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Het effect van global change op bodem C en bodem C cyclering

Global change effects on soil C and soil C cycling

Proefschrift voorgelegd tot het behalen van de graad van doctor in de wetenschappen, biologie aan de Universiteit Antwerpen, te verdedigen door

Thesis submitted for the degree of Doctor in Philosophy in the School of Earth and Environmental Sciences, James Cook University, by

Wouter DIELEMAN

Promotoren	
Prof. Dr. Ivan Janssens	Supervisor
Dr. Ir. Sebastiaan Luyssaert	Prof. Dr. Michael Bird

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STATEMENT ON THE CONTRIBUTION BY OTHERS

Nature of assistance	**Contribution**	**Names and affiliations**
Intellectual support	Conception of scientific questions and approach	Prof. dr. Ivan Janssens, University of Antwerp, Belgium Prof. dr. Sebastiaan Luyssaert, University of Antwerp, Belgium; LSCE IPSL, France Prof. dr. Michael Bird, James Cook University, Australia
	Data analysis	Prof. dr. Ivan Janssens, University of Antwerp, Belgium Prof. dr. Sebastiaan Luyssaert, University of Antwerp, Belgium; LSCE IPSL, France Prof. dr. Michael Bird, James Cook University, Australia Prof. dr. Andrew Krockenberger, James Cook University, Australia

		Dr. Sara Vicca, University of Antwerp, Belgium Dr. Sebastiaan Leuzinger, ETH ITES, Switzerland
	Cartography and GIS	Anurag Ramachandra, Conservation International Prof. dr. David Gillieson, James Cook University, Australia Prof. dr. Paul Nelson, James Cook University, Australia
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Data collection	Research assistance	Dr. Chris Wurster, James Cook University, Australia Dr. Michael Zimmermann, James Cook University, Australia Dr. Gustavo Saiz, James Cook University, Australia; Karlsruhe Institute of Technology, Germany Anthony Forsyth, James Cook University, Australia Iain Goodrick, James Cook University, Australia

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Of course I have also tested my own flexibility. A PhD is not always easy, and is also about working your way through problems, questions and challenges. In that way, the sentence ‘Aut viam inveniam, aut faciam’ (I will find a way, or I will make one) is appropriate here. In this moment I therefore also thank myself to finish this work! I am ready for the next step.

Sincerely,
Wouter

ABBREVIATIONS

AB	Aboveground Biomass
C	Carbon
CH_4	Methane
CI	Confidence Interval
CO_2	Carbon Dioxide
FACE	Free Air Carbon dioxide Enrichment
FRB	Fine Root Biomass
FRP	Fine Root Production
(F)RT	(Fine) Root Turnover
GCME	Global Change Manipulation Experiment
GHG	GreenHouse Gas
IPCC	Intergovernmental Panel on Climate Change
LAI	Leaf Area Index
LF	Litterfall
MAP	Mean Annual Precipitation
MAT	Mean Annual Temperature
MB(C)	Microbial Biomass (Carbon)
MLE	Maximum Likelihood Estimator
N	Nitrogen
NEE	Net Ecosystem Exchange
Nmin	net Nitrogen Mineralization
N_2O	Nitrous Oxide

NPK	Nitrogen Phosphorous Potassium (fertilizer)
NPP	Net Primary Production
OTC	Open Top Chamber
P	Phosphorous
PNG	Papua New Guinea
PNL	Progressive Nitrogen Limitation
Ra	autotrophic Respiration
RB	Root Biomass
REDD	Reduced Emissions from Deforestation and Degradation
Rh	heterotrophic Respiration
RS or R/S	Root – Shoot ratio
SCE	Soil CO_2 Efflux
SD	Standard Deviation
SE	Standard Error
SR	Soil Respiration
SOC	Soil Organic Carbon
SOM	Soil Organic Matter
TB	Total Biomass

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LIST OF PUBLICATIONS

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Do global change experiments overestimate impacts on terrestrial ecosystems? Trends in Ecology & Evolution, 26, 236-241.

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ABSTRACT

The concentration of carbon dioxide (CO_2) in the atmosphere represents the balance between CO_2 uptake and CO_2 release by the terrestrial and oceanic biosphere. In the absence of anthropogenic greenhouse gas emissions, CO_2 uptake and release are approximately in balance, and atmospheric CO_2 concentrations remain relatively constant over time. However, fossil fuel burning and land-use change have increased greenhouse gas emissions considerably, with an increase in global mean temperatures as a consequence. In addition, the changes in global temperatures are inducing alterations in precipitation patterns, and fossil fuel emissions are increasing the amount of reactive nitrogen deposition. These changes will, alone and in combination, affect the CO_2 exchange of terrestrial ecosystems with the atmosphere through changes in plant productivity, soil nutrient and water balances, and carbon storage in biomass and soils.

In this thesis, I have addressed this topic by evaluating the effects of four major global changes (elevated CO_2, rising temperatures, increasing N deposition, and changing water availability) on soil carbon (C) storage and soil C cycling in terrestrial ecosystems. Using a database with results from global change manipulation experiments across a range of different ecosystems, and a soil sampling campaign along an altitudinal gradient in tropical forest, I assessed whether global changes affected C storage in soils and tried to uncover the

mechanisms behind the effects of global change on soil C content and soil C cycling.

First, I have tested whether elevated CO_2 concentrations can increase soil C content through increased plant productivity, and found that elevated CO_2 accelerates soil C cycling, but does not increase soil C content. Effects of nitrogen (N) availability were important in the CO_2 response of soil C processes, as we found that soil C content did increase in elevated CO_2 when sufficient amounts of N fertilizer were added. In addition, I have shown that initial soil N content affected responses of fine roots, microbial decomposition and soil C content to elevated CO_2. Secondly, I have tested the hypothesis that N fertilization consistently decreases decomposition processes, and found a strong negative effect of N fertilization on microbial biomass and activity. I have revealed possible mechanisms behind these effects and indicated that N fertilization can thus lead to increases in soil C content. Thirdly, I analyzed a dataset of warming experiments to test whether warming consistently stimulates microbial decomposition rates. I did not find a consistent increase in microbial respiration or soil respiration, due to large variation between experiments. Here, I concluded that interactions and feedbacks related to soil water and nutrient status, and ecosystem-specific responses were causing the large range of observed effects. Further, in an analysis of combined elevated CO_2 and warming experiments, I have shown that elevated CO_2 and warming can alleviate (part) of each other's limitations, resulting in synergistic interactions between different global change drivers. But more often, effects of combined global changes are unpredictable, suggesting the need for global models to validate their models structures against results from studies combining multiple global changes, rather than against effects of individual global changes. Lastly, due to spatial variability and differences in sensitivity of different terrestrial ecosystems, I have indicated the importance of a high quality documentation of site variables to be able to explain the observed effects of global change drivers.

In this thesis, I have increased the understanding of mechanisms and patterns behind effects of N fertilization and elevated CO_2 concentrations, and have synthesized and clarified some effects and responses patterns in warming experiments. In addition, I have indicated gaps in our current understanding of soil C cycling processes due to lack of data, or low data quality. To increase the understanding of mechanisms behind global change effects on soil processes, I call for a more integrated approach where effects on soil C inputs, C pools, and C losses are simultaneously addressed, with a stronger attention for documentation of, and effects on, soil nutrient and water balances.

SAMENVATTING

De concentratie van koolstof dioxide (CO_2) in de atmosfeer is het resultaat van de balans tussen CO_2 opname door plantengroei, en CO_2 vrijgave door respiratie. Zonder de uitstoot van broeikasgassen, die veroorzaakt wordt door menselijke activiteiten, is de balans tussen CO_2 opname en vrijgave nagenoeg constant. Gedurende de voorbije decennia heeft de stijging in het verbruik van fossiele brandstoffen er echter voor gezorgd dat de concentratie van broeikasgassen in de atmosfeer aanzienlijk is gestegen. Dit heeft als gevolg dat de gemiddelde temperatuur op aarde stijgt. Daarenboven werkt deze stijging in temperatuur ook veranderingen in patronen en intensiteit van regenval in de hand, en de verbranding van fossiele energiebronnen verhoogt ook de hoeveelheid stikstof (N) depositie. Deze veranderingen in het klimaat en de atmosfeer zullen elk op zich, en in combinatie met elkaar, de uitwisseling van CO_2 tussen terrestrische ecosystemen en de atmosfeer beïnvloeden door veranderingen in de productiviteit van planten, veranderingen in beschikbaarheid van water en nutriënten in de bodem, en veranderingen in de opslag van koolstof (C) in biomassa en de bodem.

In deze thesis kom ik tegemoet aan enkele van de vragen die deze veranderingen opwerpen, door het effect van vier grote ‘Global Change’ factoren op de C inhoud van de bodem en de cyclering van C in de bodem in terrestrische ecosystemen te bestuderen. Aan de hand van een databank die resultaten uit global change manipulatie experimenten in een waaier aan verschillende types ecosystemen bevat, en een veldstudie rond bodem C langs een hoogtegradiënt in tropisch regenwoud, heb ik getest of de globale veranderingen in atmosfeer en klimaat de C opslag in de bodem beïnvloedt en heb ik geprobeerd om de mechanismen achter effecten te achterhalen.

Ten eerste ben ik nagegaan of er onder verhoogde atmosferische CO_2 concentraties een stijging in C opslag in de bodem kan worden bekomen. Daarop heb ik gevonden dat verhoogde CO_2 concentraties de cyclering van C wel versnelt, maar dat een verhoogde C opslag in de bodem onwaarschijnlijk is. Van uiterst belang hierbij zijn de effecten van N beschikbaarheid aangezien de opslag van C in de bodem in verhoogde CO_2 concentraties wel toeneemt bij bemesting met aanzienlijke hoeveelheden N. Bovendien heb ik ook aangetoond dat de initiële N inhoud van de bodem patronen in wortelgroei, microbiële respiratie en bodem C onder verhoogde atmosferische CO_2 concentraties beïnvloedt. Ten tweede heb ik de hypothese dat N bemesting de decompositie van organisch materiaal zou vertragen getest, en heb ik aangetoond dat N bemesting inderdaad negatieve effecten op microbiële groei en respiratie heeft. Daarenboven heb ik een aantal verschillende mechanismen gesuggereerd en daarbij aangetoond dat N bemesting de C inhoud van de bodem kan verhogen. Wat betreft effecten van stijgende temperaturen ben ik nagegaan of microbiële respiratie consistent wordt gestimuleerd door hogere temperaturen. Ik vond geen onvoorwaardelijk gestimuleerde microbiële activiteit, deels te verklaren door de grote variatie tussen verschillende experimenten. Hier concludeerde ik dat interacties en feedbacks gerelateerd aan beschikbaarheid van water of nutriënten, en de verschillende respons in verschillende types ecosystemen verantwoordelijk waren voor de waaier aan verschillende effecten. In een analyse waar verhoogde atmosferische CO_2 concentraties en hogere temperaturen werden gecombineerd, heb ik aangetoond dat beide global change factoren elkaars limiteringen kunnen opheffen, wat synergistische effecten mogelijk zou kunnen maken. Veel vaker echter, waren de effecten als gevolg van gecombineerde global change factoren onvoorspelbaar. Dit geeft aan dat modellen bij voorkeur zouden moeten getest worden tegen resultaten uit experimenten met gecombineerde global change factoren, in plaats van experimenten die individuele global change factoren bestuderen. Tenslotte, omdat de ruimtelijke variatie en de verschillende gevoeligheid van bepaalde terrestrische ecosystemen de interpretatie van effecten van global change bemoeilijkt, heb ik meerdere keren het belang van een kwalitatief goede beschrijving van experimentele omstandigheden aangegeven om effecten voldoende te kunnen verklaren.

If you haven’t got good quality soil, you’re rooted. – Kathryn Curlis

1. GENERAL INTRODUCTION

1.1. A changing climate in a changing world

Over the past centuries the global atmosphere has changed through increased abundance of atmospheric greenhouse gasses (GHGs) and aerosols. Atmospheric carbon dioxide concentrations ([CO_2]) have increased from pre-industrial levels of 280ppm to 393ppm in 2011 (Fig. 1.1), a concentration that by far exceeds the range over the last 650,000 years (Pearson & Palmer 2000). The primary sources for this increase are fossil fuel combustion and land use change (Houghton 2003; Foley *et al.* 2005; IPCC 2007). Because of its high atmospheric concentration, it is mainly the increased level of CO_2 that induces the so-called 'greenhouse effect'.

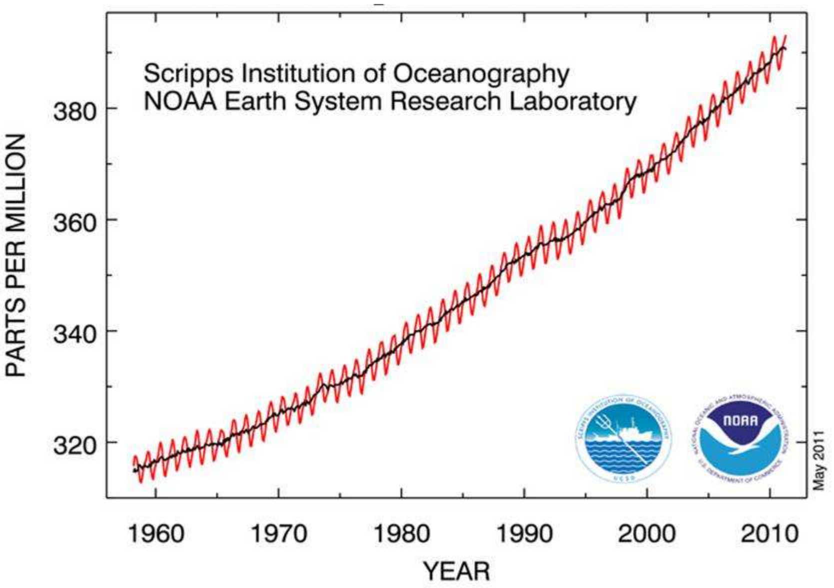


Figure 1.1: Atmospheric measurements of CO_2 concentrations at Mauna Loa, Hawaii over the last decades.

However, concentrations of methane ([CH_4]) and nitrous oxide ([N_2O]) have also increased considerably, mostly as a consequence of agricultural practices (Davidson 2009). Although far less abundant than CO_2, the greenhouse gas potential of CH_4 and N_2O is a lot stronger than that of carbon dioxide (25 and 298 times stronger than CO_2 respectively, calculated for a 100 year period), adding to their weight in the overall greenhouse effect. The total radiative forcing (measure of how much influence a factor has in changing the in- and outgoing amount of energy in the atmosphere) of CO_2, CH_4 and N_2O is 1.66, 0.48 and 0.16 W m^{-2} respectively (IPCC 2007).

The effect of this increase in atmospheric greenhouse gas concentrations has been that of global surface warming, resulting in sea level rise and reduction of global snow cover. Over the last 100 years, the global surface temperature of the earth has increased by 0.74°C, and has been increasing with 0.13°C per decade in the last 50 years (IPCC 2007). For the next two decades, the IPCC suggests an average warming of about 0.2°C per decade for a range of scenario’s. This warming will be greatest over land and at high latitudes.

In addition to a trend of global surface warming, precipitation amounts have increased in several parts of the world, while other parts have experienced significant reductions over the last century. Future precipitation amounts are likely to increase in high latitudes and decrease in subtropical land-areas, continuing the currently observed trends. But changes in rainfall distribution are still hard to predict due to large spatial and temporal variability (IPCC 2007). In addition to changes in precipitation patterns, the frequency and intensity of extreme events such as droughts, heat waves or heavy precipitation events will likely increase.

Apart from these changes to climate, deposition of reactive nitrogen has increased over the last 200 years due to fossil fuel combustion and anthropogenic production of nitrogen to meet the demand of agriculture and industry (Galloway 2008). In 1860, the amount of NOx and NH_3 deposited on the earth's surface was estimated to be 34 TgN yr^{-1}. In 1995 it had increased to 100 TgN yr^{-1}, and it is projected to be 200 TgN yr^{-1} in 2050 (Galloway 2008). Background values for N deposition are generally around 5 kgN ha^{-1} yr^{-1} or less, but there are now regions where it easily exceeds 10 kgN ha^{-1} yr^{-1}.

1.2. Global change and the terrestrial C cycle

Atmospheric CO_2 serves as the main nutrient for plant growth on our globe. Using solar energy, carbon dioxide molecules are taken up and transformed into sugars during photosynthesis (Larcher 2001). Sunlight, nutrients and water are needed to balance this process, and can become limiting over time (Larcher 2001). Thereafter, plants use these assimilated C compounds to build stuctural components, and allocate C to their different organs to optimize plant growth, i.e. light capture by increasing the size of their crown (i.e. increasing leaf area index (LAI)), nutrient and water uptake by extending their root network (Larcher 2001; Litton *et al.* 2007; Poorter *et al.* 2012).

Because terrestrial ecosystems contain approximately 500 PgC in plant biomass and about 2500 PgC in soil compartments (Fig. 1.2), with a yearly exchange of 120 PgC with the atmosphere (Lal 2008), they play a pivotal role in the global carbon cycle as a sink for atmospheric CO_2. As current atmospheric [CO_2] continues to rise, plant productivity and thus C retention may be stimulated. As such, the rising atmospheric CO_2 concentrations have changed natural C sink strengths, with about 55% of the human-induced CO_2 emissions being absorbed by land and ocean (Le Quéré *et al.* 2009). In terrestrial ecosystems, it is the direct effect of elevated CO_2 concentrations stimulating plant photosynthesis (Ainsworth & Long 2005) that drives this negative feedback on the terrestrial carbon cycle. Indeed, Eurasian boreal and North American temperate forests have been identified as large C sinks over the past 19 years (Myneni *et al.* 2001).

However, while elevated CO2 levels might induce an increased C sink capacity in terrestrial ecosystems, the concurrent rise in global temperatures is suggested to stimulate decomposition processes more than plant CO_2 uptake, thereby reducing the negative feedback on atmospheric CO_2 levels expected from the fertilizing

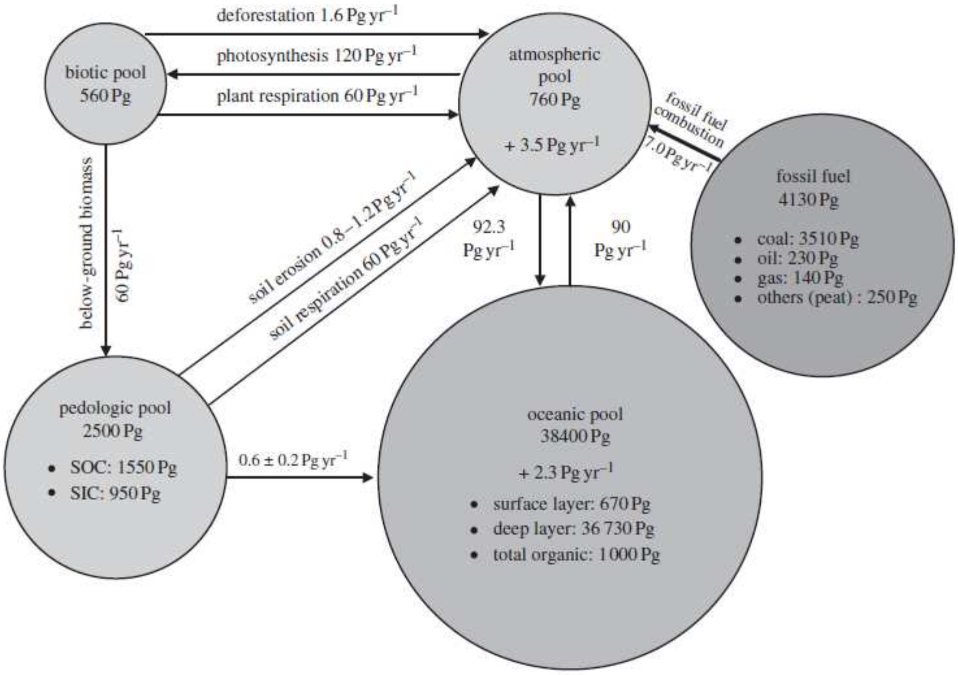


Figure 1.2: Principal global C pools and fluxes between them. *Source: Lal, 2008, Philosophical Transactions of the Royal Society*

effect of elevated CO_2 alone (Cox *et al.* 2000; Friedlingstein *et al.* 2006; Friedlingstein & Prentice 2010). In addition, as a consequence of higher temperatures and redistribution of precipitation amounts, droughts and fires can significantly reduce plant biomass production and C sequestration (Ciais *et al.* 2005; Reichstein *et al.* 2007; Arnone *et al.* 2008; Huang *et al.* 2009; Huang & Anderegg 2011) and might trigger a large C release in peatlands (Fenner & Freeman 2011).

Furthermore, because N uptake in plant biomass increases linearly with C uptake when plants develop (Yang *et al.* 2011), a gradual nitrogen limitation of plant growth is believed to reduce the stimulating effect of elevated CO_2 levels on plant growth in the long term (Luo *et al.* 2004). Increasing amounts of N deposition can possibly play an important role here by reducing this N limitation in the future.

To synthesize, terrestrial ecosystems play a key role in global change as they are intimately linked with it through the exchange of CO_2 between vegetation and the atmosphere. The large amounts of C they sequester can potentially slow down the current rise in atmospheric CO_2 levels. But clearly, terrestrial ecosystems are also affected by other global change drivers, and therefore it is highly uncertain whether terrestrial ecosystems will act as C sinks or sources in the future.

1.3. The key role of soil C

Soils are the largest pool of organic carbon in the terrestrial biosphere (Fig. 1.2), containing over 2300Pg of carbon in the top three meters (Jobbagy & Jackson 2000). Changes in the soil organic carbon (SOC) pool are driven by changes in the balance between atmospheric CO_2 uptake by plant primary production, and soil CO_2 effluxes through decomposition processes and root respiration (i.e. soil respiration) (Trumbore 2006). Because these annual effluxes of CO_2 from soils to the atmosphere are about 10 times the amount derived from fossil fuel combustion (Mooney *et al.* 1987), a change in this balance can have a strong impact on atmospheric CO_2 levels.

1.4. Two projects, one goal

As we explained in the sections above, C uptake and retention in the terrestrial biosphere are important processes with regard to global change. In particular, soil C storage is key because it stores the largest amount of C in terrestrial ecosystems. Nevertheless, in the past, scientific studies have often focused on responses of biomass C storage, and large gaps in our understanding of changes in soil C stocks and effects on soil C dynamics still remain.

This thesis addresses these questions using two different approaches: analysis of a database of global change manipulation experiments (GCMEs), and an altitudinal gradient study in tropical forest in Papua New Guinea.

1.4.1. Database of global change manipulation experiments

The first part of this PhD involved the analysis of a database containing results of global change manipulation experiments. The general aim of this part was to study the mechanistic responses of terrestrial ecosystems to the main global atmospheric change drivers: increases in atmospheric CO_2 levels, temperature and

N deposition, and changes in precipitation. We focused on the main C pools in terrestrial ecosystems and the fluxes of C in and out of the soil, determining the magnitude and direction of changes in the soil C pool.

1.4.2. Altitudinal gradient study in tropical forest

The second part involved a soil sampling campaign on an altitudinal gradient in a conservation area in Papua New-Guinea. The main aim here was to assess the average soil C stocks in the area, and to compare soil C and N stocks at different altitudes. This “time-for-space” substitution offered the possibility to estimate future effects of increasing temperatures and changing precipitation on soil C stocks.

1.4.3. The strengths of both projects

The database project constantly developed, and the vast amount of available data and individual experiments provided new and fresh data at a very fast pace. The database covers a whole range of different climatic regions and different ecosystem types, and its meta-analytical analysis offered the synthesis necessary to develop new experiments, and to validate existing models. Database analysis also indicated existing gaps and chronic problems existing in the current ongoing research.

The altitudinal gradient project is important as it is located in tropical rainforest, a biome that is understudied mainly due to logistical issues. In addition, the soil C inventory resulting from the project will be one of the first in Papua New Guinea (PNG), providing a benchmark for future work. There is a strong climate change component in the project, as 9 permanent sites along this altitudinal gradient (90-3050m) provide the possibility to analyze the influence of changing temperature and rainfall. Alongside this climate change research, PNG is also strongly involved in the ongoing discussions about Reduced Emissions from Deforestation and Degradation (REDD), and urgently needs a proper assessment of its C stocks to make REDD possible in PNG.

1.4.4. Their differences

There are some distinct differences between both projects. Most obvious is that the database is focused on GCMEs, whereas the altitudinal gradient study uses time-for-space substitution to represent future climatic changes.

A second difference is that the database study synthesized a large set of individual experiments over a large range of different environmental conditions, while the altitudinal gradient was one single experiment. Therefore, the database had the potential to lead to generalizations across a continuum of environmental conditions, while the gradient study was spatially specific.

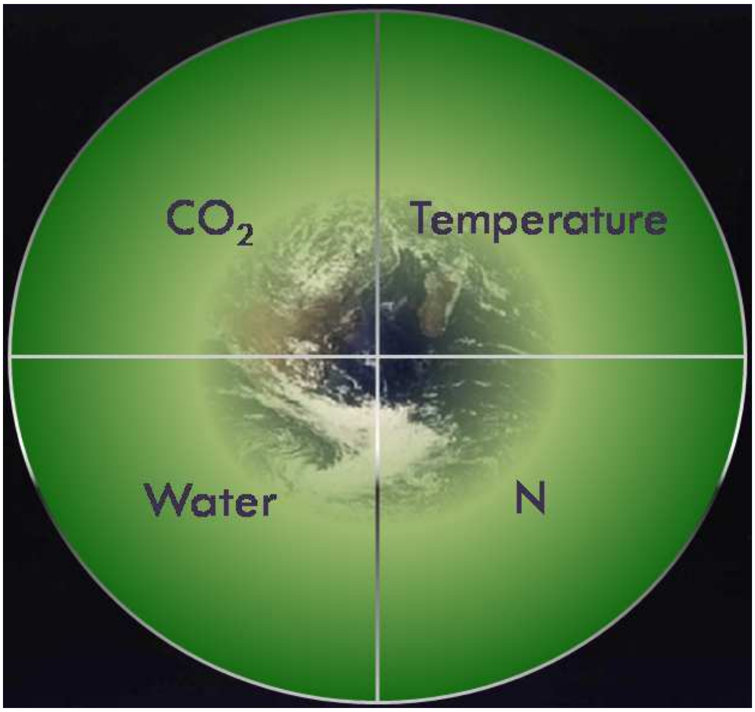
The third difference is the set of parameters that are studied. In the database study, a multitude of response variables were addressed, from plant biomass, over root dynamics, to microbial properties, respiration and soil C. In the altitudinal study, the list of parameters studied was restricted to one (i.e. soil C), but the dataset was more extensive, and has higher resolution.

A last difference is that the database represents mostly temperate or boreal areas (although tropical, arctic, and desert systems are also represented), whereas the altitudinal study was performed in a pristine tropical rainforest environment.

1.4.5. The common goal

The fundamental differences between both projects are clear, yet they offer a unique possibility to compare these different approaches, and test the robustness of each other's findings. Ultimately, the common goal is to understand how global changes will affect existing soil C pools, and to uncover the mechanisms behind those effects. It will therefore be very interesting to see whether results from both projects are similar, complement each other, or display strong differences.

2. GLOBAL CHANGE MANIPULATION EXPERIMENTS



2.1. Introduction

The effects of human-induced climate change have been the focus of a vast amount of scientific studies over the last decades. Typically, a multitude of different approaches are used, amongst them laboratory studies (e.g. Fang *et al.* 2005; Fierer *et al.* 2005; Bradford *et al.* 2008a; Liu *et al.* 2009a), long-term observations and inventory studies (e.g. Giardina & Ryan 2000; Bellamy *et al.* 2005; Luyssaert *et al.* 2007; Bond-Lamberty & Thomson 2010), environmental gradient studies (e.g. Bird *et al.* 2004; Loomis *et al.* 2006; Wynn *et al.* 2006; Oberbauer *et al.* 2007; Heckman *et al.* 2009; Girardin *et al.* 2010; Thomas *et al.* 2010; Ostonen *et al.* 2011), global change manipulation experiments (e.g. Oechel *et al.* 1994; Oren *et al.* 2001; Melillo *et al.* 2002; Neff *et al.* 2002; Loya *et al.* 2003; Mack *et al.* 2004; Dukes *et al.* 2005; Körner *et al.* 2005), or ecosystem modeling studies (e.g. Cannell & Thornley 1998; Eliasson *et al.* 2005; Knorr *et al.* 2005b; Pepper *et al.* 2005; Esser *et al.* 2011). An increased understanding and better estimation of the magnitude and direction of ecosystem responses, derived from such experiments, will increase the accuracy of model structures, and be highly relevant for the validation of these models.

In this chapter, we focused on global change manipulation experiments (GCMEs). In these experiments, one or more global change drivers are typically manipulated in a number of treatment plots or units, and a number of control plots are used to quantify the relative effects of the treatments. Manipulation experiments are performed on a range of spatial and temporal scales, but are generally <10 years in duration and study a representative sample of ecosystems rather than regional or catchment size systems.

Results of experiments manipulating CO_2 levels, temperature levels or N- and water availability were collected in a global database. We selected studies that reported above- and belowground C pools and fluxes to analyze mechanisms and identify key players in soil C cycling. We used meta-analysis to quantitatively synthesize effects of single global change drivers, and where possible their interactions.

2.2. Methods

2.2.1. Description of the database

The ‘Global Change Manipulation Experiments’ database is a comprehensive database focusing on single or multifactor experiments manipulating CO_2 levels, temperature, water-, or N availability in terrestrial ecosystems. The database comprises a wide variety of ecosystem types (e.g. forests, heathlands, grasslands, tundra systems, …), spans several climatic regions (from boreal to arid systems), and includes experiments of variable duration (from 1 growing season to 51 years of treatment). The location of experimental sites contained in the database are depicted in Appendix 1.

A general problem associated with database compilations is selection and publication bias. An example of how publication bias can result in overestimation of global change effect sizes was discussed in our New Phytologist letter (Dieleman & Janssens 2011). One can limit or largely prevent the occurrence of selection and publication bias by outlining a clear question and well-defined selection criteria before the start of data collection.

Most of the data included in the analysis were extracted from figures and tables in published papers, although some data were not published in peer reviewed literature, but obtained directly from researchers. The prerequisites for the data to be included in the database were (1) the availability of a measure of variation on the measurements for statistical purposes, and (2) a minimum of 2 replicates in the experimental design. We collected data on aboveground biomass, fine- and total root biomass, fine root dynamics, root-to-shoot ratio, aboveground litterfall, microbial biomass C and N, heterotrophic respiration (Rh), litter decomposition, soil CO_2 efflux, root respiration, net N mineralization and soil C and N content.

The database currently contains 4576 entry lines (ambient value and treatment response), from 527 experiments (defined as one treatment combination at a particular site), on 164 different sites across the globe.

The database is available online at the following link: www.ua.ac.be/wouter.dieleman, and is intended for synthesis work i.e. studying global and regional patterns, validating models, comparison of individual sites with a wide range of similar or contrasting sites, etc.

The database contains a large amount of descriptive meta-data that describes experimental treatment conditions and site properties, and provides the source of all data points entered in the database. A concise description learns that in the *'Sites'* table (Fig. 2.1), one can find information about the location, the type of system, the climate, nutritional status and structure of the soil, and general management or historical information. The *'Experiments'* table contains information on the

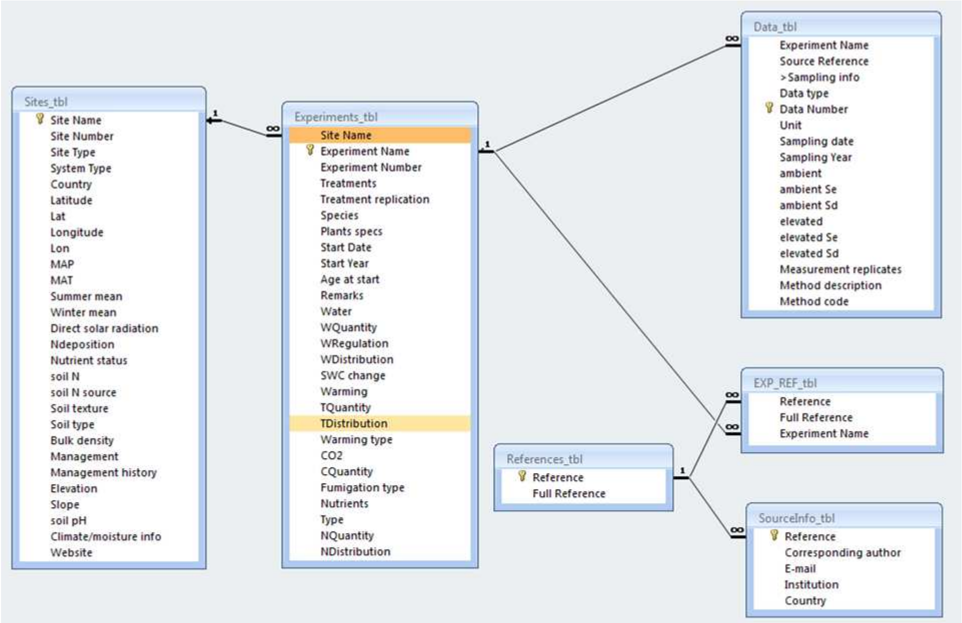


Figure 2.1: Relationships in the database that ensure easy data retrieval.

experimental treatments, the species used in the experiment, replication and start date of the experiment. The '*Data'* table contains the source reference, data type, unit, sampling date, the ambient and response values (with SE or SD), the number of sampling replicates and a description of the method used. The *'References'* table contains all full references for all data points in the database, the *'Source Information'* table provides author and contact information for all data points. A lot of this information can help in properly interpreting data. Therefore, to ensure that all this information is easily retrievable, the database has a relational structure (Fig. 2.1), where all data points are linked to the

"Experiments" table containing all individual experiments. On its turn, the experiments table is linked to the sites table.

2.2.2. Quantitative synthesis.

2.2.2.1. The concept of meta-analysis

The increasing large body of studies in experimental ecology has led to interest in methods for summarizing this evidence to reach general conclusions. In a traditional narrative literature review, results from individual studies that are representative or noteworthy are summarized qualitatively, serving as the basis for generalization, extrapolation, or other objectives of the reviewer. Although the typically qualitative narrative approach has been of fundamental importance in synthesizing many types of scientific research (e.g. Fog 1988; Ceulemans & Mousseau 1994; Zak *et al.* 2000), it does not address a quantitative integration of large numbers of research reports.

Meta-analysis is a statistical method that has been developed for the quantitative integration of research results from independent experiments. Meta-analysis has been conducted extensively in the social and medical sciences and has recently been extended to ecological studies (Gurevitch & Hedges 1999; Osenberg *et al.* 1999). The advantage of meta-analysis is the ability to place confidence limits around effect size estimates, to discriminate statistically among subsets of the data, and to compare critically the variance within and among studies.

In a meta-analysis, the result of each independent experiment is usually expressed as an index of effect, and these effect estimates are then combined across studies to produce a summary of the findings (Hedges *et al.* 1999). Therefore, it can be used to answer a great many questions. For example, how large is the effect over all studies combined? Is it positive or negative, and is it reliably different from zero? Are the results consistent across studies? If the results are not in agreement among studies, are there differences in the magnitude of the effect among meaningful categories of studies, e.g. does the effect differ among different ecosystems types?

2.2.2.2. How did we apply this in our analyses?

For each analysis, we selected a particular set of data (e.g. all soil respiration data retrieved in experiments subjected to elevated temperatures). The next step would be to select all independent studies, appropriate for the analysis (i.e. only one result for a particular experiment, generally from the most recent reference). When several years of data were reported for a particular experiment, we calculated a weighted mean, using the reciprocal of the measurement variance as weight factor.

Several studies used different species in the same experiment or included other manipulations, e.g. fertilization, ozone, different soil types, different management. Results from different treatments, plant species, soils, or measurement protocols within the same experiment were considered independent experiments and were used as different inputs in the meta-analysis.

This dataset was then analyzed with meta-analytical techniques using MetaWin 2.1 software (Rosenberg *et al.* 2000). Like in conventional meta-analysis, each individual observation was weighted by the reciprocal of the mixed-model variance (Hedges *et al.* 1999). We used standard deviation (SD) values reported in the individual studies, or calculated the SD from the reported standard error and the number of replicates. The natural log of the response ratio (r = response in treatment plots/response in control plots) was used as metric in the analyses. The use of the natural logarithm instead of the Hedges d-index has the advantage of linearizing the metric, thereby being less sensitive to changes in a small control group.

A mixed model was used to assess the overall treatment effect for the different parameters. If the number of studies used to calculate a mean and confidence interval (CI) is lower than 20, the CI can be too narrow (Hedges *et al.* 1999). Therefore, we used the CI based on resampling methods for the assessment of statistical differences (2500 iterations). As compared to standard confidence intervals, confidence intervals based on such bootstrapping tests are larger and the resulting estimates are more conservative (Adams *et al.* 1997). If the calculated 95% confidence interval did not encompass zero, the treatment response was considered significant. Significant between-group differences (e.g. categorical analyses for treatment and ecosystem type comparisons) were identified on the basis of the within- and between-group heterogeneity.

2.2.2.3. Conceptual framework

The soil C pool of a terrestrial ecosystem roughly is the result of the balance between C inputs and C outputs (Trumbore 2006). As we are interested in the mechanisms behind the response of soil C pools to a changing climate, and want to discern effects of increased plant growth from direct effects on decomposition processes in the soil, it will be key to study effects on soil C inputs (litterfall, root turnover and rhizodeposition) and soil C outputs (root respiration and SOM decomposition) in combination with responses of the soil C content.

In our analyses, we will start from a conceptual representation of C pools, and C inputs and outputs in the soil (e.g. Fig. 2.2), and how they respond to global change drivers. Trying to represent all C pools and fluxes in the scheme will make it possible to estimate responses of terrestrial systems to a changing climate, and find out the mechanisms behind them.

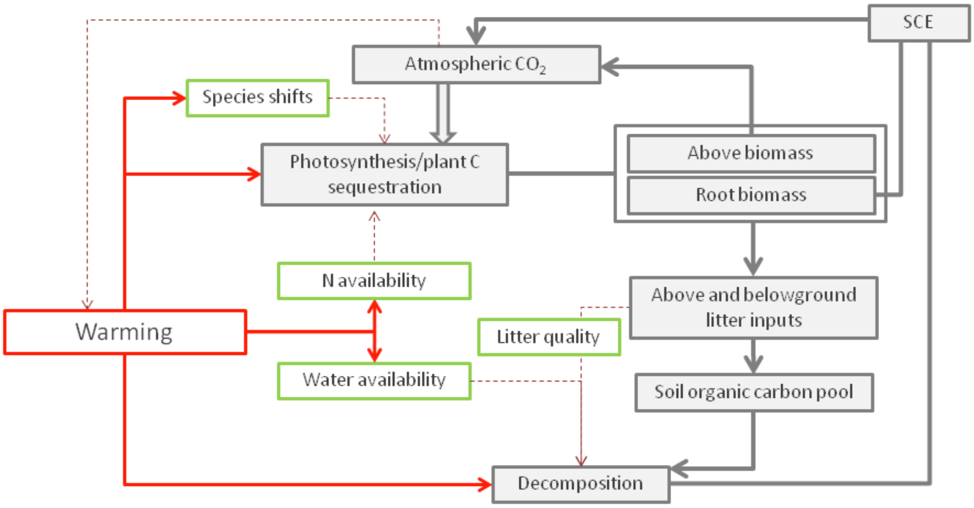


Figure 2.2: Example of a conceptual representation of the different C pools and fluxes involved in C cycling in terrestrial ecosystems (grey boxes and lines) and their relationship to climatic (red boxes and lines) and environmental feedbacks (green boxes).

2.2.2.4. Outline of results section

In the following chapters, we will discuss effects of different global change drivers on soil C cycling processes, based on different peer-reviewed publications (at the start of each section, the publication status is indicated where applicable). In section 2.3, individual papers are presented separately and grouped in the 'results' section of the GCME analyses (2.3.1 to 2.3.4). As such, each results-section includes an introduction and discussion as it is published in the original papers. To conclude the results section, we indicate some obvious subjects we did not address in this thesis (i.e. water manipulations and the role of mycorrhizae, see 2.3.5).

In section 2.4, we discuss these results with an overall synthesis and indicate some points of consideration when using GCME studies as a scientific approach.

2.3. Results

2.3.1. Soil [N] modulates soil C cycling in CO_2 fumigated tree stands: A meta-analysis.

This chapter was published in Plant, Cell and Environment:

Dieleman, W.I.J., Luyssaert, S., Rey, A., De Angelis, P., Barton, C.V.M., Broadmeadow, M.S.J., Broadmeadow, S.B., Chigwerewe, K.S., Crookshanks, M., Dufrene, E., Jarvis, P.G., Kasurinen, A., Kellomaki, S., Le Dantec, V., Liberloo, M., Marek, M., Medlyn, B., Pokorny, R., Scarascia-Mugnozza, G., Temperton, V.M., Tingey, D., Urban, O., Ceulemans, R., Janssens, I.A., 2010. Soil [N] modulates soil C cycling in CO_2-fumigated tree stands: a meta-analysis. Plant Cell and Environment 33, 2001-2011.

2.3.1.1. Abstract

Under elevated atmospheric CO_2 concentrations, soil carbon (C) inputs are typically enhanced, suggesting larger soil C sequestration potential. However, soil C losses also increase and progressive nitrogen (N) limitation to plant growth may reduce the CO_2 effect on soil C inputs with time. We compiled a dataset from 131 manipulation experiments, and used meta-analysis to test the hypotheses that 1) elevated atmospheric CO_2 stimulates soil C inputs more than C losses, resulting in increasing soil C stocks, and 2) that these responses are modulated by N. Our results confirm that elevated CO_2 induces a C allocation shift towards belowground biomass compartments. However, the increased soil C inputs were offset by increased heterotrophic respiration, such that soil C content was not affected by elevated CO_2. Soil N concentration strongly interacted with CO_2

fumigation: the effect of elevated CO_2 on fine root biomass and –production and on microbial activity increased with increasing soil N concentration, while the effect on soil C content decreased with increasing soil N concentration. These results suggest that both plant growth and microbial activity responses to elevated CO_2 are modulated by N availability and that it is essential to account for soil N concentration in C cycling analyses.

2.3.1.2. Introduction

Terrestrial ecosystems play a pivotal role in the global carbon (C) cycle and are believed to sequester 30% of the anthropogenic CO_2-emissions (Canadell *et al.* 2007; IPCC 2007). As the atmospheric CO_2 concentration ($[CO_2]$) continues to rise, plant productivity and C sequestration may further increase, either directly through enhanced photosynthesis or indirectly via increased water or nutrient use efficiency. This fertilizing effect of increasing $[CO_2]$ is well established (Eamus & Jarvis 1989; Ceulemans & Mousseau 1994; Idso & Idso 1994; Wullschleger *et al.* 1997; Norby *et al.* 1999; Norby *et al.* 2002; Temperton *et al.* 2003a; Norby & Luo 2004; Ainsworth & Long 2005; Norby *et al.* 2005; Hyvönen *et al.* 2007). However, it has been hypothesized that, as nutrients become progressively immobilized in plant biomass and soil organic matter (SOM) pools, nutrient limitations might eventually inhibit CO_2-induced increases in plant productivity, and thus limit a further increase of C sequestration in terrestrial ecosystems (Kramer 1981; Ceulemans & Mousseau 1994; Oren *et al.* 2001; Temperton *et al.* 2003b; Luo *et al.* 2004; de Graaff *et al.* 2006). In the short term, priming (a stimulation of microbial growth and activity, and decomposition of older, more recalcitrant organic matter as a consequence of increased labile C inputs (Cheng & Johnson 1998; Kuzyakov 2002; Fontaine *et al.* 2007) could serve as an alleviating mechanism, and in the longer term, nutrient-poor systems may adjust by steadily redistributing their acquired nitrogen (N) stocks (Rastetter *et al.* 1997; Cannell & Thornley 1998), but neither mechanism suffices to overcome the progressive N limitation completely.

Coinciding with the rising levels of atmospheric [CO_2], atmospheric deposition of reactive N has also increased over the last century (Denman *et al.* 2007), originating mainly from fossil fuel burning and artificial fertilizer applications (Davidson 2009). Besides a number of negative effects on terrestrial ecosystems, nitrogen fertilization significantly stimulates aboveground biomass production (Ciais *et al.* 2008; Pregitzer *et al.* 2008; de Vries *et al.* 2009; Luyssaert *et al.* 2010; Thomas *et al.* 2010), and therefore could increase the amount of plant-derived C entering the soil. While a stronger CO_2 effect on aboveground biomass in N fertilized systems compared to unfertilized ones is a general response (Curtis & Wang 1998; de Graaff *et al.* 2006), this is not the case for the belowground biomass response (de Graaff *et al.* 2006). In addition, previous meta-analysis did not reveal a clear microbial C or microbial respiration response to N fertilization in CO_2 fumigated tree stands (de Graaff *et al.* 2006).

Thus, altogether, in contrast to our knowledge of aboveground processes, belowground processes and the complex interactions between plants and microbes within the coupled carbon and nutrient cycles are still poorly understood, and it is not elucidated yet whether N constrains C cycling or C inputs stimulate N cycling (Luo *et al.* 2006a). To improve projections of changes in belowground C pools in response to global climate change, Zak et al. (2000) stressed that fine root production, longevity and biochemistry should serve as a starting point to resolve microbe-related research, because fine root dynamics and rhizodeposition are likely to exert a stronger influence than aboveground litter inputs. Van Groenigen et al. (2006) and De Graaff et al. (2006) concluded that soil C can increase significantly in elevated CO_2, but only when sufficient amounts of N fertilizer are added. In that case, the increased soil C inputs from plant material could overcome CO_2-induced increases in decomposition, resulting in a net increase of soil C.

Using meta-analysis, we investigated how soil C inputs, C pools and C losses are affected by elevated atmospheric [CO_2]. More specifically, we aim: (1) to test the hypothesis that elevated atmospheric stimulates soil C inputs more than C losses, resulting in increasing soil C stocks; and (2) to evaluate how N can modulate these responses.

Although some of the N fertilization effects in elevated CO_2 studies that were found in previous studies are small or not existing, they do not necessarily imply that nutrient availability only plays a minor role, because unfertilized soils may in fact be nutrient-rich, while nitrogen or even NPK-fertilized soils may be characterized by limitations of other nutrients. Therefore, in addition to the comparison of CO_2 effects between fertilized and unfertilized experiments, we also compared the CO_2 responses in poor and richer systems along a gradient of soil [N].

2.3.1.3. Methods

For an extensive description of methods, we refer to chapter 2.2

Data acquisition

We focus on tree stands only because many grassland sites are managed by grazing or through forage production, which plays a key role in the C balance as a large part of the primary production is removed (Soussana *et al.* 2007). Therefore, grasslands are often fertilized to sustain productivity, which is likely to modify their responses to CO_2 fumigation.

We collected data on aboveground biomass, fine- and total root biomass, fine root production, root-to-shoot ratio, aboveground litterfall, microbial biomass C, heterotrophic respiration (Rh), soil CO_2 efflux, net N mineralization and soil C content from 32 sites, resulting in 279 entries for the meta-analysis. General information about the sites is given in Table 2.1.

We included CO_2 enriched studies, using FACE (Free Air Carbon Enrichment) or OTC (Open Top Chamber) technology, where roots could proliferate freely (i.e.

Table 2.1: General information about the sites used in this meta-analysis.

SITE	TREATMENT	SPECIES	SOIL [N] (g N/kg soil)	LAT	LONG	MAP (mm)	MAT (℃)
Bily Kriz	CO_2	Picea abies	-	49.5 N	18.53 E	1400	5
Birmensdorf (calcareous)	CO_2 x Fertilization x	Picea abies, Fagus sylvatica	0.76	47.35 N	8.43 E	691	10
Birmensdorf (acidic)	CO_2 x Fertilization x	Picea abies, Fagus sylvatica	0.512	47.35 N	8.43 E	691	10
Christchurch	CO_2	Pinus radiata	0.1485	43.53 N	172.7 E	616	12.2
DukeFACE	CO_2	Pinus taeda	0.79	35.95 N	79.15 W	1140	15.5
DukeFACE	CO_2 x Fertilization	Pinus taeda	0.79	35.95 N	79.15 W	1140	15.5
EUROFACE	CO_2 x Fertilization	Populus alba, P. nigra, P. x euramericana	1.2	42.37 N	11.8 E	700	14.1
FACTS II FACE	CO_2 x Ozone	Populus tremuloides, Betula papyrifera, Acer saccharum	1.2	49.67 N	89.57 W	833	11.5
Forestry Commission	CO_2 x Water	Fraxinus excelsior, Pinus sylvestris, Quercus petraea	-	35.9 N	84.33 W	1378	14.3
Glencorse	CO_2	Betula pendula	-	55.52 N	3.2 W	850	8.2
Glendevon	CO_2xFertilization	Alnus glutinosa, Betula pendula, Picea sitchensis, Pinus sylvestris	-	56.2 N	4 W	1416	6.8
Mekrijarvi Research Station	CO_2xWarming	Pinus sylvestris	-	62.78 N	30.95 E	667	2.1

Merrit Island	CO_2	Quercus myrtifolia, Q. geminata, Q. chapmanii	0.1	28.63 N	80.7 W	1310	-
Montalto di Castro	CO_2	Quercus ilex forest	1.1	42.37 N	11.53 E	612	15
Oak Ridge FACE	CO_2	Liquidambar styraciflua	1.12	35.9 N	84.33 W	1371	13.9
Oak Ridge OTC (maple)	CO_2 x Warming	Acer saccharum, Acer rubrum	-	35.9 N	84.33 W	1378	14.3
Oak Ridge OTC (oak)	CO_2	Quercus alba	-	35.9 N	84.33 W	1378	14.3
Oak Ridge OTC (yellow-poplar)	CO_2	Liriodendron tulipifera	-	35.9 N	84.33 W	1378	14.3
POPFACE	CO_2	Populus alba, nigra, euramericana	1.2	42.37 N	11.8 E	700	14.1
Suonenjoki	CO_2	Betula pendual ROTH 4 and 80	0.46	62.65 N	27.05 E	307	12.6
Swiss Treeline FACE	CO_2	Larix decidua, Pinus uncinata	-	46.8 N	9.83 E	-	-
UA OTC	CO_2	Pinus sylvestris	1.2	51.17 N	4.38 E	770	12
UMBS - alder	CO_2	Alnus glutinosa	0.183	45.57 N	84.67 W	-	-
UMBS - aspen (eur.)	CO_2xFertilization	Populus x euramericana	-	45.57 N	84.67 W	-	-
UMBS - aspen (grand.)	CO_2	Populus grandidentata	0.079	45.55 N	84.78 W	-	-
UMBS - aspen (trem.)	CO_2xFertilization	Populus tremuloides	0.97 (rich), 0.21 (poor)	45.57 N	84.67 W	-	-

UMBS - aspen (trem.2)	CO_2xFertilization	Populus tremuloides	0.97 (rich), 0.21 (poor)	45.57 N	84.67 W	-	-
UMBS - maple	CO_2xFertilization	Acer saccharum	0.97 (rich), 0.21 (poor)	45.57 N	84.67 W	-	-
USDA - orange	CO_2	Citrus aurantium	-	33.43 N	112.07 W	-	-
USDA Placerville	CO_2xFertilization	Pinus ponderosa	0.856	38.73 N	120.8 W	1000	18
Vielsalm	CO_2xFertilization	Picea abies	-	50.28 N	5.92 E	972	7.5
WebFACE	CO_2	Mixed deciduous forest	-	47.47 N	7.5 E	990	-

pot or growth chamber studies were not included). Experimental conditions were summarized by a number of variables (Table 2.2). Studies were categorized as fertilized when any N-based fertilizer was added during the experiment or unfertilized when no fertilizer was added. Experiments were classified as irrigated when water was added and not irrigated when no water was added during the experiment. We refer to the database for detailed methodologies for the respective experiments.

Note the difference between fertilized and 'high soil N concentration'. Although both annotations can be interpreted as nutrient-rich, we tested their effects with different analyses: categorical analysis (fertilized vs. unfertilized) for the former and continuous (regression with soil [N] as variable) for the latter one.

Meta-analysis

A mixed model was used to assess the overall treatment effect of CO_2 enrichment, and the influence of fertilizer addition and soil N concentration. We also tested for differences between irrigation treatments, seasonal growth strategy (deciduous or evergreen trees), the fumigation type used, the amount of CO_2 increase and the duration of the treatments. Significant differences between groups (=categorical analyses for treatment comparisons, different seasonal strategies

Table 2.2: Treatment conditions at the experimental sites.

SITE	CO_2 INCREASE (μmol/mol)	FUMIGATION TYPE	FERTILIZATION	IRRIGATION	AGE AT START (years)
Bily Kriz	350	OTC	NF	NI	13
Birmensdorf (calcareous)	200	OTC	F and NF	I	2
Birmensdorf (acidic)	200	OTC	F and NF	I	2
Christchurch	292	OTC	F	I	seedlings
DukeFACE	200	FACE	NF	NI	13
DukeFACE	200	FACE	F and NF	NI	22
EUROFACE	180	FACE	F and NF	I	cuttings (3-year-SRC)
FACTS II FACE	180	FACE	NF	NI	1
Forestry Commission	300	OTC	NF	I and NI	1
Glencorse	350	OTC	NF	NI	seedlings
Glendevon	350	OTC	F and NF	I	1 or 2
Mekrijarvi Research Station	200	OTC	NF	NI	20-30
Merrit Island	350	OTC	NF	NI	postburn (3 months)
Montalto di Castro	350	OTC	NF	NI	30
Oak Ridge FACE	180	FACE	NF	NI	10
Oak Ridge OTC (maple)	300	OTC	NF	NI	1
Oak Ridge OTC (oak)	300	OTC	NF	NI	1

Oak Ridge OTC (yellow-poplar)	300	OTC	NF	NI	1
POPFACE	180	FACE	NF	I	cuttings (3-year-SRC)
Suonenjoki	360	OTC	F	I	7
Swiss Treeline FACE	180	FACE	NF	NI	29
UA OTC	400	OTC	NF	NI	1
UMBS - alder	345	OTC	NF	NI	cuttings
UMBS - aspen (eur.)	345	OTC	F and NF	I	cuttings
UMBS - aspen (grand.)	350	OTC	NF	I	cuttings
UMBS - aspen (trem.)	350	OTC	F and NF	I	cuttings
UMBS - aspen (trem.2)	200	OTC	F and NF	NI	cuttings
UMBS - maple	200	OTC	F and NF	NI	cuttings
USDA - orange	300	OTC	F	I	3
USDA Placerville	350	OTC	F and NF	I	3
Vielsalm	350	OTC	F and NF	I	11
WebFACE	160	FACE	NF	NI	mature

and fumigation technologies) were identified on the basis of the within and between group heterogeneity. Statistically significant differences are reported at *P*<0.05. Analyses with continuous variables (soil N concentration and amount and duration of CO_2increase) were performed when the number of studies was larger than 10. Both a weighted regression using Metawin as an unweighted regression (using Matlab 7.4.0.287 (R2007a) (MathWorks, Massachusetts, USA)) were performed and used for comparison. Statistically significant correlations were reported at *P*<0.05.

2.3.1.4. Results

Soil C inputs

Aboveground litterfall and fine root production responded to elevated CO_2 with an increase of 14 and 44% respectively (Fig. 2.3, Table 2.3). Both parameters were not affected differently by elevated CO_2 in fertilized and unfertilized plots (Table 2.4). The CO_2 effect on fine root production was positively related to soil N concentration, for both the weighted meta-analysis regression (Table 2.4) as for the unweighted regression (Fig. 2.4a). No significant effect of amount or duration of the CO_2 increase in the treated plots was observed (Table 2.4).

C pools

Aboveground biomass and total and fine root biomass, responded positively to elevated CO_2 with a 21, 39 and 43% increase respectively (Fig. 2.3, Table 2.3). This did not result in a statistically significant increase in root-to-shoot ratio in CO_2 fumigated studies, although there was a clear positive trend (Fig. 2.3, Table 2.3). Microbial biomass C and soil C were not significantly affected by elevated CO_2 (Fig. 2.3, Table 2.3).

There was no significant difference in CO_2 response between fertilized and unfertilized plots for any of the studied C pools (Table 2.4). However, aboveground biomass and soil C were significantly stimulated by elevated CO_2 in the N fertilized studies, but not in the unfertilized experiments (Table 2.3). The CO_2 effect on fine root biomass was positively related to soil N concentration, for both the weighted meta-analysis regression (Table 2.4) as for the unweighted regression (Fig. 2.4b). The CO_2 effect on soil C content demonstrated a negative relationship with increasing soil N concentration in both regressions (Fig. 2.4c, Table 2.4). There was no significant effect of amount or duration of the CO_2 increase in the treated plots (Table 2.4).

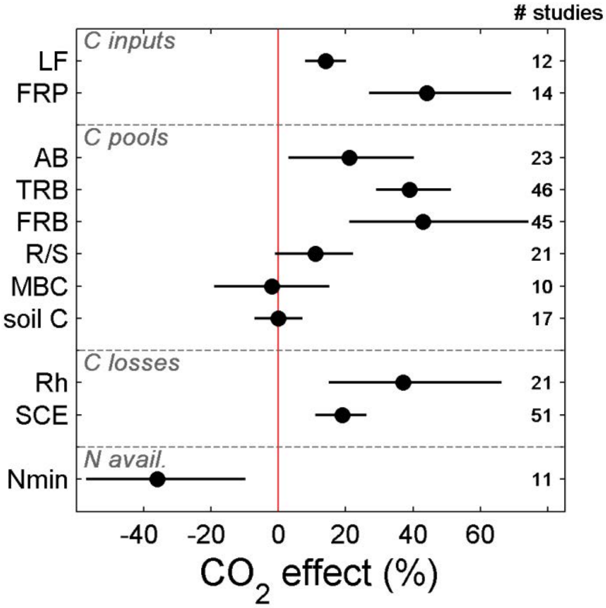


Figure 2.3: Overall CO_2-effects on soil C inputs, C pools, C losses and N availability. The effect on litterfall (LF), fine root production (FRP), aboveground biomass (AB), total root biomass (TRB), fine root biomass (FRB), root-to-shoot ratio (R/S), microbial biomass C (MBC), soil C content (soilC), heterotrophic respiration (Rh), soil CO_2 efflux (SCE) and net N mineralization (Nmin) are indicated as percentage response to elevated CO_2. Overall means and confidence intervals are given, which means a significant CO_2-effect is apparent when the zero-line is not crossed. The number of studies used for the analysis is indicated above the x-axis.

Soil C losses and net N mineralization

Heterotrophic respiration and soil CO_2 efflux increased by 37 and 19% under elevated CO_2 respectively (Fig. 2.3, Table 2.3). Net N mineralization decreased by 36% in elevated CO_2 (Fig. 2.3, Table 2.3). There was no significant difference in CO_2 response between fertilized and unfertilized plots for any of the studied parameters (Table 2.4). Both Rh and net N mineralization were significantly affected by elevated CO_2 only in the unfertilized experiments (Table 2.3). Increasing soil N concentration had a positive effect on the CO_2 response of Rh according to the meta-analysis regression (Table 2.4), while this relationship was borderline insignificant for the unweighted regression (Fig. 2.4d).

The CO_2 effect on microbial respiration increased with treatment duration (Table 2.4). None of the other parameters exhibited significant relationships with the amount of elevated CO_2 added, nor with the duration of the CO_2 treatments (Table 2.4).

2.1.3.5. Discussion

Overall CO_2 responses

The observed overall CO_2-induced stimulation of above- and belowground biomass and production agrees well with previous experimental findings (Rogers *et al.* 1994; Curtis & Wang 1998; Pendall *et al.* 2004; de Graaff *et al.* 2006). Interestingly, elevated CO_2 increased fine and total root biomass and production in all possible experimental conditions addressed in this analysis (Table 2.3), while this was not the case for the aboveground biomass response to elevated CO_2. Together with the positive trend in the root-to-shoot ratio, this provides a strong signal for a C-allocation shift towards belowground biomass compartments in CO_2

Table 2.3: Elevated CO_2 effects on aboveground biomass, litterfall, total root biomass, fine root biomass, fine root production, root-to-shoot ratio (R/S), microbial biomass, heterotrophic respiration (Rh), soil CO_2 efflux, net N mineralization and soil C, indicated as percentage response to elevated CO_2. Numbers in bold italics indicate statistically significant CO_2-effects. The CO_2-effect is considered significant when 0 is not included in the confidence interval.

	Overall	Fertilized	Not fertilized
C inputs			
Litterfall	**14**	**20**	**12**
Fine root production	**44**	**19**	**52**
C pools			
Aboveground Biomass	**21**	**30**	19
Root biomass	**39**	**50**	**38**
Fine root biomass	**43**	**36**	**46**
R/S	16	5	19
Microbial biomass	-2	2	-5
soil C	0	**14**	-5
C losses			
Heterotrophic respiration	**37**	27	**44**
Soil CO_2 efflux	**19**	**24**	**17**
N availability			
net N mineralization	**-36**	2	**-43**

fumigated systems. This is a common response in an elevated CO_2 world (Rogers *et al.* 1994; Luo *et al.* 2006b) as plants need more resources to sustain the enhanced growth (Bryant *et al.* 1983).

Increased above- and belowground litterfall in elevated CO_2 enhances the soil C input. As soil organisms tend to be C-limited (Zak *et al.* 1993; Hu *et al.* 2006), one would expect an increase in microbial biomass C as a consequence. However, this is not observed. Although microbes probably profit from the improved C availability initially, their biomass turns over relatively quickly (Heath *et al.* 2005; Lukac *et al.* 2009), possibly in part due to enhanced grazing by other soil organisms in elevated CO_2 (Zak *et al.* 2000). Moreover, the higher N immobilization in the increasing plant biomass (Luo *et al.* 2004) may impose a concomitant N-limitation of microbial growth (Hu *et al.* 2001; Hu *et al.* 2006).

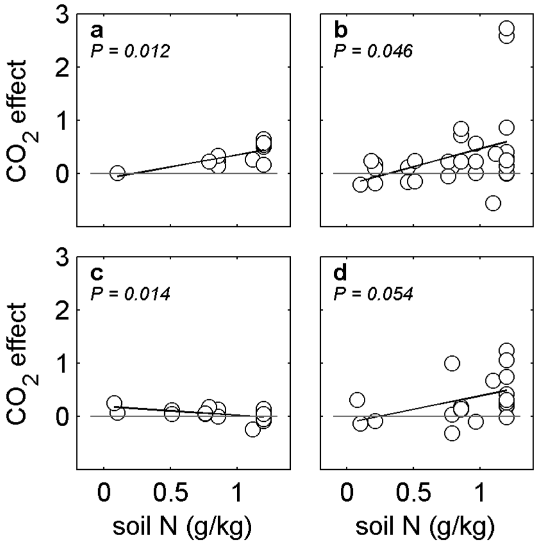


Figure 2.4: Unweighted regressions relating individual CO_2 responses (y-axis) to soil N concentration (x-axis). Responses of fine root production (a), fine root biomass (b), soil C content (c) and heterotrophic respiration (d) are depicted as the log response ratio (ln(elevated CO_2/ambient CO_2)). Soil [N] is given in gN kg $soil^{-1}$. Differences are significant at $P<0.05$.

We hypothesize that N limitation is a more plausible explanation for the lack of response of microbial biomass to elevated CO_2. Indeed, microbial biomass did not increase in spite of the increased plant C inputs. Moreover, the overall response of net N mineralization (a measure for the available inorganic N in the soil) to elevated CO_2 was negative, and net N mineralization decreased even more in elevated CO_2 when only the unfertilized experiments were included. As larger quantities of C entering the soil normally result in more N uptake, even in N-limited systems (Finzi *et al.* 2007), our results thus suggest that elevated CO_2 makes trees more efficient in immobilizing N, and that microbial growth likely becomes N-limited in elevated CO_2, at least where N availability is not very high. Our observed increase in microbial respiration is counterintuitive considering the lack of microbial biomass response in elevated CO_2 studies. However, besides the increase of biomass C inputs in the soil, plants also tend to increase root exudation in elevated CO_2 (Fitter *et al.* 1997; Drigo *et al.* 2008; Lukac *et al.* 2009). This labile C input could fuel the microbial community (Zak *et al.* 2000; Heath *et al.* 2005), but is mainly respired because the N necessary to convert these C inputs into microbial biomass is lacking. Therefore, Rh can increase despite the lack of change in microbial biomass.

Another possible mechanism is a shift in microbial community composition, towards a more fungal dominated community, which is less N demanding (Hu *et al.* 2001; Zhang *et al.* 2005; Carney *et al.* 2007). This shift may occur, but would

Table 2.4: *P*-values for the meta-analytical comparisons of CO_2 effects in different experimental treatments or conditions.

	Fertilization	CO_2 increase	Duration	soil N
C inputs				
Litterfall	0.38	0.29	0.07	0.95
Fine root production	0.22	0.13	0.28	***<0.001***
C pools				
Aboveground Biomass	0.69	0.18	0.89	0.5
Root biomass	0.4	0.47	0.87	0.27
Fine root biomass	0.63	0.48	0.17	***<0.001***
R/S	0.4	0.63	-	0.43
Microbial biomass	0.82	0.55	0.58	-
soil C	0.12	0.39	0.79	***0.02***
C losses				
Heterotrophic respiration	0.54	0.06	***0.045***	**0.02**
Soil CO_2 efflux	0.44	0.06	0.09	0.85
N availability				
net N mineralization	0.25	0.11	0.1	-

still be expected to increase microbial biomass, albeit less pronounced. Both mechanisms could play a role in explaining the positive response of Rh in elevated CO_2, but data is lacking to test which of these mechanisms is more important. As both plant litter production and Rh in CO_2 fumigated experiments increase to a similar extent, the lack of response of soil C is not unexpected (Fig. 2.5). Similar results for forests were already reported by de Graaff et al. (2006), who reported a positive response of soil C only for grasslands. Thus, any increase in C accumulation in tree stands subjected to elevated CO_2 will likely be confined to increased woody biomass production.

Influence of N fertilization

We did not find any significant differences in the response of aboveground biomass and soil C storage to elevated CO_2 between fertilized and unfertilized studies, which is in contrast to earlier studies (de Graaff *et al.* 2006; Luo *et al.* 2006b; van Groenigen *et al.* 2006). However, while both aboveground biomass and soil C are significantly stimulated by elevated CO_2 in the fertilized studies, they are not in the unfertilized ones, suggesting that the lack of a statistically significant difference might be caused by low statistical power due to a smaller dataset (in this analysis only tree stands were considered). Our focus on tree stands only may also explain part of the difference because forests and grasslands have very different C use patterns (Schulze *et al.* 2009).

Van Groenigen et al. (2006) and De Graaff et al. (2006) indicated that soil C accumulation is significantly enhanced in elevated CO_2 only when sufficient amounts of N were added. They hypothesize that as nitrogen fertilization enhances plant productivity, it therefore could increase the amount of C entering the soil, resulting in a net increase of soil C. The reason for this would be that the stimulated plant production in fertilized stands would overcome the increased decomposition in elevated CO_2. While this hypothesis also fits our results, we suggest a prominent role for fine root dynamics as soil C inputs (Zak *et al.* 2000).

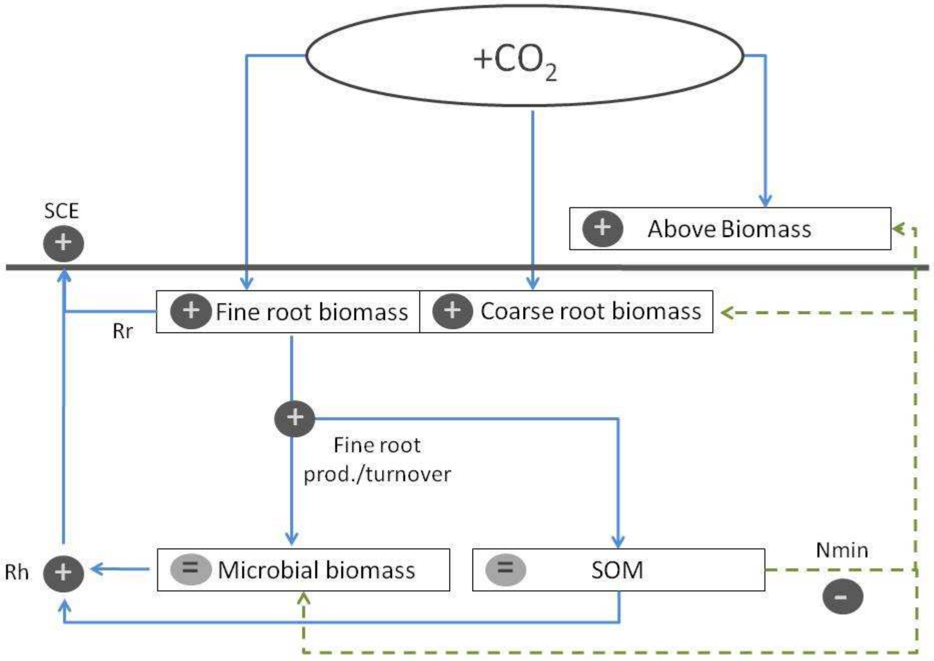


Figure 2.5: Conceptual representation of the overall CO_2 effects on C cycling in tree stands. Pools are given in boxes; fluxes are given in lines. Blue lines are C-related processes; green dashed lines are N-related. Black circles indicate a statistically significant CO_2 effect; grey circles indicate a statistically non significant trend. Results are considered statistically significant at $P<0.05$. SOM = Soil Organic Matter, Nmin = net N mineralization, Rh = microbial respiration, Rr = root respiration, SCE = Soil CO_2 efflux. Elevated CO_2 induces a C allocation shift towards belowground biomass, where the increased C inputs (fine root production/turnover) increase the CO_2 response of microbial respiration (Rh), leaving net change in soil C unaffected in elevated CO_2. A strong negative CO_2 response of net N mineralization indicates a lower N availability in elevated CO_2.

As de Graaff et al. (2006) suggested, we found a stronger CO_2 response of woody biomass compartments in the fertilized stands. However, while the fine root biomass and production response to elevated CO_2 was significantly affected in both fertilized and unfertilized tree stands, the CO_2 response is larger in the unfertilized ones. Indeed, N fertilization is known to generally stimulate woody biomass increase, without affecting soil C inputs (Pregitzer *et al.* 2008). At the same time, we observed a strongly increased Rh response to elevated CO_2 in the unfertilized stands, while the CO_2 effect is not significant in the fertilized stands. Our results therefore suggest that, due to an increased soil exploration in unfertilized stands, fine root dynamics and rhizodeposition will be more pronounced, and will serve as a direct substrate for the microbial community. As a consequence, we see an increased soil C storage in the fertilized stands subject to elevated CO_2, while a negative trend is apparent in the unfertilized stands. Aside from this C input-related feedback, retarded rates of soil organic matter decomposition in N fertilized systems are common (Fog 1988; Berg & Matzner 1997), which could also contribute to an increased soil C storage in tree stands (Janssens *et al.* 2010). The inhibitory effects of N fertilization on SOM decomposition can be obscured in CO_2 fumigated experiments (Janssens *et al.* 2010), as soil C inputs typically increase under CO_2 fumigated systems (DeLucia *et al.* 1999; Pendall *et al.* 2004; Subke *et al.* 2006; Liu *et al.* 2007b; Soussana & Luscher 2007), and CO_2 elevation stimulates root exudation and rhizodeposition, all of which affect microbial activity (Norby *et al.* 1995; Canadell *et al.* 1996; Lipson *et al.* 2005). This could also explain why N fertilization only stimulates soil C accumulation in elevated CO_2 when very large amounts of N are applied (van Groenigen *et al.* 2006).

Relationships with soil N concentration

Soil N concentration was significantly correlated to responses of fine root biomass and –production, microbial respiration and soil C to elevated CO_2 (Table 2.4). Figure 2.4 illustrated that roots and Rh show only minor responses when soil N is low, which is to be expected, as the elevated CO_2 induced growth stimulation cannot be sustained without sufficient available N. In N-rich soil, however, we see that elevated CO_2 strongly increases plant productivity, which affects belowground C cycling through a stimulation of both C inputs and losses. In N-rich soils, this accelerated C cycling under elevated CO_2 even results in a negative effect of elevated CO_2 on soil C storage with increasing soil N concentration (Table 2.4, Fig. 2.4). Overall, these results confirm that the CO_2 effect on soil C inputs is the driving factor in soil C cycling, and can be modulated by N. However, these effects of soil N on the elevated CO_2 responses in our analysis differ from the approach where N fertilization is used as a measure for soil N availability in tree systems. Therefore, responses to elevated CO_2 and interactions with N are summarized in Box 2.1.

Box 2.1: Interactive effects of elevated CO_2 and N on C cycling in tree stands.

N fertilization and soil N concentration are two different ways to approach N availability, yet interact differently with elevated CO_2 (see grey coloured parts in the table). These contrasting effects are mainly a function of the direct availability of the N. In fertilized stands, the N is added in mineral form, while in N-rich systems, the N is still embedded in organic molecules or bound to the soil matrix. CO_2-fumigated tree stands will respond to both conditions in a different way, starting with fine root dynamics. In N-fertilized stands, the readily available N reduces the need for soil exploration by fine roots, and the associated reduction in rhizodeposition decreases the stimulation of Rh in elevated CO_2. This decreased decomposition response in N fertilized tree stands provides a larger potential for soil C accumulation. When tree stands are not fertilized, the larger demand for N in elevated CO_2 elicits an increased soil exploration by fine roots. Therefore, only in the N-rich systems, plants can sustain the increased growth responses in elevated CO_2.

	Fertilized vs. unfertilized experiments	**High soil [N] vs. Low soil [N]**
C inputs		
Litterfall	↑	-
Fine root production	↓	↑
C pools		
Aboveground biomass	↑	-
Root biomass	↑	-
Fine root biomass	↓	↑
Microbial C	↑	-
soil C	↑	↓
C losses		
Heterotrophic respiration	↓	↑
Soil CO2 efflux	↑	-

Note: Arrows indicate the direction of the effect of N on the CO_2 responses of soil C inputs, C pools, and C losses.

In response to the exacerbated fine root dynamics in elevated CO_2, the Rh response to elevated CO_2 increases, resulting in a decreasing soil C response to elevated CO_2 in systems with high soil N concentrations. Our findings indicate that an approach where treatments are simply opposed is a convenient way for statistical analysis, but fails to cover important information that is often not accounted for. Unfertilized sites can be nutrient rich, and therefore partly miss the expected fertilization treatment response (e.g. POPFACE or EUROFACE experiments). Similarly, it is normal that nutrient-poor sites that are fertilized demonstrate the largest relative responses (e.g. the Birmensdorf experiments). This could be one of the reasons why we do not always find a clear effect of fertilization treatments on soil C cycling in elevated CO_2. The response to CO_2 is an issue of definition, time and resource supply (Körner 2006). Based on our results, we suggest that more attention should be given to proper descriptions and reporting of experimental characteristics and soil properties in manipulation experiments. Parameters such as soil nutrient or water availability, or stand LAI (leaf area index), age and management are often not described properly, although they play very important role in regulating plant responses to elevated CO_2 and would make evaluating tree stand responses more accurate. Therefore, we underline that a better understanding of terrestrial ecosystem responses to global change we need, could be obtained from better or more standardized reporting of experimental conditions.

Conclusion

Our results confirm the important role of fine root dynamics in soil C cycling in elevated CO_2, as the increased fine root activity induced an acceleration of SOM decomposition processes. At the same time, N availability can limit plant growth responses and can therefore influence soil C cycling responses in elevated CO_2. While we failed to indicate differences between N fertilized and unfertilized tree stands in elevated CO_2, we clearly showed that soil N concentration can modulate soil C cycling. In elevated CO_2, fine root biomass and production, and heterotrophic respiration all increase with increasing soil N, while soil C decreases with total soil N concentration, regardless of N fertilization. We can therefore conclude that soil C cycling rates and soil C sequestration potential in elevated CO_2 will be influenced by initial soil properties and fertility, since (woody) plant growth responses to elevated CO_2 are dependent on N availability, while belowground responses are more dependent on changing soil C availability.

2.3.2. Effects of elevated CO_2 and N fertilization on plant and soil carbon pools of managed grasslands: a meta-analysis.

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Sillen W.M.A. and Dieleman W.I.J., 2012. Root biomass responses to elevated CO_2 limit soil C sequestration in managed grasslands. Biogeosciences Discussions, 9, 357-386.

2.3.2.1. Abstract

Elevated atmospheric CO_2 levels and increasing nitrogen deposition both stimulate plant production in terrestrial ecosystems. Moreover, nitrogen deposition could alleviate an increasing nitrogen limitation experienced by plants exposed to elevated CO_2 concentrations. But nevertheless, an increased rate of C flux through the soil compartment as a consequence of elevated CO_2 concentrations has been suggested to limit C sequestration in terrestrial ecosystems, questioning the potential for terrestrial C uptake to mitigate the increasing atmospheric CO_2 concentrations. Our study used data from 69 published studies to investigate whether CO_2 elevation and/or nitrogen fertilization could induce an increased carbon storage in grasslands, and considered the influence of management practices involving biomass removal or irrigation on the elevated CO_2 effects. Our results confirmed a positive effect of elevated CO_2 levels and nitrogen fertilization on plant growth, but revealed that N

availability is essential for the increased C influx under elevated CO_2 to propagate into belowground C pools. However, moderate nutrient additions also promoted decomposition processes in elevated CO_2, reducing the potential for increased soil C storage. An important role in the soil carbon response to elevated CO_2 was attributed to the root response, since there was a lower potential for increases in soil C content when root biomass was more responsive to CO_2 elevation. Future elevated CO_2 concentrations and increasing N deposition might thus increase C storage in plant biomass, but the potential for increased soil C storage is limited.

2.3.2.2. Introduction

Atmospheric CO_2 concentrations have strongly increased since the pre-industrial era (IPCC 2007), resulting in the contemporary CO_2 concentration of 380 ppm that exceeds all earlier concentrations since the late Tertiary era, when most of the modern plants evolved into their present shapes (Pearson & Palmer 2000; Crowley & Berner 2001). Because of the stimulating effect of these elevated CO_2 concentrations on photosynthesis and plant productivity (Nowak *et al.* 2004; Ainsworth & Long 2005; Soussana & Luscher 2007), it has been hypothesized that plants can partly buffer human induced CO_2 emission by sequestering C (Gifford 1994). Grasslands are estimated to embody more than 10% of the carbon (C) reservoir of the biosphere (Eswaran *et al.* 1993; Nosberger *et al.* 2000), with most C (up to 98% of the total C) located in their belowground compartment (Hungate *et al.* 1997). The 3.7 billion ha of the earth's surface with permanent grasslands have an estimated potential annual C sequestration capacity of 0.01-0.3 GtC (Lal 2004), which implies that 4% of total global emissions of greenhouse gasses could be buffered by grasslands (Soussana & Luscher 2007).

Because many grasslands are managed for feeding domestic herbivores, either directly through grazing or through forage production, grassland C and N cycles might be affected because a large part of primary production is removed (Soussana *et al.* 2007). As a consequence, grasslands are often fertilized with nutrients to sustain productivity. In addition, the increased reactive nitrogen (N) deposition caused by the burning of fossil fuels and the use of artificial fertilizers (Davidson 2009) may affect large areas of the world in the future (Galloway 2008). Excessive N deposition can negatively influence ecosystem health and diversity (Aber *et al.* 1998), but lower concentrations can alleviate the N limitation that plants generally experience in grasslands, thereby stimulating plant production (Lu *et al.* 2011).

In their review, de Graaff et al. (2006) hypothesized that increased plant production in elevated CO_2 could overcome increased soil organic matter (SOM) decomposition processes when ecosystems were supplemented with additional N. However, their study focused on both woody and herbaceous systems, and indicated different trends in C sink strength between both system types. In addition, a large proportion of the elevated CO_2 effect in grasslands tends to be due to improved water relations (Morgan *et al.* 2004a; Körner 2006; Morgan *et al.* 2011), which was not found to be a dominant driver for tree stands (Körner 2006). Moreover, grasslands showed very variable responses of belowground biomass, while tree stands consistently displayed intensified belowground metabolism (Körner 2006).

Because of these functional differences between grasslands and tree stands, and the management component involved in grasslands, we focused on elevated CO_2 effects in grasslands only. More specifically, we investigated the effects of

Table 2.5: Information about the ecosystem type and the irrigation, fertilization and management practices at the sites that were used in the experiments included in our analysis. Different letters ((a) and (b)) within the fertilizer specifications are used to separate different experiments that were executed on the same site.

Site Name	Irrigation	Fertilization	Fertilizer Type	Fertilizer Amount	Biomass removal	Reference
Aberdeen	Yes	Yes & No	NaH_2PO_4, KNO_3 and $NaNO_3$	-	Clipping	(Paterson *et al.* 2008)
Amsterdam	Yes	Yes	NH_4NO_3	47 kg ha^{-1} yr^{-1}	-	(Hoorens *et al.* 2003)
Cedar Creek grassland	No	Yes & No	NH_4NO_3	(a) 40 kg ha^{-1} yr^{-1} (b) 100 kg ha^{-1} yr^{-1}	Burning	(Dijkstra *et al.* 2006; Keeler *et al.* 2009)
Canberra Phytotron	Yes	Yes	Not specified	22, 67 and 198 kg ha^{-1} yr^{-1} in different treatments		(Lutze *et al.* 2000)
Duolun	No	Yes	NH_4NO_3	100 kg ha^{-1} yr^{-1}	-	(Xia *et al.* 2009)
Gainesville grassland	Yes	Yes	NPK (and Mg and S)	70-80 kg ha^{-1} depending on year	-	(Allen *et al.* 2006)
Ginninderra	Yes	Yes	slow release fertilizer	100 kg ha^{-1} yr^{-1}	-	(Volder *et al.* 2007)
Jasper Ridge (FACE)	Yes & No	Yes & No	$Ca(NO_3)_2$	70 kg ha^{-1} yr^{-1}	-	(Dukes *et al.* 2005)
Jasper Ridge (OTC)	No	Yes & No	(a) Urea + Osmocote 120 days slow release fertilizer (b) NPK (120 day release fertilizer)	(a) 200 kg ha^{-1} (b) low: 30 kg ha^{-1}; high: 200 kg ha^{-1}	-	(Hungate *et al.* 1997; Cardon *et al.* 2001)
Jokioinen	Yes	No	-	-	Mowing	(Kanerva *et al.* 2008)

Linden-Leihgestern (FACE)	No	Yes	Thomas kali fertilizer and N	4 kg ha^{-1} yr^{-1}	Cutting	(Sonnemann & Wolters 2005)
Manawatu	No	Yes	superphosphate, K_2SO_4, $MgSO_4$, Cu and Zn	-	-	(Ross *et al.* 2004)
Moor House	No	No	-	-	-	(Fitter *et al.* 1997)
Nåntuna	No	No	-	-	Cutting	(Marissink *et al.* 2002)
NERC	Yes	No	-	-	Herbivory	(Kandeler *et al.* 1998)
New Zealand (FACE)	No	Yes	superphosphate, KSO_4	-	Grazing	(Allard *et al.* 2005)
Niwot Ridge	No	Yes	(a) urea-N as osmocote pellets (b) osmocote pellets (urea-N and P_2O_5-P)	250 kg ha^{-1} yr^{-1} for the first two years, 100 kg ha^{-1} yr^{-1} thereafter	-	(Bowman *et al.* 1993; Neff *et al.* 2002)
PHACE	No	No	-	-	-	(Dijkstra *et al.* 2010)
Swiss Central Alps	No	Yes & No	NPK (3:2:3)	45 kg ha^{-1} yr^{-1}	-	(Niklaus & Korner 1996; Schappi & Korner 1996)
Swiss Jura	No	No	low dose P fertilization (superphosphate)	-	Mowing/Clipping	(Leadley *et al.* 1999; Stocker *et al.* 1999; Niklaus *et al.* 2003; Niklaus & Korner 2004)
SwissFACE (Bromus/Carex)	No	No	-	-	-	(van Kleunen *et al.* 2006)
SwissFACE (Lolium)	No	Yes	NH_4NO_3	low: 140 kg ha^{-1} yr^{-1}; high: 420 kg ha^{-1} yr^{-1} in 1993 and 560 kg ha^{-1} yr^{-1} after 1993	Cutting	(Sowerby *et al.* 2000; de Graaff *et al.* 2004; Bazot *et al.* 2006)

SwissFACE (Trifolium)	No	Yes	NPK, N as NH_4NO_3 solution	low: 140 kg ha^{-1} yr^{-1}; high: 420 kg ha^{-1} yr^{-1} in 1993 and 560 kg ha^{-1} yr^{-1} after 1993	Cutting	(de Graaff *et al.* 2004)
TasFACE	No	No	-	-	-	(Pendall *et al.* 2011)
University of Antwerp	No	No	-	-	-	unpublished
University of Guelph	Yes	Yes	Hoagland's solution	47 kg ha^{-1} yr^{-1}	-	(Klironomos *et al.* 1998)
Univerisity of Michigan Biological Station	Yes	No	-	-	-	(Treonis & Lussenhop 1997)
USDA ARS	Yes	No	-	-	-	(Blank & Derner 2004)
USDA Central Plains	No	No	-	-	-	(Morgan *et al.* 2004b; Pendall & King 2007)

elevated CO_2 concentrations and N fertilization individually, and the influence of N fertilization, biomass removal and irrigation on the CO_2 effects on C pools in grasslands (aboveground, root and microbial biomass, and soil C) using the following hypotheses: (1) elevated CO_2 will stimulate plant production and will increase allocation of C to root compartments due to direct effects on photosynthesis and increasing depletion of nutrients, (2) addition of N will stimulate plant productivity but will leave microbial biomass unaffected because of an increasing C limitation of microbes, (3) the combined CO_2 and N treatment will strongly stimulate biomass production and will stimulate soil C storage via larger C inputs, and (4) aboveground biomass removal and irrigation will affect C allocation patterns towards aboveground plant compartments, through reduced LAI and reduced need for root production.

2.3.2.3. Methods

For an extensive description of methods, we refer to chapter 2.2

Data acquisition

We constructed a database, consisting of results from 69 manipulation experiments in grassland systems exposed to elevated CO_2 concentrations with/without nutrient additions. Here, we focus on aboveground (AB), root (RB) and microbial biomass (MB), root to shoot ratio (RS, calculated where AB and RB were available) and soil C content. This resulted in 182 entries that were used in the meta-analysis.

We extracted information about amount and type of fertilizer added (independent from the intention of creating a different treatment) and the execution of other management practices (biomass removal or irrigation) from the articles. Whenever this information was lacking, the study was considered as not including fertilization or other management. The extracted information is synthesized in Table 2.5.

Meta-analysis

We examined the effect of elevated CO_2 concentrations and fertilization separately (in experiments where single factor CO_2 and combined CO_2 and fertilization treatment effects were reported, we extracted a single factor fertilization treatment response using the control values of both CO_2 treatments), the effect of elevated CO_2 concentration in combination with fertilization, the effects of the type and the amount of N fertilizer added (classification in low and high amounts was based on a background value of 50 kgN ha^{-1} yr^{-1}, based on projected N deposition values in 2050 (Galloway 2008)), and the effects of biomass removal or irrigation on the elevated CO_2 effects.

2.3.2.4. Results

Single factor and combined treatment effects of elevated CO_2 and N addition

Aboveground biomass increased under all three treatments (i.e. elevated CO_2, N fertilization and their combination) (Fig. 2.6). Root biomass decreased when only CO_2 levels were elevated, but increased when nutrients were added, either with or without CO_2 elevation as a co-treatment (Fig. 2.6, Table 2.6). Microbial biomass increased in elevated CO_2 concentrations, both with and without fertilization, and showed an opposing trend in response to the single factor fertilization treatment (Fig. 2.6, Table 2.6). Soil C content increased in the single factor CO_2 treatment and was unaltered under the other treatments (Fig. 2.6).

In the combined elevated CO_2 and fertilization treatment, aboveground biomass responded equally strong to different fertilizer types, but was stimulated more when lower doses of N fertilizer were added (Fig. 2.7, Table 2.6). In contrast, root biomass responded strongly positively to CO_2 elevation with NPK fertilizer addition, while pure N addition did not affect root biomass (Fig. 2.7, Table 2.6).

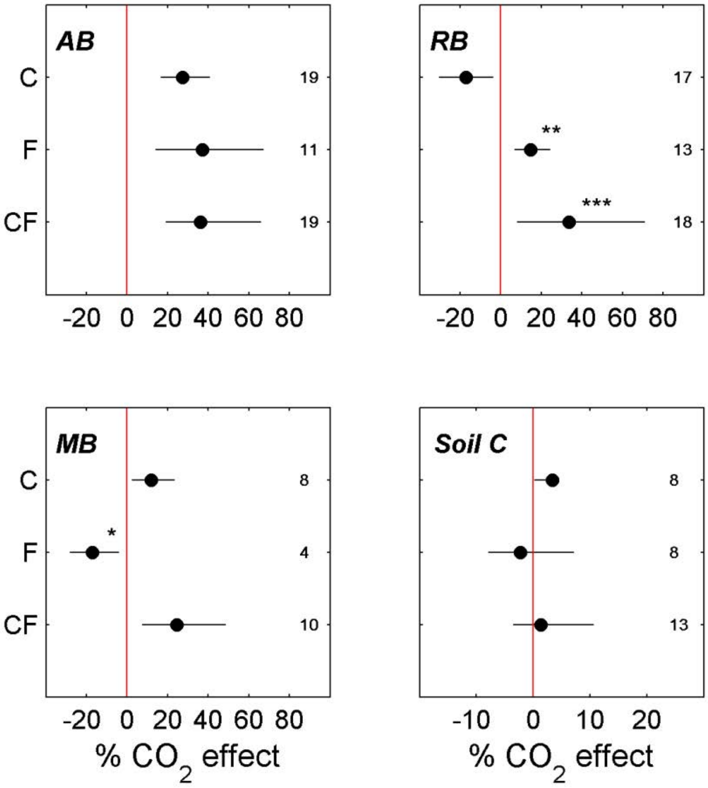

Figure 2.6: Responses of grassland C pools to three different treatments: CO_2 elevation (C), fertilization (F) and the combination of CO_2 elevation and fertilization (CF). Responses are shown as percentage increase of aboveground biomass and 95% confidence intervals (CI) for (AB), root biomass (RB), microbial biomass (MB), and soil C content (Soil C). Treatment responses were considered statistically significant when zero was not included in the 95% CI. Statistically significant differences with the single factor CO_2 treatment are indicated by: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Similar to the aboveground biomass response, root biomass increased more when low doses of N were applied (Fig. 2.7, Table 2.6). Microbial biomass and soil C responses to elevated CO_2 were not affected differently by different fertilizer

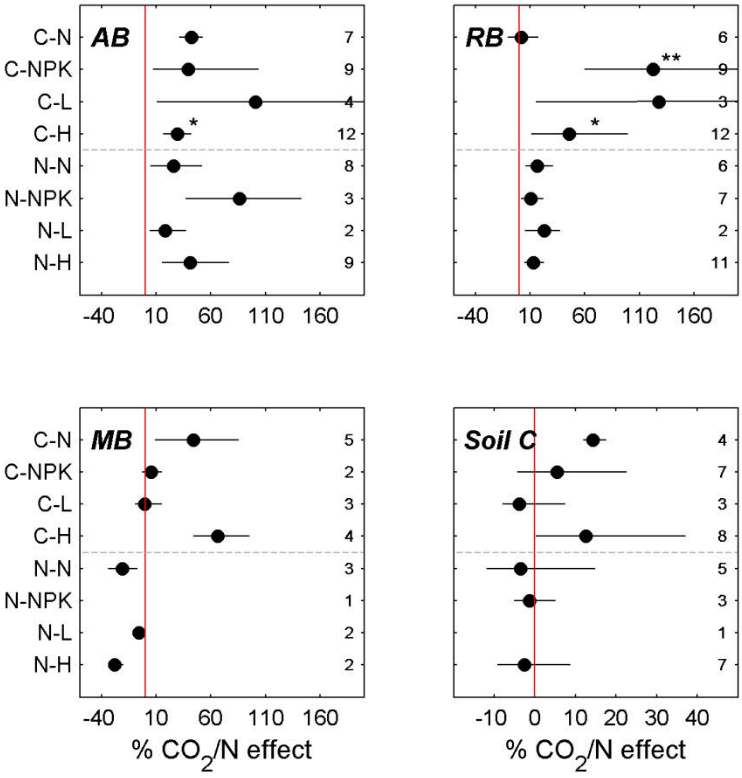

Figure 2.7: CO_2 and N fertilization responses of grassland C pools to different N fertilizer type and intensity: CO_2 elevation with pure N fertilizer (C-N), CO_2 elevation with NPK fertilizer (C-NPK), CO_2 elevation with low N fertilizer application (C-L, less than 50 kgN ha^{-1} yr^{-1}) and CO_2 elevation with high N fertilizer application (C-H, more than 50 kgN ha^{-1} yr^{-1}), N fertilization with pure N fertilizer (N-N), N fertilization with NPK fertilizer (N-NPK), N fertilization with low N fertilizer application (N-L, less than 50 kgN ha^{-1} yr^{-1}) and N fertilization with high N fertilizer application (N-H, more than 50 kgN ha^{-1} yr^{-1}). Responses are shown as percentage increase and 95% confidence intervals (CI) for aboveground biomass (AB), root biomass (RB), microbial biomass (MB), and soil C content (Soil C). Treatment responses were considered statistically significant when zero was not included in the 95% CI. Statistically significant differences between fertilizer type or intensity are indicated by: * $P < 0.05$; ** $P < 0.01$.

types or doses (Table 2.6), but interestingly they demonstrated increases only when fertilized with pure N fertilizers and at high doses of N addition (Fig. 2.6).

Weighted linear regression analysis also suggested an increase in microbial biomass in elevated CO_2 with higher N fertilization doses (Table 2.7).

The single factor N fertilization treatment effects on C pools were not significantly different between fertilizer type or dosage (Fig. 2.7, Table 2.6), although a trend towards stronger aboveground biomass responses was apparent under NPK fertilization. This trend was confirmed by weighted linear regression analysis (Table 2.7).

Table 2.6: Overview of the *P*-values for the meta-analytical comparison between the responses of grassland C pools to different treatments. Results shown for: (1) CO_2 elevation and fertilization treatments (C = elevated CO_2, CF = elevated CO_2 with fertilization, F = fertilization); (2) different fertilization specifications when CO_2 is elevated (type: fertilizer consisting of N only or of NPK; amount: low when less than 50 kgN ha^{-1} yr^{-1} is applied and high when more is applied) and (3) other management procedures when CO_2 is elevated (biomass removal and irrigation). The parameters considered are: aboveground plant biomass (AB), root biomass (RB), root-to-shoot ratio (RS), microbial biomass (MB) and soil C content (Soil C). Differences between responses for a parameter were considered statistically significant when $P < 0.05$ (***bold italics***).

	AB	RB	RS	MB	Soil C
(1) C vs. F	0.4682	***0.0044***	0.8169	***0.0128***	0.086
C vs. CF	0.6269	***0.0008***	0.1859	0.4346	0.7017
F vs. CF	0.9676	0.3255	0.1811	0.0716	0.5274
(2) CO_2 + N type	0.9736	***0.0016***	***0.0012***	0.4262	0.6809
CO_2 + N amount	***0.0172***	0.2491	0.1919	***0.0336***	0.2019
N, N type	0.1076	0.6006	***0.0344***	-	0.8477
N, N amount	0.5674	0.4702	0.1795	0.3419	-
(3) CO_2 + biomass removal	0.7889	0.0744	-	0.7093	-
CO_2 + irrigation	0.2603	0.0776	0.99	0.926	0.3503

Table 2.7: Meta-analysis results for linear regression analysis between amount of N fertilization and elevated CO_2 effects on C pools, and the relationship between biomass responses and soil C responses to elevated CO_2. Indicated are the *P*-values for regressions with aboveground biomass (AB), root biomass (RB), microbial biomass (MB) and soil C (soil C), the amount of datapoints (n) and the slope of the regressions. Regressions are considered statistically significant at *P*<0.05 (***bold italics***).

N dosage	***P*-value**	**n**	**slope**
In elevated CO_2			
AB	0.5196	16	-0.0053
RB	0.9891	15	-0.0001
MB	***0.0314***	7	0.014
soil C	0.8884	11	0.0007
In single factor N fertilization			
AB	***0.0417***	11	0.0267
RB	0.833	13	0.001
MB	***0.0183***	4	-0.0455
soil C	0.1117	8	0.0091
Soil C response			
Pure C	*P-value*	*n*	*slope*
AB	0.9004	8	-0.0269
RB	0.8183	6	0.0295
MB	0.9751	4	-0.0049
Pure C and Cf (<50 kgN ha^{-1} yr^{-1})			
AB	0.6008	11	-0.0948
RB	***0.0411***	9	-0.0866
MB	0.9269	5	-0.0139
C+Cf+CF (>50 kgN ha^{-1} yr^{-1})			
AB	0.4392	15	-0.135
RB	0.1205	13	-0.0557
MB	0.9853	7	-0.0028

Other management procedures (biomass removal and irrigation)

Biomass removal or irrigation did not significantly affect CO_2 responses, although root biomass showed a stronger trend towards a decrease in systems where aboveground biomass was removed or systems that were irrigated (Fig. 2.8, Table 2.6).

Carbon allocation shifts

The root-to-shoot ratio (RS) of grasslands decreased in single factor CO_2 and N fertilization treatments, indicating an preferential allocation of C towards aboveground biomass (Fig. 2.9). The combined CO_2 and N treatment did not change allocation patterns in grasslands (Fig. 2.9). There was a strong contrast between RS-responses to elevated CO_2 depending on the type of fertilizer added: pure N addition decreased RS, while NPK fertilizers increased RS in elevated CO_2 (Fig. 2.9, Table 2.6). Biomass removal and irrigation did not affect the overall RS response to elevated CO_2 (Fig. 2.9).

2.3.2.5. Discussion

Elevated CO_2 effects were generally in accordance with previous studies indicating increased biomass production, and small or no increases in soil C content (Fig. 2.6)

(Pendall *et al.* 2004; de Graaff *et al.* 2006; Hungate *et al.* 2009; Lu *et al.* 2011). However, we found a decrease in root biomass as a consequence of elevated CO_2 concentrations, which is in sharp contrast to most other studies (Rogers *et al.* 1994; Curtis & Wang 1998; Pendall *et al.* 2004; de Graaff *et al.* 2006), and partly refutes our first hypothesis.

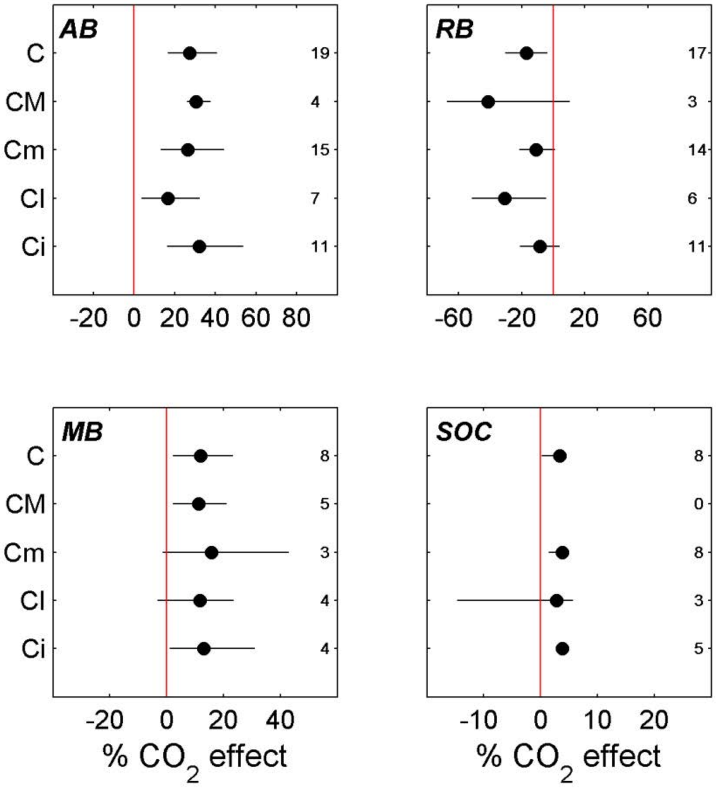

Figure 2.8: The CO_2 effect in experiments with (CM) or without (Cm) biomass removal, and irrigated (CI) and non-irrigated (Ci) experiments, compared to the full CO_2 dataset (C). Responses are shown as percentage increase and 95% confidence intervals (CI) for aboveground biomass (AB), root biomass (RB), microbial biomass (MB), and soil C content (Soil C). Responses were considered statistically significant when zero was not included in the 95% CI.

However, unfertilized systems did not always display increases in root biomass in response to elevated CO_2 (de Graaff *et al.* 2006), and showed a clear dependence on N additions (van Groenigen *et al.* 2006). In addition, several pieces of evidence in this study can help to explain the observed decrease in root biomass under elevated CO_2: Firstly, when plants are deprived of their shoots multiple times by harvest, burning or grazing, proportionally more energy has to be allocated to aboveground biomass for repair and regrowth, which could impair root growth by lowering the amount of C available for belowground biomass. Secondly, in irrigated systems, root biomass tended to decrease even more, compared to non-irrigated systems. According to Volk et al. (2000), Bunce (2004) and Morgan et al. (2004a), an increased water use efficiency (WUE) as a consequence of reduced stomatal conductance in elevated CO_2 is the major reason for increased plant biomass in higher atmospheric CO_2 concentrations. Irrigation would reduce the need for an extensive root network, and reduce the advantage based on increased WUE. Therefore, although we did not find significant direct effects of biomass removal or irrigation on C pools, we suggest grassland management might have affected root biomass responses to elevated CO_2. When we excluded all experiments that were irrigated or where biomass was removed, root biomass was no longer significantly decreased by elevated CO_2 (data not shown), offering support for our fourth hypothesis.

Nutrients regulate C allocation responses to elevated CO_2

Elevated CO_2 increased aboveground biomass in all treatments (Fig. 2.6-2.8), while root biomass was only significantly stimulated when nutrients were applied (Fig. 2.6-2.8). This was reflected in an increased allocation of C to aboveground biomass compartments in the single factor CO_2 treatment (Fig. 2.9). It was only in the combined CO_2 and fertilization treatment that C allocation was balanced between aboveground and root biomass, or increased towards root biomass (Fig. 2.9). The increased allocation to aboveground biomass could have been a consequence of increased water use efficiency of plants in elevated CO_2 (Morgan *et al.* 2004a), or a consequence of the typical annual regrowth of grassland biomass to reinstate light capture. However, our results showed that the root biomass response in elevated CO_2 was unaffected when pure N fertilizers were added, but increased strongly when NPK fertilizers were added (Fig. 2.7) and that RS decreased in elevated CO_2 with addition of pure N fertilizer, while it increased under NPK fertilization in elevated CO_2 (Fig. 2.9). In addition, in the single factor fertilization treatment, aboveground biomass tended to respond stronger to NPK fertilizers (Fig. 2.7, Table 2.6) suggesting limitation by nutrients other than N. As it has been shown before that N-fixing plant species in particular can become

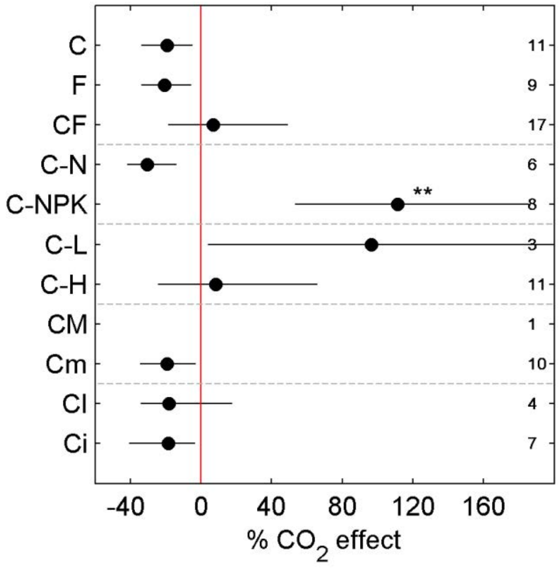

Figure 2.9: Effects on the root-to-shoot ratio (RS) in grasslands under elevated CO_2 (C), nitrogen fertilization (F), combined elevated CO_2 and N fertilization (CF), elevated CO_2 with pure N fertilizer (C-N)), CO_2 elevation with NPK fertilizer (C-NPK), CO_2 elevation with low N fertilizer application (C-L, less than 50 kgN ha^{-1} yr^{-1}), CO_2 elevation with high N fertilizer application (C-H, more than 50 kgN ha^{-1} yr^{-1}), elevated CO_2 with (CM) or without (Cm) biomass removal, and elevated CO_2 in irrigated (CI) and non-irrigated (Ci) experiments. Responses are shown as percentage increase and 95% confidence intervals (CI) and were considered statistically significant when zero was not included in the 95% CI. Statistically significant differences between fertilizer type are indicated by: ** $P < 0.01$.

limited by non-nitrogen nutrients in elevated CO_2 (van Groenigen *et al.* 2006), it seems likely that non-nitrogen nutrients might play an important role in regulating the C allocation patterns in the elevated CO_2 experiments in these grasslands.

Constructive use of C in microbial biomass

Elevated atmospheric CO_2 concentrations stimulated microbial biomass and soil C content (Fig. 2.6), confirming earlier work (Zak *et al.* 2000; Pendall *et al.* 2004; Hungate *et al.* 2009; Dieleman *et al.* 2010). The negative effect of N addition on microbial biomass is also in accordance with previous work (Treseder 2004), and our second hypothesis, suggesting microbes either became more C limited under N fertilization, or deteriorating soil conditions and chemical stabilization of SOM inhibited microbial growth (DeForest *et al.* 2004; Treseder 2004; Janssens *et al.* 2010). In addition, N additions mainly stimulate C sequestration in long-lived biomass compartments (Pregitzer *et al.* 2008), and hence the amount of C being incorporated into the soil matrix might have been limited (Lu *et al.* 2011). Because root biomass increased in N fertilized experiments (Fig. 2.6), and microbial biomass was found to further decrease at higher N fertilization rates (Fig. 2.7, Table 2.7), it seems more likely that the inhibiting effects of N fertilization dominated in the microbial biomass response. The larger amount of C being stored in longer-lived biomass might also explain why soil C content was not significantly affected, because C was retained in biomass and not added to the soil matrix.

The strong increase in the combined CO_2 and fertilization treatment for microbial biomass (Fig. 2.6), and the borderline significant difference with the single factor fertilization treatment reaffirms the general C limitation of microbial communities. Microbes use C compounds as their main source for energy, and are therefore often C-limited (Zak *et al.* 1993; Demoling *et al.* 2007). However, microbes need N to be able to accumulate C into their biomass (Niklaus & Korner 1996). So in absence of N, microbes use the energy they obtain from decomposing easily degradable C-compounds to decompose N-richer compounds, which can result in higher respiration rates. Therefore, as expected, we found the highest increase in microbial biomass in the combined CO_2 and N fertilization treatment (Fig. 2.6).

Soil C storage in grasslands under elevated CO_2

We found a striking similarity between soil C and microbial biomass responses (Fig. 2.6-2.7) and opposing trends between microbial biomass and soil C responses on the one hand, and root biomass responses to elevated CO_2 on the other hand (Fig. 2.6-2.7). As we show in figure 2.10, elevated CO_2 concentrations without addition of mineral fertilizer reduced root biomass but stimulated microbial biomass. However, aboveground biomass increased, which suggests that the increase in microbial biomass possibly resulted as a consequence of priming, which is more likely to happen in nutrient-poor systems (Hoosbeek *et al.* 2004). This mechanism might have provided the nitrogen to maintain plant growth. In contrast, when purely N was added to grasslands, root biomass increased and microbial biomass

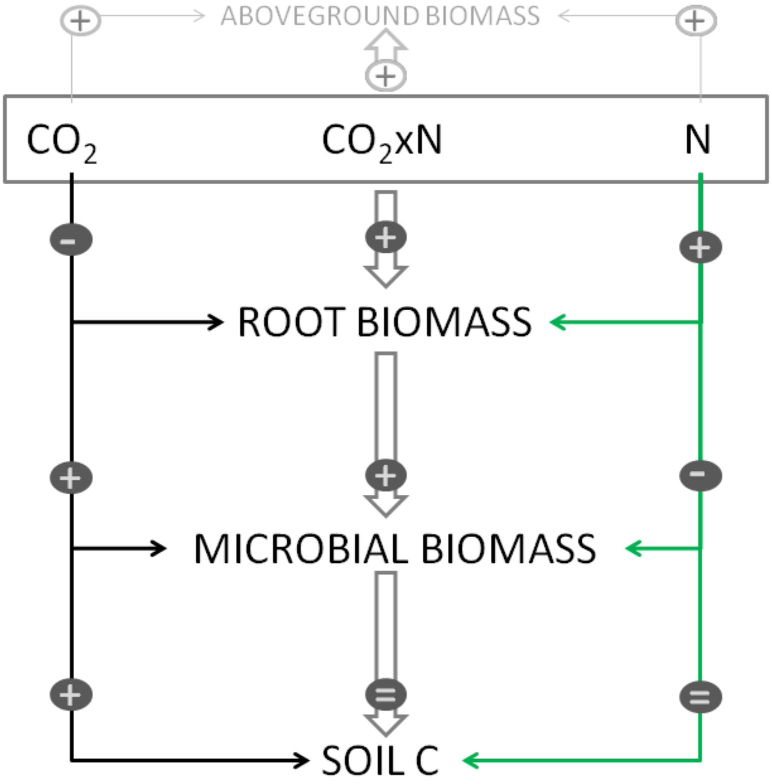

Figure 2.10: Synthesis of elevated CO_2 effects in grasslands. When no N fertilizer was added, elevated CO_2 stimulated aboveground biomass, but reduced root biomass. An increased root death as a consequence might have served as substrate for microbes and a C input for soil C pools. When only N fertilizer was added, both aboveground and root biomass were stimulated but microbial biomass was decreased, suggesting C limitation or chemical inhibition of microbial communities. When grasslands in elevated CO_2 were fertilized with N (CO_2xN), C storage was largest and both root biomass and microbial biomass were stimulated. Increased cycling of C left soil C pools unaffected.

decreased (Fig. 2.10). In that case, C might have been stored in root tissues with longer turnover times, and hence the C accumulation in plant biomass was not transferred to soil compartments limiting microbial growth. In the combined CO_2

and N fertilization treatment, both root biomass and microbial biomass significantly increased in elevated CO_2. The combination of elevated CO_2 and N fertilization resulted in excess C that was allocated to root biomass. In the soil compartment this increased C input in concert with sufficient N availability translated into higher microbial biomass. However, despite the positive effects on microbial and root biomass in the combined treatment, the soil C pool remained unaffected, possibly because of an increased cycling of C in the soil compartments (Körner *et al.* 2005; Lukac *et al.* 2009; Dieleman *et al.* 2010).

We suggest an important role for root biomass and dynamics and their response to nutrients under elevated CO_2 concentrations, based on our findings above (see Fig. 2.6-2.7). To further support this, we found a neutral effect of root biomass responses to soil C sequestration in ‘pure’ CO_2 experiments, but found a significant correlation between the root biomass response and the soil C response in elevated CO_2 when realistic amounts of N fertilizer (i.e. max. of 50 kgN ha^{-1} yr^{-1}) were added (Fig. 2.11, Table 2.7), suggesting lower potential for increases in soil C content when root biomass becomes more responsive to elevated CO_2. In this case, the C inserted in the soil matrix by root exudation or root turnover might promote more rapid cycling of C inputs into the soil. In support of our findings, Cardon et al., (2001) showed that microbes in nutrient-poor environments are forced to decompose older soil organic matter for N supply, but when excess C is available in nutrient-rich situations, the newly sequestered C inputs into the soil become preferential C substrates for microbial decomposition in elevated CO_2.

For experiments with higher rates of N fertilization, soil C did tend to increase regardless of root responses (Figs. 2.7 and 2.11), in accordance with Van Groenigen et al. (2006), who reported that soil C only increased at high rates of N fertilization (>30 kgN ha^{-1} yr^{-1}). Moreover, respiration rates can be reduced when terrestrial systems are fertilized with large amounts of N (Fog 1988; Janssens *et al.* 2010). So at high fertilization rates, the effects on decomposition might have overpowered the CO_2 effects on roots, promoting an increasing soil C response in elevated CO_2. We thus cannot confirm, nor refute our third hypothesis, as soil C did not increase in combined CO_2 and fertilization manipulation. Instead, we propose that the soil C response will be determined by the nutrient-dependant root biomass response and the associated feedbacks to soil C decomposition in elevated CO_2.

Implications

Both CO_2 elevation and N addition appeared to be limited in their effect by the presence of the other resource: N respectively C. Elevated CO_2 concentrations stimulated plant productivity, but in a less powerful way compared to when N was

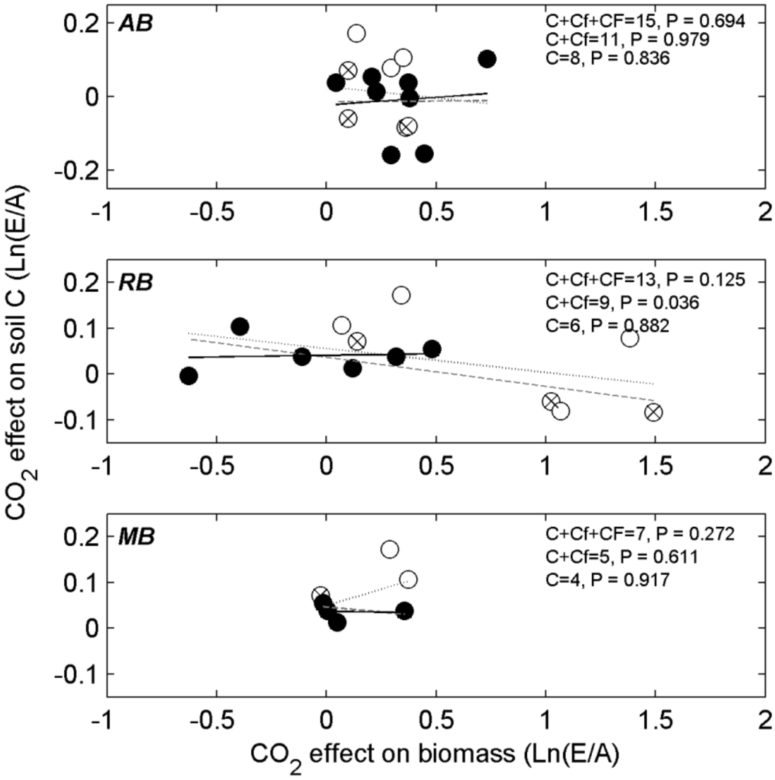

Figure 2.11: Relationships between the CO_2 response of soil C content and the aboveground biomass (AB), root biomass (RB) and microbial biomass (MB) response. Data shown are 'pure' CO_2 experiments (black circles, C), elevated CO_2 experiments with moderate N additions (<50 kgN ha^{-1} yr^{-1})(crossed circles, Cf), and elevated CO_2 experiments with high N additions (>50 kgN ha^{-1} yr^{-1}) (white circles, CF). The number of data points, the *P*-value for the regressions and the R^2-value for all regressions are indicated. Regressions are considered statistically significant at *P*<0.05.

added. The excess C that plants thus acquired was transferred to the soil microbial community, where an increased rhizodeposition might have alleviated the C limitation of soil microorganisms. Addition of nitrogen only, on the other hand, created a strong plant growth response. However, the excess C that is provided by

CO_2 elevation is lacking for the stimulus to propagate into the soil community. Consequently, as indicated by our results, it is the combination of CO_2 elevation and N addition that increased the C pool of plant biomass and that stimulated the soil community.

Conclusion

In grasslands, different management strategies did not affect the overall stimulating effect of elevated CO_2 on aboveground biomass production. However, CO_2 elevation only increased root biomass significantly when aboveground biomass production was optimized (i.e. when N fertilization was applied). We have shown here that, while other nutrients might become important in the future, N availability is essential for the increased C influx under elevated CO_2 to propagate into belowground C pools. However, moderate nutrient additions also promoted decomposition processes in elevated CO_2, reducing the potential for increased soil C storage. The close relationship between root dynamics and soil C storage is a crucial link in plant-soil interactions in terrestrial ecosystems, and determines the potential for increased soil C storage in elevated CO_2. In conclusion, while future elevated CO_2 concentrations and increasing N deposition might increase C storage in plant biomass, increases in soil C storage are small.

Because most of the biomass in non-forest ecosystems is short-lived, we suggest the capacity of grasslands to buffer human CO_2 emissions is limited.

2.3.3. Reduction of forest soil respiration in response to nitrogen deposition

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2.3.3.1. Abstract

In recent decades, many industrialized regions have been subject to enhanced atmospheric deposition of reactive nitrogen. This unintended fertilization is recognized to have stimulated forest growth, but nitrogen also strongly affects soil microbial activity and thus the recycling of carbon and nutrients in soils. Here, we present evidence from a meta-analysis that nitrogen deposition typically impedes carbon cycling through both rhizospheric and saprophytic pathways, except where the additional nitrogen remains insufficient to meet the nitrogen needs of trees and microbes. The associated, nitrogen-induced reductions in respiratory carbon losses are often of the same order of magnitude as the stimulation of wood production. These large reductions have a substantial effect on the net exchange of carbon with the atmosphere. Whether nitrogen deposition also affects soil carbon cycling in nitrogen-saturated and tropical forests remains an open question.

2.3.3.2. Introduction

Atmospheric deposition of reactive nitrogen (N), originating mainly from fossil fuel burning and artificial fertilizer applications (Davidson 2009), has increased three- to five-fold over the last century (Denman *et al.* 2007). In many areas of the globe, N deposition is expected to increase further (Galloway *et al.* 2004; Dentener *et al.* 2006), with global deposition rates projected to increase by a factor of 2.5 by the end of the century (Lamarque *et al.* 2005). Atmospheric deposition of N has many negative ecological effects in terrestrial and aquatic ecosystems, such as eutrophication and loss of biodiversity (Aber *et al.* 1989; Vitousek *et al.* 1997; Maskell *et al.* 2010). Excessive N deposition can also result in soil acidification, loss of base cations and nitrate leaching into groundwater (Högberg *et al.* 2006; de Vries *et al.* 2007; Dise *et al.* 2009). However, low levels of atmospheric N deposition can have one positive effect: it stimulates plant growth and the associated uptake of carbon (C) from the atmosphere contributes to climate change mitigation (Ciais *et al.* 2008; Pregitzer *et al.* 2008; Reay *et al.* 2008; Thomas *et al.* 2010). In 2007, Magnani and co-workers even revealed N deposition as the dominant driver of forest ecosystem carbon sequestration (Magnani *et al.* 2007).

The publication by Magnani and colleagues generated an intense debate on the magnitude and sustainability of the N-induced C sink and its underlying mechanisms (de Vries *et al.* 2008; Sutton *et al.* 2008; Janssens & Luyssaert 2009).

Two responses contribute to the enhanced C sink strength of forests under elevated atmospheric N deposition: increased wood formation (Elvir *et al.* 2003; Ciais *et al.* 2008; Pregitzer *et al.* 2008) and accumulation of surface litter and soil organic matter (SOM) (Olsson *et al.* 2005; Hyvönen *et al.* 2007; Pregitzer *et al.* 2008). Accumulation of SOM could originate from increased soil C inputs, but this is not commonly observed in forests exposed to N deposition (Pregitzer *et al.* 2008). In contrast, decreased rates of plant litter and SOM decomposition by enhanced soil N inputs have frequently been reported (Fog 1988; Berg & Matzner 1997). Through a meta-analysis of measurements in N-addition experiments and a comparison of study sites exposed to elevated or background atmospheric N deposition (Appendix 2), we show that the negative effect of N on below-ground C fluxes is widespread, albeit not universal, in forest ecosystems. Despite the long history of evidence that N deposition slows decomposition, this effect has not been included in current C cycle models.

2.3.3.3. Methods

For an extensive description of the meta-analysis methods, we refer to chapter 2.2

Meta-analysis of manipulation experiments

DATA ACQUISITION

We collected data on total biomass (TB), litterfall (LF), fine root production (FRP), root respiration (Rr), microbial biomass (Cmic), microbial respiration (Rh), litter decomposition rates (LD), soil CO_2 efflux (SCE) and soil C content, resulting in 255 entries for the meta-analysis originating from 57 manipulation experiments (see database). We only included studies performed in woody systems, where nitrogen (N) was added as a fertilization treatment.

META-ANALYSIS

A mixed model was used to assess the overall treatment effects on the different C pools and fluxes. Similarly, we tested our dataset for differences between additional treatments, types and amount of N fertilizer and tree seasonal strategy (evergreen vs deciduous).

If the calculated 95% confidence interval did not overlap with zero, then a significant response was considered. Significant differences between groups (=categorical analyses for treatment comparison, different tree seasonal strategies and type and amount of fertilizer) were identified on the basis of the within and between group heterogeneity. Significant differences are reported at $P<0.05$.

Analysis of field sites

DATA ACQUISITION

To assess the contributions of changes in Rh and SCE to NPP, we conducted a literature and database search (Luyssaert *et al.* 2007) to determine the fate of the C sequestered under different N-deposition levels. Observation-based estimates were compiled for C cycle components, including NPP, SCE and Rh. Observed and modeled estimates of wet, dry and total N-deposition were derived from published datasets (Galloway *et al.* 2004; Holland *et al.* 2005; Dentener *et al.* 2006). An arbitrary threshold of 5.5 kgN ha^{-1} yr^{-1} from wet deposition was used to separate a low from a high deposition group. However, the conclusions of this study hold for wet deposition thresholds ranging from 5.5 up to 10 kgN ha^{-1} yr^{-1}. We used wet rather than total deposition because the estimates for dry deposition are based on a limited set of observations and questionable assumptions, resulting in large uncertainties (Galloway *et al.* 2004; Holland *et al.* 2005).

Although a wide range of management practices is applied globally, all of them share one key characteristic: woody biomass is exported from the site. Among sites, information on management was of variable quality and detail. Therefore, a coarse classification was used to distinguish between managed and unmanaged sites. We excluded all sites that were recently disturbed, fertilized, irrigated or for which no management information was available. Nevertheless, better data allowing refined management classifications is likely to result in more detailed understanding of the management effect on C-cycling and its interactions with N-deposition.

UNCERTAINTIES

Uncertainties of flux estimates are rarely reported in the literature, although measurement errors or gapfilling uncertainties have been sometimes addressed (Oren 2006; Papale 2006; Richardson 2006; Moffat 2007) (typically ~15-20%). Therefore, we estimated the total uncertainty for every flux quantity contained in the data set using a consistent framework based on expert judgment (Taylor & Kuyatt 1994). This framework was designed to account for differences in data quality between sites due to length of time series, methodology and conceptual difficulties (i.e. separation of autotrophic and heterotrophic respiration, etc.). Subsequently, uncertainties were fully accounted for in the statistical analyses by means of 1000 simulations based on Monte Carlo principles (Rubinstein 1981). Within each of the 1000 Monte Carlo simulations performed, normally distributed random errors, based on the uncertainty framework of the database, were added to the observed fluxes. Therefore, all results that are based on flux data are reported as the median value and the 68% confidence interval of the probability distribution (± standard deviation).

STATISTICAL ANALYSIS

We used a maximum likelihood estimator (MLE) to estimate the parameters of a linear relationship between NPP and Rh. The MLE accounts for uncertainties in both NPP and Rh when estimating the parameters of the relationship.

2.3.3.4. Results: Empirical evidence for a reduction of soil C cycling by N

Litter quality modulates N effects on litter decomposition

It is well established that leaf litter with higher N (or high N to lignin ratios) decomposes faster than its lower N counterpart (Swift *et al.* 1979; Aber & Melillo 1980; Melillo *et al.* 1982), although this difference may revert during later stages of the decomposition process (McClaugherty & Berg 1987). However, adding N to low N litter does not accelerate its decomposition. In an early review of more than 60 experiments on the effect of N application on decomposition of various types of organic matter, Kåre Fog (1988) concluded that “when all these papers are considered together, it is evident that no effect, or even a negative effect, of the addition of N to decomposing organic material is a very widespread phenomenon“.

Literature reviews have indicated distinct decomposition responses to N addition in litter with low versus high lignin content (Fog 1988; Knorr *et al.* 2005a). In agreement with earlier studies on leaf litter decomposition, N addition accelerates decomposition of low lignin litter. In contrast to the response of low-lignin litter, Fog (1988) concluded that: “in recalcitrant substrates the situation is completely different. Here negative effects of the addition of N are much commoner, especially if the lignin content is high, in direct contrast to the case above. Thus, the higher the lignin content, the smaller the amount of N tolerated”. Almost exactly the same results were obtained in a recent comprehensive review with quantitative meta-analysis on the effects of N addition on litter decomposition (Knorr *et al.* 2005a). Moreover, the overall mean response to N addition tended to be positive in short-term studies, but a significant decrease in decomposition rate (17%) was detected in studies lasting longer than two years.

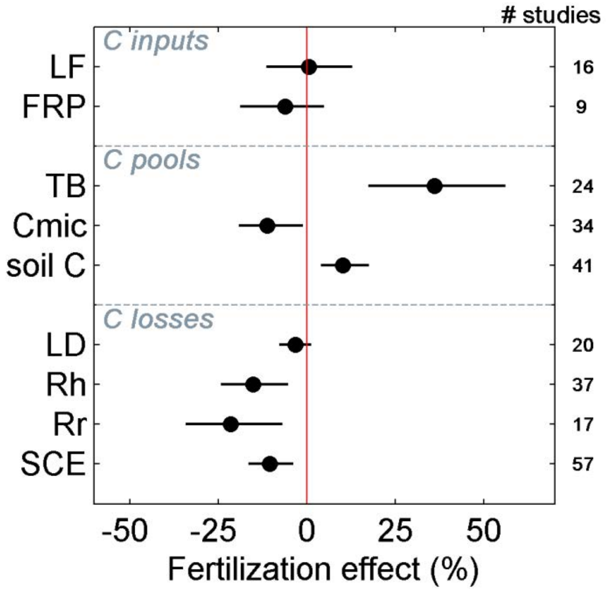


Figure 2.12: Effect of experimental N-addition on various forest C pools and fluxes as calculated by meta-analysis. Positive values indicate that N addition increased the factor, negative values indicate a decrease. Error bars indicate the 95% confidence interval. Data are the weighted means for n data points (n is listed along the righthand axis). Parameters listed are C inputs: litterfall (LF) and fine-root production (FRP), C pools: total tree biomass (TB), microbial biomass (Cmic) and soil C content (soil C), and C losses: litter decomposition (LD), heterotrophic respiration (Rh), root respiration (Rr) and soil CO_2 efflux (SCE). Site information, data and references to all studies included in this analysis are given in the GCME database.

Given that forest litter contains a substantial fraction of low quality litter (litter with high lignin and low N contents that decomposes very slowly) and the clear evidence that low quality litter is negatively affected by N addition, it is to be expected that atmospheric N deposition would typically reduce litter decomposition in forest ecosystems. Our statistical meta-analysis (Rosenberg *et al.* 2000) applied to data from 20 N-manipulation experiments in forests suggested only a small and statistically insignificant decline in leaf litter decomposition rate (Fig. 2.12). However, in agreement with the previous studies, we found that N addition tends to accelerate decomposition in genera producing easily degradable leaf litter (e.g. Acer, Populus, Betula), whereas it clearly reduced decomposition rates in species with more recalcitrant litter (Pinus, Picea, Fagus, Quercus; Fig. 2.13). Moreover, all forests produce large quantities of lower quality litter, such as twigs, branches, and seed pods, which may be even more strongly affected by added N, but data are not available to test this hypothesis.

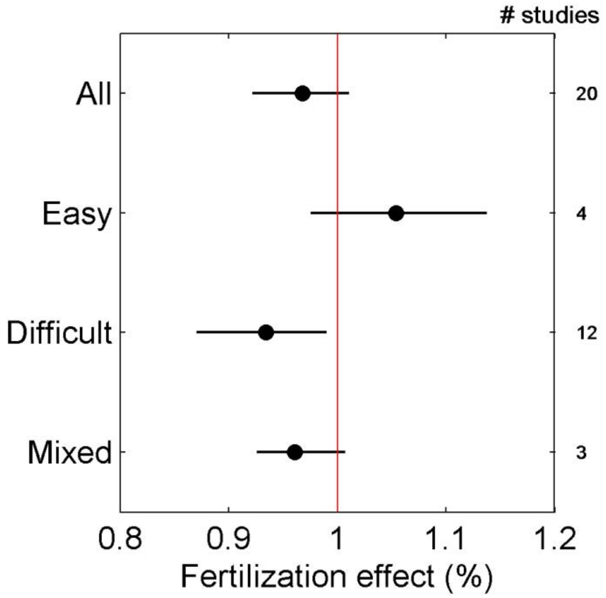


Figure 2.13: Effect of experimental N-fertilization on decomposition of different litter types, as calculated by meta-analysis. Positive values indicate that N addition increased the factor, negative values indicate a decrease. Error bars indicate the 95% confidence interval. Data listed are the weighted means for n data points, which is listed along the y-axis. Listed are all datapoints, easily decomposable litter from Betula, Acer and Populus, litter that is difficult to decompose from Quercus, Pinus, Larix, Picea and Fagus, and forest litter that was not separated between species (Mixed). Site information, data and references to all studies included in this analysis are given in the GCME database.

Heterotrophic respiration is commonly reduced

Respiration in aerobic soil involves the breakdown or organic molecules with, in aerobic soil, CO_2 as the main end-product. Release of CO_2 is therefore commonly used as a proxy for respiratory activity. Here, we define respiration by organisms obtaining their energy from the decomposition of litter and SOM as heterotrophic respiration. Heterotrophic respiration is thus an integrator of decomposition of organic matter in all stages of decomposition and in all soil layers, making its response to N addition more relevant to soil C cycling than that of leaf litter decomposition alone.

To mitigate variation due to methodological differences (Hanson *et al.* 2000; Subke *et al.* 2006), we focus this review on heterotrophic respiration estimates obtained either from lab incubation or with the trenching technique (Hanson *et al.* 2000), both of which physically separate the soil from the root inputs. Our statistical meta-analysis revealed that the average response of heterotrophic respiration to N addition is much more pronounced than that of leaf-litter decomposition alone. Averaged over 36 N-manipulation studies in forest ecosystems, heterotrophic respiration declined by 15% when N was added (Fig. 2.12). Variation among experiments was very high, with responses ranging between a reduction of 57% and stimulation by 63% (Fig. 2.14, left panel). Forests exposed to elevated atmospheric N deposition are also observed to have lower heterotrophic respiration than forests receiving background N deposition (wet deposition < 5.5 kgN ha^{-1} yr^{-1}; Fig. 2.15 left panel). For forests with an NPP around 600 gC m^{-2} yr^{-1}, the reduction amounts to roughly 100 gC m^{-2} yr^{-1} (Fig. 2.15 left panel). The different slopes of the two regressions in Figure 2.15 further suggest that at highly productive sites, where N is unlikely to be the most limiting nutrient, N deposition has a stronger negative effect than at less productive sites, where N immobilization is likely higher and the negative effect on heterotrophic respiration is only marginal.

It can be concluded that both episodic addition of high fertilizer amounts and chronic deposition of small amounts of N induce a decline in heterotrophic respiration in most –but not all– forest ecosystems.

Soil CO_2 efflux response depends on productivity response

Soil CO_2 efflux (SCE) is an important indicator for belowground C cycling (Kutsch *et al.* 2009). Although heterotrophic respiration constitutes a substantial part of SCE, two important C fluxes, related to the presence of roots in soils, differentiate SCE from heterotrophic respiration. First, a major component of SCE is autotrophic in nature (root-, mycorrhizal- and rhizosphere respiration), coupling temporal variation in SCE to that in belowground C allocation and, ultimately, photosynthesis (Ekblad & Högberg 2001; Högberg *et al.* 2001; Sampson *et al.* 2007). Second, rhizodeposition, the transfer of root-derived C compounds to soil, can stimulate microbial activity and thus prime decomposition of SOM (Cheng & Johnson 1998; Kuzyakov 2002; Fontaine *et al.* 2007). This priming mechanism alters heterotrophic respiration, but could not have made a contribution to the heterotrophic respiration measurements presented in the previous section, which were all made in the absence of live roots. As with heterotrophic respiration, there is convincing evidence that SCE declines following N addition, either through fertilization (-10%; Fig. 2.12) or through atmospheric N deposition (Fig. 2.15 right panel). In most studies, this negative effect appears almost instantaneously, while the negative responses persist for years after the addition of nitrogen has ceased (Martikainen *et al.* 1989; Berg & Matzner 1997; Ågren *et al.* 2001). Although the average response of SCE in fertilization experiments was clearly negative, SCE was found to be positively affected by N addition in roughly 25% of the 57 manipulation studies (Fig. 2.14, right panel). A closer look at the experiments where SCE increased following N fertilization revealed that these were mainly studies where N addition has the potential to strongly enhance photosynthesis: very young plantations (<4 years), where N addition may have accelerated canopy development, and CO_2-fumigated forest stands, where extra N helps to sustain the stimulatory CO_2 effect on photosynthesis (Figure 2.14, Ceulemans & Mousseau 1994; Oren *et al.* 2001; Butnor *et al.* 2003). Part of the increased C

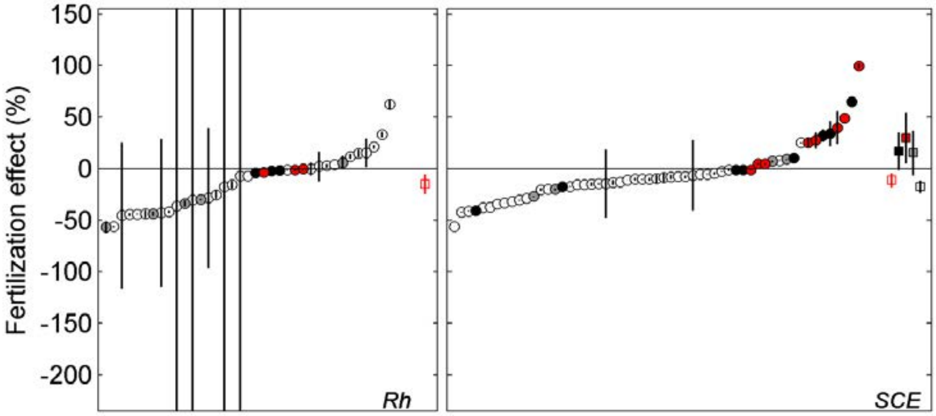


Figure 2.14: Relative effect of N addition on heterotrophic respiration (Rh, left panel) and soil CO_2 efflux (SCE, right panel) in the manipulation experiments included in the statistical meta-analysis (circles) and their overall mean effect size (open red squares). Open black circles are forest stands more than four years old that received no additional CO_2 and their mean is represented by the open black square. Grey symbols are forest stands more than four years old exposed to elevated atmospheric CO_2 concentrations, solid black symbols are forests of four years old or younger, and solid red symbols are forest stands of four years old or younger that also received CO_2 fumigation. Error bars indicate the 95% confidence interval. In these young and/or CO_2 fumigated forests, N deposition favours SCE (positive response), whereas in forests older than five years, N deposition has a negative effect on SCE almost consistently. The effect of N addition on Rh, in contrast, is more consistently negative and does not differ between very young and/or CO_2 fumigated and older forests. Site information, data and references to all studies included in this analysis are given in the GCME database.

availability was probably allocated belowground, stimulating rhizosphere respiration and microbial activity. We thus hypothesize that the increases in SCE observed in a sub-set of studies (Fig. 2.14, right panel) are attributable to enhanced photosynthesis following N enrichment.

Excluding the very young and CO_2-fumigated sites, the average decline in SCE amounted to –17%, roughly 150-200 gC m^{-2} yr^{-1}. Sites receiving more than 50 kgN ha^{-1} yr^{-1} exhibited a stronger decline in SCE (-21%) than sites receiving doses of less than 50 kgN ha^{-1} yr^{-1} which better mimics the effects of atmospheric N-deposition. Nevertheless, the response of SCE in this latter group remained significantly negative (-10%). Per unit N added, this reduction in SCE amounts to 36 gC per gN added. At a small number of sites older than five years and not enriched with CO_2, SCE responded positively (or did not respond) to N addition. At these few sites, we speculate that N might be limiting microbial growth and thus heterotrophic respiration (see section on heterotrophic respiration above).

Soil C increases, microbial biomass decreases

The response of plant-litter inputs (leaf-litter fall and fine-root production) to N addition is variable; both increases and decreases are frequently observed, and on average litter inputs are not affected (Fig. 2.12). In N fertilization experiments, the combination of reduced heterotrophic C losses and unaltered plant-litter inputs results in substantial soil C accumulation (relative increase of 10%; Fig. 2.12), corresponding to 19 gC per gN added.

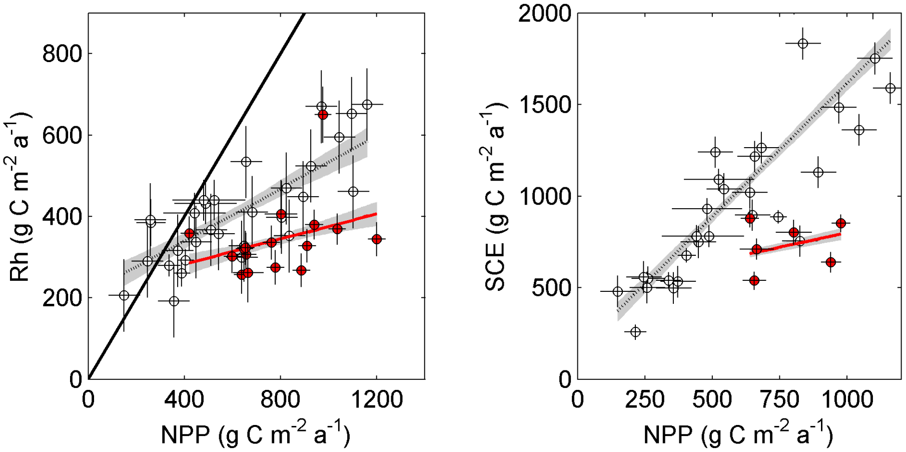


Figure 2.15: Observed annual heterotrophic respiration rates (Rh) as a function of annual biomass production (NPP) in forests exposed to elevated or background N deposition. Left pane: open black symbols and dotted black line (Y = 212 + 0.32 X) represent forests with wet N deposition < 5.5 kgN ha^{-1} yr^{-1} (the current average deposition rate in unaffected forests). Solid red symbols and solid red line (Y = 217 + 0.16 X) reflect forests with N deposition > 5.5 kgN ha^{-1} yr^{-1} (affected by N deposition). The shaded areas surrounding the regression lines represent the 67% confidence intervals (1 SD). The data support the hypothesis that intercepts are equal (p = 0.475) but reject the hypothesis that slopes are equal (p = 0.965). Right pane: observed annual soil CO_2 efflux (SCE) as a function of annual biomass production (NPP) in forests exposed to elevated or background N deposition. Open black symbols and dotted black line (Y = 153 + 1.47 X) represent forests with wet N deposition < 5.5 kgN ha^{-1} yr^{-1} (the current average deposition rate in unaffected forests). Solid red symbols and solid red line (Y = 483 + 0.32 X) reflect forests with N deposition > 5.5 kgN ha^{-1} yr^{-1} (affected by N deposition). The shaded areas surrounding the regression lines represent the 67% confidence intervals (1 SD).

Thus, on average, about half of the reduction in SCE in the manipulation experiments may be due to C accumulating in the soil. This substantial increase in soil C should be interpreted with caution, because most studies report C pool changes only for the uppermost soil layers. In a comprehensive review of results from ^{15}N field experiments, long-term low dose N fertilizer experiments and ecosystem models, de Vries and co-workers (de Vries *et al.* 2009) also reported that N deposition substantially stimulated soil C sequestration. Taking all evidence into account, soil C sequestration in European forests in response to N deposition was estimated to range from 5 to 23 gC gN^{-1} (de Vries *et al.* 2009), agreeing well with our estimate. Sequestration rates of similar magnitudes were also reported in other, more limited multi-site analyses (Hyvönen *et al.* 2008; Pregitzer *et al.* 2008).

The efficiency of soil C storage per unit N deposited declines with the amount of N added or deposited to the forest (Hyvönen *et al.* 2008). This trend is expected, because as N availability increases, a larger fraction of it will be lost to groundwater and atmosphere, and the probability for adverse effects of N saturation increases proportionally.

Caution is, however, warranted when interpreting spatial correlations between N deposition and soil C sequestration across forests (Peter Högberg, personal communication). Within Europe, for example, N-deposition co-varies with natural soil nutrient availability. Human population centres have developed in the more fertile regions with good climate for N mineralization (not too dry, not too cold) and N deposition increased with population. Hence, soil C accumulation may be partly attributable to the more favorable, natural nutrient availability.

In agreement with the declining heterotrophic respiration and SCE, we observe a statistically significant decline (-11%) in microbial biomass (Fig. 2.12). A review on the effects of N deposition on microbial biomass showed that the average decline exceeded 20% in both temperate and boreal forests (Treseder 2008). When excluding very young forests and elevated CO_2 treatments from our analysis, we observe an average reduction of microbial biomass of 16%, in line with the review by Treseder (2008) and with the response of SCE.

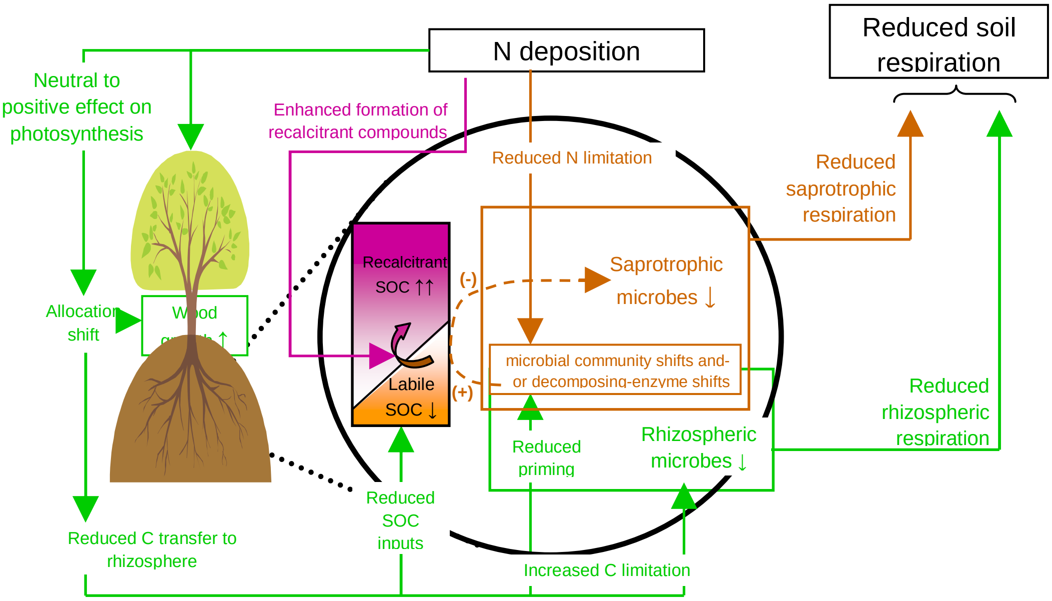
2.3.3.5. Processes underlying the reduction of soil respiration

Empirical evidence thus tells us that various aspects of belowground C cycling are negatively affected by N addition, that these effects are widespread, but that there are also conditions under which N addition accelerates decomposition and soil respiration (severe N-limitation, young forests, elevated CO_2 experiments). The following mechanisms have been put forward in the literature to explain these observations (Box 2.2).

Reduced belowground carbon allocation

Nitrogen is the most important macro nutrient. With the exception of regions where other nutrients are more limiting, a large increase in N availability to trees reduces the need for investment of C in their nutrient-absorbing system. This induces a shift in C allocation in favour of production of aboveground tissues at the expense of the root system (Litton *et al.* 2007). In absolute values our data do not show clear differences in fine root NPP between N-fertilized and control plots (Fig. 2.12). However, the nutrient acquisition system is not limited to the root system and there is abundant evidence that N enrichment spectacularly affects the activity of the rhizosphere, and of mycorrhizal root symbionts in particular (Treseder 2004). In a N-supply gradient study, C allocation to fungal symbionts was the process that responded most to N addition (Högberg *et al.* 2003). Drastic declines in the production of fruiting bodies by mycorrizal fungi (Ruhling & Tyler 1991), in the contribution of mycorrhizae to total microbial biomass (Tietema 1998), in mycorrhizal diversity (see Treseder (2008) for review), in mycorrhizal infection rates and survival (Egerton-Warburton & Allen 2000), and in arbuscular mycorrhizal biomass, hyphal length and storage structures (Johnson 1993; van Diepen *et al.* 2007) have all been reported in response to N addition. These results reflect reduced reliance of trees on fungal symbionts under high N deposition, which underlies the strong reduction in belowground C allocation. The mere reduction in belowground C allocation has been shown to produce a direct negative effect on rhizosphere respiration and thus SCE (Phillips & Fahey 2007). The reduction of C inputs into the soil is an important mechanism via which also decomposition and soil respiration may be altered (Box 2.2).

Box 2.2: conceptual scheme depicting the mechanisms that explain the N-induced response of below-ground C cycling and its variation (see also main text). The effects of N deposition on the saprotrophic system may be related to two, mutually non-exclusive mechanisms: (1) enhanced chemical stabilization of organic matter into compounds recalcitrant to microbial decay (magenta arrows) and (2) shifts in microbial enzyme synthesis and activity towards preferential decomposition of labile, energy-rich compounds, coupled with reduced decomposition of recalcitrant substrates (brown arrows). In the presence of roots, N-effects via altered rhizosphere C inputs (green arrows) influence rhizosphere respiration, but also C cycling through the saprotrophic system. Under increasing N availability, wood production is typically promoted at the expense of belowground C allocation, reflecting the reduced need for an elaborate nutrient acquisition system. Declining availability of energy-rich compounds, combined with excess nitrogen affects the functioning of the microbial community, producing a different enzyme spectrum. The reduced amount of C available for mineralization results in further reduced saprotrophic biomass, and the associated increase in net N mineralization thus aggravates the above-mentioned, direct negative N effects on decomposition of recalcitrant soil organic matter.



Although retarded belowground C cycling in response to N addition is the general rule in forests, there are exceptions where soil respiration is enhanced by N enrichment. In severely N-limited forests, such as in the northern boreal zone, microbial biomass may grow following N addition, resulting in enhanced enzyme production and increased soil respiration. More commonly, however, N deposition may stimulate canopy photosynthesis. In N-limited, open forests, as well as in very young, accruing plantations, N deposition can accelerate canopy closure and thus enhance light interception and photosynthesis. In N-limited systems (e.g. boreal forests or CO_2-enriched trees), leaf-level photosynthesis may also be enhanced by N addition. This increase in photosynthesis may offset the relative decline in belowground C allocation, such that more labile C enters the soil, fueling not only rhizosphere microbes but also saprotrophic microbes and their decomposition of recalcitrant SOC. Hence, although negative responses to N deposition and addition clearly dominate the literature, positive responses do occur. The scheme presented in this Box 2.2 is, however, not valid for N-saturated systems where adverse effects such as acidification, cation leaching and altered vegetation composition may induce very different responses.

Mycorrhizal root symbionts have the capacity to degrade organic matter (Gadgil & Gadgil 1971) and their decline would therefore be expected to directly reduce decomposition. Additionally, mechanisms exist that are potentially even more relevant for the response of decomposition to the shift in belowground C allocation. Fine roots and especially mycorrhizal hyphae exude substantial amounts of soluble organic compounds (Högberg & Högberg 2002) and these compounds serve as C and energy sources for saprotrophic organisms that subsequently decompose organic matter in search of nutrients (van Groenigen *et al.* 2006). This mechanism, known as the priming effect, is widely accepted to exert a major control over soil organic matter decomposition (Wu *et al.* 1993; Cheng & Johnson 1998; Kuzyakov *et al.* 2000; Fontaine *et al.* 2004; Hoosbeek *et al.* 2004; Subke *et al.* 2004). By supplying less substrate to the decomposers, the N-induced reduction of belowground C allocation can thus be expected to impede decomposition of SOC.

The reduction in belowground C allocation thus contributes to the reduction in SCE (on average 36 gC per gN; section on SCE responses), but cannot explain the substantial increase in soil C (on average, 19 gC per gN; section on soil C responses). Such large soil C accumulation can only be explained by other mechanisms directly reducing the activity of saprotrophs.

Shifts in saprophytic community structure or function

There are multiple mechanisms through which alteration of microbial community structure or function may affect decomposition of soil organic matter. First, addition of N could make saprotrophic organisms switch from decomposing N-containing recalcitrant SOM to energy-rich rhizodeposits (preferential substrate theory (Merckx *et al.* 1987; Lekkerkerk *et al.* 1990; Liljeroth *et al.* 1990; Fontaine *et al.* 2004)). Alternatively, less efficient microbial populations (less C assimilated in biomass and more CO_2 released per gram litter decomposed) that require little N could be outcompeted by populations that are currently nitrogen limited, but are more efficient (Ågren *et al.* 2001). Ågren and co-workers postulated that such a microbial community shift towards more efficient, N-demanding species could explain the observed reductions in SCE. Their model would also be consistent with the observed increases in soil C stocks and reduced microbial biomass if the new microbial populations also produced more recalcitrant organic molecules.

In the early 1960’s Mangenot & Reymond (1963) had observed that by adding N to sawdust, several species of wood-decomposing basidiomycetes lose their competitive ability, with different species dropping out at sequentially higher N levels. There is ample recent evidence that N addition to forest soils induces such shifts in microbial community composition (Compton *et al.* 2004; Frey *et al.* 2004; Feng *et al.* 2008; Treseder 2008).

Whatever the exact mechanism explaining the reduced decomposition of recalcitrant SOC or of litter in final stages, alterations in the activity or expression of enzymes involved in the decomposition process are likely to play a key role. Cellulose decomposing – and phosphate acquiring enzymes were reported to increase following N fertilization, especially in N-limited ecosystems (Carreiro *et al.* 2000; Sinsabaugh *et al.* 2005; Keeler *et al.* 2009), often accelerating cellulose decomposition (Carreiro *et al.* 2000; Sjoberg *et al.* 2004). In contrast, lignin-degrading enzymes, such as phenol oxidases and peroxidases are frequently down-regulated ((Fog 1988; Carreiro *et al.* 2000; Saiya-Cork *et al.* 2002; DeForest *et al.* 2004; Frey *et al.* 2004), but see (Keeler *et al.* 2009)). Several white-rot fungi were found not to synthesize their lignin-degrading enzymes in the presence of low molecular weight N compounds (Keyser *et al.* 1978; Tien & Myer 1990; Berg & Matzner 1997), but this response varies among species (Fog 1988; Waldrop & Zak 2006). One potential reason for this reduced synthesis of lignin-degrading enzymes is that lignolitic fungi have low efficiency and growth rate, and are therefore likely to be out-competed by other microbes, resulting in reduced ligninase activity and, hence, lignin degradation (Mangenot & Reymond 1963; Ågren *et al.* 2001; DeForest *et al.* 2004). Many easily decomposable substrates are embedded in the lignin matrix within fresh plant litter (Fog 1988). Thus, by degrading lignin more slowly, decomposition of all compounds within the lignin matrix is reduced (DeForest *et al.* 2004).

These shifts in the expression and/or activity of various enzymes are thus likely to explain the observation that more labile litter types or compounds (such as cellulose) decompose faster following N addition, especially in the short term (Fog 1988; Knorr *et al.* 2005a). In the long-term, the reduced production or activity of specific enzymes involved in the degradation of more recalcitrant compounds may be responsible for the negative effect of N addition. Although the concepts of altered microbial community structure and enzymatic spectra are appealing, Keeler et al (2009) were not able to find any correspondence between the N-addition effects on decomposition rates and on the activities of six key-enzymes involved in decomposition. Moreover, despite the strong evidence that lignin degrading enzymes are suppressed by N addition, this mechanism may only be valid in organic surface layers; in mineral soils neither lignin nor lignin-derivatives accumulate in soils exposed to N addition (e.g. Feng *et al.* 2008). It is thus obvious that the molecular transformations in soils and the role of exo-enzymes in the degradation of the produced recalcitrant compounds are far from elucidated.

The role of stabilizing mechanisms

A major fraction of SOM is chemically or physically protected from microbial decay (Oades 1984). If N addition were to interact with these stabilization mechanisms, a major effect on decomposition and heterotrophic respiration could be expected. One hypothesis often brought forward to explain the reductions in decomposition or heterotrophic respiration is abiotic stabilization of SOM (Fog 1988; Berg & Matzner 1997). There is evidence of direct chemical incorporation of added nitrogen into organic matter, producing heterocyclic forms of N (indoles and pyroles) (Thorn & Mikita 1992) or phenolic compounds polymerized by N-bridges (Nömmik & Vahtras 1982), two groups of compounds that are highly resistant to degradation by microbial enzymes. By chemically protecting part of the organic matter available for decomposition, these abiotic reactions could thus reduce decomposition and enhance C sequestration (see also Fog 1988; Berg & Matzner 1997). However, ^{15}N NMR spectrometry indicates that most of the retained N is in the amide form, indicating dominance of biotically mediated formation of recalcitrant SOM (Clinton *et al.* 1995; Aber *et al.* 1998; Burdon 2001; Sutton & Sposito 2005), possibly in response to an N-induced shift in microbial community composition. Although this downplays the role of abiotic reactions postulated to play an important role (Fog 1988; Berg & Matzner 1997), it does not rule them out.

Incorporation of SOM within soil aggregates could also constitute an important physical stabilization mechanism. Aggregate formation correlates positively with rhizodeposition and microbial biomass and -activity, which all decline in response to N deposition. Hence, aggregate formation is not expected to explain the reduced SOM cycling.

Finally, soil acidification is also a stabilization mechanism through which decomposition of plant litter and SOM could be reduced. Given that soil pH is crucial to enzyme functioning (Fog 1988), acidification could have a detrimental effect on microbial activity and thus on decomposition of SOM. Especially after chronic N deposition, poorly buffered soils where nitrification occurs will tend to exhibit lower pH (Fog 1988; Bowden *et al.* 2004). Nonetheless, even where soil acidification does not occur, negative effects of N addition are frequently observed (Fog 1988). Hence, acidification may aggravate the response, but not explain it completely.

2.3.3.6. Implications

Both the N-fertilization manipulation experiments and the comparison between forest ecosystems subjected to background versus elevated N deposition provide evidence for a decline in SCE and heterotrophic respiration of the same order of magnitude as net ecosystem productivity (the actual net CO_2 uptake or release by ecosystems) reported elsewhere (Janssens *et al.* 2001; Ciais *et al.* 2005; Luyssaert *et al.* 2007; Piao *et al.* 2008; Schulze *et al.* 2009). It is thus not surprising that Magnani and co-workers (2007) identified N deposition as a better determinant of forest net ecosystem productivity than climate or site productivity. Networks of ecosystem CO_2 flux measurements are commonly used to construct greenhouse gas balances (Schulze *et al.* 2009) or to extract information on the determinants of these fluxes (Janssens *et al.* 2001; Ciais *et al.* 2005; Luyssaert *et al.* 2007; Magnani *et al.* 2007; Reichstein *et al.* 2007; Piao *et al.* 2008). The fact that N deposition often reduces heterotrophic respiration and SCE by such vast amounts implies that syntheses of CO_2 flux networks have to take N deposition into account when analyzing effects of e.g. climate on C cycling.

Eventually, all forests receiving elevated N deposition for decades or longer, will run into N saturation, a state in which the forest N cycle is no longer closed (Aber *et al.* 1998). Although the exact effects remain unclear, a decrease in productivity could be anticipated through the loss of base cations and lower phosphorus availability (Schulze *et al.* 1989; Högberg *et al.* 2006; de Vries *et al.* 2007). Long-term responses may thus differ from the overall response reported here.

To date, N deposition has been elevated mainly in regions with relatively young, nutrient-rich soils, where N is often limiting plant growth (Eastern US, Europe, China). It remains to be tested whether N deposition will affect C cycling (stimulate wood growth and retard SOM decomposition) similarly in tropical (but also other) regions with older, severely weathered soils, where N may not be the most limiting nutrient (Matson *et al.* 1999). If N enrichment were to slow down decomposition, then nutrient immobilization in accumulating SOC could negatively affect tropical forest productivity. Because 70% of the global primary productivity is realized in the tropics, a shift in N-deposition towards these regions might alter the global C-balance differently to that which might be expected by extrapolating the responses in temperate regions. Current understanding of C-nutrient interactions in severely weathered soils or soils with shortages of other nutrients remains extremely limited and N-addition manipulation studies in these areas too rare to allow statistical meta-analysis

The evidence for altered belowground C cycling presented here highlights the need to incorporate N cycling and N deposition into terrestrial C cycle models; not only the potential N effects on productivity and interactions with increasing atmospheric CO_2 levels (Bonan 2008; Zaehle *et al.* 2010), but especially the negative effects on decomposition, belowground C allocation and the coupling of C and N cycling in ecosystems. To do this properly, more work is needed to fully understand the relative importance of the different mechanisms at play (Box 2.2), on the long-term responses to chronic N deposition, as well as on the responses in tropical areas. Given that the size of the N-induced inhibition of below-ground respiration is of the same order of magnitude as the forest C sink, a better understanding of N deposition effects should be a strong, future research priority.

2.3.4. Simple additive effects are rare: a quantitative review of plant biomass and soil process responses to combined manipulations of CO_2 and temperature.

This chapter is in press at Global Change Biology:

Wouter I.J. Dieleman, Sara Vicca, Feike A. Dijkstra, Frank Hagedorn, Mark J. Hovenden, Klaus S. Larsen, Jack A. Morgan, Astrid Volder, Claus Beier, Jeffrey S. Dukes, John S. King, Sebastian Leuzinger, Sune Linder, Yiqi Luo, Ram Oren, Paolo De Angelis, David Tingey, Marcel R. Hoosbeek, Ivan A. Janssens. 2012. Simple additive effects are rare: a quantitative review of plant biomass and soil process responses to combined manipulations of CO_2 *and temperature.*

2.3.4.1. Abstract

In recent years, increased awareness of the potential interactions between rising atmospheric CO_2 concentrations ([CO_2]) and temperature has illustrated the importance of multi-factorial ecosystem manipulation experiments for validating Earth System models. To address the urgent need for increased understanding of responses in multi-factorial experiments, this paper synthesizes how ecosystem productivity and soil processes respond to combined warming and [CO_2] manipulation, and compare with those obtained in single factor [CO_2] and temperature manipulation experiments.

Across all combined elevated [CO_2] and warming experiments, biomass production and soil respiration were typically enhanced. Responses to the combined treatment were more similar to those in the [CO_2]-only treatment than

to those in the warming-only treatment. In contrast to warming-only experiments, both the combined and the CO_2-only treatments elicited larger stimulation of fine root biomass than of aboveground biomass, consistently stimulated soil respiration, and decreased foliar nitrogen (N) concentration. Nonetheless, mineral N availability declined less in the combined treatment than in the [CO_2]-only treatment, possibly due to the warming-induced acceleration of decomposition, implying that progressive nitrogen limitation (PNL) may not occur as commonly as anticipated from single factor [CO_2] treatment studies. However, because the warming induced stimulation of decomposition might be transient, long-term effects on PNL remain uncertain.

Responses of total plant biomass, especially of aboveground biomass, revealed antagonistic interactions between elevated [CO_2] and warming, *i.e.* the response to the combined treatment was usually less-than-additive. This implies that productivity projections might be overestimated when models are parameterized based on single factor responses.

Our results highlight the need for more (and especially more long-term) multifactor manipulation experiments. Because we found a stronger similarity in response patterns between combined treatments and single factor [CO_2] treatments, our results also suggest that projected responses to global warming in

Earth System models should not be parameterized using single factor warming experiments.

2.3.4.2. Introduction

Increasing concentrations of greenhouse gases in the atmosphere are expected to increase global surface temperatures in the coming decades. The latest IPCC report projects a warming of 0.2 °C per decade in the next two decades, and of 0.6 – 4 °C by the end of the 21st century (relative to observations in 1980 - 1999). At the same time, increased variability and regional changes in precipitation patterns are very likely and the atmospheric CO_2 concentration ($[CO_2]$) may continue to rise, up to 600 - 1550 ppm by the end of this century (IPCC 2007). These climatic and atmospheric changes influence ecosystem functions, whereby uptake or emission of greenhouse gases constitute an important climate-controlling feedback mechanism (Friedlingstein *et al.* 2006; Van Groenigen *et al.* 2011).

An important approach increasing our understanding of the effects of global changes on terrestrial ecosystems is manipulation of these driving variables in ecosystem experiments (e.g. Beier *et al.* 2004; Rustad 2008). Apart from providing enhanced understanding of ecosystem responses to global change, the data provided by these manipulation experiments can help to parameterize and evaluate Earth System models, as well as ecosystem models that predict future ecosystem functioning and ecosystem services. Because multi-factor manipulation experiments test the anticipated changes in climate and $[CO_2]$ simultaneously as opposed to single factor manipulative experiments, these can be expected to yield more relevant benchmarking data (e.g. Shaw *et al.* 2002; Dukes *et al.* 2005; Larsen *et al.* 2011).

Multifactor manipulation experiments are more expensive than single factor experiments, because the number of study plots double for each additional factor involved. Therefore these experiments are still scarce (14 sites in this analysis, see also Rustad, 2008), and most ecosystem models are therefore currently parameterized and tested with results obtained in single factor experiments. This paper addresses this restriction by synthesizing the published effects of warming and elevated atmospheric CO_2 concentration, applied separately and in combination, on terrestrial ecosystem biomass production (main terrestrial carbon (C) pool taking up atmospheric CO_2), soil respiration (main flux releasing CO_2 to the atmosphere), and nitrogen (N) mineralization (main limiting factor of plant CO_2 uptake in most natural northern ecosystems).

2.3.4.3. Methods

For an extensive description of the meta-analysis methods, we refer to chapter 2.2

We collected data from 150 manipulation experiments across a range of different ecosystems and climates, reporting data on total biomass, aboveground biomass, root biomass, fine root biomass, soil C, heterotrophic respiration, soil respiration,

Table 2.8: Sites that applied single factor CO_2 and warming treatments, and a simultaneous CO_2 and warming treatment.

SITE NAME	COUNTRY	SYSTEM	TREATMENTS	SOURCE REFERENCES
Natural/Outdoor systems				
Brandjberg	Denmark	temperate heathland	CO_2xWarmingxDrought	(Andresen *et al.* 2009; Larsen *et al.* 2011; Selsted *et al.* 2011)
Flakaliden	Sweden	Picea forest	CO_2xWarming	(Comstedt *et al.* 2006; Kostiainen *et al.* 2009)
Ginninderra	Australia	Phalaris aquatica swards	CO_2xWarming	(Volder *et al.* 2007)
Jasper Ridge	USA	California annual grassland	CO_2xWarmingxFertilizationxWater	(Dukes *et al.* 2005)
Mekrijarvi	Finland	Pinus forest	CO_2xWarming	(Pajari 1995; Niinistö *et al.* 2004)
Oak Ridge grasslands	USA	Model grassland	CO_2xWarmingxWater	(Wan *et al.* 2007; Kardol *et al.* 2010)
Oak Ridge maples	USA	Acer rubrum, Acer saccharum	CO_2xWarming	(Edwards & Norby 1999; Norby *et al.* 2000; Wan *et al.* 2004)
PHACE	USA	Northern mixed-grass prairie	CO_2xWarming	(Dijkstra *et al.* 2010; Morgan *et al.* 2011)
Stillberg	Switzerland	Treeline larch and pine system	CO_2xWarming	(Hagedorn *et al.* 2010; Dawes *et al.* 2011)
TasFACE	Australia	Species-rich temperate grassland	CO_2xWarming	(Hovenden *et al.* 2008; Pendall *et al.* 2011)
Controlled mescocosms/phytotrons				
Duke Phytotron	USA	Pinus and Robinia seedlings	CO_2xWarmingxFertilization	(Larigauderie *et al.* 1994; King

				et al. 1996; Uselman *et al.* 2000)
NERC Ecotron	UK	Grassland model ecosystem	CO_2xWarming	(Kandeler *et al.* 1998)
Risø	Denmark	Pisum sativum	CO_2xWarming	(Gavito *et al.* 2003)
USEPA	USA	Pseudotsuga seedlings	CO_2xWarming	(Lin *et al.* 2001; Olszyk *et al.* 2003; Tingey *et al.* 2006)

nitrogen availability and foliar N concentration, resulting in 821 entries for the meta-analysis. General site information, source references and sampling methods are described in the GCME database.

In addition to the typical meta-analysis in which all available data were used, we also performed a more robust comparison of the single factor and combined factor manipulation experiments by taking into account only those experiments where all three treatments were tested simultaneously: elevated CO_2 only, warming only, and combined CO_2-enrichment + warming. Experiments included in this analysis are listed in Table 2.8.

2.3.4.4. Results and discussion

What single factor experiments revealed

Because the effects of single factor elevated [CO_2] and elevated temperature are relatively well understood and have previously been synthesized (Ceulemans & Mousseau 1994; Curtis & Wang 1998; Rustad *et al.* 2001; Nowak *et al.* 2004; Pendall *et al.* 2004; Ainsworth & Long 2005; Norby *et al.* 2005; de Graaff *et al.* 2006; Hyvönen *et al.* 2007; Dieleman *et al.* 2010; Way & Oren 2010; Wu *et al.* 2011; Elmendorf *et al.* 2012), we will only briefly touch upon these single factor manipulation effects.

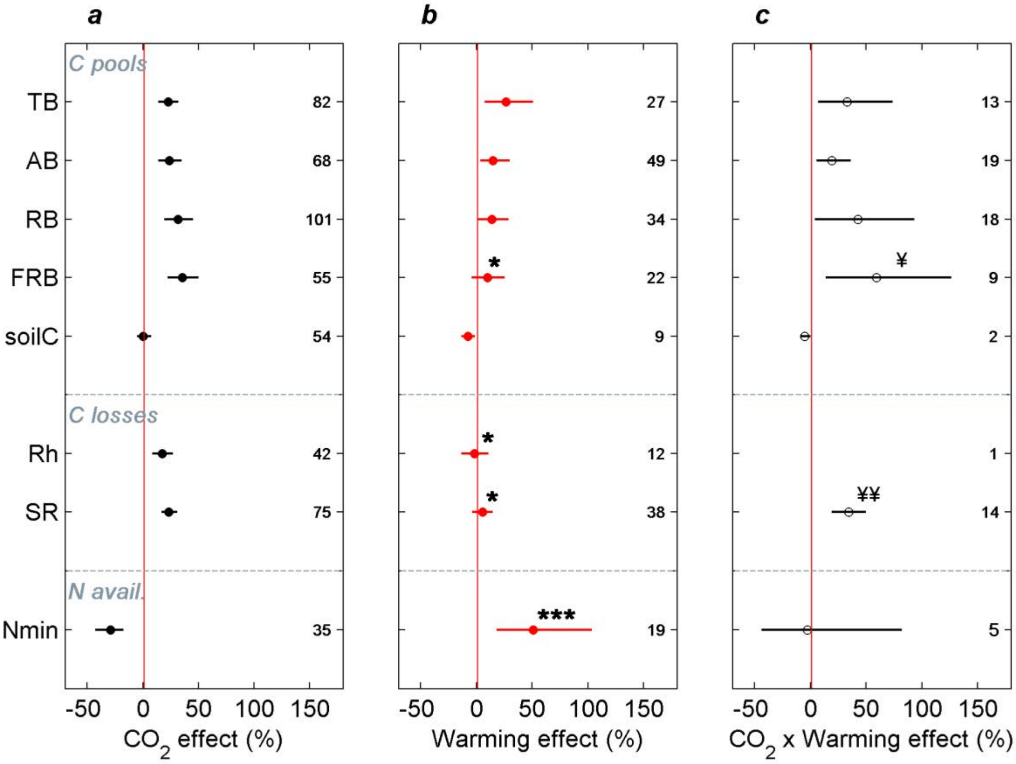


Figure 2.16: Overall meta-analysis effect sizes for the elevated CO_2 (a), elevated temperature (b) and the combined elevated CO_2 and temperature treatments (c) reported as a percentage change relative to the control. Data listed are total biomass (TB), aboveground biomass (AB), root biomass (RB), fine root biomass (FRB), soil C content (soilC), heterotrophic respiration (Rh), soil respiration (SR) and mineral nitrogen availability (Nmin). Positive values indicate a positive treatment effect, negative values indicate a decrease. Error bars represent the 95% confidence interval. Data are the weighted means for n data points. The number of studies is given along the Y-axis. Significant differences in the response to CO_2 enrichment versus the warming response are indicated (* indicates differences with the CO_2 responses, ¥ indicates differences with the warming responses. * or ¥ indicates a significant difference at $P<0.05$; ** or ¥¥ indicates a significant difference at $P<0.01$, *** or ¥¥¥ indicates a significant difference at $P<0.001$).

Elevated [CO_2] increases plant photosynthesis and growth, although responses can be limited in mature tree stands and nutrient-limited systems (Körner 2006), and tend to decline with the duration of the experiment (Leuzinger *et al.* 2011). In many ecosystems, the indirect [CO_2] effect via reduced stomatal conductance and subsequent water savings (Volk *et al.* 2000; Morgan *et al.* 2004a) could be much more sustainable than the direct [CO_2] effect on photosynthesis (Holtum & Winter 2010). As a consequence of the enhanced plant production, the increased demand for nutrients stimulates belowground C allocation and fine root and mycorrhizal growth (Fig. 2.16a) (Rogers *et al.* 1994; Curtis & Wang 1998; e.g. Ceulemans *et al.* 1999; de Graaff *et al.* 2006; Litton *et al.* 2007; Dieleman *et al.* 2010). The associated increase in fine root turnover and rhizodeposition generally enhances substrate availability to soil organisms (Dieleman *et al.* 2010), whose greater biomass and/or activity may stimulate the decomposition of organic material (Zak *et al.* 2000; Heath *et al.* 2005; Dijkstra & Cheng 2007; Fontaine *et al.* 2007; Hagedorn *et al.* 2008; Kuzyakov 2011). Results of the current meta-analysis support this pattern: heterotrophic- and total soil respiration generally increased under [CO_2] enrichment (Fig. 2.16a). The simultaneous increase in soil C inputs and soil C losses under elevated [CO_2] leaves soil C stocks largely unchanged (Schlesinger & Lichter 2001; Dieleman *et al.* 2010) (Fig. 2.16a, but see Hungate *et al.* 2009, who showed that various meta-analyses indicated increased soil C in N fertilized ecosystems), confirming that elevated [CO_2] mainly accelerates soil C cycling in terrestrial ecosystems (Lukac *et al.* 2009).

As a consequence of increasing plant and microbial biomass stocks in elevated [CO_2], more nutrients are immobilized, which may result in progressive N limitation (PNL, for concept see Oren *et al.* 2001) (Hungate *et al.* 2003; Luo *et al.* 2004) in unfertilized and infertile ecosystems. Our meta-analysis supports this hypothesis, showing a substantial reduction in mineral N availability (Nmin; NO_3^- and NH_4^+ concentration) in response to increased [CO_2] (Fig. 2.16a), which suggests either a gradual depletion of soil N levels, or a more conservative use of N in an increasingly more closed N cycle. In general, plant biomass and soil C fluxes thus respond positively to elevated [CO_2] in manipulation experiments, but nutrient availability is expected to constrain this stimulation in the long run.

In contrast to elevated [CO_2], which affects different terrestrial ecosystems quite uniformly at the stomatal level, resulting in increased photosynthesis, biomass production, and resource demands, warming is a more complex driver that directly affects multiple processes (e.g. photosynthesis, respiration, evapotranspiration, N mineralization) in various direct as well as indirect ways. Plant productivity, for example, can be influenced by warming directly through accelerated reaction rates, cell division and elongation, but is also affected indirectly through altered water and nutrient availabilities or a lengthening of the growing season (Jarvis & Linder 2000; Saxe *et al.* 2001). In addition, optimal growth temperatures differ between biomes at different latitudes, which will largely determine the responsiveness of different ecosystems to different degrees of warming (Way & Oren 2010).

This greater complexity of the warming response results in average response patterns that differ strongly from those to [CO_2]-enrichment, not only in size and statistical significance, but especially in the direction of the responses. Firstly, N availability declines substantially under elevated [CO_2], while warming typically stimulates nutrient availability via enhanced net N mineralization rates (Rustad *et al.* 2001; Pendall *et al.* 2004; Hyvönen *et al.* 2007) (Fig. 2.16a,b). Secondly, in contrast to elevated [CO_2] experiments, higher temperatures enhance aboveground biomass, but do not stimulate (fine) root biomass (Fig. 2.16b) (Way & Oren 2010). This apparent difference in the impact on biomass distribution patterns may be partly due to the increased N availability in warming experiments (Fig. 2.16b). Higher nutrient availability reduces the need to develop an elaborate belowground nutrient acquisition system (Melillo *et al.* 2011). A third striking difference is the lack of stimulation of soil respiration in the warming experiments (Fig 2.16b). Whereas elevated [CO_2] typically increases soil respiration (Zak *et al.* 2000; Dieleman & Janssens 2011; Selsted *et al.* 2011) (Fig. 2.16a), soil respiration does not show a consistent long-term response to warming (Fig. 2.16b). Although soil respiration generally increases when temperature rises on a short time scale (Rustad *et al.* 2001), several mechanisms can prevent a persistent positive warming effect on microbial and soil respiration (Davidson & Janssens 2006).

Particularly important in this regard are depletion of labile soil organic matter pools following extended stimulation of microbial decomposition during earlier phases in the warming experiments (Kirschbaum 2004; Eliasson *et al.* 2005; Knorr *et al.* 2005b; Hartley *et al.* 2007; Bradford *et al.* 2008b), warming-induced water limitation of microbial activity (Suseela *et al.* 2012), and thermal acclimatization of root and/or microbial respiration (Atkin & Tjoelker 2003; Vicca *et al.* 2010).

Elevated [CO_2] and warming thus elicit very different ecosystem responses, but in situ these two global change factors are changing concurrently. Therefore, combined [CO_2] enrichment and warming experiments are crucial to test whether ecosystem processes in a warmer, [CO_2]-enriched world will mirror those observed in warming, those in elevated [CO_2], or whether their responses will be additive, antagonisitc or even synergistic.

Average responses to combined CO_2 and warming

To date, combined warming and [CO_2] enrichment studies have been rare and only few C-cycle related variables were reported for at least five manipulation experiments (Fig. 2.16c). Because of this we performed a more extensive analysis on response variables: a first analysis based on conventional meta-analysis with weighted effect estimates (Fig. 2.16c), and a second analysis based on histograms and median effect estimates to study the distribution of effect sizes in our dataset (Fig. 2.17).

For total, aboveground and belowground biomass, the weighted meta-analysis estimates of the responses to elevated [CO_2] and warming were considerably higher compared to the median effect estimates (Fig. 2.17). As meta-analysis gives

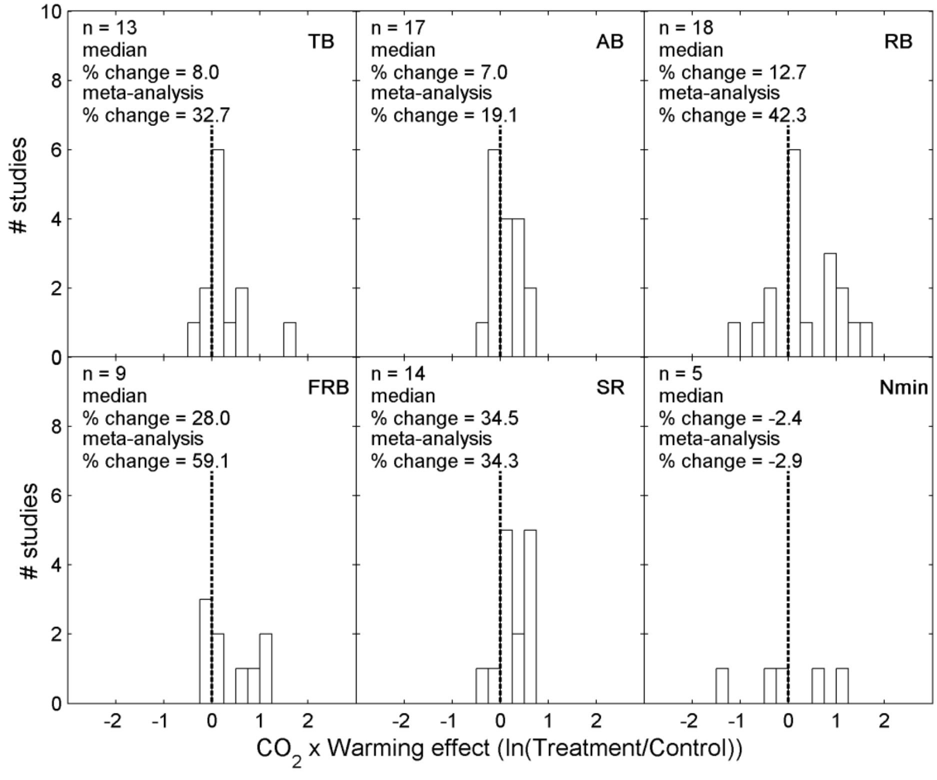


Figure 2.17: Histograms for the combined warming and elevated CO_2 treatment effect on total biomass (TB), aboveground biomass (AB), root biomass (RB), fine root biomass (FRB), soil respiration (SR) and mineral N availability (Nmin). The number of studies, the median effect estimates and the meta-analysis effect estimates are given for each dataset.

more weight to larger studies (*i.e.* studies with more treatment replicates), this indicates that larger studies tended to report larger responses. However, while the median effect estimates were smaller, also the number of studies reporting positive responses was consistently larger than the number of studies reporting negative responses (Fig. 2.17). Hence, a positive effect of a combined warming and elevated [CO_2] treatment on biomass production was supported by both analyses. In addition, the trend for a larger belowground biomass response compared to the aboveground biomass response was also apparent in both analyses. The increased allocation to belowground biomass translated into a strong positive fine root biomass response in both analyses (Fig. 2.17).

Both the median effect estimate and the meta-analysis estimate (Figs. 2.16c and 2.17) indicated a stimulated soil respiration in response to combined warming and elevated [CO_2] treatment. Indeed, out of 14 studies, only two reported a negative response to the combined treatment, adding confidence to the observation of a consistently higher soil respiration rate in a warmer climate and higher [CO_2]. Effects on mineral N availability, on the other hand, were very variable (Fig. 2.17), from very negative to very positive, suggesting a large dependence on site-specific circumstances.

To summarize, effects of combined elevated [CO_2] and warming on plant biomass compartments tended to be variable, but nevertheless positive, with a tendency for greater increase in C allocation to belowground biomass. Feedbacks related to availability of nutrients might be part of the explanation, but we could not conclusively test this mechanism. Most convincing, however, is the consistent increase in soil respiration in the vast majority of experiments. While our results thus suggest a consistent increase of C release to the atmosphere, we reported relative changes here, precluding inferences on the actual C balance. Our results are consistent with a study comparing four ecosystem models that found that combined [CO_2] and warming treatment stimulated net primary production (NPP) and decomposition (Rh) (Luo *et al.* 2008). However, these authors found that combined [CO_2] and warming generally resulted in a net increase of C storage in a range of different terrestrial ecosystems, suggesting that effects on plant net primary production and soil C inputs are proportionally larger than effects on decomposition of soil organic matter. The currently available data from combined [CO_2] and warming experiments is, however, too small to test this model outcome.

Which factor dominates response to combined CO_2 and warming?

Direct comparison of the average meta-analytical effect estimates for combined warming and CO_2-fumigation (as shown in Fig. 2.16c) with those for single factor manipulation (Fig. 2.16a,b) was difficult, because of the low number of experiments combining elevated [CO_2] and warming treatments as opposed to a multitude of single factor experiments (14 *versus* 130 experimental sites). Therefore, we performed the meta-analysis taking into account only those ecosystem manipulation experiments that tested all three treatments (*i.e.* [CO_2] only, warming only and the combined treatment, hereafter 'paired meta-analysis') (Fig. 2.18, Table 2.8). Responses to warming-only and [CO_2]-only treatments (Fig. 2.18a,b) were very similar to those in the much larger, comprehensive dataset (Fig. 2.16a,b), indicating that the experiments used in this more robust, but restricted paired meta-analysis provided a representative sample.

A first observation is that the uncertainties in the combined treatment yielded by the meta-analysis, tended to be larger than those in the single factor experiments (error bars in Fig. 2.18c *versus* those in 2.18a,b). In other words: responses to the combined treatment were much less consistent across experiments. Secondly, across all tested variables, the response pattern in the combined treatment appeared to be more similar to that of the [CO_2]-only treatment than to the warming-only treatment: root biomass (+41% in the combined treatment) and especially fine root biomass (+58%) were stimulated more than aboveground biomass (only +15%), and soil respiration increased substantially (Fig. 2.18). This suggests that, averaged over all multifactor manipulation experiments, [CO_2] manipulation affected ecosystem carbon cycling more strongly than warming. The impact of CO_2 enrichment may have been greater because the degree of CO_2 alteration in the experiments was typically larger in relative terms than the imposed temperature change (*i.e.* [CO_2] is often (nearly) doubled, while temperature treatments generally remain within the temperature range plants experience in the current climate). This is, however, not a drawback or artifact,

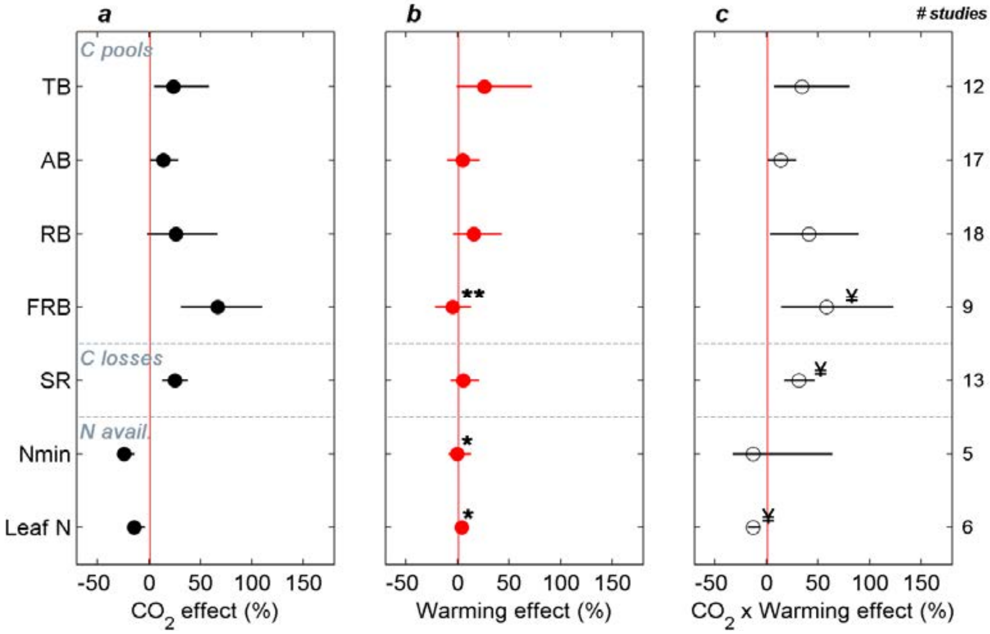


Figure 2.18: Meta-analysis effect sizes for experiments where all three treatments were tested simultaneously: elevated CO_2 (a), elevated temperature (b) and the combined elevated CO_2 and temperature treatment (c), reported as a percentage change relative to the control. Data listed are total biomass (TB), aboveground biomass (AB), root biomass (RB), fine root biomass (FRB), soil respiration (SR), mineral nitrogen availability (Nmin) and foliar N content (Leaf N). Positive values indicate a positive treatment effect, negative values indicate a decrease. Error bars represent the 95% confidence interval. Data are the weighted means for n data points. The number of studies is given along the Y-axis. Significant differences in the response to CO_2 enrichment versus the warming response are indicated (* indicates differences with the CO_2 responses, ¥ indicates differences with the warming responses. * or ¥ indicates a significant difference at $P<0.05$; ** or ¥¥ indicates a significant difference at $P<0.01$).

because the much larger relative increase in [CO_2] than in temperature is consistent with the projected future scenarios. As mentioned before, the effect of a warming treatment will depend on optimal growth temperature and the magnitude of the treatment relative to the prevailing temperatures at a particular site (Way & Oren 2010). That is why for example in high latitude/altitude ecosystems, warming could become proportionally more important than [CO_2] increases.

The similarity between the response to the combined treatment and that to the [CO_2]-only treatment, as suggested by the general mean response pattern in figure 2.18, is not that clear when looking across individual experiments (Fig. 2.19). For aboveground and fine root biomass, the response to the combined treatment was clearly more similar to the [CO_2]-only treatment rather than to the warming-only treatment (Fig. 2.19). The dominance of the [CO_2] impact in the response of fine root biomass to the combined treatment may explain why the response of soil respiration to the combined treatment also agrees better with [CO_2] alone than to warming alone, both across experiments (Fig. 2.19) as well as when averaged over all experiments (Fig. 2.18).

Is there evidence for progressive nitrogen limitation in combined elevated [CO_2] and warming?

While the stimulation of plant productivity in elevated [CO_2] is known to gradually decline due to progressive nitrogen limitation (PNL, Oren et al. 2001) (Hungate *et al.* 2003; Luo *et al.* 2004), warming is known to accelerate organic matter mineralization and thus to enhance nutrient availability. The combined warming and elevated [CO_2] experiments provide the opportunity to test whether or not PNL occurs when these contrasting determinants of soil N availability are combined.

As expected, our meta-analysis reveals that elevated [CO_2] decreased soil N availability (Figs. 2.16a and 2.18a), but that it increased (or tended to increase) under warming (Figs. 2.16b and 2.18b). On average, these opposite responses counterbalanced each other in the combined elevated [CO_2] and warming experiments, resulting in little change in N availability relative to the control treatments (Figs. 2.16c and 2.18c). Further, across individual experiments, responses of soil N availability in the combined [CO_2] and warming experiments were positively correlated with responses in the warming-only experiments, but were not statistically significantly correlated with the responses in the [CO_2]-only experiments (Fig. 2.19). These two observations are highly relevant, because they imply that results of [CO_2]-only experiments overstate the likelihood that PNL will occur in future.

However, we also assembled a dataset for foliar N concentrations as a more direct indication of PNL (Figs. 2.18c and 2.19 (inset)). Plant nutrient concentrations are not only influenced by the soil nutrient availability, but also by the dilution effects of the enlarged biomass and by the competitive immobilization by soil microbes. Interestingly, our meta-analysis indicated that foliar N concentration did decline statistically significantly in combined elevated [CO_2] and warming (Fig. 2.18c). Moreover, across the individual experiments, foliar N concentration responses showed a contrasting pattern compared to the response of soil nutrient availability: a positive correlation between the combined treatment effects and the single factor [CO_2] effects (thus also in line with the responses of soil respiration and biomass production), compared to a marginally significant negative correlation for single factor warming effects (Figs. 18 and 19 (inset)). These analyses of the response of foliar N concentration to the combined treatments both suggest that PNL can be expected to occur also when [CO_2] and temperature increase together.

In line with the latter results, many soil warming studies have exhibited only short-lived stimulation of organic matter decomposition (Luo et al. 2001; Melillo et al. 2002), implying that also the stimulatory effect of warming on net N

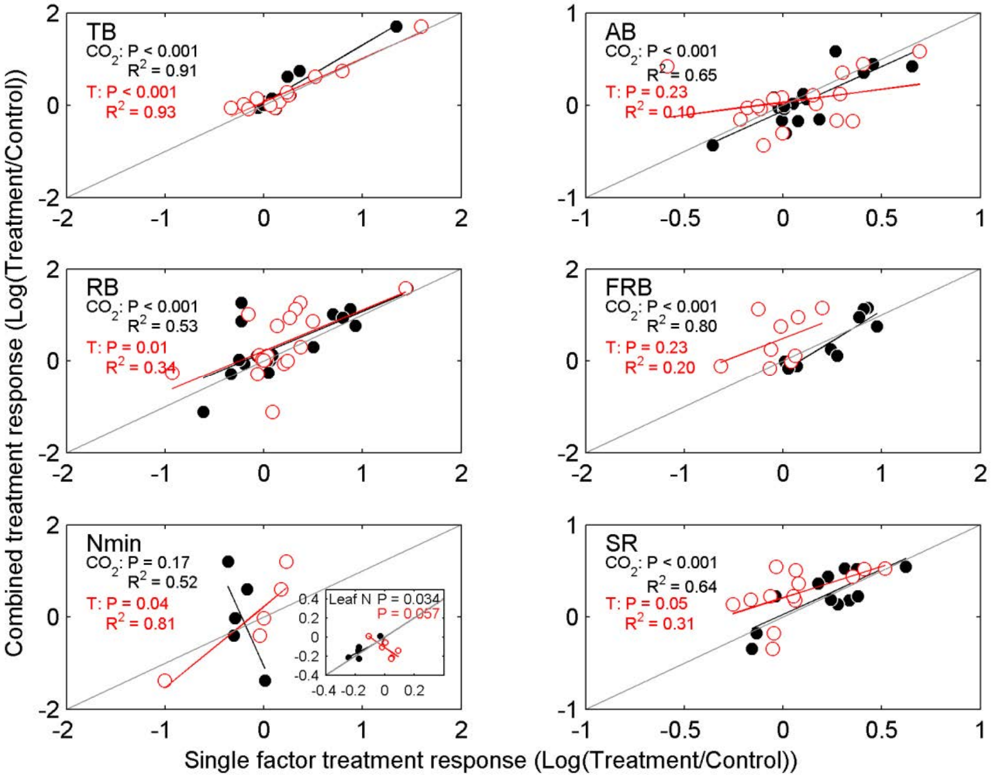


Figure 2.19: Meta-analysis effect sizes for single factor CO_2 effects (black circles) and single factor warming effects (red circles) plotted against the combined CO_2xwarming effect sizes. Data are reported as log(Treatment/Control). Data listed are total biomass (TB), aboveground biomass (AB), root biomass (RB), fine root biomass (FRB), mineral nitrogen availability (Nmin), foliar N content (Leaf N) and soil respiration (SR). *P*-values and R^2 values for linear regressions between single factor and combined treatment effects are given. Significant correlations were assessed at $P<0.05$.

mineralization might not be sustainable in the long term. Our dataset for mineral N availability is, however, dominated by short-term experiments (2 - 5 years). At this stage, there is clearly insufficient long-term data available to support a robust conclusion on the occurrence of PNL in a future warmer and [CO_2]-enriched world; knowledge that is crucial for the parameterization of models.

Are responses synergistic, antagonistic, or additive?

When applied in combination, interactive effects of elevated [CO_2] and warming may not simply result in additive responses, but might elicit synergistic or antagonistic responses. For example, accelerated nutrient mineralization caused by warming could counterbalance [CO_2]-induced nutrient limitations and allow the full [CO_2] fertilization effect to be expressed. In this case, the CO_2 effect could be larger in the combined treatment than in the [CO_2]-only treatment. Similarly, increased water use efficiency (WUE), caused by elevated [CO_2], could overcome warming-induced water limitation and thereby allow the full warming effect on biomass production (Morgan *et al.* 2011). Hence, synergistic responses of biomass production to warming and elevated [CO_2] are therefore not unrealistic (Norby & Luo 2004).

The responses of plant biomass in combined treatment experiments relative to the sum of the single factor treatment responses are shown in Fig. 2.20. In the case of synergistic responses, individual experiments should be situated above the 1:1 line, which is clearly the exception rather than the rule. However, this is not

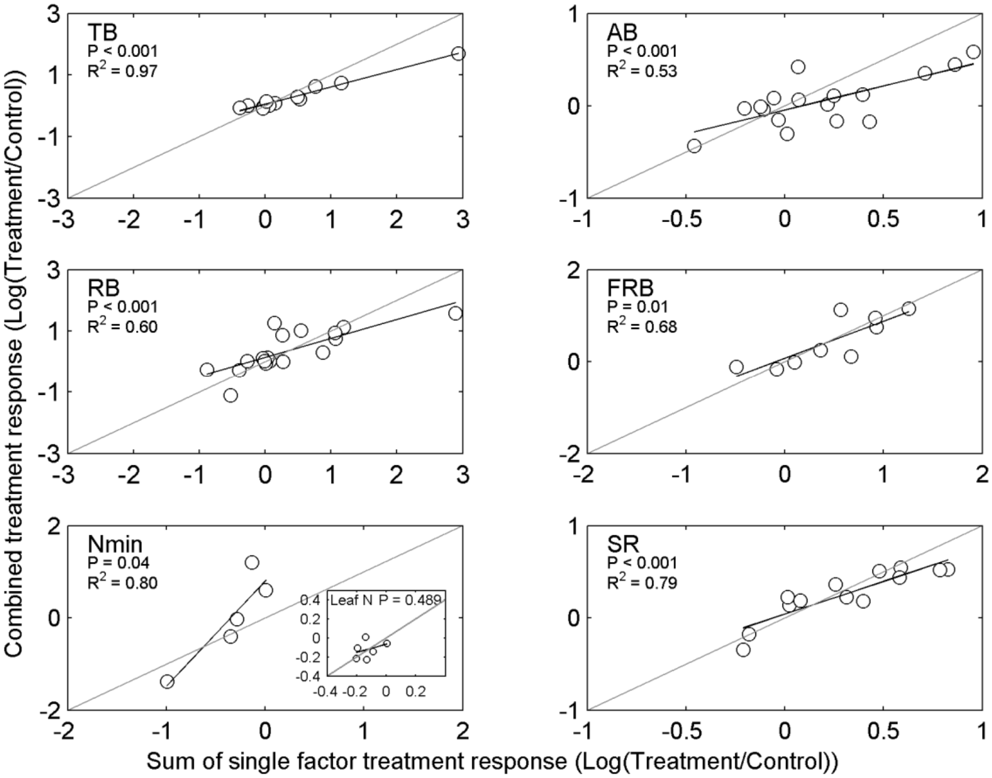


Figure 2.20: Meta-analysis effect sizes for the calculated sum of single factor CO_2 effects and single factor warming effects, plotted against the combined CO_2xwarming effect sizes. Data are reported as log(Treatment/Control). Data listed are total biomass (TB), aboveground biomass (AB), root biomass (RB), fine root biomass (FRB), mineral nitrogen availability (Nmin), foliar N content (Leaf N) and soil respiration (SR). *P*-values and R^2 values for linear regressions between single factor and combined treatment effects are given. Significant correlations were assessed at $P<0.05$.

that surprising *per se*, since warming will only tend to enhance the [CO_2] response if the response is actually suppressed by nutrient limitations. Similarly, elevated [CO_2] will only tend to increase the warming response if it helps to overcome a warming-induced water limitation. Therefore, synergistic responses should only be expected where nutrient limitation is currently suppressing the [CO_2] response and where water constraints are currently limiting the growth stimulation by warming. The general absence of a synergistic response therefore suggests that either current experiments rarely involve nutrient- or water-limited ecosystems, or that warming does not sufficiently mitigate nutrient limitation and/or elevated [CO_2] does not sufficiently mitigate drought.

In combination, warming and elevated [CO_2] clearly increase total and belowground biomass (Fig. 2.18c), but this effect is less than synergistic (Fig. 2.20), especially for aboveground biomass. This suggests the possible occurrence of antagonistic mechanisms. One such potential antagonistic mechanism is that both elevated [CO_2] and warming typically increase leaf area (Wullschleger *et al.* 2002; McCarthy *et al.* 2007; Way & Oren 2010). Consequently, the evapotranspiration in the combined treatment might be increased in response to the higher vapour pressure deficit and higher leaf area, despite the improved WUE as a consequence of elevated CO_2 alone. In this case, the combined CO_2 and warming treatment would deplete soil water reserves more rapidly than warming alone (but see Morgan *et al.* 2011). Similarly, a reduction of root biomass (relative to the increased leaf area) as a consequence of higher nutrient availability due to the warming treatment might make plants more susceptible to periodic droughts (Way & Oren 2010), possibly limiting the balancing effect of the increasing WUE in elevated [CO_2].

Differences in plant growth strategies might also affect the capacity of ecosystems to respond to a combined treatment. For example, Medlyn *et al*. (2001) have shown that stomatal conductance in evergreen trees is less responsive to elevated [CO_2], and therefore water savings might be lower, increasing their susceptibility to warming-induced droughts. If other biogeochemical processes (e.g. root exudation) differ in similar ways between species and ecosystems types, this might affect responses in a combined treatment as well. Further, increased competition in a mixed C_3 (favored by elevated [CO_2]) and C_4 (favored by warming) plant community might increase competition for resources and limit the full effect of both drivers (see Morgan *et al.* 2011for shift towards more C4). Lastly, co-limitation of primary productivity by N and phosphorus (P) (Harpole *et al.* 2011) may constrain combined effects of [CO_2] and warming when reduced N availability with [CO_2] in counteracted by reduced P availability with warming (Dijkstra et al., in prep.). Such antagonistic responses appear to be the rule rather than the exception in the response of aboveground biomass, where the response to combined [CO_2] and warming is smaller than the response to [CO_2] alone in the vast majority of experiments (Fig. 2.18). Fine root biomass responded much less to warming than to [CO_2] enrichment, whether analyses encompassed all studies (Figs. 2.16b and 2.17b), or when only studies that included both the single and combined treatments were evaluated (Fig. 2.18). Small responses of root biomass to warming are consistent with increased nutrient availability, which typically affects fine root biomass very little, but induces a substantial allocation shift in favor of aboveground biomass (Litton *et al.* 2007; Brassard *et al.* 2009).

Limitations and recommendations

Although the current set of experiments indicated a statistically significant increase in biomass and soil respiration in response to elevated [CO_2] and warming, this may not be robust because the current set of experiments may not be large enough to equally represent the range of different ecosystem types. The amount of available data did not allow us to test for treatment duration effects, for differences among manipulation types or intensities, for differences among vegetation types, etc. This highlights the need for more multi-factorial experiments. Nevertheless, with the currently available data, we have shown that the combined treatments elicited responses that were more similar to [CO_2]-only than to warming-only experiments. Therefore, model testing with data from [CO_2] enrichment-only experiments is to be preferred over testing with data from warming-only experiments.

Secondary effects of warming and [CO_2] enrichment (*i.e.* changes in water and nutrient availability) determine the ultimate response of terrestrial ecosystems. We were not able to test their influence due to a lack of data, or differences in reported parameters. Therefore more attention should be given to standardized protocols for experimental design and measurements, not only for biomass production and ecosystem C fluxes, but especially for water availability/stress, as well as for nutrient availability.

We did not include precipitation changes in this analysis although they form an essential part of the changing climate and have been shown to affect responses in combined elevated [CO_2] and warming (e.g. N mineralization response at Brandbjerg, Larsen *et al.* 2011). Similarly, increasing amounts of reactive N deposition could strongly affect the responses observed here. The replication of combined [CO_2] and warming experiments on dry *versus* wet, and nutrient-poor *versus* –rich sites could be an alternative approach where both water and nutrient effects can be incorporated in analyses like ours.

Conclusions

Elevated [CO_2] and warming exert fundamentally different effects on C storage and C and nutrient cycling in terrestrial ecosystems. In a combined treatment, effects of elevated [CO_2] often dominated the response, suggesting a larger sensitivity of terrestrial ecosystems to rising [CO_2] compared to rising temperatures. This dominance of [CO_2] in the combined treatments is probably attributable to the larger imposed relative changes in [CO_2] than in temperature, as is consistent with projected changes. Responses to single factor treatments were rarely additive, and interactions may lead to overestimation of effects based on the single factor results. Our results suggest that ecosystem models should ideally be tested against results from multifactor experiments to optimize their model structures.

2.3.5. What we didn't address

2.3.5.1. Precipitation changes

Unfortunately, we were not able to analyze effects of changing precipitation amounts and soil water availability. Therefore, we present a short qualitative review of the major effects of rainfall manipulations here.

A meta-analysis on mostly temperate systems indicated that generally, decreased water availability reduces plant growth (net primary production (NPP)) and ecosystem C fluxes (net ecosystem exchange (NEE)), while supplemental precipitation increases NPP and NEE (Wu *et al.* 2011). Individual studies indicated that soil respiration is generally reduced by drought treatments (Chimner & Welker 2005; Harper *et al.* 2005; Borken *et al.* 2006; Sowerby *et al.* 2008), and increased by irrigation treatments (McCulley *et al.* 2007). Moreover, the proportion of root respiration in total soil CO_2 efflux increased with drought, suggesting drought mainly affected microbial respiration (Jensen *et al.* 2003; Borken *et al.* 2006) and biomass (Jensen *et al.* 2003). Microbial diversity also decreased after multiple or extreme droughts (Schimel *et al.* 1999; Taylor *et al.* 2004), and an increase in fungal dominance of soil communities in response to drought has been reported (Jensen *et al.* 2003; Curiel Yuste *et al.* 2010).

Root biomass and production was reduced in some species (Meier & Leuschner 2008; Nikolova *et al.* 2009), and root turnover increased by drought (Meier &

Leuschner 2008), suggesting a larger C input into soil. As a consequence, drought could potentially lead to increased soil C storage. However, it is important to notice that in experiments where water availability was not limited, induction of drought was beneficial for microbial respiration rates, and resulted in a considerable C release (Jensen *et al.* 2003; Sowerby *et al.* 2008).

Taken together, effects of precipitation changes are therefore very dependent on site-specific water balances, and increases in precipitation might be beneficial in arid plant communities, but inhibit soil processes in non-water limited systems (Weltzin *et al.* 2003). In addition, the magnitude and direction of effects of rewetting and soil water repellency on SOM decomposition are still very uncertain (Schimel *et al.* 1999; Borken *et al.* 2006; Sowerby *et al.* 2008; Goebel *et al.* 2011). And besides changing amounts of precipitation, the altered frequency and intensity of precipitation events might play an important role as well (Knapp *et al.* 2002; Harper *et al.* 2005; Gerten *et al.* 2008), adding to the complexity of precipitation change as a global change driver.

2.3.5.2. Mycorrhizae

A somewhat obscure factor in the plant-soil interface are mycorrhizal fungi. Mycorrhizae form a symbiotic relationship with plant roots, improving nutrient uptake for the plant, in return for C supply by the plant. A considerable amount of

C can thus be allocated to growth and maintenance of these fungi, and it has been estimated that up to 25% of soil respiration can be attributed to mycorrhizal respiration (Heinemeyer *et al.* 2007). Mycorrhizae can therefore play an important role in plant and soil responses in a changing environment.

A short description of responses to global changes learns that mycorrhizal growth and infection is generally stimulated by elevated CO_2 (Rillig *et al.* 1998; Treseder 2004; Alberton *et al.* 2005), as it is highly dependent of substrate supply (Rillig *et al.* 2002; Heinemeyer *et al.* 2007). Mycorrhizal abundance generally decreases in N or P fertilized systems (Treseder 2004), confirming their symbiotic role in relation to nutrient uptake. Mycorrhizal root infection increased with increasing latitude (Ostonen *et al.* 2011), but in manipulation experiments, responses to temperature and soil moisture changes were found to be variable (Rillig *et al.* 2002; Heinemeyer *et al.* 2007). In addition to these direct effects on soil C cycling, mycorrhizal growth can affect soil aggregate stability and soil structure (Rillig *et al.* 1999; Rillig *et al.* 2002), with possible consequences for soil C storage. Altogether, responses are variable, and a lot of work still needs to be done to uncover the role of mycorrhizal dynamics in a changing climate.

2.4. Discussion

2.4.1. All global change drivers affect soil C cycling differently

There were a number of clear differences in the response patterns to the different global change drivers we tested. For example, N fertilization clearly stimulated long(er) lived C pools (above and root biomass, and SOC), while soil C inputs (litterfall and root turnover) were reduced (Fig. 2.21). As a consequence, there was no stimulation of microbial communities, and decomposition decreased. In addition, we have shown in previous chapters that inhibition of microbes by excess N will also add to this reduction of decomposition rates. In contrast to the N fertilization response, CO_2 elevation did not strongly increase plant C pools, but markedly stimulated soil C inputs (possibly related to increased need for N). Because of a lack of N availability, microbes are precluded to accumulate extra C into biomass, resulting in a strong increase in microbial decomposition rates (Fig. 2.21). Elevated CO_2 thus mainly stimulated labile C inputs and soil C cycling, while N fertilization promoted C storage in C pools. When CO_2 elevation and N fertilization were combined, they relieved each other’s limitations, and we found that all C pools and fluxes were stimulated (Fig. 2.21). Warming moderately stimulated plant C pools, and also stimulated soil C inputs (Fig. 2.21). Nevertheless, soil C decreased suggesting increased microbial decomposition rates.

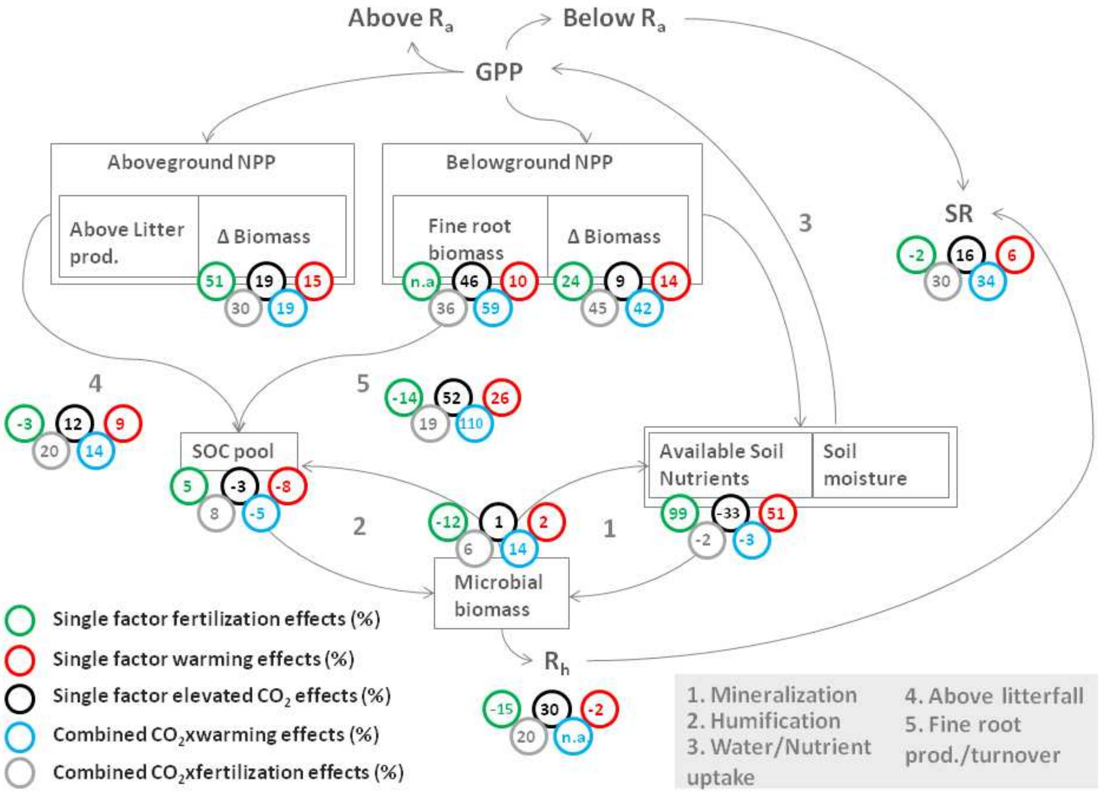


Figure 2.21: Overall average effects of tested global change drivers using the GCME database. Data given are the percentage effects over all ecosystem types or treatment designs.

However, microbial activity remained unaffected (Fig. 2.21). This failure to connect responses of different soil variables to warming in our theoretical scheme suggests our analysis might not be robust, or other factors also influenced responses of soil variables to warming.

Indirect effects and feedbacks related to nutrient and water availability are very likely a part of the explanation, as they affect all of the above relationships (Fig. 2.22). Excessive nitrogen availability has potential direct effects on decomposition, but an adequate amount of available nitrogen will likely be more important for a sustained stimulation of plant growth. Similarly, reduced water availability could limit plant growth, but water availability can also play an important role via direct inhibition of microbial decomposition due to drought (lack of medium for substrates to diffuse to enzymes) or in water-saturated ecosystems (oxygen limitation of decomposition). In a combined elevated CO_2 and warming treatment, increased WUE and increased N mineralization as a consequence of elevated CO_2 and warming respectively might relieve some of these limiting feedbacks. Our data partly confirm this as we found a strong stimulation of plant C pools and soil C inputs in our combined elevated CO_2 and warming analyses. Unfortunately, numbers were too low to infer on SOC or microbial biomass and decomposition (2, 3 and 1 studies respectively). This lack of datapoints for soil responses in warming experiments (single or combined treatments) is still one of the largest gaps in the GCME database.

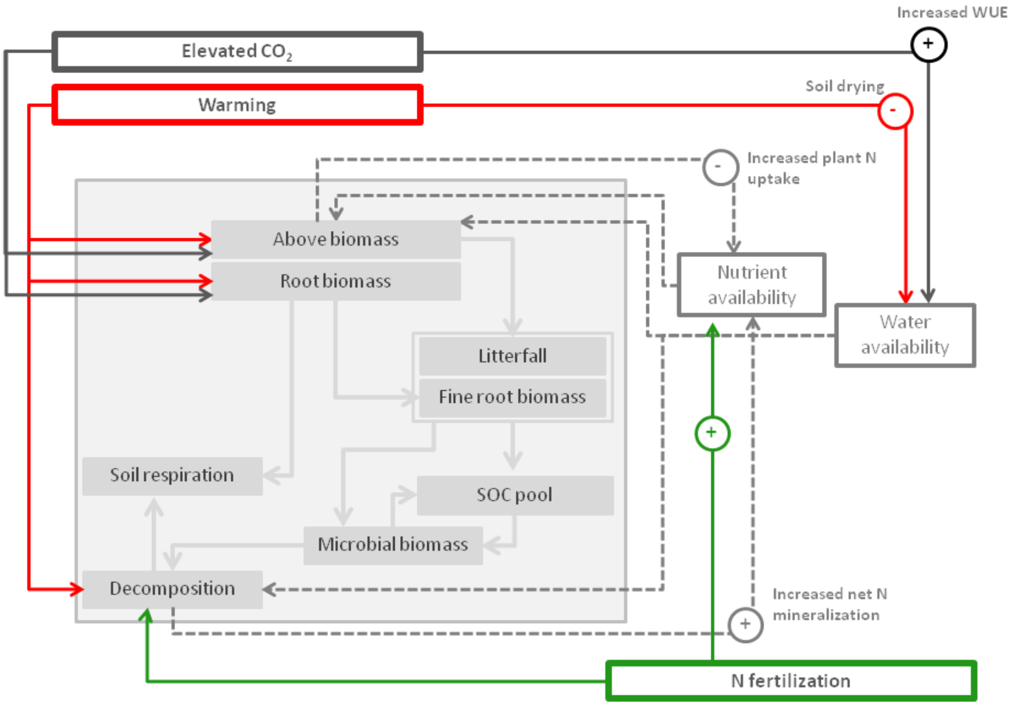


Figure 2.22: Theoretical scheme of direct effects of global change drivers (full line arrows) on nitrogen and water availability, and the indirect effects and feedbacks related to water and nutrient availability (dotted arrows). For clarity, direct and indirect effects on plant and soil C pools are not depicted. For these effects we refer to figure 2.21.

2.4.2. Interactions and feedbacks

While effects of elevated CO_2 were generally more consistent across experiments, we found larger variability for responses to warming treatments, suggesting a much stronger influence of initial site conditions on ecosystem responses. Over time, feedbacks and interactions related to biotic and abiotic environmental variables (water, nutrients, community species composition) might develop or have developed (see also Fig. 2.22), making generalizations over multiple studies a challenging task.

For example, it is well established that elevated CO_2 effects might become limited by nutrient availability over time (Oren *et al.* 2001; Luo *et al.* 2004), and multiple studies have focused on reviewing the effects of nitrogen fertilization in high atmospheric CO_2 conditions (Curtis & Wang 1998; de Graaff *et al.* 2006; van Groenigen *et al.* 2006). With the data in our database, we found that N additions in elevated CO_2 experiments had large effects on aboveground biomass, soil C and soil respiration (Fig. 2.23). No effect of N additions was observed on microbial biomass or the heterotrophic respiration response to elevated CO_2. Thus, because we found large effects on plant biomass but not on microbial biomass or respiration, we conclude that an increased root growth (and hence respiration) might account for a large part of the N effect on soil respiration. In addition, we found that soil C increased in elevated CO_2 experiments receiving N additions, in agreement with the lack of an increase in heterotrophic respiration, and confirming findings by earlier reviews (de Graaff *et al.* 2006; van Groenigen *et al.* 2006).

In contrast to experiments applying elevated CO_2, where an increased WUE is one of the main effects, the response of plant growth and decomposition to elevated temperatures can become limited by water availability more easily, especially in

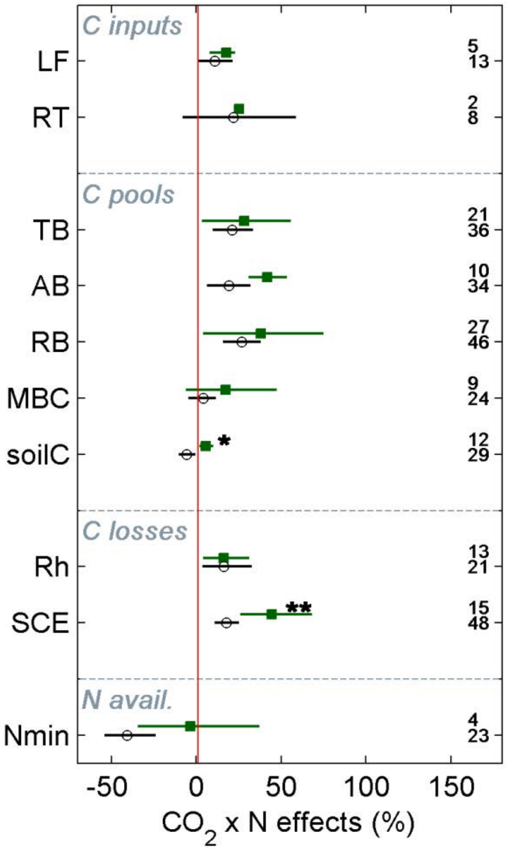


Figure 2.23: Elevated CO_2 effects with (green squares), or without N additions (white circles). Data presented are littefall (LF), root turnover (RT), total biomass (TB), aboveground biomass (AB), root biomass (RB), microbial biomass (MBC), soil C content (soil C), heterotrophic respiration (Rh), root respiration (Rr), soil respiration (SCE) and mineral N availability (Nmin). Data are the response in percent change compared to a treatment control, error bars are the 95% confidence intervals. The number of studies is given along the Y-axis. Statistically significant differences are reported at *P*<0.05 (* *P*<0.05; ** *P*<0.01).

grassland systems (Casella *et al.* 1996; De Boeck *et al.* 2008; Sherry *et al.* 2008; Liu *et al.* 2009b; De Boeck & Nijs 2011). In addition, microbes obtain most of their energy out of C compounds and are therefore mostly limited by C supply. An increased rate of SOM decomposition rate can deplete pools of easily decomposable C compounds, which is why a warming-induced stimulation of decomposition has often been found to be short-lived unless an increased supply of C compounds is maintained (Eliasson *et al.* 2005; Knorr *et al.* 2005b; Bradford *et al.* 2008a; Allison *et al.* 2010). Because elevated CO_2 increases water use efficiency, and provides an increased flux of C towards soil compartments, it has been hypothesized that elevated CO_2 has the potential to alleviate limitations to plant growth in elevated temperatures (Norby & Luo 2004). However, as we have shown in a previous chapter (2.3.4), observations for combined treatments are few and interpretation is hardly straightforward. The limited amount of work on interactive treatments often yielded unexpected results (Shaw *et al.* 2002; Shen *et al.* 2009), and has indicated that additive effects are the exception rather than the rule (Larsen *et al.* 2011; Wu *et al.* 2011). As such, the magnitude of multifactor effect estimates yielded by models might be overestimated (Fig. 2.24, see Leuzinger *et al.* 2011), indicating we need more multifactor experiments to validate model structures.

In addition to interactions between global change drivers, feedbacks induced by changing litter quality and species composition might arise on a longer time-scale. Elevated CO_2 is known to increase plant N uptake and can change the C:N ratio of plant tissue (King *et al.* 2005; Liu *et al.* 2007a). Therefore, because of increasing competition within soil microbial communities for available soil N under elevated CO_2, a lower litter quality might decrease (litter) decomposition rates (Hu *et al.* 2001). Expansion of shrubs producing recalcitrant litter in the arctic might

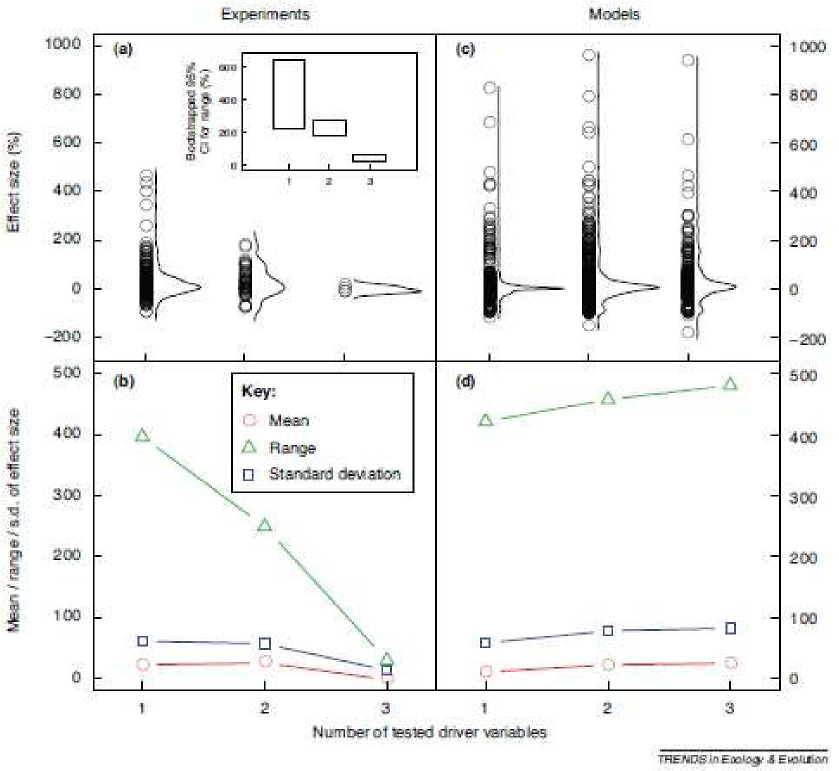


Figure 2.24: Effect sizes plotted against the number of tested driver variables irrespective of the nature of both the driver variables and the response variables. **(a)** The raw data from 160 experimental sites plus a relative density distribution using a spline function are shown (n=638, 132, 8 for one-, two- and three-factor experiments). **(b)** The corresponding mean, range (spread of 99% of data) and standard deviation of effect sizes. Nitrogen addition was excluded as a treatment to improve comparability between experiments and models, but the inclusion of those studies would not change the overall picture. **(c and d)** The samples plots for data from a modeling analysis with six ecosystem models, run for 17 driver variable combinations and seven sites: n=1176, 1176 and 504, respectively, as in **(a)**. The insert in **(a)** shows the bootstrapped 95% confidence intervals for the ranges at each level. *SOURCE: Leuzinger et al., 2011*.

therefore represent a negative feedback to atmospheric CO_2 levels (Cornelissen *et al.* 2007). However, a shift towards a more fungal dominated community, which is less N demanding (Zhang *et al.* 2005; Carney *et al.* 2007) might affect this feedback. In addition, an increased decomposition due to higher temperature sensitivity of recalcitrant compounds (Fierer *et al.* 2005; Davidson & Janssens 2006) can alter the relationship between litter quality and decomposition rates.

Clearly, when elevated CO_2 and changing climatic drivers induce shifts in species composition of plant (Dukes & Field 2000; Berendse *et al.* 2001; Luo *et al.* 2009; Langley & Megonigal 2010) and/or microbial communities (Carney *et al.* 2007; Rinnan *et al.* 2007; Briones *et al.* 2009), the expected response of ecosystem C cycling based on simples plant-soil relations might become a lot more complicated.

2.4.3. Responses in different ecosystem types

Shifts in community species composition are induced by a larger sensitivity of one species or ecosystem type to a particular global change driver. Therefore, to infer on different responses between ecosystem types, we tried to stratify our overall mean effect estimates between tree stands, herbaceous communities, and tundra systems.

Effects of elevated CO_2 on plant biomass are commonly suggested to be strongest for forest communities due to their lower photosynthetic acclimation as a consequence of larger sink capacity (i.e. increase in LAI and C storage in woody biomass) (Ainsworth & Long 2005). Although we did not find significant differences between woody and herbaceous systems in our overall dataset for elevated CO_2 experiments (Fig. 2.25), our work indicated that, in response to elevated CO_2, forests and non-forest systems prioritize differently in their allocation of C compounds, i.e. larger increases in belowground biomass in tree stands and a preferential allocation of C to aboveground biomass in non-forest systems respectively (see chapters 2.3.1 and 2.3.2, Fig. 2.25a). This difference can arise from functional differences between both system types: (i) the long-lived nature of aboveground woody biomass enables an allocation of C to other structural components (e.g. roots), compared to the typical investment of most herbaceous plants in annual aboveground biomass regrowth (Körner 2006); (ii) the larger uptake and storage of N in woody biomass might promote soil N depletion and a need to explore the soil matrix in search for nutrients, whereas in herbaceous communities, effects of increased water use efficiency (WUE) are dominant (Morgan *et al.* 2004a; Körner 2006), which might reduce the need for an extensive root network.

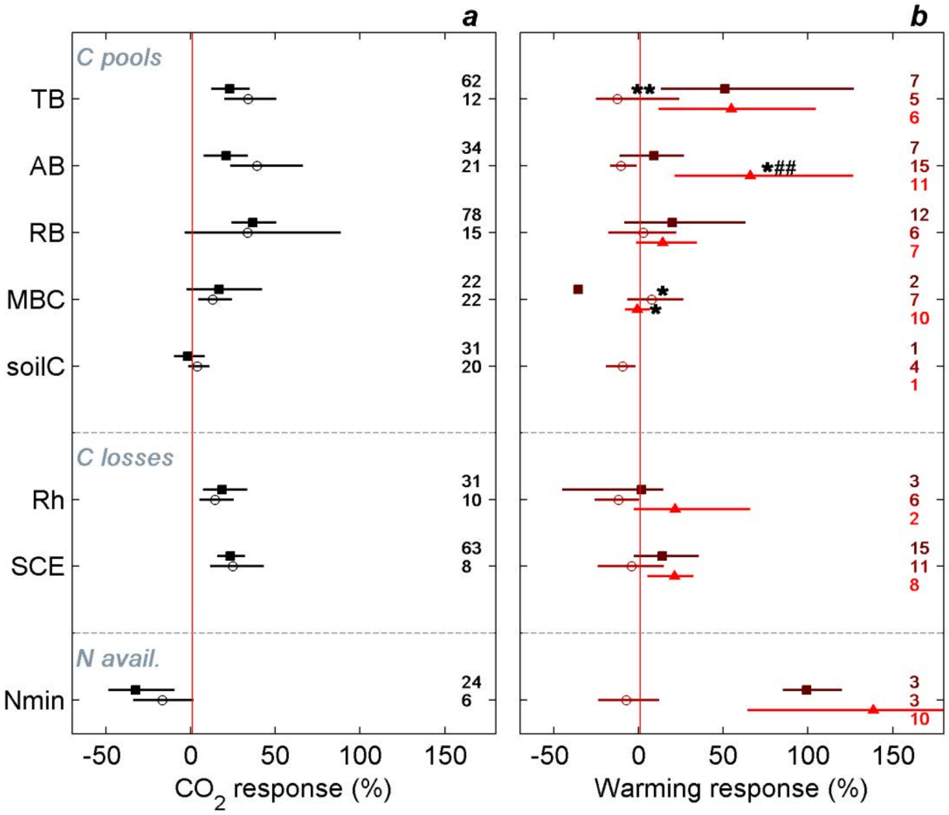


Figure 2.25: Effects of elevated CO$_2$ (a) and temperature (b) on C pools and fluxes in woody (squares), herbaceous (circles) and tundra (triangles) systems. Data presented are total biomass (TB), aboveground biomass (AB), root biomass (RB), microbial biomass (MBC), soil C content (soil C), heterotrophic respiration (Rh), soil respiration (SCE) and mineral N availability (Nmin). Data are the response of single factor treatments in percent change compared to a treatment control, error bars are the 95% confidence intervals. The number of studies is given along the Y-axis. Statistically significant differences are reported at *P*<0.05. * represents a statistically different response compared to woody species (* *P*<0.05; ** *P*<0.01), # represents differences compared to herbaceous species (# *P*<0.05; ## *P*<0.01).

In response to warming, we found strongly different responses in tundra systems in comparison with responses in temperate forest and herbaceous systems, which were broadly similar. This is in agreement with a meta-analysis performed by Rustad et al. (2001), who suggested large differences between ecosystem types. While aboveground biomass was generally not affected in woody systems or even reduced in grasslands, aboveground biomass in tundra systems was strongly stimulated in elevated temperatures (Fig. 2.25b). High-latitude and –altitude systems are generally limited by temperature because of shorter growing seasons. Because growing season length has been shown to increase in response to elevated temperatures, the increase in plant growth is not unexpected (Saxe *et al.* 2001). In addition, photosynthesis rates increase until an optimum temperature is reached (Larcher 2001), suggesting larger potential for positive responses in colder biomes. Lastly, an increased SOM decomposition rate might result in higher availability of N, possibly stimulating plant growth even further (Rustad *et al.* 2001). Because boreal regions are generally extremely limited by N, this might also explain their dominant growth response to increasing temperatures.

As we have shown in previous chapters and highlight here, global change effects on several response variables differ depending on the type of ecosystem or climatic region considered. Therefore, it is important to acknowledge these differences as a source of residual variability in large synthesis studies, and appreciate the role of species- and site-specific differences between experiments. In regard to this, the content of our database is clearly dominated by global change manipulation experiments (GCMEs) in temperate regions, with only a limited amount of experiments in boreal and tropical regions (Fig. 2.26). This is one of the major limitations of our study, and by extrapolation, of the GCME community. A better representation of different ecosystems types in a variety of climatic regions will be essential to arrive at robust estimates of ecosystems response variables.

In addition to this, it is well perceived that species diversity, age-structure and successional changes in plant communities can affect C dynamics (Carey *et al.* 2001; Asshoff *et al.* 2006; Bradley & Pregitzer 2007; Way & Oren 2010; Drake *et al.* 2011). Because manipulation experiments are often performed in model ecosystems, the extrapolation of results from non-natural ecosystems to larger scales should thus be done with the necessary caution.

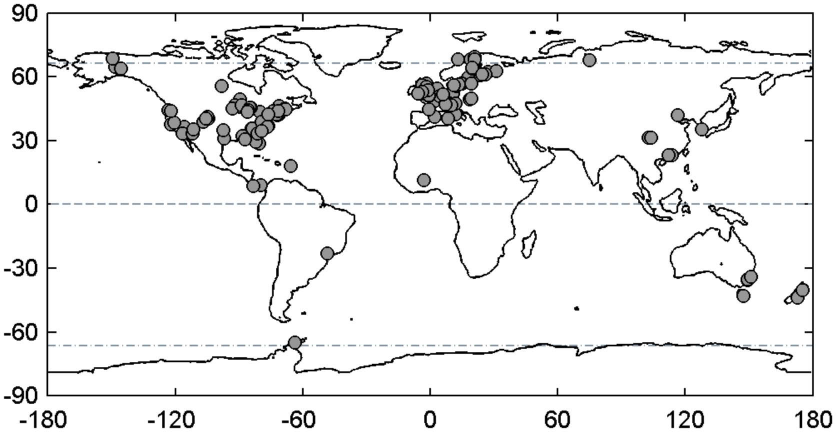


Figure 2.26: Location of global change manipulation experiments in our database.

2.4.4. Step increase treatments and temporal trends

The design of GCMEs inherently involves some disadvantages. Global change manipulations are often performed as ‘shock’ treatments, applying a step increase rather than a gradual increase in CO_2 concentrations or temperature. A consequence of this approach is an overestimation of the treatment effect in the early stages of experiments (Luo & Reynolds 1999; Shen *et al.* 2009), followed by a reduced effect when ecosystems have found a new equilibrium. Because of this artifact, the temporal extent of global change effects often contrasts between models and experiments. For example, in contrast to results from GCMEs, modeling exercises indicated that the suggested progressive N limitation of elevated CO_2 effects might not evolve under the natural trend of increasing atmospheric CO_2 concentrations, due to natural redistribution of N in vegetation and soils (Cannell & Thornley 1998; Esser *et al.* 2011). Similarly, while models predict an increased release of C towards the atmosphere due to rising temperatures (Friedlingstein *et al.* 2006; Luo 2007; Friedlingstein & Prentice 2010), global change experiments generally show an increased decomposition in the short-term, after which decomposition falls back to baseline rates (Melillo *et al.* 2002).

In our database, we found that the elevated CO_2 effect on aboveground and root biomass was stronger for higher concentrations of CO_2. In contrast to the effect on aboveground biomass, which stayed constant over time, the effect on root biomass decreased with time (e.g. Fig. 2.27, Table 2.9), suggesting the commonly observed increase in C allocation belowground might be overestimated by the current evidence based on predominantly short-term studies. Soil respiration was not affected by the treatment intensity, but the elevated CO_2 effect tended to decrease with time (possibly as a results of decreasing root biomass, Table 2.9).

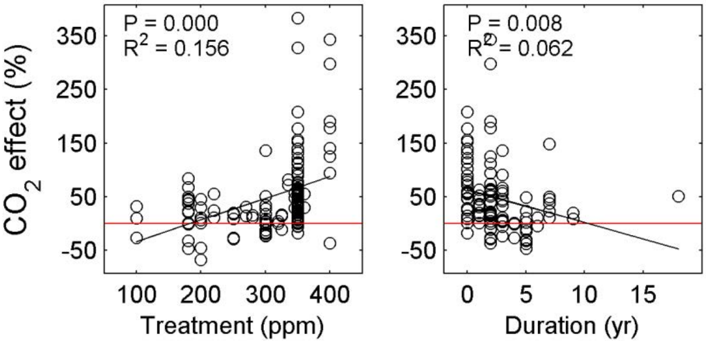


Figure 2.27: Correlation between treatment intensity and duration of elevated CO_2 treatments on root biomass. Regressions with treatments intensity were performed against the average effect for all available data per experiment, the regressions with treatment duration were performed by plotting the last year measurement average against the total year of treatment. Linear regression *P*-values and R^2-values are given. Regressions are considered statistically significant at *P*<0.05. Effect sizes are weighted means, and treatment duration is given as total years of treatment.

Considering the overall neutral mean effect of elevated CO_2 concentrations on soil C (see sections 2.3.1 and 2.3.2), it was not surprising we found no correlation between the soil C response and treatment intensity or treatment duration. Warming treatments stimulated aboveground biomass more with increasing treatment intensity, and this positive effect of higher temperature on aboveground biomass also increased with treatment duration (Table 2.9). The temperature effect on root biomass was also larger in higher treatment intensities, but the effect decreased with time (Table 2.9). Mineral N availability was not affected by treatment intensity, but was stimulated more in long-term experiments (Table 2.9), possibly explaining the reduced root biomass response over time. While soil respiration was not affected by treatment intensity or duration at all, soil C content decreased more with increasing treatment intensity, indicating increasing decomposition rates in higher temperatures (Table 2.9). This last finding was only based on ten data points, and hence should be interpreted with caution. In response to N fertilization, we did not find trends for above- or (total) belowground biomass related to treatment intensity or duration. However, the fine root biomass response displayed a negative correlation with treatment duration (Table 2.9). As expected, the mineral N availability in the soil increased with treatment intensity (Table 2.9). We also found a stronger decrease in soil respiration in longer-term experiments, and probably associated with this, a stronger increase in soil C content with treatment duration (Table 2.9).

These trends broadly reflect the overall mean effect sizes we discussed in previous chapters: larger biomass and root growth in elevated CO_2, increased plant growth and possible increase in decomposition in response to warming, and reduction in decomposition and an increase in soil C in N fertilization experiments.

Table 2.9: P-values and signs of the slopes for linear regressions between treatment effects and treatment intensity and duration for total biomass (TB), aboveground biomass (AB), root biomass (RB), fine root biomass (FRB), mineral N availability (Nmin), soil respiration (SR) and soil C content (SOC). Regressions with treatments intensity were performed against the average effect for all available data per experiment, the regressions with treatment duration were performed by plotting the last year measurement average against the total year of treatment. Linear regressions are considered statistically significant at $P<0.05$ (bold italics).

	Elevated CO_2				***Warming***				***N fertilization***			
	Intensity		*Duration*		*Intensity*		*Duration*		*Intensity*		*Duration*	
	P-value	slope	P-value	slope	P-value	slope	P-value	slope	P-value	slope	P-value	slope
TB	0.102	+	0.15	-	***0.018***	+	0.639	-	0.774	-	0.669	-
AB	***0.036***	+	0.369	+	***0.014***	+	***0.006***	+	0.602	-	0.527	+
RB	***<0.001***	+	***0.008***	-	***0.049***	+	***0.004***	-	0.692	+	0.167	-
FRB	***0.017***	+	***0.029***	-	0.078	+	0.334	-	0.913	+	***0.014***	-
Nmin	0.12	-	0.353	+	0.978	-	***0.027***	+	***0.049***	+	0.72	+
SR	0.884	-	***0.022***	-	0.78	+	0.209	-	0.272	+	***<0.001***	-
SOC	0.596	+	0.154	+	***0.013***	-	0.972	+	0.45	-	***0.001***	+

However, the correlations in table 2.9 indicate that some of the trends we found are in fact related to the intensity of the step-increase treatments, and that some of these effects gradually reduce over time (e.g. root biomass response in elevated CO_2, root biomass response to warming, fine root biomass response to N fertilization). In other words, these effects might be a result of the experimental design of global change experiments. In contrast, the aboveground biomass response to warming increased with treatment intensity, and also increased with treatment duration, adding confidence that this might be a ‘real’ effect. Similarly, the increasingly negative soil respiration response and the increasingly positive soil C content response to N fertilization over time also suggest accumulated effects and therefore a ‘real’ effect.

Extrapolating these findings to a global scale, baring region- or ecosystem specific responses in mind, would mean that high-latitude systems would respond strongest to warming and that these effects would be maintained on longer timescales. Similarly, N deposition across the globe might increasingly promote reduced decomposition of SOM. Elevated CO_2 on the other hand, might promote more rapid cycling of C through terrestrial ecosystems, but this would not be a permanent effect. These findings agree well with changes we already see in the present (Beck *et al.* 2011).

2.4.5. Conclusions

In the previous chapters, we have shown that global change experiments can be very useful to better understand terrestrial C cycling in a future climate and a changing atmosphere. However, some inherent limitations arose over the last decennia, and are highlighted in these last paragraphs. These limitations are inevitable, as the design of GCMEs is limited by logistical and financial issues. Laboratory studies can help to determine the sensitivity of C pools and fluxes to climatic changes, and environmental gradient studies can represent long-term effects and complexity through time-for-space substitutions. A better integration of these different approaches might facilitate the link between studies on the sensitivity of individual response variables (lab studies), mechanistic studies on several C pools and fluxes (GCMEs) and their natural complexity related to temporal and spatial variability (gradient studies).

3. SOIL C STOCKS ON AN ALTITUDINAL GRADIENT IN TROPICAL FOREST

3.1. Introduction

Altitudinal gradients offer steep environmental clines, and are therefore excellent natural laboratories in which to perform ecological research. There are four major atmospheric changes associated with altitude (Körner 2007): (1) decreasing total atmospheric pressure and partial pressure of gasses, (2) reduction of atmospheric temperature (with implications for humidity), (3) increasing radiation under a cloudless sky, and (4) increased UV-B radiation. Of these four, the only direct variable that affects soil C pools is the reduction in temperature, as decomposition of soil organic matter is generally reduced at lower temperatures due to slower enzymatic reaction rates (Larcher 2001). Indirectly, the other three variables might play a role in aboveground productivity of plants, thereby affecting the C inputs into the soil compartments. Consequently, altitudinal transects provide a powerful way to provide a better insight in the major drivers behind the impact of changing climatic conditions on soil C pools (Körner 2007; Malhi *et al.* 2010).

The interpretation of the effects of climatic changes along altitudinal gradients and the extrapolation towards future global change effects effectively comes down to 'space-for-time' substitutions. One major advantage of this approach is that the ecosystem studied has inherently integrated environmental changes over long periods of time. Therefore, possible feedbacks that might have evolved over time are also accounted for.

In this chapter, we will discuss soil C stocks along an altitudinal gradient in primary tropical forest in north-eastern Papua New Guinea. Soil samples were gathered over an altitudinal range of roughly 3000m, encompassing a temperature difference of about 16° C (10-26.3). In addition, several grassland plots were sampled to compare grassland soil C stocks with forest soil C stocks.

3.2. Methods

3.2.1. The YUS conservation area and the altitudinal gradient

The study was carried out in the YUS (Yopno – Uruwa - Som, the three major river valleys in the area) conservation area (76000 ha), located in the Saruwaged mountain range on the Huon Peninsula in Papua New Guinea's northeastern Morobe province (6°04'S, 146°48'E) (Fig. 3.1).

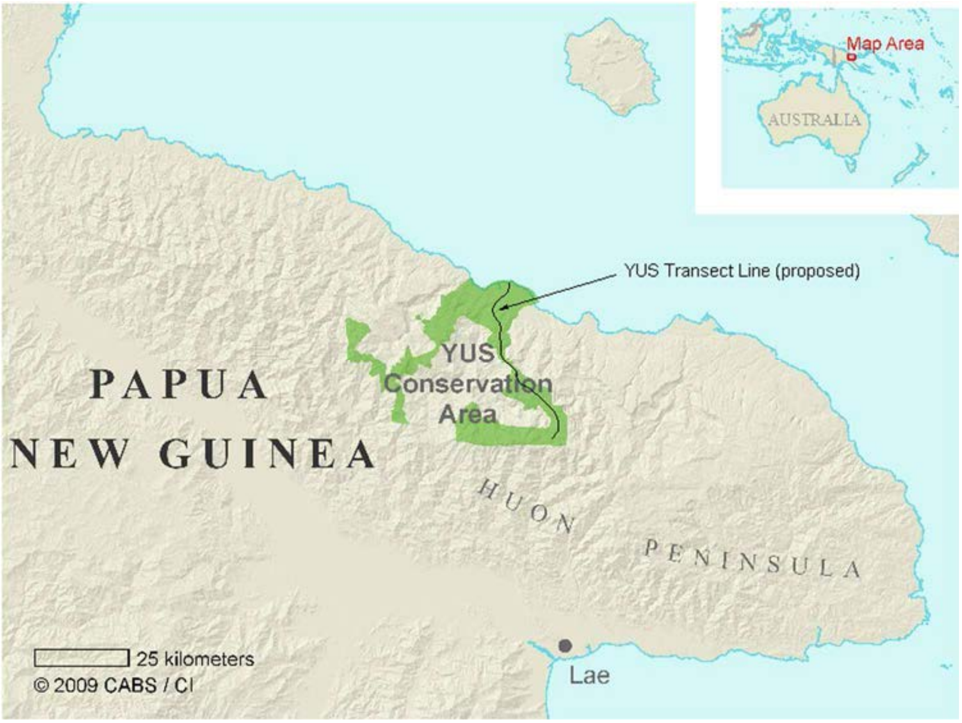


Figure 3.1: Location of the YUS conservation area. YUS - conservation project in NE Papua New Guinea. Involved partners: James Cook University, Conservation International, Tree Kangaroo Conservation Project.
Website: http://www.conservation.org/sites/gcf/portfolio/asia_pacific/Pages/yus.aspx

Plant species richness on the Huon-peninsula is believed to be one of the highest in New Guinea. Lowland alluvial plains are dominated by *Pometia*, *Ficus*, *Alstonia* and *Terminalia* forests interspersed with *Saccharum* and *Imperata* grasslands (Paijmans 1976). The vegetation on hills below 1000m consists of mixed Dipterocarp, *Casuarina*, *Auracaria*, and *Themeda*, *Imperata* and *Heteropogon* grasslands, and Eucalypt savannas (Paijmans 1976). These hill forests are characterized by a lower canopy, lower emergent heights, lower frequency of large girth trees and buttresses, and less variable height, closure, and crown size, than in forest on alluvium on the coastal plain. However, species richness is great (Paijmans 1976). Vegetation in the lower montane zone (1000-3000m) consists of *Castanopsis* and *Nothofagus* forest, coniferous forest, and *Miscanthus* grasslands. Mixed lower montane forest is smaller crowned, more even in height, more densely closed and regular, has smaller tree girth than lowland hill forest, averaging between 20 and 30m with high tree density (Paijmans 1976). Although species richness declines with increasing altitude, it is high in the lower part of the upper montane forest (>3000m) and common families include *Myrsinaceae*, *Ericaceae*, *Myrtaceae*, *Rubiaceae* (Paijmans 1976).

Nine permanent 1ha plots were established along an altitudinal gradient ranging from 100 up to 3050m above sea level by Conservation International as a part of the YUS project (Table 3.1, Fig. 3.2). Plots were established along the ridgeline, and as a result slopes were often gentle or flat, compared to the usually much

Table 3.1: Climatic description of the altitudinal gradient.

Site number	Altitude (m a.s.l.)	Tmax* (°C)	Tmin* (°C)	MAT* (°C)	MAP* (mm)
6	100	30.9	21.7	26.3	2598
7	611	27.8	19.0	23.4	2806
8	900	26.6	17.8	22.1	2911
9	1345	23.0	14.8	18.8	3207
10	1753	20.1	12.5	16.2	3484
11	2195	18.3	10.7	14.4	3643
12	2293	16.9	9.5	13.0	3788
13	2800	14.3	7.2	10.5	4090
14	2939	13.8	6.7	10.0	4218

*Climatic data are retrieved from WorldClim

steeper adjacent topography. The transect covers lowland tropical forest (below 1000m), lower montane forest (1000-3000m) and upper montane forest (above 3000m) (Paijmans 1976). Anthropogenic grasslands are found near local villages situated in valleys and coastal plain areas (Fig. 3.2), and are subject to frequent burning. The area receives between 2600 and 4200mm of precipitation each year (WorldClim, Hijmans et al. 2005) and experiences temperatures ranging from a minimum of 6.7°C at the highest altitude sites to a maximum of 30.9° at the lowest altitude sites (WorldClim, Hijmans *et al.* 2005).

All soils at our sites developed on limestone bedrock, with exception of the lowermost site, which was a limestone soil with a 50cm A-horizon directly sitting on alluvial deposits. With increasing altitude, an increased degree of soil development and generally deeper soils were observed. The organic top layer

Figure 3.2: Schematic representation of sampling locations along the altitudinal gradient. Ninety one forest locations (54 in 1ha plots and 37 in other forest plots) and 14 grasslands sites were sampled. Forest sampling locations are clustered according to the 1ha plot number (i.e. site 6 to 14). One cluster contains six locations in each hectare plot and 3-6 additional forest locations within 200m altitude of the 1ha plot. Grassland sites are identified by the name of the closest village.

increased in thickness from the lowland sites towards the highest sites (up to 30 cm thickness), and generally overlaid deep A-horizons. Some of the lower montane sites had relatively thin A-horizons overlaying mixed layers of weathering mineral soil and rocks in the top 50-70cm. At the higher sites, evidence of B-horizons was emerging. According to the PNGRIS database (Bryan &

Shierman 2008), soils at the lowland sites are classified as Hapludolls and Rendolls, lower montane forest soils are classified as Troporthents, and the upper montane forest soil are classified as Cryorthents.

3.2.2. Soil C survey

3.2.2.1. Sampling campaign

In total, 297 soil samples and 54 litter samples were collected in the nine permanent 1ha plots. In addition, 209 soil samples and 38 litter samples were collected in 37 other forest plots along the altitudinal gradient to capture natural variability, and 70 soil samples in 14 grassland plots were collected in order to compare soil C stocks between forests and grasslands at different altitudes.

In each of the 1ha plots, soil profiles were sampled at 6 locations. At each location, litter was sampled in three 20x20cm squares, located in line and 5-10m from each other. These three samples were bulked, weighed and a subsample retained for analysis. At the central litter sampling location, soil samples were taken at 0-10, 10-20, 20-30, 45-50, 65-70 and 95-100cm depth. For each 1ha plot, this resulted in a total of 18 (bulked to 6) replicates for the litter layer, and 6 replicates for all sampled layers of the soil. A soil pit to 1m depth was excavated outside the hectare plots at a location representative of the sampling locations.

In each of the other forest/grassland plots, soil profiles were sampled at 3 locations. At each (forest) location, litter was sampled in three 20x20cm squares, located in line and 5-10m from each other. These three samples were bulked, weighed and a subsample retained for analysis. At the central litter sampling location, soil samples were taken at 0-10, 10-20, 20-30, 45-50, 65-70 and 95-100cm depth. At both other sampling locations, soil samples were taken at 0-10, 10-20 and 20-30cm depth. The three samples for the 0-10, 10-20 and 20-30cm interval were bulked, weighed and a subsample retained for analysis. For each sampling location, this resulted in a total of 3 (bulked to 1) replicates for the litter layer, 3 (bulked to 1) replicates for the 0-30cm layers and one sample for the deeper layers of the soil.

Soil cores were taken using metal tubes of 5cm in diameter and 10cm length. Samples were taken between August and November 2010. The depth of the soil was estimated by pushing a wooden rod through the base of the sampling hole to a maximum of two meters.

3.2.2.2. Sample processing

The wet weight of all samples was recorded in the field, and all samples were oven-dried in the lab at 60°C. Dry mass was quantified and samples were crushed and sieved to remove all stones and roots larger than 2mm in diameter. Samples were then reweighed, and a subsample was ground to estimate organic C and N concentrations using dry combustion in an elemental analyser (Costech, Costech Analytical Technologies, CA - USA).

To remove possible inorganic C from the samples and make sure we only measured organic C, a subsample of the 65-70 and 95-100cm samples was treated with a 6N HCl solution and assessed for presence of carbonates. Where carbonates were present, prior to analysis, we also treated samples from the overlying layers with the acid solution until no response was observed.

Bulk soil densities were calculated using sample dry weights and the sampling tube volumes. SOC and N densities were then determined using the bulk densities for each layer. Total SOC and N stocks for the 30cm profiles were calculated by summing stocks for the individual 10cm layers. SOC and N stocks for the 100cm profile were obtained by calculating sampling location-specific relationships using the stocks for the sampled layers, using both an exponential and a power function. SOC and N stocks for the deeper layers not directly sampled were then interpolated using these relationships, and total 100cm profile stocks calculated by summing numbers for all layers. The mean and standard deviation (SD) of the exponential and power function was used in the statistical analyses. Average values and standard deviations for hectare plots were obtained by taking the mean of the 6 sampling locations.

The pH of all individual samples was measured in a 0.01M $CaCl_2$ solution. Soil textural analysis was performed on pooled samples. A subsample of each sample at each sampling location was taken to obtain 20g bulk samples for each layer at all hectare plots. Aggregates were dispersed by submerging samples in a sonicator overnight. After sonication, 10g of sample was mixed into a 5% sodium hexametaphosphate solution and left to stand overnight. The sample was then sieved at 63µm and dried in the oven at 60°C. The dried sample was used to calculate the proportion of sand-sized particles in the samples.

3.2.3. Outline of results section

In the following chapters, we will discuss results of our soil C survey in different sections. Part of these sections are in the process of manuscript preparation or submission (3.3.1 and 3.3.2) and include an introduction and discussion. In section 3.3.3 and 3.3.4, we indicate where our results can play an important role and place these results in a broader perspective related to climatic effects on soil C stocks in the YUS area.

3.3. Results

3.3.1. Do soil C stocks vary predictably with altitude in tropical forest?

Manuscript in preparation

Authors: Wouter I.J. Dieleman, Michelle Venter, Anurag Ramachandra, Andrew Krockenberger, Michael Bird

3.3.1.1. Introduction

Soil is the largest pool of organic carbon in the biosphere, containing over 2300Pg of carbon (C) in the top three meters (Jobbagy & Jackson 2000). Changes in the soil organic carbon (SOC) pool are driven by changes in the balance between atmospheric CO_2 uptake by plant primary production, and soil CO_2 effluxes through decomposition processes and root respiration (i.e. soil respiration) (Trumbore 2006). Because these annual effluxes of CO_2 from soils to the atmosphere are 8-10 times the amount derived from fossil fuel combustion (Mooney *et al.* 1987; Lal 2008), a change in this balance can have a strong impact on atmospheric CO_2 levels. But despite the significance of potential changes in the SOC pool in its effects on the global carbon cycle, we continue to lack robust estimates of the spatial and temporal variability of SOC pools.

One of the largest gaps in global datasets are tropical forests. These forests play a key role in the global C cycle storing a total of 471 Pg of C (Pan *et al.* 2011), with

36-60% of their ecosystem C stored in soil (Don *et al.* 2011). Most studies addressing soil C storage in the tropics sampled in lowland tropical forests, while it has been shown that montane forest soils can also contain large amounts of C (e.g. Moser *et al.* 2011). Indeed, carbon storage in soils is determined by a number of factors, including climate, vegetation, soil texture, interaction with soil organisms, but also topography (Townsend *et al.* 1995; Trumbore 1997; Jobbagy & Jackson 2000; Bird *et al.* 2004). In this regard, it is unclear whether soil C stocks vary predictably with altitude or slope.

Earlier studies of tropical montane forests have indicated that while aboveground biomass and productivity generally decreases with increasing altitude (Raich *et al.* 1997; Kitayama & Aiba 2002; Raich *et al.* 2006; Leuschner *et al.* 2007; Wilcke *et al.* 2008; Girardin *et al.* 2010), SOC stocks tend to increase with altitude (Townsend *et al.* 1995; Kitayama & Aiba 2002; Raich *et al.* 2006; Girardin *et al.* 2010), although some studies have reported a lack of a continuous increase above a certain altitude (Schrumpf *et al.* 2001; Schawe *et al.* 2007), and others have even failed to find any significant trend with increasing altitude (Soethe *et al.* 2007; Zimmermann *et al.* 2010).

In this study we focus on spatial patterns in SOC stocks along an altitudinal gradient spanning 2950m in the Morobe-province of Papua New Guinea (PNG). The tropical forests of the island of New Guinea are part of the third largest expanse of rainforest on the planet (Shearman & Bryan 2011), a region that has received far less attention than the tropics of the Amazon basin or Africa. More specifically, we tested whether SOC stocks varied predictably with altitude, slope or aspect in our study area and compared our data with a larger dataset of published SOC stocks on altitudinal gradients to identify possible general trends.

3.3.1.2. Methods

For an extensive description of the methods, we refer to chapter 3.2

In this chapter, we have only used the forest plot data.

Statistical analysis

Mean values for different soil layers and hectare plots were compared using one-way ANOVA with a Tukey-Kramer adjusted post hoc test to indicate between group differences. The relation between SOC stocks and altitude, slope or aspect was assessed using linear regression analysis. ANCOVA analysis of means and slopes was performed to analyze the effect of different aspect along the altitudinal gradient. We investigated the correlation between SOC stocks and altitude, mean annual precipitation (MAP), mean annual temperature (MAT), soil depth, soil pH, soil texture (= %sand), root mass, litter mass and CN-ratio using a pairwise linear correlation analysis.

Statistical differences or correlations were considered statistically significant at $P<0.05$. Statistical analysis was perfomed using Matlab 7.4.0.287 (R2007a) (MathWorks, Massachusetts, USA).

3.3.1.3. Results

Soil profiles

Soil bulk densities did not change significantly with soil depth at most altitudes (Fig. 3.3a, Table 3.2), although we found a trend for higher bulk densities in deeper layers for the lower altitude sites (Fig. 3.3a, Table 3.2). Mean bulk densities and bulk densities for individual soil layers decreased significantly with altitude (Fig. 3.3a, Table 3.2), indicating more compacted soil at lower altitudes. Mean bulk densities for different layers ranged from 0.266 to 0.449 g cm^{-3}, with individual samples ranging between 0.147 and 0.856 g cm^{-3}.

SOC concentration decreased with soil depth at all altitudes (Fig. 3.3b, Table 3.2). Mean C concentration and C concentration of individual soil layers significantly increased with altitude (Fig. 3.3b, Table 3.2). Mean SOC concentration for

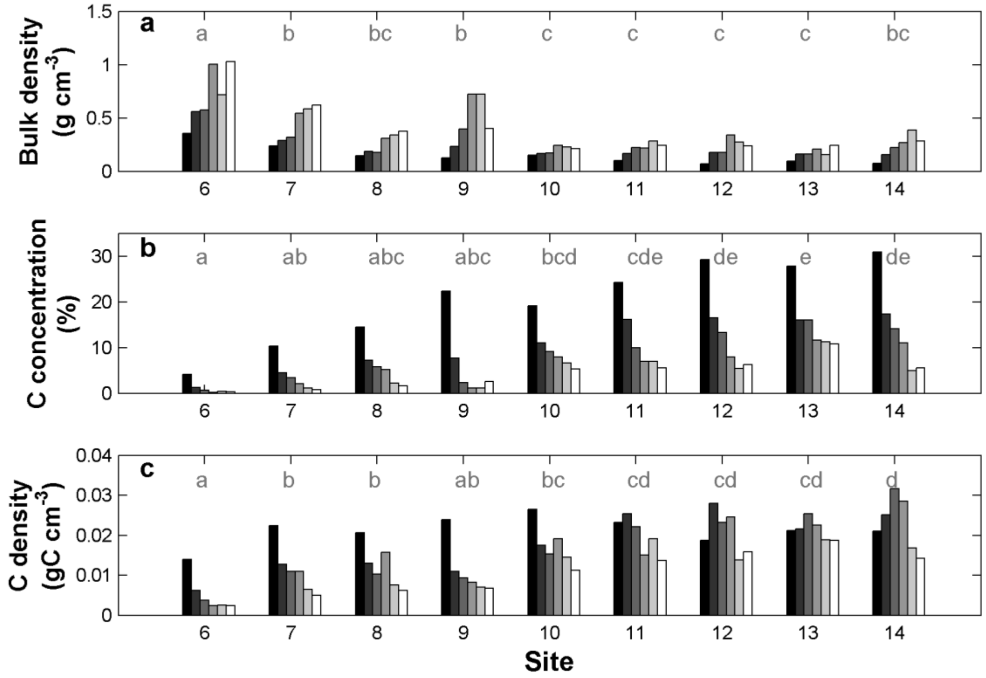


Figure 3.3: Distribution of mean bulk density (a), C concentration (b), and C density (c) for 100cm profiles in all hectare plots. Values are means of 6 samples for 0-10, 10-20, 20-30, 45-50, 65-70, 95-100cm layers in black towards white respectively. Different letters indicate significant differences between group means according to Tukey-Kramer post hoc tests. Significant differences are assessed at *P*<0.05. Error bars are left out for clarity. The altitude for the sites presented in the graph range between 100m (site 6) and 2939m (site 14). For a more detailed description, see Table 3.1 and Figure 3.2.

different layers ranged from 3.70 to 20.13%, with individual samples ranging between 0.8 and 29.1%.

Carbon densities did not change significantly with altitude for the top 20cm of the soil. For the deeper layers, C density increased with altitude (Fig. 3.3c, Table 3.2), indicating higher amounts of C stored in deeper layers at higher altitudes. Mean C

Table 3.2: *P*-values and between-group significant differences for the different layers of the soil profile within and between sites. Significant differences between hectare plot means and significant differences between soil layers are assessed at *P*<0.05. The altitude for the sites presented range between 100m (site 6) and 2939m (site 14). For a more detailed description, see Table 3.1 and Figure 3.2. Different layers: a=0-10cm, b=10-20cm, c=20-30cm, d=45-50cm, e=65-70cm, f=95-100cm

Bulk density

SITES	**6**	**7**	8	**9**	**10**	**11**	**12**	**13**	**14**
Overall *P*-value	0.097	<0.001	0.881	0.007	0.254	0.006	0.028	0.118	0.113
Significant diff. within sites	=	a<def; bc<ef	=	a<de	=	a<c	a<bc	=	=

LAYERS	**a**	**b**	c	**d**	**e**	**f**
Overall *P*-value	<0.001	0.043	0.077	<0.001	<0.001	<0.001
Significant diff. between sites	6>10-14	6>11,13	=	6>7-8,10-14; 7>10-11,13-14; 9>8,10-14	6-7,9>8,10-14; 7>10-11,13-14	6>8-14; 7>10-14; 8>10-13

C concentration

SITES	**6**	**7**	8	**9**	**10**	**11**	**12**	**13**	**14**
Overall *P*-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Significant diff. within sites	a>rest	a>rest; b>def	a>rest; b>cdef; cd>ef	a>rest; b>e	a>rest; b>ef	a>rest; b>cdef; c>f	a>rest; b>def; c>f	a>rest	a>rest; b>def; c>ef

LAYERS	**a**	**b**	c	**d**	**e**	**f**
Overall *P*-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Significant diff. between sites	6<9-14; 7<10-14; 8<12-14	6,7<10-14; 8<11-14; 9<11,13-14	6<8,10-14; 7-9<10-14; 10-12<13-14	6-7,9<10-14; 8<10,13-14	6-9<10-14; 11-12<13;	6-8<13

C density

SITES	**6**	**7**	8	**9**	**10**	**11**	**12**	**13**	**14**
Overall *P*-value	0.009	<0.001	<0.001	0.001	0.002	<0.001	<0.001	0.002	0.006
Significant diff. within sites	a>def	a>rest	a>cdef	a>def	a>ef	a>f, bc>def	a,c>ef; b>def	c>ef	c>f
LAYERS	**a**	**b**	c	**d**	**e**	**f**			
Overall *P*-value	0.763	0.019	<0.001	<0.001	<0.001	<0.001			
Significant diff. between sites	=	=	6-8<14	6<10,12-14; 7<12-14; 8-9<14	6-9<11-14	6-7<12-13; 8<11-14; 9<13			

density over all soil layers therefore was higher at higher altitudes (Fig. 3.3c). Mean C densities for different layers ranged from 0.010 to 0.042 gC cm^{-3}, with individual samples ranging between 0.005 and 0.055 gC cm^{-3}.

Carbon stocks

Litter C stocks were similar across the whole altitudinal gradient (Fig. 3.4a). In contrast, SOC stocks increased with altitude for both the top 30cm of the soil (Fig. 3.4b), as well as the total 1m soil profile (Fig. 3.4c). In addition, soil C stocks were distinctly different between lower altitude sites (altitude clusters 6-9) and the sites further upslope (clusters 10-14) for the total 1m profile C stocks (Fig. 3.4c), indicating different patterns of C storage in deeper layers of the soil. Mean litter C stocks ranged from 0.25 to 0.52 kgC m^{-2}, with individual samples ranging between 0.10 to 0.87 kgC m^{-2}. Mean soil C stocks for the total 1m profile ranged from 4.8 to 19.4 kgC m^{-2}, with individual samples ranging between 2.2 and 26.2 kgC m^{-2}.

Relationship with topography

We found a clear linear relationship between soil C stocks with altitude on our transect, indicating larger SOC stocks at higher altitude (Fig. 3.5a). No relationship was found between SOC stocks and slope (Fig. 3.5c), and SOC stocks were not significantly different due to different aspect along the altitudinal gradient (Fig. 3.5d).

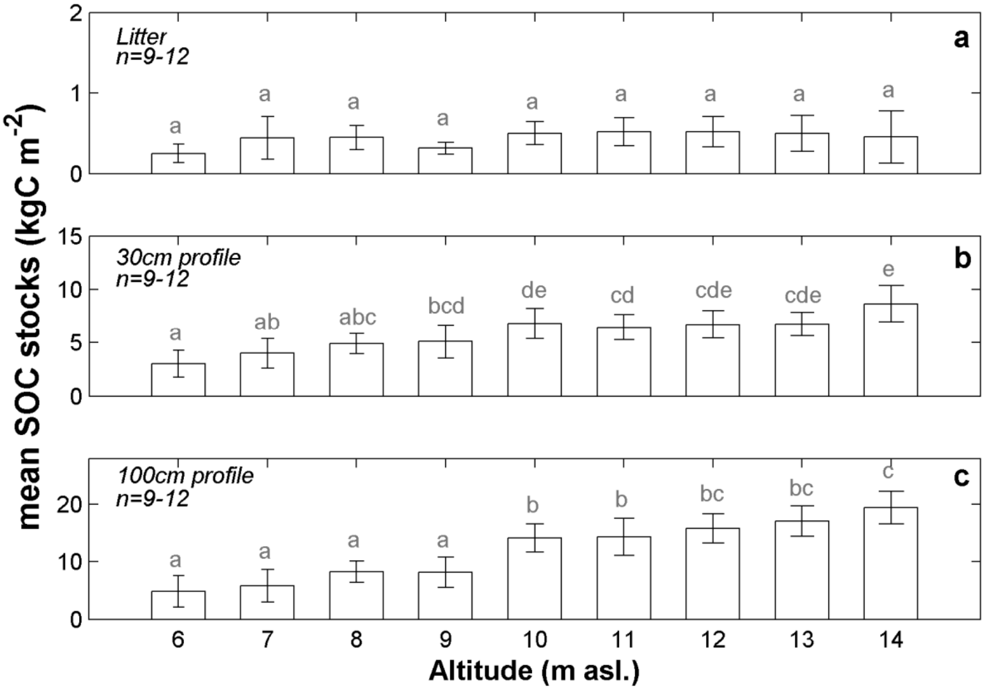


Figure 3.4: C stocks for the different altitude clusters along the gradient. Data presented are Litter C stocks (a), SOC stocks for the top 30cm of soil (b), and SOC stocks for the 1m soil profile (c). Different letters indicate significant differences between group means according to Tukey-Kramer post hoc tests. Significant differences are assessed at *P*<0.05. The altitude for the sites presented in the graph range between 100m (site 6) and 2939m (site 14). For a more detailed description, see Table 3.1 and Figure 3.2.

Correlation with environmental drivers

Soil C stocks were correlated to many environmental variables (Table 3.3), indicating a strong dependence to climatic variables (MAP, MAT) and soil characteristics (soil depth, pH and texture). Soil C stocks were correlated to litter mass, but not to root mass (Table 3.3). Many environmental variables were also correlated to each other, indicating a strong covariance of driver variables along our altitudinal gradient.

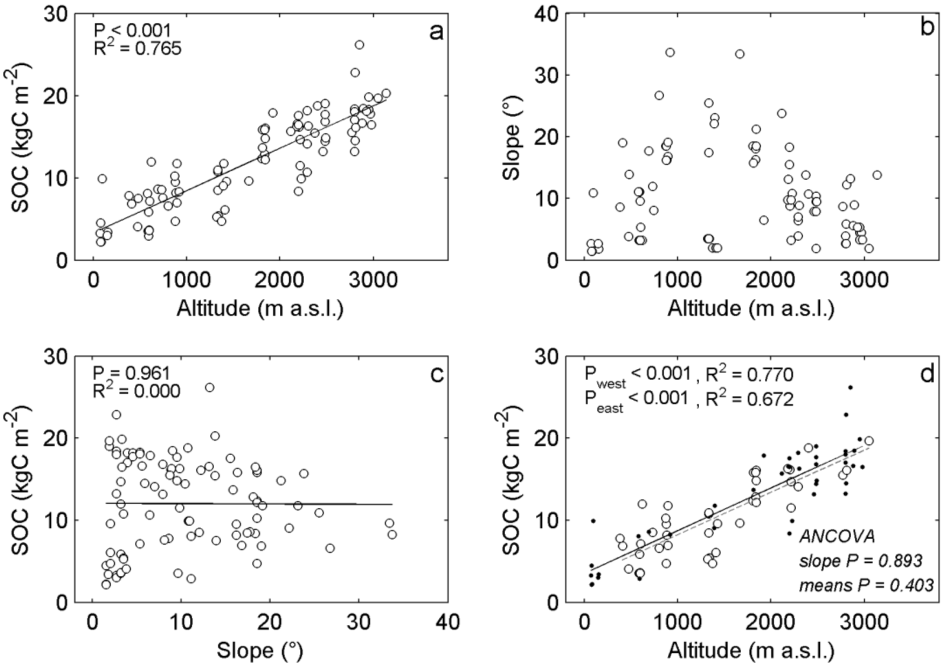


Figure 3.5: Relationship of soil organic carbon (SOC) stocks with topographic variables. Data displayed are the relationship of altitude with soil organic carbon (SOC) stocks (a), of altitude with slope (b), of slope with SOC stocks (c), and the relationship between altitude and SOC stocks for East aspect sites (white circles) and West aspect sites (black dots). Significant linear correlations and ANCOVA differences for slopes and group means are considered significant at *P*<0.05.

Table 3.3: Summary of pairwise linear correlation analysis for all considered variables affecting SOC stocks. Correlations are considered statistically significant at $P<0.05$.

	Altitude	*MAP*	*MAT*	*soil depth*	*pH*	*soil texture*	*root mass*	*litter mass*	*CN-ratio*
MAP	<0.001								
MAT	<0.001	<0.001							
soil depth	<0.001	<0.001	<0.001						
pH	<0.001	<0.001	<0.001	<0.001					
soil texture	<0.001	<0.001	<0.001	<0.001	<0.001				
root mass	0.09	0.1	0.15	0.62	0.1	0.03			
litter mass	<0.001	0.01	0.009	0.83	0.001	0.05	0.004		
CN-ratio	<0.001	<0.001	<0.001	0.08	<0.001	0.28	0.014	0.24	
SOC stocks	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.21	0.004	<0.001

3.3.1.4. Discussion

Along our transect, we found higher soil bulk densities in lower altitude sites compared to higher altitude sites, suggesting a stronger compaction of soils at lower altitudes. However, larger amounts of stones in the lower altitude sites (Table 3.4) partly diluted this trend when we corrected soil bulk densities for stone content (data not shown). We observed a clear difference in C densities along the soil profile for the lowland sites (6-9) compared to sites further upslope (10-14). At the higher sites, C storage was more evenly spread across the top 30cm layers, whereas at the lower altitude sites, C densities steadily decreased with soil depth (Fig. 3.3c). Because C concentrations and C densities demonstrated more similar trends with altitude, we suggest that C concentration dominantly explains the major trends in C density along our gradient. On the other hand, the more even spread of C storage in the top layers of the soil might be explained by lack of a decresing trend in bulk density with soil depth at the higher altitude sites. This is in accordance with the buildup of a thicker organic layer at the higher altitude sites on our and other gradients (Leuschner *et al.* 2007; Graefe *et al.* 2008; Wilcke *et al.* 2008; Zimmermann *et al.* 2010).

The stronger difference in SOC stocks along our altitudinal gradient for the full 1m soil profile compared to the 30cm topsoil, indicates that considerably larger amounts of C are stored in the deeper layers of the soil at higher altitudes

Table 3.4: Soil characteristics at different altitudes. Sites presented in the graph range between 100m (site 6) and 2939m (site 14). For a more detailed description, see Table 3.1 and Figure 3.2.

Altitude cluster	CN ratio	pH (CaCl2)	Average soil depth (cm)	Sand content (% particles > 63 µm)	Stone content (%)
6	13.31	7.83	96.6	50.2	34.5
7	17.33	7.09	92.2	40.7	69.7
8	14.24	6	128.1	79.3	50.7
9	15.32	6.61	117.7	68.7	35.1
10	15.9	5.25	134.1	75.0	22.3
11	17.22	5.21	>200	82.0	21.5
12	17.98	4.93	175	70.7	20.3
13	21.6	4.74	180.5	70.7	26.1
14	20.09	4.9	194.4	80.7	19.3

compared to lower altitudes. The closer proximity of the limestone parent material at lower altitudes induced higher pH values (Table 3.4) in the soil, creating more optimal conditions for microbial breakdown of organic matter at higher temperature. At higher altitudes, soils were increasingly more acid, and the bedrock was generally found deeper down. As such, soil depth and pH play an important role in determining SOC stocks along our gradient, which is confirmed by their highly significant correlation with SOC stocks (Table 3.3).

The importance of controlling for lithology

The mean SOC stocks along our transect varied between 4.8 and 19.4 kgC m^{-2}, which falls well within the range reported by other altitudinal transect studies in the tropics (Table 3.5). Our study showed that SOC stocks increased linearly with increasing altitude, confirming findings of some earlier tropical forest transect studies (Townsend *et al.* 1995; Kitayama & Aiba 2002; Raich *et al.* 2006; Girardin *et al.* 2010). However, in other tropical transects there were no changes with altitude (Raich *et al.* 1997; Soethe *et al.* 2007; Zimmermann *et al.* 2010), or no consistent pattern was found (Schrumpf *et al.* 2001; Schawe *et al.* 2007).

The vast majority of our sites along the transect were established on soil that developed on limestone bedrock (the lowermost sites were overlying carbonate-containing alluvial sediments), and this eliminated much of the variability that a varying bedrock lithology can create (Körner 2007). The majority of longer tropical transects (spanning >1000m in altitude) that established their sites on gentle slopes (<25°) and similar soil types (Kitayama & Aiba 2002; Girardin *et al.* 2010, see Table 3.5) also reported an increase of SOC stocks with increasing altitude. In contrast, transects with sites on steep slopes and changing soil types or soil properties did not (Schrumpf *et al.* 2001; Schawe *et al.* 2007; Soethe *et al.* 2007, see Table 3.5), suggesting that variation in soils and steepness of slopes also affects patterns in SOC stocks along altitudinal gradients.

Schawe et al. (2007) found an increase in SOC stocks between 1800m and 2600m, after which SOC stocks leveled for the next 700m, due to charcoal in some samples and different soil forming processes (hydromorphic soils) above 2500m

Table 3.5: Comparison with other tropical forest transects.

Reference	Location	Altitude range (m asl.)	depth sampled (cm)	Increase in SOC stocks	Range of C stocks (kgC m^{-2})	Soil description of selected sites	Slopes of selected sites
YUS	Papua New Guinea	90-3050	100	yes	5-19	All limestone soils, lowermost site overlying sedimentary deposits	96% of sites <25°
Kitayama & Aiba, 2002	Borneo	700-3100	100	yes	ca. 7-28	sedimentary substrate (sandstone/mudstone), pH 4.1-4.9	gentle (17°-27°)
	Borneo	700-3100	100	no	ca. 8-10	ultrabasic substrate (serpentinized peridotite), pH 4.5-5.4	gentle (11°-24°)
Townsend *et al.,* 1995	Hawaii	900-1500	20	yes	11.39-15.36	allophanic soils (Udands), extremely similar along sites	relatively level
Girardin *et al.,* 2010	Peru	194-3025	40	yes	1.4-7	all except one on Paleozoic shalesslates), below 1000m clay rich soils on alluvial sediments, lower at higher sites	ridgetop
Schawe *et al.,* 2007	Bolivia	1700-3400	100	not consistent	22-53	Ordovician metasiltstone, slates and sandstones, pH 3-4.5	Steep (>25°)
Soethe *et al.,* 2007	Ecuador	1900-3000	110	no	13.1-40.2	gleyic Cambisols, Podzols at highest altitude plot, pH < 3.5 to 30cm depth	27°-31°
Zimmermann *et al.,* 2010	Peru	2994-3860	90	no	mean of 11.8	Histic Lithosol	-

Schrumpf *et al.,* 2001	Ecuador	1100-3050	100	not consistent	ca. 7-35	mainly phyllites, partly metamorphic sandstones as well as quartzites, pH 3-5	very steep (30°, with maxima >60°)
Raich *et al.,* 1997	Hawaii	290-1660	50	no	0.51-14.5	All on pahoehoe lava, consistently very acid	gentle
Raich *et al.,* 2006	Meta-analysis of 6 transects	ca. 0-4000	5 x 100, 1 x 50	yes	ca. 6-60		

compared to lower altitudes. Similarly, Schrumpf et al. (2001) reported an increase in SOC stocks between 1850 and 2350m, and an inconsistent trend for higher altitudes, coinciding with distinctly different composition of the clay fraction of the respective parts of the transect. Studies using locations with soils that were persistently very acid (Raich *et al.* 1997; Soethe *et al.* 2007, see Table 3.5) did not show any relationship between SOC stocks and altitude either.

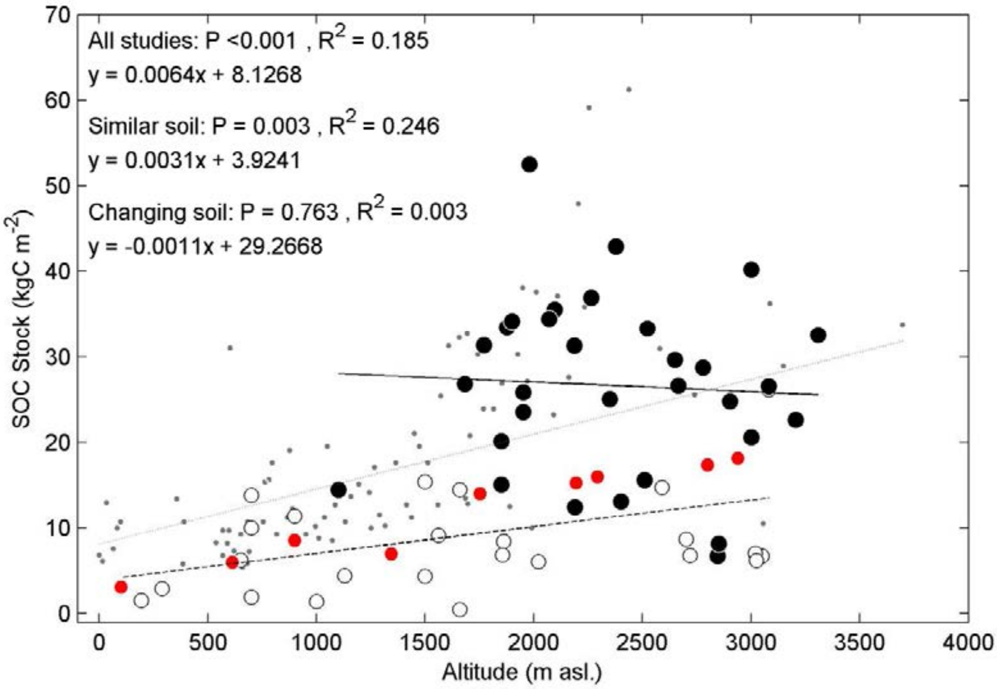


Figure 3.6: Relationship between SOC stocks and altitude for all currently available data for SOC stocks along tropical altitudinal transects (grey dots, dotted line). Transects were categorized as having similar lithology along their whole transect (white circles, dashed line, red dots for our study), or changing lithology and/soil characteristics (black circles, solid line). No soil data were available for the transects in Raich et al, 2006. Linear regression *P*-values, R^2 values, and equations are given.

We tested this pattern by extracting and plotting all soil C stock data for the tropical altitudinal transects in table 3.5. For sites with consistent lithologies along the majority of their altitudinal gradient, we found a clear relationship between altitude and SOC stocks. In contrast, for those that reported data for sites on changing soil types and properties, no relationship was observed (Fig. 3.6). This finding stresses the importance of careful site selection, and indicates the importance of the soil parent material when studying SOC dynamics. Indeed, Heckman et al. (2009) found that SOC varies predictably among soil types, and indicated pH as an overarching control of SOC dynamics. Accordingly, SOC stocks were suggested to be low on limestone soils (Heckman et al. 2009), consistent with our findings.

Relationship with topography

The differences in insolation period or intensity of rain and wind events that may occur with changing site aspect did not seem to be important in our study area. Similarly, the differences in microclimate due to variation in soil moisture and the intensity and duration of available sunlight on different slope forms did not affect SOC stocks significantly. In contrast, environmental drivers associated with altitude were strongly correlated to SOC stocks along our gradient.

Several previous studies have suggested slower decomposition rates to be the main driver behind larger SOC stocks at high altitudes. Most studies attributed this reduction to changes in climate, and to declining temperature in particular (Vitousek *et al.* 1994; Townsend *et al.* 1995; Kitayama & Aiba 2002; Neto *et al.* 2011; Salinas *et al.* 2011). Other studies suggested adverse soil conditions (e.g. waterlogged soils, low pH values, high aluminium concentration), inhibiting soil fauna and microbial activity, as the main driver for slower SOC decomposition at higher altitude. Reduced mineralization of SOC is associated with reduced nitrogen availability, which is in agreement with observed decreases in leaf and litter N concentration at higher altitudes in several studies (Tanner *et al.* 1998; Kitayama & Aiba 2002; Graefe *et al.* 2008). Plants generally respond to reduced nitrogen availability by allocating more C to root compartments (Kitayama & Aiba 2002; Leuschner *et al.* 2007; Soethe *et al.* 2007; Girardin *et al.* 2010) to increase soil exploration. While both aboveground standing biomass and productivity tend to decrease with increasing altitude (Raich *et al.* 1997; Tanner *et al.* 1998; Kitayama & Aiba 2002; Raich *et al.* 2006; Leuschner *et al.* 2007; Girardin *et al.* 2010), the partitioning between above and belowground biomass compartments seems to remain constant (Girardin *et al.* 2010). This suggests an increased root C residence time at higher altitudes (Graefe *et al.* 2008; Girardin *et al.* 2010) providing another mechanism that could ultimately lead to increasing SOC stocks with altitude.

Along our gradient, we observed that climatic changes (temperature and precipitation) were of major importance (Table 3.3): SOC stocks decreased with increasing temperatures, and increased with increasing amounts of precipitation. However, we derived temperature and precipitation data from a grid based dataset (i.e. WorldClim, Hijmans *et al.* 2005, grid-size 1km), so while the sign and overall relationships are probably correct, the relatively large WorldClim grid-size might not have captured the frequent steep increases in altitude on our gradient, and therefore might limit the accuracy of these relationships.

In addition to climatic variables, soil characteristics also co-varied strongly with altitude (soil depth, pH and texture, Table 3.3) and thus likely played an important role in explaining SOC stocks. Soil depth and pH were closely correlated with each other, reflecting the influence of the soil parent material. As indicated in table 3.4, soil depth increases with increasing altitude in our study, which means the limestone bedrock is closer to the soil surface in the lower altitude sites. This is clearly illustrated in the higher pH values in the lowland sites and might explain the strong correlation of SOC stocks with pH along our gradient.

Because so many variables co-varied with altitude along our gradient (Table 3.3), we could not tease out the main drivers behind the relationship with SOC stocks. Nevertheless, the individual correlations we found agree well with existing hypotheses about SOC stocks at high altitudes: a warmer and drier climate in concert with (close to) neutral pH at lower altitude provides favorable conditions for microbial decomposition. In contrast, at higher altitudes, cold and wet conditions and an acidic soil likely inhibited high microbial decomposition rates, and stimulated a buildup of a thicker organic layer. More controlled laboratory experiments will therefore be needed to identify sensitivity of SOC stocks and decomposition of organic matter to different environmental changes along our gradient.

Conclusion

We have illustrated here that SOC stocks vary predictably with altitude on moderate slopes (<25°) in tropical forests, provided sites selection takes lithology into account. We have shown that this altitudinal trend was due to a larger buildup of organic matter in the top layers of the soil, but that in addition to this, the deeper layers also play an important role in C storage. High altitude sites thus clearly contain larger amounts of carbon, which can be explained by several environmental drivers. However, many drivers co-varied with altitude, stressing the complexity of teasing out controlling variables in gradient studies.

3.3.2. Contrasting distribution of soil organic C along the soil profile in tropical forests and grasslands in Papua New Guinea.

3.3.2.1. Introduction

With about 438 Gt of C stored in biomass and soils, tropical forests represent one of the largest C pools in the terrestrial biosphere (Dixon *et al.* 1994; Malhi *et al.* 1999). The high temperatures and abundant rainfall in these systems offer optimal conditions for large C uptake through photosynthesis (Luyssaert *et al.* 2007; Beer *et al.* 2010), but also promote rapid C release through autotrophic respiration and SOM decomposition (Luyssaert *et al.* 2007). In addition, while tropical areas represent more than half of the global C-sink (Pan *et al.* 2011), fire, extensive deforestation and land use change also return a large amount of CO_2 back into the atmosphere (Cochrane *et al.* 1999; Nepstad *et al.* 1999; Houghton 2003; Aragao & Shimabukuro 2010). Further, the increasing frequency of droughts and drought-related fires might limit forest C uptake and storage in the future (Zarin *et al.* 2005; Toomey *et al.* 2011). As a consequence, despite the large uptake of tropical systems, the current net C-balance of tropical forests is near neutral (Pan *et al.* 2011), and future trends are uncertain.

Papua New Guinea (PNG) is an extensively forested country where deforestation and degradation commenced a lot later than in most of the Asia-Pacific region, and therefore still has large stretches of intact rainforest (Shearman & Bryan 2011). Deforestation and degradation has mainly taken place in the lowland areas, but the highlands on the Huon peninsula will potentially suffer the same fate in the future (Shearman & Bryan 2011). In addition, increasing population has started to affect larger areas of forest through land-use change (McAlpine & Freyne 2001). Land use change, deforestation and forest degradation have been shown to decrease soil C stocks (Don *et al.* 2011), affect microclimate (e.g. through increased solar incidence stimulating evaporation), and change soil properties such as bulk density and soil stability. Forest disturbance can thus not only affect the overall quality of the soils in these systems, but can also reduce C storage potential.

To increase our understanding of C storage patterns in tropical forests, and to be able to estimate potential future effects of forest degradation and land-use change on C storage, it is crucial to establish baseline C stock data for the forests of PNG (Fox *et al.* 2010). To address this need, we have collected 576 soil samples in tropical forest and grassland sites in the YUS conservation area in northeastern Papua New Guinea (PNG) to evaluate differences in soil C stocks between forests and grasslands at different altitudes, and to infer on potential effects of land use change.

3.3.2.2. Methods

For an extensive description of the methods, we refer to chapter 3.2

Statistical analysis

The relation between SOC stocks, bulk density (BD) and C concentration and altitude was assessed using linear regression analysis. ANCOVA analysis of means and slopes was performed to analyze the differences between grassland and forest SOC stocks along the altitudinal gradient. Statistical differences or correlations were considered statistically significant at $P<0.05$. Statistical analysis was perfomed using Matlab 7.4.0.287 (R2007a) (MathWorks, Massachusetts, USA).

3.3.2.3. Results and discussion

Soil C storage in grassland and forest soils

Changes in soil C stocks are dominantly explained by altitude for forest plots in the YUS area as we indicated in the previous chapter. In addition, we found that there is generally no difference between forest and grassland SOC stocks at comparable altitudes for soils to 1m depth (Fig. 3.7). In the previous chapter, we have also indicated that soil depth was strongly correlated to SOC stocks in forests. But because very often the grassland soils were shallow in our study area, particularly on steep slopes and frequently burned sites, an analysis for the top 30cm layer was more appropriate here.

Soil C stocks in the top 30cm of the soil profile were higher in grassland plots compared to forest plots (Fig. 3.8a) as indicated by ANCOVA analysis of means and slopes. The main reason for this difference in soil C stock is the consistently higher soil bulk density of the top soil layers in grasslands along the altitudinal gradient (Figs. 3.8b and 3.9).

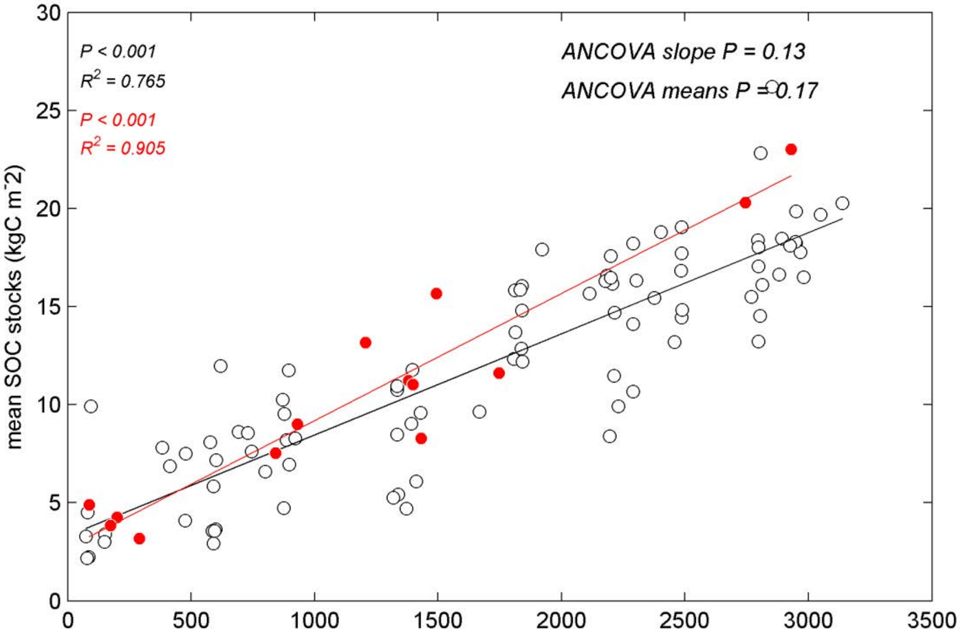


Figure 3.7: Relationship between altitude and soil C stocks for the 100cm profile in grassland (red circles) and forest (white circles) plots. The P-value and R^2-value of the individual linear regressions are given. Significant correlation is assessed at $P<0.05$. P-values for ANCOVA analysis are given to assess differences between means and slopes of both regressions. Statistical differences are considered at $P<0.05$.

The C concentration of the topsoil layers was higher in forest soils (Fig. 3.8c), likely due to the larger amount of litter production and higher C:N value of tree litter tissues and the usual absence of fire in the forests. Because the C concentration was lower in grassland soils compared to forest soils, this indicates that differences in soil bulk density were largely responsible for the higher SOC stocks

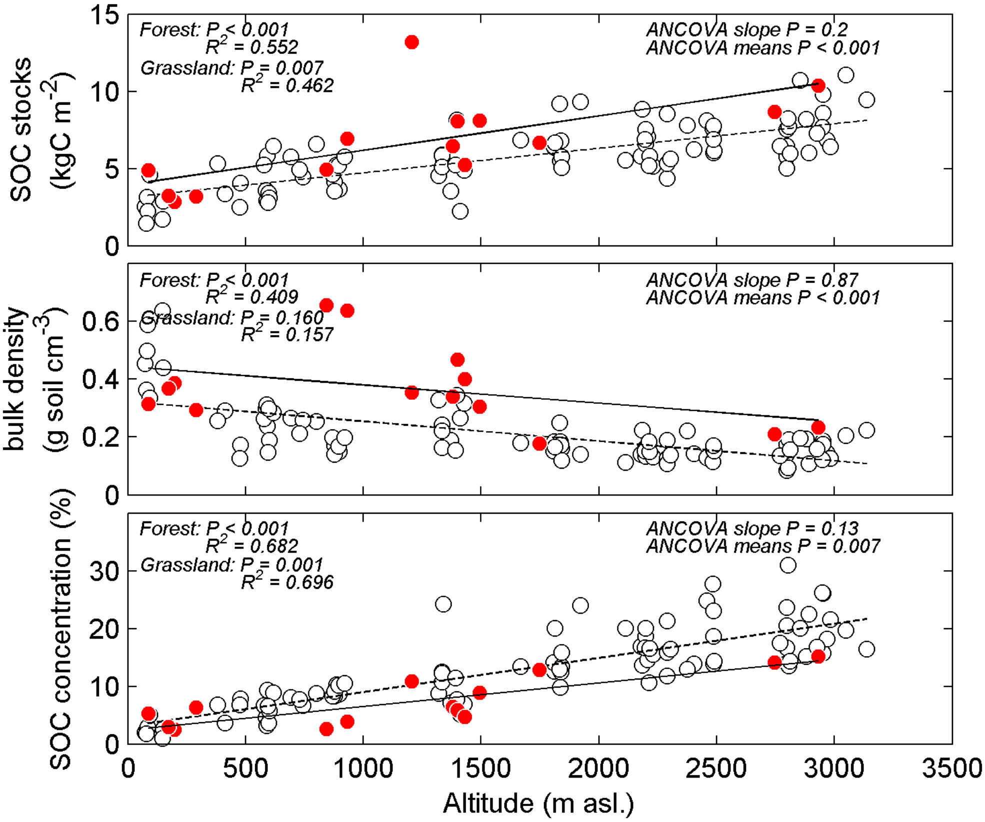


Figure 3.8: Relationship between altitude and soil C stocks (a), mean bulk density (b) and soil C content (c) for the 30cm soil profiles in grassland (red circles) and forest (white circles) plots. The P-value and R^2-value of the individual linear regressions are given. Correlations are considered statistically significant at $P<0.05$. *P*-values for ANCOVA analysis are given to assess differences between means and slopes of both regressions. Statistical differences are considered at $P<0.05$.

in grassland soils (Fig. 3.8). A likely explanation for the higher soil compaction in grassland sites compared to forest sites might be associated with land-use patterns.

Human-induced fires and subsistence agriculture are common practice in the study area and have been shown to induce stronger compaction of the top layers of soil (Prober *et al.* 2008; Schrumpf *et al.* 2011). Unfortunately, we did not find a relationship between grassland SOC stocks or soil bulk density, and time since the

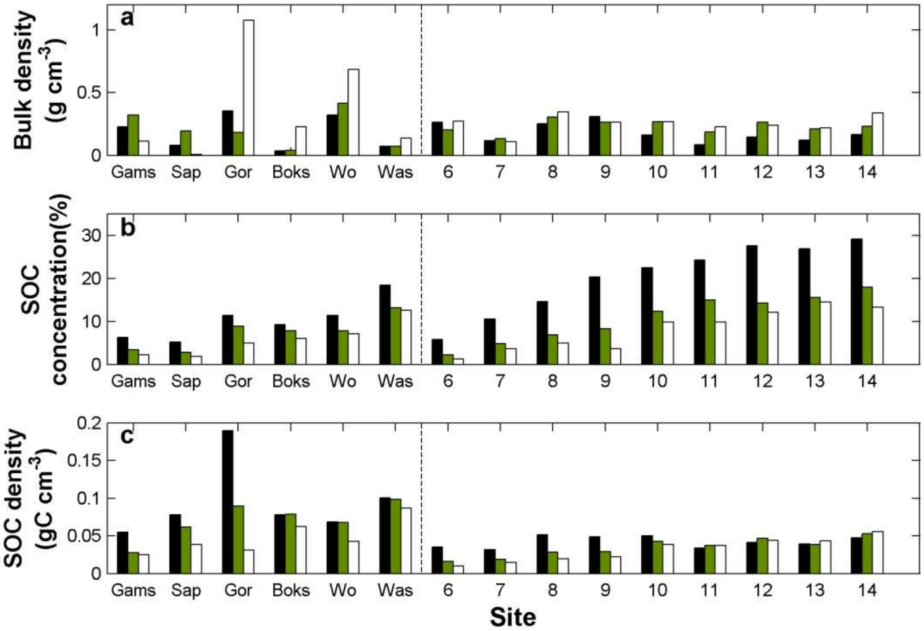


Figure 3.9: Bulk density (a), C concentration (b) and C density (c) of the 0-10 (black), 10-20 (green) and 20-30cm (white) layers of the soil in grassland (left of line) and forest plots. Forest sites presented in the graph range between 100m (site 6) and 2939m (site 14). Grassland sites are presented according to their altitude (from Gams (lowest) to Wasaunon (highest)). For a more detailed description, see Table 3.1 and Figure 3.2.

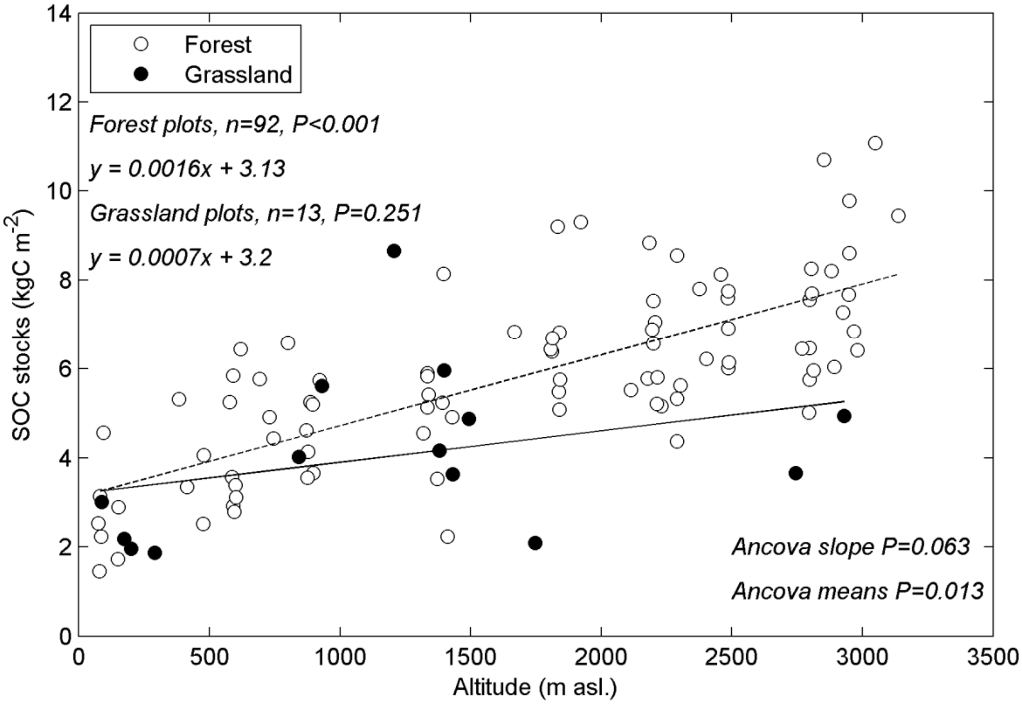


Figure 3.10: Relationship between altitude and bulk density corrected soil C stocks for the 30cm profile in grassland (red circles) and forest (white circles) plots. The P-value and R^2-value of the individual linear regressions are given. Significant correlation is assessed at *P*<0.05. *P*-values for ANCOVA analysis are given to assess differences between means and slopes of both regressions. Statistical differences are considered at *P*<0.05.

last burning event (data not shown), but this might be due to the relatively low amount of grassland sites that were sampled.

In contrast to our findings, forest conversion to grassland is generally thought to lead to a reduction of soil C stocks (Don *et al.* 2011). However, in their review, Don and colleagues point out that soil bulk density changes with land-use type, as was also found in our study. Therefore, SOC stocks need to be corrected for differences in bulk density in order to directly compare SOC stocks on the same basis of soil mass (Don *et al.* 2011). Based on ANCOVA results for a comparison of bulk density between grassland and forest along the altitudinal gradient (Fig. 3.8a), we corrected soil C stocks for the grassland plots with a factor BD_{corr}/BD (BD_{corr} = BD-0.12 (difference between intercept of regression for grassland and forest)). As a result, we found lower soil C stocks for grassland compared to those in forest (Fig. 3.10), and this difference tended to increase with altitude. The marginally significant difference in slope is important because it suggests that conversion of forest to grasslands at higher altitude sites might lead to a larger loss of soil C than expected from land-use change studies in lowland areas.

Effects of reforestation and implications for biomass C storage

Although we have not tested this in our study area, indirect effects of land use change on soils have also been shown to be important. For example, a comparison of pastures and forests in different stages of regrowth indicated that root C inputs in pastures and forests were not different, but litter inputs where significantly higher in forests (de Camargo *et al.* 1999). Another study observed higher soil respiration rates in forests, possibly associated with higher litter inputs (Sayer *et al.* 2011). Increased C inputs through above- and belowground litter in reafforested grasslands were also found to rapidly restore the normal C cycle (de Camargo *et al.* 1999; Wolf *et al.* 2011).

This faster cycling of plant material can restore soil biogeochemistry through nutrient cycling, because large amounts of non-nitrogen nutrients (P and K in particular) are stored in biomass (Hartemink 2005). In this regard, while grasslands tend to show rapid recovery into secondary forest when left undisturbed (Yassir *et al.* 2010), multiple cycles of fallowing or fire will progressively reduce aboveground biomass and litter inputs (Zarin *et al.* 2005; Eaton & Lawrence 2009), and thus affect the nutrient status of tropical systems. Therefore, the positive effects associated with the presence of trees on soil development, biogeochemistry and associated nutrient cycles will affect the potential for future biomass growth and C storage (de Camargo *et al.* 1999; Liu *et al.* 2011).

Another advantage of reforestation is the restoration of the hydrological cycle (de Camargo *et al.* 1999), with changes in microclimate (affecting decomposition) and feedbacks to global warming through evaporative cooling (Arora & Montenegro 2011). In addition, reforestation can stabilize soils on steep slopes, and prevent erosion and landslides through soil development, stronger soil aggregation and higher C content promoted by encroaching tree root networks in deeper layers of the soil (Don *et al.* 2011). This could also directly increase the soil C storage as soil thickness was a main determinant of SOC storage on slopes in our study area and other studies (Yoo *et al.* 2006).

Conclusion

We have shown here that differences in soil physical properties promote different C storage patterns in forests and grasslands. Taking changing bulk density into account, tree stands have the largest potential to store C in soils. When corrected for density, the difference in soil C storage between forests and grasslands increases with altitude, implying that land use change at higher altitude might lead to larger C losses than in lowland sites. Because large amounts of C are stored in aboveground biomass, the indirect advantages of soil development in tree stands compared to grasslands (improvement of soil development, quality, and nutrient cycles) are also important, especially at lower altitudes, where the difference in soil C stocks between forests and grasslands is small.

3.3.3. Towards C management in YUS

3.3.3.1. Total C storage in YUS

As we have shown in the previous chapters, soil C stocks increase with increasing altitude (chapter 3.3.1). But the altitudinal gradient in our study area correlates to climatic gradients as well as a gradient in soil pH. Both sets of variables suggest that the higher C pools at higher altitude are promoted by reduced SOM decomposition rates. Combining our soil C dataset with aboveground biomass data (Venter et al., in prep), indicates that the proportion on C stored in soil increases with altitude (Fig. 3.11), suggesting a shift of C storage towards

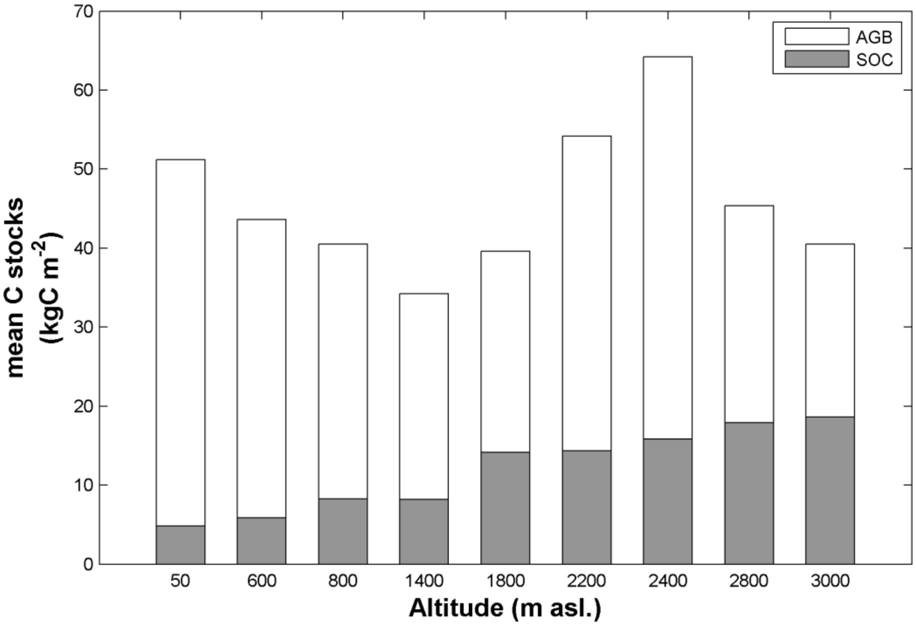


Figure 3.11: Mean C stocks in aboveground biomass (AGB) and soil (SOC) for different altitude clusters along the gradient. Data presented are means for 12-18 sites (AGB) and 9-12 sites (SOC).

belowground compartments, and confirming the more prominent role of soil C storage at higher altitudes. Explaining what controls this relationship between aboveground biomass C and soil C storage will be key to understanding the controls of C cycling in the YUS area. A large number of previous studies have indicated that nutrient availability and soil texture control plant biomass production in the tropics (Aragao *et al.* 2009; Cleveland *et al.* 2011), agreeing with our suggestion of reduced SOM decomposition rates. However, other studies have indicated that climate is a stronger predictor of forest growth compared to soils and disturbance (Toledo *et al.* 2011), and topography strongly predicts aboveground biomass (AGB) in other studies (Mascaro *et al.* 2011). In reality, as we also found for the SOC stocks, the control of NPP in tropical forest is likely to be a combination of these variables, with site-specific factors determining the dominant driver amongst them.

The irregular altitudinal pattern in the aboveground biomass data (Fig. 3.11) refutes a linear relationship with nutrients on our transect, and preliminary analysis indicates that solar radiation has a strong correlation with a large part of the AGB estimates along the altitudinal gradient (Venter et al., personal communication). Future steps will have to elucidate how different variables control aboveground biomass and might bring a better understanding of the plant-soil connection.

3.3.3.2. Carbon mapping

For successful implementation into climate change mitigation policies, robust estimates of carbon stocks over large geographical areas are needed (Asner *et al.* 2010; Saatchi *et al.* 2011). Therefore, one of the final outcomes of the C component of the YUS program will be the integration of soil C stocks and aboveground biomass C estimates into C maps.

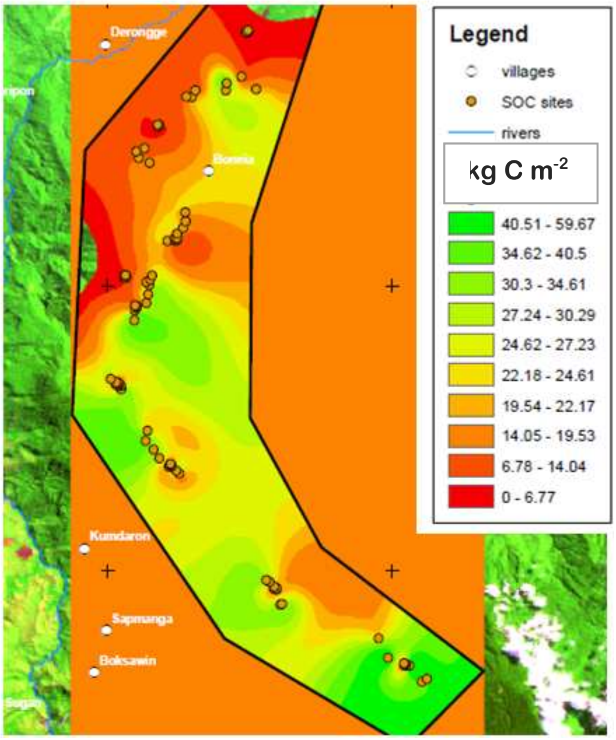


Figure 3.12: Soil C stock map for the YUS altitudinal transect, based on the relationship with altitude using a spline interpolation fit.

In figure 3.12, an example map is given for SOC stocks that is based on the relationships found in previous chapters. In addition to this map, estimates for the different vegetation types in the YUS area will be produced (Table 3.6). A combination of the topographic relationship with soil C stocks and the different estimates between vegetation types represents a first step towards providing a benchmark for policy makers (Gibbs *et al.* 2007). Future efforts should focus on quantifying (re)growth rates, C stocks in secondary forests and fallows, and evaluating eligible locations for C management (Gibbs *et al.* 2007).

Table 3.6: Soil C stocks for different vegetation types, classification in vegetation types is based on a vegetation classification map for the area (Gillieson *et al.* 2011).

Vegetation type	**SOC ± SD (kg C m^{-2})**	**SOC range (kg C m^{-2})**	**Elevation range**	**N**
Medium Crowned Forest	6.5 ± 2.8	2.2-12.0	73-921	31
Small Crowned Forest	13.5 ± 4.4	3.2-22.8	1206-2812	55
Very Small Crowned Forest	19.4 ± 2.8	16.5-26.2	2852-3136	12
Regrowth (fallow)	7.9 ± 5.2	3.8-15.7	290-1747	2
Grassland	10.5 ± 4.5	4.3-11.6	88-1494	5

3.3.4. Climatic effects on soil C cycling in YUS

In the previous chapters, we were able to identify clear patterns in the distribution of SOC stocks in our study area. In addition, a range of environmental variables were demonstrated to be correlated to SOC stocks. These variables might thus be part of the controlling factors of soil C and soil C cycling processes in the YUS area. However, we are not yet in a position to discuss direct effects of climatic changes along the gradient.

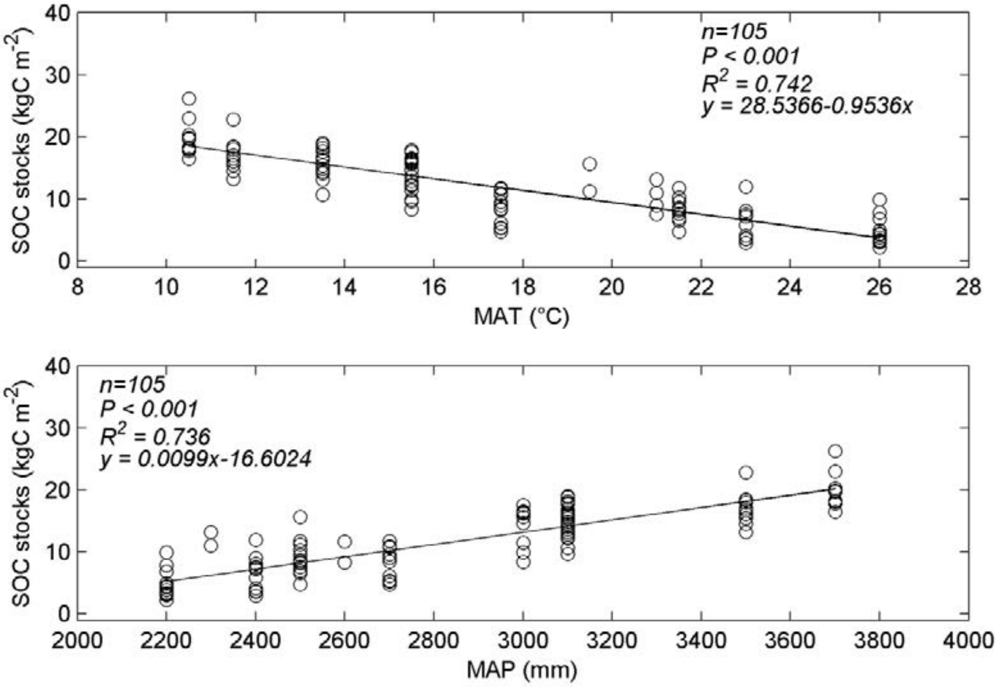


Figure 3.13: Linear regression between SOC stocks and mean annual temperature (MAT) (top panel), and SOC stocks and mean annual precipitation (MAP) (bottom panel). *P*-value, R^2-value and regression equations are given.

Since this thesis addresses effects of climatic changes on soil C and soil C cycling, we nevertheless calculated an average change in SOC stocks per unit of change in temperature or precipitation (Fig. 3.13). Our data suggest that SOC stocks decreased with 0.95 kgC m^{-2} per °C increase in temperature, and increased by 0.01 kgC m^{-2} per mm increase in rainfall along our gradient. This means that following the predictions of the IPCC (2-4°C increase in temperature and 20% increase in rainfall for the YUS area by the end of the century) (IPCC 2007), an average of 1.9-3.8 kgC could be lost due to increasing temperatures, and an average of 4.5-7.7 kgC could be gained through increases in precipitation. Of course, whether a particular patch of soil will lose or gain C due to changes in temperature and rainfall will differ strongly on the altitude of a particular location. Although the accuracy of our climatic data might be limited at this point, these relationships are still valuable as a benchmark for future analyses. In addition, weather stations have been installed along the altitudinal gradient and will provide more accurate weather data in the future.

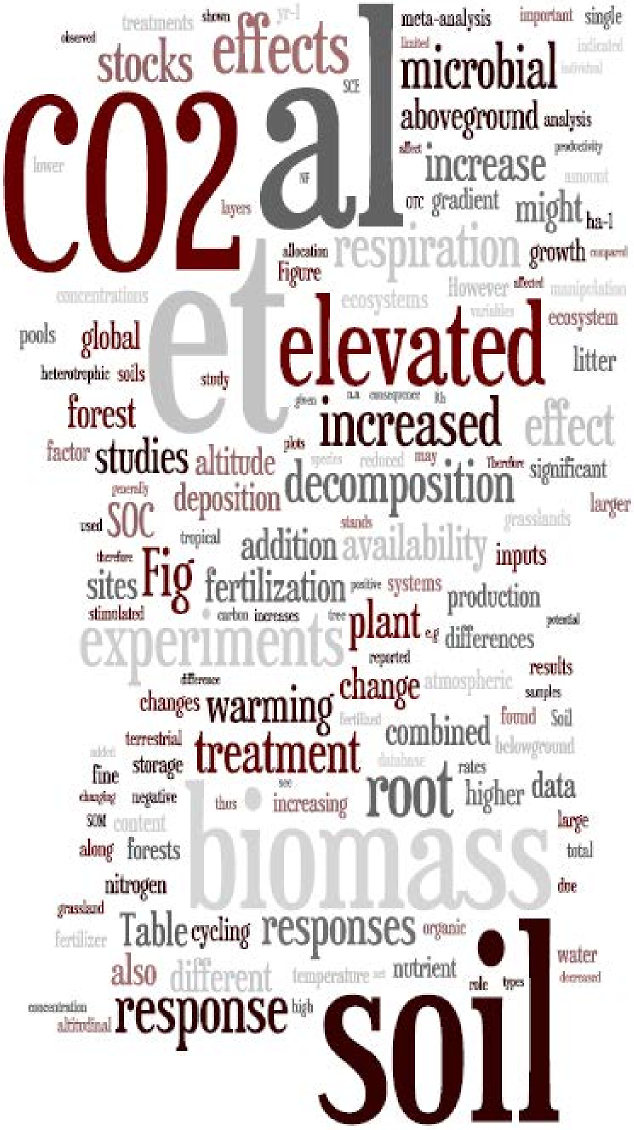
To further analyze mechanisms behind changes in SOC stocks in the YUS area, we need to know how root biomass and dynamics, litterfall, and decomposition respond with changing altitude (and thus climate) (e.g. Salinas *et al.* 2011). The installation of litter traps, and minirhizothrons or root ingrowth bags in the 1ha plots (e.g. Graefe *et al.* 2008; Girardin *et al.* 2010), and a soil transplantation experiment between the 1ha plots (e.g. Zimmermann *et al.* 2009) might address these questions. However, due to logistical limitations and the restricted accessibility, we have not addressed this possible next step in this thesis. In addition, due to the strong covariation of both temperature and precipitation with altitude in the current dataset, respiration measurements should be performed in controlled laboratory experiments to be able to separately assess the effects of temperature and water availability.

Within this altitudinal gradient study, effects of elevated CO_2 concentrations or increasing N deposition were not addressed. A small number of studies have indicated that biomass growth in tropical systems was not very responsive to elevated CO_2 concentrations (Körner & Arnone 1992), but fine root dynamics (Körner & Arnone 1992) and soil respiration (Körner & Arnone 1992; Deng *et al.* 2010) were strongly stimulated. In contrast, N additions have been shown to strongly reduce soil respiration in tropical forest (Mo *et al.* 2007). It is therefore likely that rising CO_2 levels and N deposition will also influence the relationship between SOC stocks and changes in temperature in rainfall.

Conclusion

In the previous chapters (3.3.1 and 3.3.2), we have shown that SOC stocks along our gradient are mainly controlled by altitude, and thus by the environmental changes that are prevalent along this gradient. Differences between forest and grassland SOC stocks are mainly caused by changes in soil bulk density that are likely due to changing land use (e.g. burning, farming). We identified changing temperature, precipitation, pH and soil depth as important co-variants with altitude, indicating they could play an important role determining SOC stocks. Reduced SOM decomposition at higher altitude due to lower temperature, higher rainfall and more acidic soils can explain the larger buildup of SOC at higher altitudes. However, our analysis also indicated the difficulties associated with separating key drivers behind SOC cycling processes, due to the large number of co-varying environmental variables. A combination of in situ studies on litter inputs and root dynamics, and controlled experiments focusing on microbial respiration rates would help to find out the mechanisms behind SOC changes along this altitudinal gradient.

4. GENERAL DISCUSSION



4.1. Synthesis of previous chapters

In this thesis, I have addressed direct and indirect effects of global changes on soil C storage and soil C cycling. I have found a variety of responses for different C pools and fluxes for all tested global change drivers and their combinations (Table 4.1). In this variety of responses, the common pattern is that they all reflect effects on microbial growth and functioning in some way: i.e. How do substrate supply, substrate quality, enzyme kinetics, nutrient availability or water availability determine whether microbial growth and respiration will be stimulated or inhibited? In turn, this will determine the changes in soil C and N pools, and whether C will be lost or sequestered. At least two of these five factors are affected in each treatment we addressed (Table 4.2). Under elevated CO_2 concentrations (Chapter 2.3.1 and 2.3.2), the supply of easily degradable C compounds increased, yielding higher microbial decomposition rates. Litter quality, while not addressed in this thesis, is known to decrease in elevated CO_2 concentrations, reducing rates of decomposition. Nutrient availability generally decreased, thereby reducing the ability of microbes to constructively use C inputs, resulting in increased microbial respiration rates. Feedbacks related to this decreasing N availability might ultimately lead to a reduced soil C input due to reduced aboveground biomass and litter production. And finally, increased water use efficiency (WUE) can positively affect microbial activity, especially in ecosystems where soil moisture is limiting.

Table 4.1: Synthesis of the findings in this thesis. All directly and indirectly involved C pools and fluxes in soil C cycling are presented, and the observed effect in our analyses indicated where applicable. + represents an increase, - a decrease, = indicates no change, +/= indicates a positive trend.

	CO_2	NITROGEN FERTILIZATION	WAR-MING	CO_2xN	CO_2xWARMING	GRADIENT STUDY
Above Biomass	+/=	+	+	+	+	+
Above Litterfall	+	=	n.a.	+	n.a.	n.a.
Root Biomass	+	+	+/=	+	+	n.a.
Root Turnover	+	n.a.	n.a.	+	n.a.	n.a.
Rr	n.a.	-	n.a.	n.a.	n.a.	n.a.
SOC	=	+	-	+/=	n.a.	-
Microbial Biomass	+/=	-	=	+/=	n.a.	n.a.
Rh	+	-	=	+/=	n.a.	n.a.
SR	+	-	=	+	+	n.a.
Mineral Nutrients	-	+	+	=	=	n.a.

n.a. - not addressed

Nitrogen fertilization (Chapter 2.3.2 and 2.3.3) did not affect substrate supply directly, as most of the increased C sequestration was stored in plant tissues with long(er) turnover times. Litter quality might have increased due to a larger availability of N. Due to small or reduced amount of labile C inputs, N fertilization did not always stimulate decomposition, and inhibitory effects on microbial growth and functioning also arose, depending on the amount of N fertilization.

Table 4.2: Synthesis of global change effects on drivers of microbial growth and activity. Qualitative effects are indicated: + indicates an increase, - a decrease, = indicates no change.

	CO_2	NITROGEN FERTILIZATION	WAR-MING	CO_2xN	CO_2xWARMING	GRADIENT STUDY
Substrate supply	+	C storage in long-lived tissues	+	+	+	n.a.
Litter Quality	-	+	n.a.	n.a.	n.a.	n.a.
Enzyme kinetics	n.a.	n.a.	+	n.a.	+	+
Nitrogen availability	-	+	+	=	=	P more important?
Water availability	Increased WUE	=	Soil drying	Increased WUE	Soil drying + Increased WUE	Soil drying

n.a. - not addressed

Warming (Chapter 2.3.4, 3.3.1 and 3.3.4) stimulated C inputs through greater plant productivity, although in some cases productivity might have been limited by water availability. In general, SOM decomposition was higher in a warmer environment due to direct effects on enzymatic reaction rates, but depletion of labile substrate or soil drying might limit this increase. Nitrogen availability increased as a consequence of increased SOM decomposition, which can stimulate plant productivity and provide higher soil C inputs.

In combined treatments (Chapters 2.3.1, 2.3.2, 2.3.4, 3.3.1 and 3.3.2), effects of single global change drivers often cancelled each other out, or interactions complicated the interpretation of results. The large range of differences in initial site properties (e.g. soil nutrient status, water balance, plant and microbial species composition, age and successional status) likely played an important role here. Nevertheless, I found that a combined CO_2 and N fertilization treatment stimulated soil C inputs, but because microbes were no longer limited by N availability, these C compounds were less easily respired and decomposition of SOM was limited. As a consequence, soil C content tended to increase. In a combined CO_2 and warming treatment, increased C supply and an increased WUE as a consequence of elevated CO_2 might have enhanced the direct stimulatory effect of warming on decomposition rates, and conversely, warming might have reduced the progressive decrease in N availability as a consequence of elevated CO_2 concentrations.

The comparison of the GCME's with the altitudinal gradient study, though limited, offered some confirmation of each other's findings. Aboveground biomass did not linearly increase with increasing temperatures, as other variables might also have played an important role (nutrients, light capture, optimal temperature limit). And while soil C stocks decreased very clearly with higher temperatures in the gradient study, I only had a limited dataset to address this question in the GCME analyses. Both approaches left some questions unanswered, and demonstrated the importance of a holistic approach: incorporation of plant-soil interactions, adequate documentation of site variables (i.e. soil nutrients and water availability, soil texture), and inclusion of representative entities of the particular system studied (i.e. to capture effects of plant species diversity and spatial variability).

4.2. Global change effects on soil C cycling

In this thesis, I have increased the understanding of mechanisms and patterns behind effects of N fertilization and elevated CO_2 concentrations, and have synthesized and clarified some effects and response patterns in warming experiments. To increase the understanding of mechanisms behind responses to elevated temperatures, but also behind responses to multifactor treatments, a larger number of experiments is necessary, with attention to documentation of N and water availability, and an approach where effects on C inputs, C pools, and C losses from soil are simultaneously addressed.

In figure 4.1 I present a conceptual scheme that indicates the necessary variables to understand global change effects on soil C content and soil C dynamics. In addition, I have added the direct effects of the global change drivers addressed in this thesis. With initial ecosystem properties in mind, effects on water availability and nutrient availability will determine long-term effects of global atmospheric changes on plant productivity. Depending on whether plant production is limited by water- or nutrient feedbacks, C allocation patterns will determine whether soil C inputs will mainly originate from aboveground, or belowground litter production. Similarly, effects on chemical and physical stability of organic matter, and substrate quality, will determine the sensitivity of organic matter to microbial

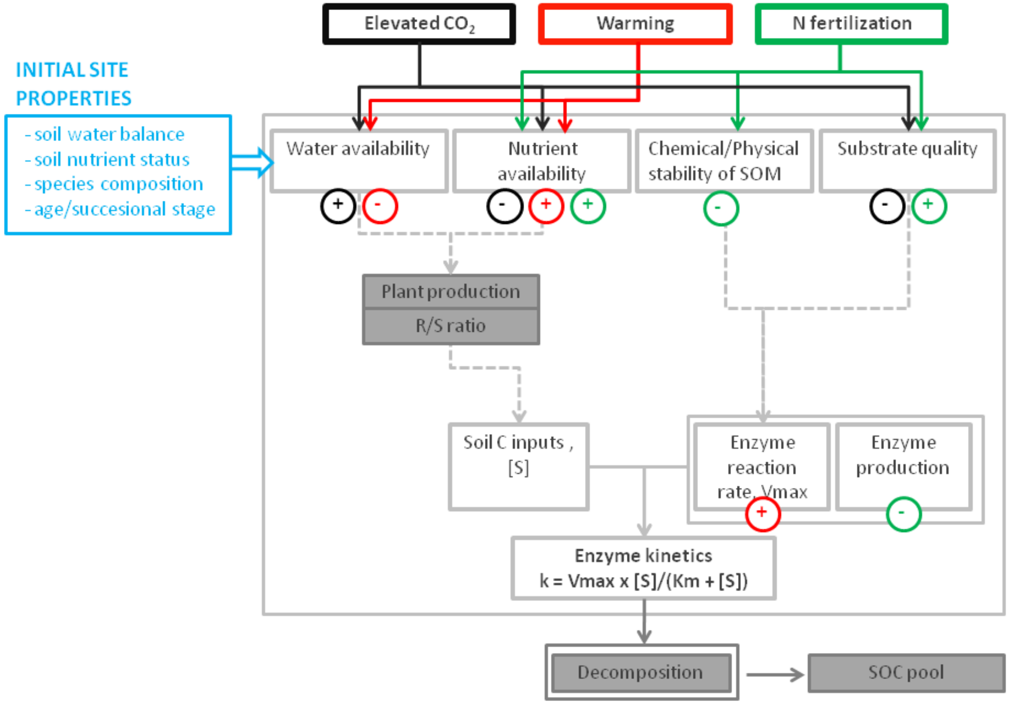


Figure 4.1: Conceptual representation of variables affecting soil C and soil C cycling. Direct effects of global changes on plant production and SOM decomposition are indicated in colored circles, and will determine short-term effects. Initial site-properties will determine long-term effects and the development of feedbacks related to nutrient- or water availability, or community species composition.

decomposition. Direct effects of enzymatic reaction rates or enzyme production can modify this step. When we then take the amount of available substrate and the sensitivity of this substrate to global changes together, we can use the Michaelis-Menten equation for enzyme kinetics to determine the effects on SOM decomposition, and predict changes in soil C content.

4.3. Important drivers in the future

4.3.1. Shifts in vegetation and microbial community species composition

As I have shown and discussed, effects of global change on soil C dynamics are inevitably connected to plant productivity responses. As such, changes in plant community species composition will have an impact on soil C dynamics through changing soil C inputs, changing litter quality, or changing soil community species composition.

Of course, global change can also directly affect soil community species composition, as has been shown for elevated CO_2 concentrations (Carney *et al.* 2007), N and NPK fertilization (Rinnan *et al.* 2007), warming (Briones *et al.* 2009) and drought (Curiel Yuste *et al.* 2010), often reporting shifts towards more fungal dominated soil communities. As such, in a world where litter quality is suggested to decrease, a less N-demanding, fungal-dominated soil community might have important implications for soil C cycling.

The spatial and temporal scale of the dominantly short-term studies in this thesis might not always represent the scale on which global change induced effects will happen. As a consequence, it is hard to upscale our results to regional and global scales, or predict in which timeframe they will happen. Gaudnik *et al.* (2011) addressed this question and found that over a period of 25 years, climatic changes had a stronger impact on regional scale plant composition changes, and N deposition had a more local effect. Their study provides a good example of how long-term monitoring can be combined with outcomes of GCME's to identify the temporal and spatial scale of predicted future effects on terrestrial ecosystems.

4.3.2. Land use change and disturbance

Croplands and pastures have become the most important terrestrial biomes on the planet, rivaling forest cover in extent and occupying ca. 40% of the land surface (Asner *et al.* 2004). This has partly led to a net loss of 7-11 million m^{-2} of forest in the past 300 years (Foley *et al.* 2005). In addition, land-use practices (e.g. fuel-wood collection, grazing) can degrade forest ecosystem conditions in terms of productivity, biomass, stand structure and species composition. However, forest regrowth in Europe has stimulated C uptake (Churkina *et al.* 2010), and recent reforestation and afforestation projects, and forest management are improving forest conditions by nitrogen fertilizing, peatland drainage or direct management efforts.

Forests are the largest terrestrial sink of C in the biosphere (Pan *et al.* 2011), followed by pastures, while agricultural areas are generally C sources (IPCC 2007). Due to this ecosystem type-specific C-sink strength, long-term trends in global land use change and disturbance will also determine the potential of terrestrial ecosystems to buffer the rise of global atmospheric CO_2 concentration.

4.4. Conclusions

Based on the findings in this thesis, elevated CO_2 will mainly accelerate soil C cycling, unless ecosystems are fertilized with nitrogen. Nitrogen fertilization will promote storage of C in soil and long-lived biomass compartments, but when soil becomes saturated with N, detrimental effects on plant survival and ecosystem diversity will start to dominate. Warming is a very complex driver as it interacts with water availability and nutrient cycling, and differs strongly between climatic regions. Where water is not limiting and C supply is abundant, decomposition will likely be stimulated. However, if one of these factors is limiting, the increase in soil respiration is short-lived. Warming consistently stimulated net N mineralization, with possible positive feedbacks to plant growth. Soil C content was not consistently affected in short-term manipulation experiments, apart from stimulating effects of N fertilization. In the altitudinal gradient study, a clear change in soil C storage was observed as a consequence of changes in climatic variables and soil properties (pH, depth and density).

I have outlined the difficulties associated with manipulation experiments (differences between ecosystems types, interactions and feedbacks, and inherent problems with experimental design), and have indicated the challenges associated with trying to determine the role of individual drivers in gradient studies.

To conclude, I launch a strong call for better documentation of individual studies, and well-designed experiments to identify key mechanisms and players in soil C cycling. In particular, the soil C input – soil C output cascade should be investigated including all relevant components (litterfall and root turnover - microbial biomass and soil C and nutrient pools - soil CO_2 effluxes) to elucidate possible pathways and mechanisms. When considering one single global change driver, the effects on soil C cycling and soil C storage are relatively easy to predict. But when several factors are combined, it becomes difficult to tease apart the proportional role of different drivers. A combination of laboratory studies testing the sensitivity of individual processes, manipulation experiments linking individual response variables, and gradient studies integrating feedbacks and long-term effects promises to deliver the best results to understand and predict global change effects on soil C and soil C cycling.

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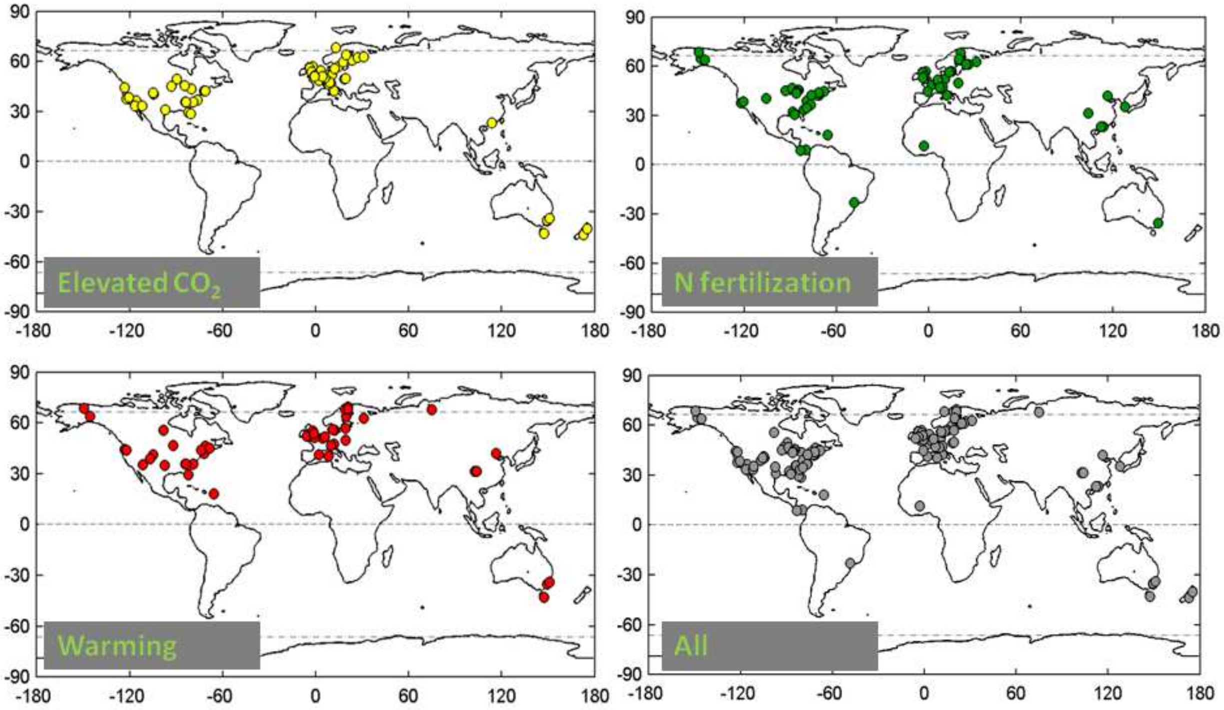
6. APPENDICES

Appendix 1: Location of experimental sites in the GCME database according to manipulated global change driver.



Appendix 2: Site information and data used in the comparison of forests exposed to low versus high N deposition. Background information and data for the sites used in the comparison of heterotrophic respiration and soil CO_2 efflux between forests exposed to low and high N deposition (threshold: wet deposition = 5.5 kg N ha^{-1} a^{-1}; Figures 3 and 4). Data provided by Law should not be used without permission (bev.law@oregonstate.edu).

Plot name	**Climatic region**	**Species**	**NPP** **g C m^{-2} a^{-1}**	**SCE** **g C m^{-2} a^{-1}**	**Rh** **g C m^{-2} a^{-1}**	**Latitude**	**Longitude**	**Wet deposition**	**Source**
CA Thompson 1	Boreal	Picea mariana Mill.	261	551	385	55.88 N	98.33 W	2.7	(Bond-Lamberty *et al.* 2004)
DE Bornhoved	Temperate	Fagus sylvatica L.	656	539		54.10 N	10.23 E	9.9	(Kutsch *et al.* 2001)
DE Hesse	Temperate	Fagus sylvatica L.	939	646	370	48.67 N	7.07 E	12.4	(Bascietto *et al.* 2003)
DE Lei 1	Temperate	Fagus sylvatica L.	649		321	51.33 N	10.36 E	9.8	(Bascietto *et al.* 2003)
DE Lei 2	Temperate	Fagus sylvatica L.	886		268	51.33 N	10.36 E	9.8	(Bascietto *et al.*

									2003)
DE Lei 3	Temperate	Fagus sylvatica L.	599		302	51.33 N	10.36 E	9.8	(Bascietto *et al.* 2003)
DE Lei 4	Temperate	Fagus sylvatica L.	909		328	51.33 N	10.36 E	9.8	(Bascietto *et al.* 2003)
DE Tharandt 1	Temperate	Picea abies L.	655		323	50.91 N	13.46 E	11.3	(Bascietto *et al.* 2003)
DE Tharandt 2	Temperate	Picea abies L.	1199		344	50.93 N	13.48 E	11.3	(Bascietto *et al.* 2003)
DE Tharandt 3	Temperate	Picea abies L.	761		336	50.93 N	13.46 E	11.3	(Bascietto *et al.* 2003)
DE Waldstein	Temperate	Picea abies L.	665	711	262	50.20 N	11.88 E	12.7	(Bascietto *et al.* 2003)
DE Wetstein	Temperate	Picea abies L.	777		275	50.45 N	11.46 E	11.7	(Bascietto *et al.*

									2003)
DK Soroe	Temperate	Fagus sylvatica L.	1035		369	55.49 N	11.65 E	9.0	(Bascietto *et al.* 2003)
IT Collelongo	Temperate	Fagus sylvatica L.	639	879	257	41.85 N	13.59 E	10.0	(Bascietto *et al.* 2003)
IT Monte di Mezzo	Temperate	Picea abies L.	801	803	406	41.75 N	14.88 E	8.2	(Schulze 2000)
JP Takayama	Temperate	Quercus crispula Bl.	744	886		36.10 N	137.41 E	5.4	(Ito *et al.* 2005; Ohtsuka *et al.* 2005)
NL Loobos	Temperate	Pinus sylvestris L.	420		359	52.17 N	5.74 E	13.3	(Bascietto *et al.* 2003)
RU Zotino	Boreal	Pinus sylvestris L.	214	258		60.72 N	89.13 E	2.7	(Lloyd *et al.* 2002)
SE Flakaliden 1	Boreal	Picea abies L.	389		260	64.12 N	19.45 E	3.1	(Bascietto *et al.*

									2003)
SE Flakaliden 2	Boreal	Picea abies L.	156	1214	476	64.12 N	19.45 E	3.1	(Olson *et al.* 2001; Eliasson *et al.* 2005a)
US Andrews 1	Temperate	Pseudotsuga menziesii Franco	1102	1752	461	44.26 N	122.20 W	1.3	Law (unpublished)
US Andrews 2	Temperate	Pseudotsuga menziesii Franco	1095	2054	653	44.25 N	122.20 W	1.3	Law (unpublished)
US Andrews 4	Temperate	Pseudotsuga menziesii Franco	1160	1590	675	44.25 N	122.20 W	1.3	Law (unpublished)
US Andrews 5	Temperate	Pseudotsuga menziesii Franco	969	1483	670	44.23 N	122.18 W	1.3	Law (unpublished)
US Andrews 6	Temperate	Pseudotsuga menziesii Franco	1044	1362	595	44.25 N	122.18 W	1.3	Law (unpublished)

US Andrews 7	Temperate	Pseudotsuga menziesii Franco	836	1834	352	44.27N	122.22 W	1.3	Law (unpublished)
US Andrews 8	Temperate	Pseudotsuga menziesii Franco	926	2288	524	44.27 N	122.23 W	1.3	Law (unpublished)
US Andrews 9	Temperate	Pseudotsuga menziesii Franco	804	2079	398	44.26 N	122.19 W	1.3	Law (unpublished)
US Cascade Head 4	Temperate	Tsuga heteropylla Sarg.	682	1265	411	45.11 N	123.88 W	3.7	Law (unpublished)
US Cascade Head 5	Temperate	Tsuga heteropylla Sarg.	511	1240	367	45.11 N	123.88 W	3.7	Law (unpublished)
US Cascade Head 6	Temperate	Tsuga heteropylla Sarg.	824	754	469	45.09 N	123.88 W	3.7	Law (unpublished)
US Cascade Head 7	Temperate	Tsuga heteropylla Sarg.	649	896	328	45.09 N	123.88 W	3.7	Law (unpublished)

US Cascade Head 8	Temperate	Tsuga heteropylla Sarg.	893	1131	448	45.09 N	123.88 W	3.7	Law (unpublished)
US Cascade Head 9	Temperate	Tsuga heteropylla Sarg.	657	1216	534	45.07 N	123.89 W	3.7	Law (unpublished)
US Metolius	Temperate	Pinus ponderosa Laws.	405	677	292	44.42 N	121.67 W	1.0	Law (unpublished)
US Metolius 1	Temperate	Pinus ponderosa Laws.	258	501	392	44.44 N	121.57 W	1.0	Law (unpublished)
US Metolius 3	Temperate	Pinus ponderosa Laws.	149	480	206	44.43 N	121.61 W	1.0	Law (unpublished)
US Metolius 4	Temperate	Pinus ponderosa Laws.	356	498	192	44.43 N	121.59 W	1.0	Law (unpublished)
US Metolius 5	Temperate	Pinus ponderosa Laws.	372	533	316	44.44 N	121.59 W	1.0	Law (unpublished)

US Metolius 6	Temperate	Pinus ponderosa Laws.	448	749	337	44.45 N	121.56 W	1.0	Law (unpublished)
US Metolius 7	Temperate	Pinus ponderosa Laws.	543	1039	357	44.43 N	121.67 W	1.0	Law (unpublished)
US Metolius 8	Temperate	Pinus ponderosa Laws.	246	558	290	44.45 N	121.67 W	1.0	Law (unpublished)
US Metolius 9	Temperate	Pinus ponderosa Laws.	639	1019	300	44.46 N	121.66 W	1.0	Law (unpublished)
US Metolius young	Temperate	Pinus ponderosa Laws.	337	541	280	44.43 N	121.57 W	1.0	Law (unpublished)
US Morgan Monroe	Temperate	Acer saccharum Marsh.	976	852	650	39.32 N	86.42 W	5.7	(Curtis *et al.* 2002)
US UMBS 1	Temperate	Populus grandidentata Michx.	442	782	409	45.59 N	84.71 W	5.4	(Gough *et al.* 2007)
US UMBS 2	Temperate	Populus grandidentata Michx.	488	782	432	45.59 N	84.71 W	5.4	(Gough *et*

									al. 2007)
US UMBS 3	Temperate	Populus grandidentata Michx.	524	1090	441	45.59 N	84.71 W	5.4	(Gough *et al.* 2007)
US UMBS 4	Temperate	Populus grandidentata Michx.	480	930	440	45.59 N	84.71 W	5.4	(Gough *et al.* 2007)

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