the beginning of the POPFACE experiment.

A similar tree species-specific effect was observed for ECM. A large significant increase of ECM colonisation was observed only in *Populus alba* (+78%), the amount of colonised root tips in *Populus nigra* increased by +12% under elevated CO_2 and ECM colonisation decreased (-26%) in *Populus euramericana* as a result of FACE. It has been shown that all three *Populus* species have been colonised by the same ECM fungus. The fact that the poplars were colonised with dissimilar intensities, appears to be due to various strategies of nutrient and water acquisition employed by the different *Populus* species.

It is unlikely, that all mycorrhizal fungal species are equally efficient in nutrient acquisition (Ravnskov & Jakobsen, 1995) and in acting as a C-sink (Bidartondo *et al.*, 2001). Several studies indicate a change in the species composition of mycorrhizal communities brought about by elevated CO_2. Very little is currently known about this change in the

assemblage of VAM, however Godbold *et al.* (1997) indicate that such a change is possible. An attempt has been made to assess the effect of FACE on VAM species structure, and although the results indicate an increase in the number of *Glomaceae* fungal species in *Populus alba* under elevated CO_2, due to low reliability of the technique utilised the results are not conclusive. Nevertheless, these findings merit further study, perhaps with the usage of species-specific primers and thus improved resolution.

Due to their easier to recognise morphological characteristics, studies of ECM revealed that the proportion of fungal species colonising the roots can vary with different levels of atmospheric CO_2 (Godbold & Berntson, 1997; Staddon *et al.*, 1999a). Since only one ECM species was found to colonise *Populus* root tips in this experiment, these claims of ECM assemblage shift as a result of elevated CO_2 could not be evaluated.

It needs to be pointed out, although it has not been measured in this study, the reaction of extra-radical hyphae to elevated CO_2 can have significant influence on the character of the mycorrhizal symbiosis. Functionally, these hyphae represent an extension of the root system (Rillig & Allen, 1999) and their proliferation can have a profound effect on the plant host itself and on the plant community as a whole. An increase in the length of extra-radical mycorrhizal hyphae is not always accompanied by an increase in percentage root colonisation. Sanders *et al.* (1998) reported a five-fold increase of extra-radical hyphae under elevated CO_2, while mycorrhizal colonisation inside roots increased only by a factor of two. The implications of this activity are remarkable, especially in systems with low N and P availability and high proliferation of mycorrhizas. By penetrating even the smallest crevices of soil aggregates (Friese & Allen, 1991) and extracting N and P, mycorrhizas reduce the availability of these nutrients to saprophytic microbes and to non-mycorrhizal plants. Firstly, limiting growth of saprophytes might retard decomposition of soil organic matter resulting in longer retention of C in the soil (Treseder & Allen,

2000; Jakobsen *et al.*, 1994) and secondly, decreased availability of nutrients in the soil could favour plants with extensive mycorrhizal networks (Sanders, 1996; Rillig *et al.*, 1999).

In view of these circumstances, it is of interest to compare the response of the root systems of *Populus* trees and of mycorrhizal symbionts colonising them to FACE treatment. Elevated CO_2 induced the production of roots involved in nutrient acquisition (i.e. fine roots) in all three *Populus* species studied, albeit by different magnitudes[13]. Likewise, mycorrhizal colonisation was affected by FACE, but the response is different and appears to be species specific. Table 7.3 shows percentage effect of elevated CO_2 on root colonisation by VAM, ECM and on fine root biomass.

Table 7.3 Effect of FACE on mycorrhizal colonisation and fine root biomass (%, ± st. error)

	Populus alba	*Populus nigra*	*Populus euramericana*
VAM	+34 (±11)	+45 (±12)	+2 (±1)
ECM	+79 (±17)	+14 (±3)	-21 (±10)
Fine roots	+35 (±24)	+86 (±22)	+67 (±22)

It is clear from the comparison that *Populus alba* has shown the smallest increase of fine root biomass under elevated CO_2 conditions, the proportion of fine roots in total root biomass was actually decreased by FACE (Table 4.1). At the same time the rate of colonisation of fine roots of *Populus alba* both by VAM and ECM increased markedly, unlike in the other two *Populus* species. This could be explained by different strategies of nutrient acquisition being employed by *Populus* species studied and this difference being amplified by elevated CO_2.

[13] See section 4.3.2

It has been shown that in some plants C-sink capacity is limiting C-fixation (Tinker *et al.*, 1994) and that one of the causes of photosynthetic acclimation to elevated CO_2 is overload of C-sinks available to plants (Bazzaz, 1990). Increased mycorrhizal colonisation and growth of hyphae in the soil is likely to affect the sink-source relationship in plants. Rillig *et al.* (1999) have reported, with some caution, that the responses of root-colonising fungi are likely to persist even after long-term exposure to elevated CO_2. However, Kasurinen *et al.* (1999) found that the initial positive response of tuber-like mycorrhizas on roots of young *Pinus sylvestris* to elevated CO_2 diminished with time. Nevertheless, it is possible that plants with extensive mycorrhizal symbioses which positively react to elevated CO_2 might have a competitive advantage due to their ability to utilise the increase in the rate of photosynthesis for a longer period of time.

7.5 *Conclusion*

It has been shown that mycorrhizal colonisation of *Populus* is affected by FACE treatment; however this effect appears to be species specific and different even among such closely related species. In this study, the colonisation of root tips of *Populus alba* grown under elevated CO_2 treatment significantly increased both for VAM and for ECM. *Populus nigra* displayed a statistically significant increase only in VAM colonisation and FACE treatment did not affect mycorrhizal colonisation of *Populus euramericana*. Assessment of shift in the structure of the mycorrhizal community resulting from elevated CO_2 treatment did not bring any conclusive results. It seems likely that due to their importance in soil C cycling and in nutrient and water acquisition, the effect of elevated CO_2 on mycorrhizas might have a significant role in determining ecosystems' responses to future levels of atmospheric CO_2.

8 Below-ground C and elevated CO_2

The need to assess the role of terrestrial ecosystems in the global C cycle and the potential change of this role as the atmospheric concentration of CO_2 increases attracted considerable scientific attention over the past decade. Higher level of CO_2 may cause profound changes in the structure and function of ecosystems, including those dominated by trees (Ceulemans *et al.*, 1999). Many impact studies have focused on above-ground tree responses and have shown that through altered physiology, development and growth, elevated atmospheric CO_2 does lead to changed functioning of an ecosystem (Jarvis, 1998). However, in order to assess ecosystems' response as a whole and to evaluate the potential role of forests and tree communities as a C sink, the below-ground response to increasing levels of CO_2 must be addressed. An increase in C assimilation, for example, might result in an altered C allocation pattern within plants (Bazzaz, 1990), changed nutrient content of organic matter entering the soil (Rastetter *et al.*, 1992) and a shift in the quality and quantity of microbial communities dependent on the input of organic matter derived from the autotrophs (Klironomos *et al.*, 1996).

All these implications have to be considered if a reliable and realistic prediction about the response of tree ecosystems to elevated CO_2 and its potential role in C sequestration is desired. This chapter summarises the results presented in previous sections and evaluates them in view of the present state of knowledge.

8.1 'Extra' C in the soil

Because the assimilation of C during photosynthesis is the entry point for inorganic C into the organic biosphere, it represents the single link through which growth, physiology, phenology and C allocation of green plants can respond to increasing levels of CO_2 (Wullschleger *et al.*, 1997). Due to this fact, a great deal of attention has been paid to studying

photosynthetic responses to elevated CO_2. An undisputed response to increased atmospheric CO_2 is an initial increase in the rate of photosynthesis (Norby *et al.*, 1999; Paterson *et al.*, 1997). However, it is important to distinguish between short- and long-term responses when considering the function of plants in future climatic conditions. Many plant species have frequently been observed to acclimatise to higher level of CO_2 by down-regulation of the rate of photosynthesis (Ceulemans & Mousseau, 1994). It is likely that photosynthetic acclimation is caused by a limited capacity to generate new sinks for additional C (Paterson *et al.*, 1997; Bazzaz, pers com). Pot or controlled-chamber experiments with trees showed speedy down-regulation of photosynthesis, but the slow-down in the rate of assimilation could almost always be attributed to restrictions on root growth imposed by pots (McConnaughay *et al.*, 1993). Studies on trees grown in open soil confirmed this hypothesis; Curtis & Wang (1998) concluded that only 10% down-regulation was observed in long-term studies carried out on a variety of trees. If this is true, it is reasonable to assume that in ecosystems where formation of new below-ground sinks is not restricted, a larger amount of C assimilated through increased photosynthesis will be added to the soil C pool. Apart from decomposition of surface detritus, C enters the soil in the form of root biomass, rhizodeposition, root respiration and assimilates transferred to symbiotic organisms. If elevated CO_2 affects any of these pathways, whether quantitatively or qualitatively, it is likely to have an effect on the amount of C stored in the soil.

8.1.1 C derived directly from roots

When considering the impact enlarged root systems might have on the amount of C entering the soil, it is important to separate the response of long-living woody root biomass from that of ephemeral fine roots (Norby, 1994). Only a few datasets detailing the response of woody roots to elevated CO_2 are available. While some authors consider static

measurements of root biomass as representative of below-ground C allocation, the prevailing conclusion is that standing root biomass is a very poor indicator of total root production and thus of C allocation below-ground (Norby *et al.*, 1999; Kubiske & Godbold, 2001). Carbon released into the soil during continuous production and death of fine roots can account for as much as 30% of the C allocated to roots (Hendrick & Pregitzer, 1993) and therefore it is important to include it in the calculation of C allocated below-ground. When both measures were taken into account, there was no significant change in root:shoot ratio in *Quercus alba* (Norby *et al.*, 1995a) or *Betula pendula* (Rey & Jarvis, 1997). Curtis & Wang (1998), in their meta-analysis, provided evidence that there is no statistically significant change in root:shoot ratio of woody plants due to elevated CO_2. This suggests that the root:shoot ratio, at least when estimated on this static level, is not altered by elevated CO_2 and that the root systems are growing bigger in size in proportion to increasing above-ground biomass. In line with these findings, below-ground C allocation by root systems of *Populus* species was calculated in two separate steps. Firstly, long-lived coarse root biomass and secondly, fine root biomass turnover were estimated to take into account different characteristics of each root category with regard to C allocation.

8.1.1.1 Coarse roots

As a measure of woody root biomass present in the soil for longer periods of time, mean coarse dry root biomass was calculated for each growing season of the POPFACE experiment (Table 8.1). It is clear, as shown in section 4.3.1, that elevated CO_2 increased standing root biomass in all three *Populus* species studied by a statistically significant margin. The fact that the observed increase in root biomass under FACE relative to control does not diminish with time can be attributed to the ability of *Populus* trees to utilise larger root systems as an additional C sink.

Table 8.1 Mean standing biomass of coarse roots (> 2 mm) in each growing season (gm^{-2}, ± st. error)

	Populus alba		*Populus nigra*		*Populus euramericana*	
Season	FACE	Control	FACE	Control	FACE	Control
1st	42 (±7.6)	30 (±12.8)	74 (±7.5)	40 (±4.5)	99 (±16.2)	56 (±13.0)
2nd	115 (±19.0)	101 (±25.8)	176 (±36.5)	110 (±7.0)	326 (±39.7)	147 (±25.2)
3rd	239 (±20.6)	135 (±12.1)	419 (±13.0)	281 (±14.0)	355 (±16.0)	220 (±10.7)

8.1.1.2 Fine roots

Subsequently, the amount of C entering the soil through turnover of fine roots was calculated. In most field studies in which fine root biomass and turnover have been examined, fine roots have been found especially responsive to elevated CO_2. Norby *et al.* (1999) in their review present six studies done with trees exposed to elevated CO_2 in which fine root density (mass of roots per unit ground area) increased by 60% to 140% as a result of elevated CO_2. Fine root length production was increased in *Fraxinus excelsior* (240%), *Pinus sylvestris* (202%) and *Quercus petraea* (95%) when grown in OTC under elevated CO_2 conditions (Crookshanks *et al.*, 1998). In an earlier review Rogers *et al.* (1994) concluded that in the vast majority of approximately 150 plant species studied, fine root growth increased under elevated CO_2. A similar response to elevated CO_2 was observed in all three *Populus* species utilised in POPFACE. Standing fine root biomass increased by 35% in *Populus alba* on average over three growing seasons. *Populus nigra* and *Populus euramericana* responded to elevated CO_2 with an increase of 84% and 53%

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3rd	239 (±20.6)	135 (±12.1)	419 (±13.0)	281 (±14.0)	355 (±16.0)	220 (±10.7)

8.1.1.2 Fine roots

Subsequently, the amount of C entering the soil through turnover of fine roots was calculated. In most field studies in which fine root biomass and turnover have been examined, fine roots have been found especially responsive to elevated CO_2. Norby *et al.* (1999) in their review present six studies done with trees exposed to elevated CO_2 in which fine root density (mass of roots per unit ground area) increased by 60% to 140% as a result of elevated CO_2. Fine root length production was increased in *Fraxinus excelsior* (240%), *Pinus sylvestris* (202%) and *Quercus petraea* (95%) when grown in OTC under elevated CO_2 conditions (Crookshanks *et al.*, 1998). In an earlier review Rogers *et al.* (1994) concluded that in the vast majority of approximately 150 plant species studied, fine root growth increased under elevated CO_2. A similar response to elevated CO_2 was observed in all three *Populus* species utilised in POPFACE. Standing fine root biomass increased by 35% in *Populus alba* on average over three growing seasons. *Populus nigra* and *Populus euramericana* responded to elevated CO_2 with an increase of 84% and 53%

respectively. Mean fine root biomass present in the soil during each growing season is shown in Table 8.2.

Although the direct impact of an increase in fine root biomass on the whole plant root:shoot C allocation ratio might be small, elevated CO_2 might affect functionality of fine roots, which in turn will have consequences for long-term ecosystem functioning (Norby *et al.*, 1999).

Table 8.2 Mean standing biomass of fine roots (< 2 mm) in each growing season (gm^{-2}, ± st. error)

	Populus alba		*Populus nigra*		*Populus euramericana*	
Season	FACE	Control	FACE	Control	FACE	Control
1st	16 (±3.4)	15 (±1.6)	30 (±4.2)	18 (±2.4)	40 (±3.9)	41 (±4.2)
2nd	54 (±5.2)	69 (±4.5)	92 (±20.4)	54 (±8.0)	113 (±11.2)	90 (±15.3)
3rd	160 (±12.6)	103 (±5.8)	202 (±26.4)	111 (±4.6)	256 (±24.4)	140 (±9.2)

Therefore, in addition to the static measurement of fine root biomass, it is important to consider fine root dynamics, which can significantly influence the estimate of the amount of C transferred below-ground. Due to methodological difficulties, little is known about effects of elevated CO_2 on fine root turnover (Ceulemans *et al.*, 1999; Pregitzer *et al.*, 2000). Nearly all studies carried out so far, which assess root turnover rate, report an increase in root production and mortality as a result of CO_2 enrichment (Kubiske & Godbold, 2001). However, the evidence gathered so far is inconclusive with some authors reporting a decrease of fine root mortality under elevated CO_2 (Tingey *et al.*, 1995). In POPFACE, elevated CO_2 increased fine root turnover by 57%, 27% and 31% in *Populus alba*, *Populus nigra* and *Populus euramericana* respectively (Table 5.2).

Consequently, in order to calculate the amount of C transferred below the soil surface during production and mortality of fine roots, mean standing fine root biomass (Table 8.2) was multiplied by fine root turnover rate (Table 5.2) resulting in the amount of dry biomass produced during each growing season of the POPFACE experiment (Table 8.3).

Table 8.3 Biomass of fine root (< 2 mm) produced in each growing season [gm^{-2}, ± st. error]

	Populus alba		*Populus nigra*		*Populus euramericana*	
Season	FACE	Control	FACE	Control	FACE	Control
1st	28 (±5.8)	18 (±1.8)	42 (±6.0)	19 (±2.4)	52 (±5.0)	41 (±4.2)
2nd	203 (±19.3)	97 (±6.3)	249 (±55.1)	114 (±16.8)	227 (±22.3)	99 (±16.8)
3rd	256 (±20.2)	217 (±12.1)	405 (±52.7)	201 (±8.2)	513 (±48.9)	308 (±20.2)

For the final calculation of C allocated below-ground the data for standing coarse root biomass and fine root biomass produced per season were summed, corrected for ash content[14] and converted from dry biomass to C. Estimates of organic matter present in coarse roots were based on standing root biomass only, because no coarse root turnover was observed during the course of the experiment. Conversion of dry biomass to C was carried out using a C:dry biomass ratio of 0.48 (Nadelhoffer & Raich, 1992). Table 8.4 displays the amount of C transferred into the soil by each *Populus* species as a result of root system growth.

[14] Ash content of dried root samples was established by burning randomly selected coarse and fine root samples at 500 ^{0}C for 8 hours. The measurement had been carried out to account for soil partic les attached to fine roots even after thorough washing.

Table 8.4 C invested in fine and coarse roots [gm^{-2}]

	Populus alba		*Populus nigra*		*Populus euramericana*	
Season	FACE	Control	FACE	Control	FACE	Control
1st	22 (±3.8)	17 (±4.1)	40 (±1.6)	21 (±1.7)	52 (±7.0)	34 (±4.8)
2nd	100 (±10.5)	68 (±9.4)	147 (±24.0)	80 (±8.5)	189 (±20.9)	86 (±3.1)
3rd	156 (±10.2)	122 (±7.9)	284 (±14.0)	171 (±7.3)	297 (±11.6)	184 (±3.4)
Total	**278** (±10.1)	**206** (±19.8)	**472** (±34.6)	**272** (±1.0)	**538** (±21.7)	**304** (±5.4)
Effect	**+35%**		**+74%**		**+77%**	

Identical ratio for conversion of root biomass to C was used for both CO_2 treatments, because no difference in C content was found to have resulted from FACE. From September until November 2000, total non-structural C content of fine roots was sampled in all three *Populus* species[15]. Figure 8.1 illustrates the build-up of non-structural C at the end of the second growing season in *Populus nigra*, however no effect of elevated CO_2 was observed. Similar results were obtained for the remaining two species, there was no difference both between the treatments and among the species.

[15] Total non-structural C measurement was carried out alongside minirhizotron observation of roots. C content was measured by application of phenol-sulphuric acid technique (Farrar, 1993).

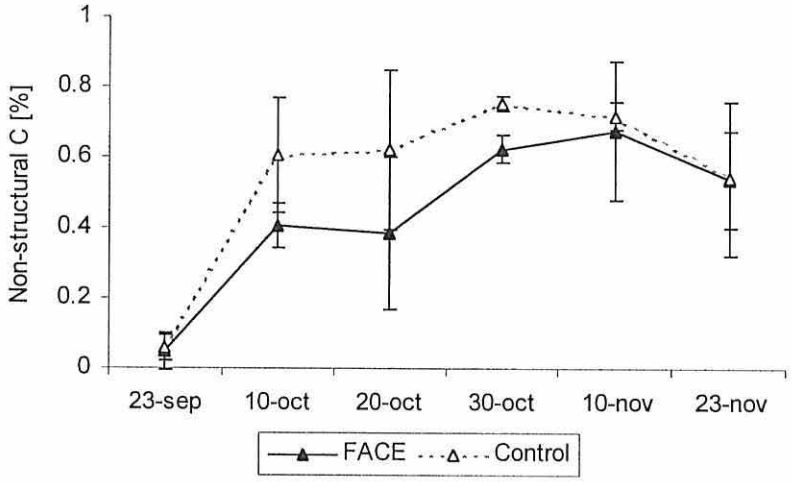

Figure 8.1 Total non-structural C content of fine roots - *Populus nigra* (% dry weight, ± st. error)

Elevated CO_2 clearly resulted in an increase of C transferred below-ground in all three *Populus* species. FACE treatment enlarged the amount of C *Populus alba* invests into its root system under the conditions present at POPFACE by 35% (Table 8.4). Even greater and almost identical enhancement of C allocation to roots was observed for the other two species, 74% and 77% for *Populus nigra* and *Populus euramericana* respectively. Such boosted investment of assimilated C below ground could be the means by which trees obtain additional resources necessary to sustain increased growth (Tingey *et al.*, 2000). It is worth mentioning, that FACE treatment resulted in more root growth in deeper soil horizons in *Populus alba* and *Populus nigra*, the consequences this might have on C storage in the soil are not yet clear. Whether this increased influx of C into the soil is likely to result in C storage, and hence in sequestration of atmospheric CO_2, or in increased C cycling is still an unanswered question (Ross *et al.*, 2000).

8.1.1.3 Root exudation

Although this process of organic matter input into the soil has not been investigated in POPFACE, due to its importance it is worthwhile to briefly discuss its response to elevated CO_2. Defined as passive loss and

active secretion of organic compounds by living roots (Cardon, 1996), root exudation can account for a sizeable proportion of assimilates allocated below-ground (Paterson *et al.*, 1997). If elevated CO_2 alters the state of root exudation, whether quantitatively or qualitatively as suggested by Norby *et al.* (1995b), the change could have a considerable effect on below-ground C storage by affecting the soil microbial activity it partially fuels (Scaglia *et al.*, 1985). It has been shown that varying input of organic exudates causes microbial community structure to change consistently and that at high substrate loading rates symbiotic fungi dominate over bacteria (Griffiths *et al.*, 1999). It is speculated that increased availability of assimilated C might result in more exudates being released from root systems (McMurtrie *et al.*, 2000). There is a possibility that this process took place in *Populus* tree grown under the elevated CO_2 treatment in POPFACE. This can be indicated, although only indirectly, by increased ash residue of roots from trees grown under FACE (Figure 8.2).

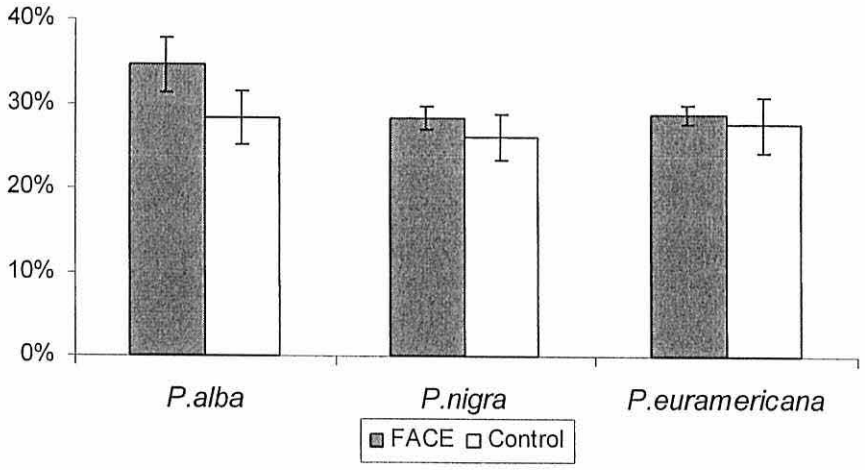

Figure 8.2 Ash content of *Populus* roots [% dry weight] (± st. error)

Increased exudation might cause soil particles to better adhere to fine root surface (D.Jones, pers. com.) affecting their removal during the washing process. Consequently, ash content of roots grown under FACE appears to be higher due to more soil particles attached to the roots even after washing. However, according to the studies published to date the

effect of elevated CO_2 on root exudation is not clear, some reporting no effect (Uselman *et al.*, 2000; Luo *et al.*, 2001) and some even a decrease (Hodge & Millard, 1998).

8.1.2 C transferred to mycorrhizas

In most plants the fungal partner forming mycorrhiza resides at the interface between plant and soil and forms the link through which part of assimilated C is transferred from plant to soil (Staddon *et al.*, 1999b). It was estimated that trees allocate 10-30% of assimilated C to their mycorrhizal symbionts (Markkola *et al.*, 1996), which makes the effect of elevated CO_2 on formation, growth and turnover of mycorrhizal fungi an important factor in any assessment of ecosystem C cycling (Norby & Jackson, 2000). The results obtained so far on mycorrhizal colonisation and functioning under elevated CO_2, as reviewed among others by Kubiske & Godbold (2001) and Fitter *et al.* (2000), have been inconclusive. Although decrease of mycorrhizal activity is rare, increases and null responses are similarly common. The results suggest comparable response by VAM and ECM, the response to elevated CO_2 being specific to the species of plant host and environmental conditions, notably nutrient availability (Treseder & Allen, 2000). Percentage root colonisation is the most frequently used measure of mycorrhizal colonisation (Ceulemans *et al.*, 1999), but is considered to be a poor indicator of fungal activity if not accompanied by detailed fine root measurement (Hodge, 1996).

In correspondence with these established theories, the effect of FACE on mycorrhizal colonisation of *Populus* roots was found to be species specific. Percentage root colonisation by VAM hyphae increased in *Populus alba* (+29%) and *Populus nigra* (+36%), while VAM colonising roots of *Populus euramericana* showed little response (+2%) to elevated CO_2. However, since elevated CO_2 generally enhances fine root biomass (Rogers *et al.*, 1994), even an unaltered percentage root colonisation under elevated CO_2 conditions could imply increased amount of intra-radical

fungal biomass. With the aim of assessing the effect of FACE on the total amount mycorrhizal hyphal biomass present in the roots, the effect of FACE on VAM percentage colonisation was combined with the effect on fine root biomass. This comparison reveals, that elevated CO_2 resulted in 72% more mycorrhizal hyphae in *Populus alba*, 156% more in *Populus nigra* and 70% more in *Populus euramericana*.

It is expected that even this adjusted estimate of mycorrhizal biomass does not necessarily reveal the real extend to which elevated CO_2 affects C cycling through mycorrhizas. A vital part of mycorrhizal functioning is the formation of extensive extraradical mycelium, because it is here where the majority of C obtained from the host is invested and where nutrient and water acquisition takes place (Klironomos *et al.*, 1997). The biomass of extraradical hyphae has been found to be positively affected by elevated CO_2 (Ineichen *et al.*, 1995), sometimes even more so than intraradical biomass (Sanders *et al.*, 1998). Therefore, without an assessment of the effect elevated CO_2 has on extraradical mycelium it is difficult, if not impossible, to estimate a change in the amount of C channelled through mycorrhizas.

Although not conclusively proven in *Populus* trees investigated in this research, a change in species composition of mycorrhizal community mediated by elevated CO_2 (Godbold *et al.*, 1997; Klironomos *et al.*, 1998) could have an effect on C cycling in the soil. As mycorrhizal species vary in tissue quality, growth rate and C demand from their host, any change in species assemblage is bound to have implications for the amount of C stored in or cycled through the soil.

8.2 *Interactions with other environmental variables*

It has been established, that documented effects of elevated CO_2 cannot be applied universally and that an interaction with other environmental variables such as the impact of nutrient and water availability, temperature and plant stress caused by pollutants takes place

(Runion *et al.*, 1994). The amount of assimilated C transferred to roots and through the host/mycorrhiza interface is not solely dependent on the increased supply of organic C due to photosynthetic stimulation (Bassirirad *et al.*, 2001). Studies examining the combined effect of elevated CO_2 and high/low N availability found that more often that not plants respond to elevated CO_2 with increased root production only in high N conditions (Sigurdson *et al.*, 2001). Likewise, mycorrhizal fungi may greatly respond to CO_2 fertilisation, but the extent of the response is mediated by N availability in the soil (Cheng, 1999). Implications of these interactions for the C pool present and cycled through the soil are not yet clear (Cardon, 1996; Staddon & Fitter, 1998). In view of these circumstances and considering that no N treatment had been carried out, it is important to bear in mind that the CO_2 effects observed might be valid only for the set of environmental conditions present in POPFACE.

8.3 Fate of additional C in the soil

In terrestrial ecosystems, where about two-thirds of C is in the soil (Schimel *et al.*, 1995), any effect of elevated CO_2 on soil C pool is likely to be indirect and mediated by plant response (van Veen *et al.*, 1991). As elevated levels of atmospheric CO_2 stimulate plant production, litterfall and rhizodeposition are expected to increase (Ceulemans *et al.*, 1999). Dead organic matter entering the soil decomposes, part of the C it contains is released back to the atmosphere and part remains as soil organic matter (SOM) (Norby & Jackson, 2000). It is the proportion of C locked up in the soil as SOM that is likely to determine the role plant dominated ecosystems will play in sequestering CO_2 from the atmosphere.

To date, no consensus has been reached on the effect elevated CO_2 might have on soil C cycling and storage (Tate & Ross, 1997). The increase in delivery of labile organic matter into the soil could result in stimulation of soil microbial activity and hence in increased nutrient mineralisation and availability (Zak *et al.*, 1993; Curtis *et al.*, 1994) or stimulation of

microbial biomass could result in more nutrients being locked in microbes and thus made unavailable to plants (Diaz *et al.*, 1993). It is also widely accepted that, apart from increased quantity, organic matter deposited under elevated CO_2 conditions has higher C/N concentration (Cotrufo *et al.*, 1998). Reduced concentration of N is thought to slow down decomposition (Cotrufo & Ineson, 1995), resulting in a build up of SOM (Berg *et al.*, 1995). Observations from POPFACE seem to support this hypothesis for *Populus nigra* and *Populus euramericana*. The increase of soil CO_2 efflux does not match the increase of soil C input under elevated CO_2 through root production only (Table 8.5). The amount of C transferred below-ground increased by 74 to 77% due to increased root production under elevated CO_2 in two species, while soil CO_2 increased only by approximately half that amount. This leads to conclusion that additional C released from rhizodeposition in *Populus nigra* and *Populus euramericana* was locked-up in slowly decomposing SOM at least for the period of three years.

Table 8.5 Effect of FACE on soil C input and soil CO_2 efflux.

	Populus alba	*Populus nigra*	*Populus euramericana*
C input	+35%	+74%	+77%
CO_2 efflux	+43%	+29%	+39%

Source: CO_2 efflux - Hocine Larbi (unpublished data).

Since *Populus* trees grown in POPFACE were found to be mycorrhizal, it is safe to assume that a substantial proportion of C transferred to soil was channelled through fungal symbionts (Rillig & Allen, 1998). A considerable amount of this C invested in symbiosis can be long-lived in the soil once the hyphal biomass was turned over. Although the rate of mycorrhizal turnover remains unclear (Fitter *et al.*, 2000), mycorrhizas are known to produce large quantities of chitin (Gooday,

1994) and glomalin (Wright *et al.*, 1998), both slowly degradable organic compounds remaining in the soil for long periods of time. Klironomos *et al.* (1998) reported that soil arthropods preferentially graze on non-mycorrhizal fungi, relatively slowing down cycling of C locked in mycorrhizal biomass. However, observed increases of mycorrhizal colonisation, especially in *Populus alba*, contradict this hypothesis when soil CO_2 efflux is taken into account. The amount of C entering the soil as root biomass increased by +36% in *Populus alba*. At the same time, soil CO_2 efflux increased by +43% as a result of elevated CO_2 treatment. This suggests that the extra C released from the soil in FACE must have come from source other than decomposing root biomass.

8.4 Carbon balance

Elevated CO_2 has caused increased below-ground production in all *Populus* species utilised in POPFACE experiment resulting in greater input of C into the soil. The effect of FACE on root growth and turnover has been apparent since the initial stages of the experiment and did not subside until the end of the third growing season. Evidence of CO_2 efflux from the soil did confirm a significant difference between the treatments; however the difference is species specific.

Similarly, mycorrhizal colonisation of fine roots was positively affected by elevated CO_2, although not in *Populus euramericana*. The plantation started growth at high soil N availability, slowly exhausting N supply and showing signs of N deficiency after three years (M. Sabatti, pers com). Klironomos *et al.* (1997) have shown that low N availability results in a mycorrhizal-based soil food web as opposed to a saprophytic/ pathogenic-based web at high N. Hence, if C transferred through mycorrhizas is mineralised at a slower pace, mycorrhizal symbionts might have contributed to C storage in the soil.

In conclusion, relying on the presented findings it is suggested that *Populus* plantations under conditions present in POPFACE will act as a C

sink under elevated CO_2 sequestering more atmospheric CO_2 below-ground, at least when *Populus nigra* and *Populus euramericana* are utilised.

9 Concluding remarks

9.1 *Does elevated CO_2 affect root processes and mycorrhizal functioning?*

After three seasons of growing three *Populus* species in a plantation under both ambient and elevated levels of atmospheric CO_2 it is possible to conclude that increased concentration of CO_2 does have a significant effect on below-ground processes.

Elevated CO_2 was found to increase the amount of root biomass in all three *Populus* species utilised in this research. *Populus* trees invest more assimilate into their root systems resulting in greater root biomass under FACE. The difference between treatments is statistically significant for *Populus nigra* and *Populus euramericana*. Both coarse and fine root biomass was increased, however their relative proportion of total standing root biomass did not change, suggesting that the functional basis of roots did not change - elevated CO_2 resulting in greater root systems only. Two of the species, *Populus alba* and *Populus nigra* significantly increased the depth allocation of root biomass as a result of exposure to FACE treatment, which might have implications for soil C cycling.

Investigation of fine root growth revealed a significant increase of fine root production in all three *Populus* species as a result of FACE. A difference in the level of increase of fine root production, although not significant, has been found among the species utilised, suggesting that CO_2 rich world might favour the species better able to utilise CO_2 fertilisation to explore the soil. Fine root turnover, expressed as a ratio of root production and maximum standing biomass, was increased by elevated CO_2. Similarly to fine root production, the change in the rate of turnover is different for each species utilised, resulting in diverse amounts of organic matter transferred below-ground.

Due to the close spacing between plot sections planted with the trees of different species, the results of root production investigation had to be verified. An application of the RAPD method of DNA analysis has been applied to the three *Populus* species utilised in POPFACE indicating that none or minimal growth of roots occurred between the species.

To examine the effect of elevated CO_2 on mycorrhizal colonisation an observation of VAM and ECM fungal activity has been carried out. The results show that mycorrhizal colonisation of *Populus* is affected by FACE treatment, however this effect is species specific and differences even among such closely related species occur. The colonisation of fine roots and root tips of *Populus alba* grown under elevated CO_2 treatment significantly increased both for VAM and for ECM. *Populus nigra* displayed a statistically significant increase only in VAM colonisation and FACE treatment did not affect mycorrhizal colonisation of *Populus euramericana*. When comparing the FACE effect on mycorrhizal colonisation with that on fine root production, it can be hypothesised that *Populus alba* utilised additional assimilated C to explore the soil through higher investment in its mycorrhizal symbionts. On the contrary, *Populus nigra* and *Populus euramericana* satisfied their need for more nutrients by investing C in growth of fine root systems. Assessment of the impact of elevated CO_2 on mycorrhizal community structure did not bring conclusive results and merits further study.

When considering the effects of elevated CO_2 on growth and functioning of a *Populus* plantation and especially when assessing its potential for C sequestration, these findings must also be taken into account. The amount of C transferred underground, its depth allocation and its form were shown to be affected by elevated CO_2 and are likely to have profound consequences for its fate within the soil. It is not clear whether increased C input into the soil results in greater C storage or C

cycling; influence of other environmental factors will play an important role in determining the amount of C sequestered by this type of fast growing ecosystem. Moreover, when considering the impact of elevated CO_2 on an ecosystem or when assessing long-term plant response to this phenomenon, its effects on intra- and inter-specific competition must be taken into account.

9.2 *Limitations of the research*

As outlined in this report on numerous occasions, nutrient status of the soil - notably N availability, is an important factor influencing the response of root systems and their mycorrhizal symbionts to elevated CO_2. Although originally planned, high and low N treatment was not carried out in the POPFACE experiment due to high soil N content at the onset of the experiment. Had an N fertilisation treatment been executed, the importance of the CO_2 X N interaction on below-ground processes would have been revealed.

Due to constraints of a technical nature placed on the size of the plants, it has been possible to carry the POPFACE experiment only over a period of three growing seasons. Although one of the overall aims of the study - achieving a closed canopy- has been reached, the evidence implies that below-ground this has been so only towards the end of the third growing season. One, or possibly two, more growing seasons could shed some light on the long-term effects increased root production has on soil processes and competition once the available soil space has been fully colonised by roots.

In cooperation with Wageningen University, The Netherlands, an attempt to estimate the effect of FACE on C flow through mycorrhizas by measuring a change of δ13C signature of C4 soil cores inserted into the soil profile in POPFACE was made. However, due to the complexity of the measurement technique, the soil samples are still being analysed.

9.3 *Areas of future research*

Better understanding of the influence of elevated CO_2 on allometric relationships within root systems is desirable in order to clarify whether increased levels of atmospheric CO_2 result in greater and functionally different or just in proportionately bigger root systems. A detailed analysis of fine root functioning and turnover and the role of coarse structural roots coupled with an investigation of non-structural carbohydrate content in roots and amount and character of root exudates, although notoriously difficult to study in natural ecosystems, under elevated CO_2 could help explain how elevated CO_2 affects soil C cycles.

The potential of mycorrhizal fungi to sequester C under elevated CO_2 conditions has not been fully investigated. More emphasis should be placed on studying the response of extra-radical hyphae, since it is the production of recalcitrant organic compounds such as chitin and glomalin that is likely to affect long-term C storage in the soil. In addition, shifts in mycorrhizal assemblage resulting from elevated CO_2 might affect C passage through the soil ecosystem and should be investigated with greater emphasis.

It is important to bear in mind that any effect future levels of atmospheric CO_2 might have on below-ground C sequestration will be consequences of a complex web of interactions between various types of organisms inhabiting the soil. Therefore, any future investigation attempting to explain the fate of additional C in the soil must take into account all components of this system.

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