The impact of increased atmospheric carbon dioxide on microbial community dynamics in the rhizosphere

Drigo, B.

Chapter 1

General introduction

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Increasing levels of atmospheric CO_2 concentrations and ecosystem responses

Due to human activity, global greenhouse gas emissions have grown since pre-industrial times, with an increase of 70% between 1970 and 2004 (IPCC 2007). Carbon dioxide (CO_2) is the most important anthropogenic greenhouse gas. The concentration of atmospheric CO_2 in 1750 was 280 ppm and had increased to 379 ppm by 2005. For comparison, at the end of the most recent ice age, there was approximately an 80 ppm rise in CO_2 concentration. This rise took over 5,000 years, and higher values than at present have only occurred many millions of years ago (IPCC 2007).

It is estimated that about two-thirds of anthropogenic climate change CO_2 emissions comes from fossil fuel burning (coal and petroleum) and about one-third from land use change (mainly deforestation and agricultural). With fossil fuels maintaining their dominant position in the global energy production, the concentration of atmospheric CO_2 is expected to rise further by an average of 1.5 ppm per year, reaching approximately 700 ppm by the end of the century (IPCC, 2007).

The primary influence of anthropogenic increase of atmospheric CO_2 concentrations on ecosystems is expected to be through the rate and magnitude of changes in climate. It has been hypothesized that extremes climate changes will occur more rapidly than the rate at which ecosystems can adapt and adjust.

The secondary influence of increased levels of atmospheric CO_2 is expected to occur through changes in the carbon cycle. Carbon, in the form of organic and inorganic compounds, notably CO_2, is cycled between the atmosphere, oceans, and the terrestrial biosphere. The largest natural exchanges occur between the atmosphere and terrestrial biota and between the atmosphere and ocean surface waters.

The terrestrial carbon cycle is already changing in response to increased atmospheric concentrations of CO_2. The amount of organic carbon in earth's vegetation and soil is estimated to be 600 Pg and 1500 Pg, respectively, and due to increasing atmospheric CO_2 concentrations this amount is likely to increase (Van Ginkel, 1999).

Over the past two decades, experiments in pots, growth chambers, open-top chambers and FACE (Free Air Carbon Dioxide Enrichment) experiments have demonstrated that enrichment of atmospheric CO_2 can have direct and indirect effects on terrestrial ecosystems and interact with the C cycling belowground (Ainsworth *et al.* 2005). The direct effect of elevated CO_2 concentration is an increase in net primary production, i.e. 'CO_2 fertilization', by enhancing the efficiency of ribulose 1, 5-bisphosphate carboxilase/oxidase (Rubisco), which consequently will increase net ecosystem production. In particularly, C_3 plants respond markedly to elevated CO_2 atmospheric concentrations, and in the coming century a 30-50% increase of C_3 plant photosynthetic activity is expected (Long *et al.*, 2004; Kuzyakov & Domanski 2000). In contrast, C_4 plants respond little to rising atmospheric CO_2 because their physiology dictates that their photosynthesic machinery is already saturated at the current ambient atmospheric CO_2 concentration.

Increased photosynthesis under elevated atmospheric CO_2 concentrations stimulate the production of the above-ground vegetation and has a strong effect on carbon fluxes from above-ground parts into the soil. Carbon input to soil generally increases in response to elevated CO_2 concentrations, owing to improved plant carbohydrate status (Barron-Gafford *et al.* 2005), even if there is no significant CO_2 stimulation of above-ground growth (Körner & Arnone, 1992). Thus, the amount of carbon entering the soil as root exudates and

rhizodeposition will increase in response to enhanced concentrations of CO_2 in the atmosphere with concomitant changes in the chemical composition of litter and root-released substrates (Pendall *et al.,* 2004). The increase of soil C availability will enhance the biomass and activity of microbes, which are generally C-limited. This increased microbial activity will enhance organic C turnover as well as the turnover of nutrients such as N , P and S, whose dynamics are strongly associated with C turnover. One of the main consequences of the enhanced microbial activity will be the increase in microbial immobilization of these nutrients (Fig 1). As plant production is often limited by quantities of N made available during the decomposition of fresh litter and organic matter in soil (Bernston & Bazzaz 1997; Hungate *et al*. 2000; Zak *et al*., 2000), the enhanced immobilization of N by microbes could reduce or eliminate the plant's ability to respond to CO_2, thereby potentially leading to long-term decreases in plant productivity.

Consequences of increased levels of CO_2 for microbial communities in the rhizosphere

Plant survival and adaptation to adverse soil conditions are strictly dependent on the capability of roots to interact with biotic and abiotic components of soil. Processes at the root-soil interphase concern a very limited area of soil surrounding the root tissue, defined as the rhizosphere. In this zone, exchanges of energy, nutrients, and molecular communication signals occur, rendering the chemistry and biology of this environment different from bulk soil.

The flow of C substrates through the microbial communities that live in the rhizosphere is a key factor influencing C storage and soil N availability. Thus, understanding the turnover of microbial biomass (biomass/assimilation rate) and the potential shifts in soil-borne community structure is central to predicting changes in soil C and N cycling under elevated CO_2 conditions.

Soil microorganisms are commonly C-limited. Therefore, increased C availability due to enhance rhizodeposition, resulting from increased level of atmospheric CO_2 concentrations, might stimulate microbial growth and activity. However, studies examining effects of elevated CO_2 concentrations on microbial biomass, particularly in the rhizosphere, have yielded mixed results. Alterations in carbon supply have been shown to decrease (Diaz *et al.* 1993; Ebersberger *et al.* 2004), increase (Zak *et al.* 1993) or not affect (Randlett *et al*. 1996; Kandeler *et al*. 1998) the growth and activities (*e.g.* decomposition and nutrient cycling) of soil-borne communities (Jones *et al.* 1998; Hu *et al.* 1999).

Among the soil biota, mycorrhizal fungi play a special role because they provide a direct link between plant roots and the soil, functioning more as an extension of the root system as opposed to a distinct part of the soil microbiota. Numerous short-term greenhouse experiments (reviewed in Staddon & Fitter 1998; Rillig & Allen 1998; Rillig *et al.* 1999) have shown that increased atmospheric CO_2 concentrations tend to increase the root internal colonization and extraradical phase (soil hyphal lengths) of the fungal symbionts giving mycorrhizal fungi a central role in the sequestration of atmospheric CO_2 on annual to decadal timescales (Staddon 2005; Staddon *et al.* 2003; Pendall *et al.* 2004).

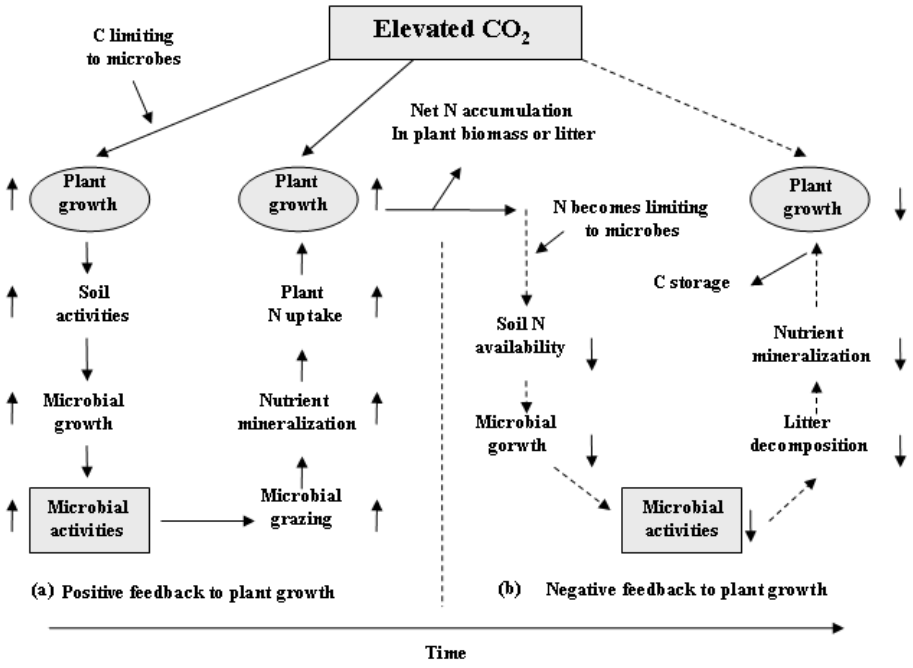

Fig. 1: Elevated CO_2 concentration alters the relative availability of carbon (C) and nitrogen (N) for microbes over time, which affects microbial feedbacks on further plant growth. (a) When C is limiting to microbes, enhanced C inputs caused by elevated [CO_2] stimulate microbial activities and increase N availability for plant growth (unbroken lines). (b) As N accumulates in less labile pools, N deficiency limits microbial growth and activities, and retards N mineralization, negatively feeding back to plant growth (dashed lines). The dashed arrow from 'elevated [CO_2]' to 'plant growth' depicts the notion means that elevated [CO_2] will not have a net effect on plant growth if nutrient limitation constrains the plant's response (figure adapted from Hu *et al.* 1999)

The dominant from of mycorrhizae in non-woody plants are formed by arbuscular-mycorrhizal fungi (AMF). These organisms are obligate biotrophs (Smith & Read 1997), meaning that energy and C for biosynthesis is necessarily provided solely by the host, *i.e.* the plant. Therefore, AMF might be the key to the consequences of elevated CO_2 on the plant and nutrient uptake, carbon input into the soil and resulting changes in the microbial activity (Norby 1994; Rillig *et al* .2000).

It has become increasingly evident that general biomass and activity measures are inadequate to fully understand the consequences of global change effects on soils (Janus *et al.* 2005), as these parameters do not address the responses of specific soil-borne microbial communities to elevated atmospheric CO_2 concentrations. Molecular-based methodologies (Tiedje *et al.* 1999; Hugenholtz *et al.* 1998; Pace 1997) now allow for more detailed and comparative analyses of microbial community. Using a variety of molecular community

analysis methods, mixed results have been reported regarding the effects of increased atmospheric CO_2 levels on the structure of bulk soil and rhizosphere microbial communities. Observations range from pronounced effects (Janus *et al.* 2005; Jossi *et al.* 2006; Mayr *et al.* 1999; Montealegre *et al.* 2000; Rillig *et al.* 1997) to subtle or undetectable effects (Bruce *et al.* 2000; Ebersberger *et al.* 2004; Griffiths *et al.* 1998; Insam *et al.* 1999; Klamer et al. 2002; Montealegre *et al.* 2000; Zak *et al.* 2000). However, these contrasting results must be viewed in the light of the different systems studied, and the different methods applied to assess community structure and diversity. Moreover, most of the relevant studies conducted under field conditions (Janus *et al.* 2005; Jossi *et al.* 2006; Marilley *et al.* 1999; Sonnemann & Wolters 2005) have focused on specific bacterial groups, showing CO_2-related shifts in the composition of *Pseudomonas* spp. (Marilley *et al.* 1999), or *Rhizobium* species (Schortemeyer *et al.* 1996; Montealegre *et al.* 2000) or stimulation of *Proteobacteria* (Jossi *et al.* 2006).

The information reported during the past two decades on the impact of global change on terrestrial ecosystems has resulted in a "patchwork", making it difficult to draw general conclusions about the full effects of elevated CO_2 on terrestrial ecosystems. In particular, this holds for information on the dynamics of microbial communities in the rhizosphere and in the bulk soil and even more on the functional consequences of potential community changes.

Objectives

The aim of this study was to assess the plant-driven impact of elevated atmospheric CO_2 concentrations on microbial communities in the rhizosphere and root-free soil. The primary focus was on understanding the mechanisms and consequences of altered C fluxes from the plant, through AMF, to soil microbial communities. By tracking changes in, and consequences of functional diversity in the soil and rhizosphere habitats, I sought to address some of the main consequences of global change for plant/soil systems.

Approach

To address my objectives, I assessed the plant-driven impact of elevated CO_2 on changes in rhizosphere communities of two dominant coastal sand dune plant species, *Festuca rubra* ssp. *arenaria* (sand fescue) and *Carex arenaria* (sand sedge). Coastal dune systems were chosen as a model due to their relative simplicity and particular relevance to the issue of global climate change. Plants were grown under controlled temperature and moisture conditions, while subjecting the aboveground compartment to defined atmospheric conditions with either ambient (350 ppm) or elevated (700 ppm) CO_2 concentrations in three different sandy (dune) soils.

Using a variety of molecular approaches, I examined the structure and abundance of the communities of bacteria, fungi and nematodes, as well as the dynamics of specific groups, such as arbuscular mycorrhizal fungi (AMF), actinomycetes, *Pseudomonas*, *Burkholderia*, *Bacillus, Trichoderma, Fusarium* and phloroglucinol-, phenazine- pyrrolnitrin-producing organisms, in the rhizosphere of *F. rubra* and *C. arenaria*.

In order to track the fate of plant-assimilated C to the belowground microbial community, and to examine the impact of elevated atmospheric CO_2 levels on the structure and the functioning of microbial communities, I conducted both short (6 months) and longer-term

(3 years) $^{13}CO_2$ pulse-chase labelling experiments. ^{13}C-Stable Isotope Probing (SIP) and its applications for tracking plant-derived C fluxes into microbial nucleic acids (RNA-SIP) or biomarkers (^{13}C- P(N)LFA) allowed for the identification of the microbial communities in the soil that were actively assimilating plant-derived substrates, thereby improving our understanding of the microbial community dynamics associated with rhizosphere carbon flow under ambient and increased CO_2 conditions.

Research questions

To investigate the above-mention objectives of the study, I formulated the following research questions:

1. What is the plant-driven effect of enhanced atmospheric CO_2 concentrations on the composition of the bacterial, fungal and nematode communities in the rhizosphere?

2. Do enhanced CO_2 concentrations result in shifts in the composition of specific bacterial and fungal rhizosphere groups?

3. What is the impact of elevated CO_2 on the capability of the soil microbial community to incorporate plant-assimilated C?

4. What are the functional consequences of changes in the community structure of some of the major microbial players brought about by elevated concentrations of atmospheric CO_2?

5. What are the consequences of elevated CO_2 on C-incorporating soil communities in the longer term?

Outline of the thesis

In **chapter 2,** I review the information available in literature on the effects of elevated atmospheric CO_2 on microbial community structure and activities in the rhizosphere. To gain further insight into the effects of elevated atmospheric CO_2 on soil-borne communities, **chapter 3** describes the plant-driven impact of elevated CO_2 on the rhizosphere communities of two dominant coastal sand dune plant species, *F. rubra* and *C. arenaria*. Analyses focused on bacterial, fungal and nematode abundance and community structure and the ratios between these groups. Bacterial, fungal and nematode community sizes were determined by real-time PCR and biomarker analyses, and molecular community profiles were generated for these communities by PCR-DGGE. Subsequently, multivariate statistical analyses were used to compare the relative impact of elevated CO_2 treatment versus plant and soil effects on these communities. In **chapter 4**, I examined effects of elevated CO_2 on root exudation, microbial community structure and functional, the size of specific functional groups, using respectively HLPC, PCR-DGGE community fingerprinting analysis and real-time PCR. For specific groups, analyses targeted *Pseudomonas* spp. and *Burkholderia* ssp. as bacteria well adapted to the rhizosphere environment and actinomycetes and the genus *Bacillus* as groups exhibiting typical bulk soil ecological strategies. The community size of two typical fungi, *Trichoderma* ssp. and *Fusarium* ssp., inhabiting the *F. rubra* and *C. arenaria* rhizosphere in costal dune

ecosystems were also determined using real-time PCR assays. Moreover the density of genes involved in the production of phloroglucinol, phenazine and pyrrolnitrin were quantified by real-time PCR. In order to track the fate of plant-assimilated C to the belowground microbial community and to examine the impact of elevated atmospheric CO_2 levels on these processes, a $^{13}CO_2$ pulse-chase labelling experiment was conducted and reported in **chapter 5**. To gain insight into the flow of carbon to different soil-borne microbial groups, specific fatty-acid biomarkers for AMF, total bacteria, *Pseudomonas* spp., *Burkholderia* spp., *Bacillus*, actinomycetes and protozoa were used to track the ^{13}C allocation from the atmosphere into these communities. Using the *F. rubra* model system, the RNA stable isotope probing technique (^{13}C-RNA-SIP), PCR-DGGE and real-time PCR were applied to identify the truly active rhizosphere microbial community species in **chapter 6**. I linked the *in situ* microbial activity to the affected total bacterial, total fungal, *Pseudomonas* spp., *Burkholderia* spp. and AMF under elevated CO_2 conditions. **Chapter 7** describes a long-term greenhouse experiment, using the same plant system, in which the adaptation capacities of C-incorporating rhizosphere communities were studied over a 3-year period of elevated CO_2 exposure. During the three years of incubation, I applied four $^{13}CO_2$ pulse labelling events. The C flow and the subsequent shifts in community composition were again tracked by using RNA-SIP, biomarkers analysis, real-time PCR and PCR-DGGE approaches. Changes in the structure and composition of AMF, bacterial and fungal rhizosphere communities were assessed as well. The results of the different studies are summarized, discussed and evaluated in **chapter 8**.