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Pathways of C and N turnover in soil under elevated atmospheric CO_2

Pfade des C- und N-Umsatzes im Boden unter erhöhter CO_2-Konzentration

Пути трансформации C и N в почве под воздействием повышенных концентраций CO_2 в атмосфере

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I) Introduction

1) The impact of elevated atmospheric CO_2 on terrestrial ecosystems

The atmospheric carbon dioxide (CO_2) concentration has been increasing continuously since the industrial revolution from 280 μmol mol^{-1} to the current level of 380 μmol mol^{-1}, and it is expected to continue rising up to 700 μmol mol^{-1} by the end of this century (Houghton and Ding, 2001; IPCC, 2007). During the past 200 years, the CO_2 concentration in the atmosphere has increased by 35% mostly due to extensive burning of fossil fuels and land-use changes (IPCC, 2007). The concern over the possible consequences of increasing concentrations of atmospheric CO_2 has created renewed interest initiating the studies exploring ecosystem responses to elevated CO_2 (Paustian et al., 1997; Pendall et al., 2004; van Kessel et al., 2006).

The increasing concentrations of CO_2 in the atmosphere have a direct effect on the plant biomass resulting in (i) higher net primary productivity (Melillo et al., 1993; Schimel, 1995) through accelerated photosynthetic rates (Paterson et al., 1997), (ii) changes in plant chemistry such as higher C/N ratio, higher concentrations of starch, sugars, and carbohydrates (Cotrufo et al., 1994), (iii) reduced stomatal conductance of plants resulting in higher water use efficiency (Körner, 2000), and (iv) stimulated root growth (Paterson et al., 1997; Rogers et al., 1998), which increases rhizodepositions including root exudates (van Veen et al., 1991; Cheng, 1999).

In contrast to the response of plant biomass to elevated CO_2, the feedback of belowground processes in soils to atmospheric CO_2 enrichment occurs indirectly through plant-derived deposits; this process is less well studied compared to the aboveground biomass response. Furthermore, there are contradictory results reported describing the fate of extra carbon (C) in soil derived from the enhanced plant photosynthesis. Thus, Diaz et al. (1993) proposed a negative feedback mechanism, where increased C input to the soil from increased productivity in elevated atmospheric CO_2 caused C and nutrient accumulation in soil organic matter (SOM). On the contrary, Zak et al. (1993) found decomposition rate to increase after exposure of litter to elevated CO_2, suggesting that a positive feedback might occur, which would increase rates of carbon and nitrogen (N) cycling through the ecosystem.

Clearly, the responses of ecosystems to elevated CO_2 are divergent and C and N dynamics in terrestrial ecosystems depend on a set of complex interactions between soil and plants. It is not clear whether soil C input and soil C mineralization could bring to soil C accumulation (sequestration) under elevated CO_2. Also, to reach the equilibrium between SOM input and decomposition can take up to decades or longer, so we need long-term

experiments under realistic field situations to predict changes in ecosystems under future CO_2 levels.

2) Free Air CO_2 enrichment (FACE) experiments

FACE is a technique for exposing crops, forest plantations and natural vegetation to elevated atmospheric CO_2 concentrations without an enclosing structure. FACE provide an opportunity to examine many aspects of elevated CO_2 effects on ecosystems, and to study the pathways of C and N in SOM under realistic field conditions (Leavitt et al., 2001; Miglietta et al., 2001; Kimball et al., 2002). FACE avoids most of the microenvironment effects imposed by the chamber technique and therefore more reliably reproduces soil processes under elevated CO_2 (van Kessel et al., 2000b). FACE technology has developed considerably since the first experiences made by Harper and coworkers in the 1970s (Harper et al., 1973). At present more than 20 FACE sites are operating all around the world from Northern and Central America to Europe, Asia and Oceania. The size of the FACE plots varies from 1-2 meters of diameter for the MiniFACE (Miglietta et al., 2001; Erbs and Fangmeier, 2006) to the 30 m of the larger FACE systems that have been used to fumigate with CO_2 the plots of forest plantations (Hendrey, 1999).

To study the fate of C in soils under CO_2 enrichment, most FACE experiments use stable-C isotopic tracers (Hungate et al., 1997; Torbert et al., 1997; van Kessel et al., 2000a, 2000b; Leavitt et al., 2001; Niklaus et al., 2001; Hagedorn et al., 2003). Namely, C has two naturally occurring stable isotopes, ^{13}C and ^{12}C. Approximately 98.89% of all carbon in nature is ^{12}C, and 1.11% is ^{13}C (Boutton, 1991). The natural $^{13}C/^{12}C$ ratio is denoted as $\delta^{13}C$ in per mil PDB [‰]:

$$\delta^{13}C = [(R_{sample} - R_{PDB})/ R_{PDB}] \cdot 1000‰ \quad (1),$$

where R is the isotopic ratio of $^{13}C/^{12}C$. R_{PDB} is the defined isotope ratio of the limestone fossil *Belemnitella americana* from the Cretaceous PeeDee Formation in South Carolina, which is set to $\delta^{13}C = 0‰$ as basis of the scale. A negative $\delta^{13}C$ value indicates that the sample is “lighter”, i.e. contains less ^{13}C than the standard.

So, the additional CO_2 is usually derived from fossil fuel, which is depleted in ^{13}C ($\delta^{13}C$ varies from −35‰ to −50‰) compared with atmospheric CO_2 ($\delta^{13}C$ −8‰) (Hungate et

al., 1996; Nitschelm et al., 1997; van Kessel et al., 2000b; Jones and Donnelly, 2004). The isotopic composition of CO_2 in the elevated CO_2 treatment is determined by the proportions of atmospheric and fossil fuel-derived CO_2, and provides a continuous C-isotope tracer. Plants grown in the elevated CO_2 atmosphere are depleted in $\delta^{13}C$ and, as the litter and rhizodeposits from these plants decompose and become incorporated into the SOM, the soil $\delta^{13}C$ will decrease. The contribution of the new FACE-derived C to total SOM can be calculated based on the $\delta^{13}C$ of the SOM under ambient conditions and the period after initiating CO_2 enrichment (see Calculations of SOM turnover). This new C contribution and the CO_2 treatment duration then allow the SOM turnover rates to be estimated (Balesdent and Mariotti, 1987). Beyond investigating the C pathways in SOM under elevated atmospheric CO_2, the study of N cycling provides insights into the transformation processes of these two elements in soil. Thus, the application of N fertilizers in FACE experiments was used to estimate N flows and plant uptake under elevated CO_2 (Hungate et al., 1997; van Kessel et al., 2006; Zanetti et al., 1997). The use of N fertilizers labeled with ^{15}N provides an opportunity to study N turnover and to separate SOM N from the fertilizer N.

3) Calculations of SOM turnover

Calculation of the C and N turnover in experiments with FACE is based on the similar approach designed for SOM turnover calculation due to C_3-C_4 vegetation change. In brief, the natural abundance of the ^{12}C and ^{13}C stable carbon isotopes between plant species with different photosynthetic pathways can be used to track C flows in SOM. Plants with the C_3 pathway of photosynthesis reduce CO_2 to phosphoglycerate, a 3-carbon compound, via the enzyme RuBisCO. In turn, C_4 plants reduce CO_2 to aspartic or malic acid, both 4-carbon compounds, via the enzyme phosphoenolpyruvate (PEP) carboxylase. Approximately 85% of all plant species use the C_3 pathway of photosynthesis (Ehleringer et al., 1991). Plants possessing the C_3 pathway have $\delta^{13}C$ values ranging from approximately –32‰ to –22‰, with a mean of –27‰ (Boutton, 1991). Plant with C_4 photosynthesis discriminate less against $^{13}CO_2$ during photosynthesis, thus they have greater $\delta^{13}C$ values than C_3 plants. C_4 plants have $\delta^{13}C$ values ranging from approximately –17‰ to –9‰, with a mean of –13‰ (Boutton, 1991).

Since the photosynthetic pathway type of the vegetation growing on a certain soil determines the isotopic composition of SOM, changes from an initial C_3 vegetation to a C_4 vegetation or *vice versa* can hence be used as 'natural ^{13}C labelling technique' (Balesdent

and Mariotti, 1996). For example, after C_3 to C_4 vegetation change the $\delta^{13}C$ value of SOM starts to change slowly from the original $\delta^{13}C$ value, which is closer to that of C_3 vegetation, to a new steady state $\delta^{13}C$ value, which is closer to that of C_4 vegetation. When the new steady state is not reached (as in most studies) and the period after the $C_3 - C_4$ vegetation change is known, the contribution of the new C_4-derived C to the total SOM can be calculated (Balesdent and Mariotti, 1996):

$$P\text{-}C_4\ [\%] = (\delta^{13}C_{4\ SOM} - \delta^{13}C_{3\ SOM}) / (\delta^{13}C_{4\ Plants} - \delta^{13}C_{3\ Plants}) \cdot 100 \qquad (2),$$

where P-C_4 a portion of new C_4-derived C (in %), $\delta^{13}C_{4\ SOM}$ is the $\delta^{13}C$ value of the SOM under C_4 plants (‰); $\delta^{13}C_{3\ SOM}$ is the $\delta^{13}C$ value of the SOM of the corresponding reference soil with continuous C_3 vegetation (‰); $\delta^{13}C_{4\ Plants}$ is the $\delta^{13}C$ value of a C_4 plant tissues (‰) and $\delta^{13}C_{3\ Plants}$ is the $\delta^{13}C$ value of a C_3 plant tissues (‰).

In respect to FACE conditions, where "artificial ^{13}C labeling" is used (see above) the Eq. (2) was adapted:

$$P\text{-}C_{FACE}\ [\%] = (\delta^{13}C_{elev} - \delta^{13}C_{amb}) / (\delta^{13}C_{elev.theor.} - \delta^{13}C_{amb}) \cdot 100 \qquad (3),$$

where P-C_{FACE} is a portion of FACE-derived C in SOM (in %); $\delta^{13}C_{elev}$ is a $\delta^{13}C$ value of the SOM under elevated CO_2 treatment (‰); $\delta^{13}C_{amb}$ is a $\delta^{13}C$ value of the SOM under ambient CO_2 treatment (‰); $\delta^{13}C_{elev.theor.}$ is a theoretical $\delta^{13}C$ value of the SOM developed under vegetation grown in continuously elevated CO_2 conditions (‰). Isotopic signatures of theoretical SOM were calculated based on the difference between the $\delta^{13}C$ of the plants growing under elevated CO_2 and the $\delta^{13}C$ of SOM. The values were corrected for isotopic fractionation during humification by subtracting the differences between $\delta^{13}C$ of vegetation growing under ambient conditions and $\delta^{13}C$ of the corresponding SOM pool of the soil developed under ambient CO_2 treatment:

$$\delta^{13}C_{elev.theor.} = \delta^{13}C_{elev.plant} - (\delta^{13}C_{amb.plant} - \delta^{13}C_{amb}) \qquad (4),$$

where $\delta^{13}C_{elev.plant}$ is $\delta^{13}C$ of the plants under elevated CO_2 treatment (‰); $\delta^{13}C_{amb.plant}$ is $\delta^{13}C$ value of the plants under ambient CO_2 treatment (‰); and $\delta^{13}C_{amb}$ is $\delta^{13}C$ of the SOM developed under ambient CO_2 treatment (‰).

The stable ^{15}N isotope which labels the N fertilizers applied in FACE experiments allows to estimate N flows and plant uptake under elevated CO_2. The portion of N derived from labelled fertilizers (P-N_{fertil}, in %) could be calculated using similar approach for FACE-derived C calculation (Eq. 3):

$$P\text{-}N_{fertil}\ [\%] = (\delta^{15}N_{soil\ actual} - \delta^{15}N_{soil\ initial})/(\delta^{15}N_{fertil} - \delta^{15}N_{soil\ initial}) \cdot 100 \qquad (5),$$

where $\delta^{15}N_{soil\ actual}$ is a $\delta^{15}N$ value of the SOM after fertilization (‰); $\delta^{15}N_{soil\ initial}$ is a $\delta^{15}N$ value of the SOM before fertilization (‰); and $\delta^{15}N_{fertil}$ is a $\delta^{15}N$ signature of N fertilizers (‰).

A simple exponential approach by Balesdent and Mariotti (1996) could be used to calculate the annual turnover rates (TR) of SOM based on the contribution of new FACE-derived C and N from fertilizers in SOM for a particular period of the FACE experiment:

$$TR = -\ln(1 - M/100)/t \qquad (6),$$

where M is a portion of C_{FACE} or N_{fertil} (Eq. 3 and 5) in SOM, and t is the time of soil exposure to elevated CO_2 concentrations and the period of N fertilization.

The mean residence time (MRT, years) of FACE-derived C and N from fertilization in SOM could be calculated as a reciprocal to the turnover rates (Gregorich et al., 1995):

$$MRT = 1/TR \qquad (7)$$

The above mentioned approaches of calculating C_4-derived C, C_{FACE}-derived C and N_{fertil} in SOM, as well as their MRTs were used for experiments comprising the current thesis (see Chapters II, III, IV).

4) SOM fractionation techniques

Due to high spatial variability and the large size of the soil C and N pools compared with soil C and N inputs, the sensitivity of individual experiments to detect changes in total soil C and N is low (Hungate et al., 1996, Six et al., 2001). Hence, to track the new C and N in SOM pools, different fractionation techniques are commonly used. Generally, the methods of SOM fractionation could be divided into three groups according to properties of isolated SOM fractions:

1. *Chemical*: methods fractionate SOM into specific chemical compounds (for example, aliphatic and aromatic constituents);
2. *Physical*: methods isolate SOM fractions attributed to different locations in soil matrix (SOM attributed to sand or clay particles, SOM located between or occluded within soil aggregates of different sizes);
3. *Biological*: methods determine SOM fractions according to availability for decomposition by soil microorganisms (integrate both chemical and physical properties of SOM).

Since the availability of SOM for decomposition by soil microorganisms is a crucial property regarding to SOM turnover in terrestrial ecosystems, the SOM fractions isolated based on their chemical and/or physical properties should be considered in respect of availability for decomposition by the microorganisms. Thus, the higher contents of polysaccharides and O-alkyl compounds (i.e. carbohydrates) vs. aromatic and alkyl structures in SOM indicate higher availability of the SOM to microbial degradation (Chefetz et al., 2002). However, those labile organic compounds within plant residues when to be occluded in soil aggregates become physically protected from decomposition by soil microorganisms (Jastrow et al., 2007).

To test the interrelations between chemical and biological characteristics of SOM an approach for SOM partitioning based on its thermal stability was chosen (Leinweber and Schulten, 1992; Siewert, 2001, 2004; Lopez-Capel et al., 2005a, 2005b; Plante et al., 2005; Kuzyakov et al., 2006). Thermogravimetry analysis coupled with differential scanning calorimetry involves a continuous, gradual temperature increase which decomposes (mainly oxidizes) different organic compounds according to their thermal stability. Such a temperature increase coupled with measuring of weight losses is termed thermogravimetry (TG). Simultaneously to temperature increase, energy released or consumed by decomposition of organics is measured by differential scanning calorimetry (DSC).

TG-DSC is extensively applied to various soil science studies. Leinweber and Schulten (1992), Leinweber et al. (1992) and Schulten and Leinweber (1999) used DSC to characterize organo-mineral complexes; Provenzano and Senesi (1999) have used DSC to study humic substances; Lopez-Capel et al. (2005a), Francioso et al. (2005), and Plante et al. (2005) applied the technique to evaluate the humification state of SOM. Studying correlations between thermal stability of SOM pools in various temperature ranges and CO_2 production by classical soil incubations, Siewert (2001) suggested the idea of a relationship between thermal stability of SOM pools and their biological degradability. It assumes that SOM pools decomposable at lower temperatures are more biologically accessible and utilizable compared to organics, which are decomposed at higher temperatures. Thus, one of the main objectives of the thesis was to test the hypothesis of close relationship between thermal degradability and biological stability of SOM.

Before applying the thermogravimetric approach for SOM fractionation and testing the hypothesized relationship between thermal and biological properties of SOM as influenced by elevated atmospheric CO_2 concentrations, we attempted to test the above mentioned relationship in regard to land use change. Namely, the technique of thermogravimetric fractionation of SOM was applied for soil after C_3 (grassland) to C_4 (*Miscanthus x gigantheus*) vegetation change. Since the period of *Miscanthus* cultivation (10 years) was much longer than the duration of the FACE experiment (3 years to the date of sampling) we expected higher contribution of new C to SOM under *Miscanthus* vs. under elevated CO_2. The broader isotopic difference between control soil and treatment after C_3 to C_4 vegetation change as compared to ambient and elevated CO_2 treatments would allow us to calculate MRTs with high accuracy. Hence, we assume to reveal the hypothesized relationships between thermal and biological properties of SOM after C_3 to C_4 vegetation change and compare them with those between ambient and elevated CO_2 treatments (see Chapters II, III, IV).

To evaluate the interactions between availability of SOM for decomposition by soil microbial biomass (biological characteristic) and protection of SOM due to the occlusion within aggregates of different sizes (physical property) we measured the activity of microbial biomass (indicated by enzyme activities) and growth strategies of soil microorganisms (fast- vs. slow growing organisms) in isolated macro- and microaggregates. We chose aggregate separates because of their contrasting properties (Jastrow et al., 2007). Thus, microaggregates (<250 μm diameter) formed by primary particles coupled together by plant and microbial debris and by humic materials or polysaccharide polymers better protect plant

residues against decomposition than macroaggregates do (>250 μm diameter) (Denef et al., 2001; Bossuyt et al., 2002; Six and Jastrow, 2002). In turn, the microbial community associated with macroaggregates (fungi and bacteria) actively mineralizes C and N as compared to microaggregates (Guggenberger et al., 1999). In contrast to macroaggregates, microaggregates having more small pores enhance survival of microorganisms, mostly bacteria, by protecting them from predation by Protozoa and soil animals (Vargas and Hattori, 1986; Postma and Van Veen, 1990; Wright et al., 1993) or from desiccation (Nishiyama et al., 1992).

So, along with hypothesized relationship between thermal and biological properties of SOM, we wanted to study the effect of elevated atmospheric CO_2 on the one hand, and soil structure represented by the sum of differently sized aggregates on the other hand, on the activity and growth strategy of microbial biomass. The reported increase in rhizodepositions including root exudates under elevated atmospheric CO_2 (van Veen et al., 1991; Cheng, 1999; Phillips et al., 2006) could stimulate the activity of soil microbial biomass, resulting in increase of rhizomicrobial respiration (Zak et al., 1993; Freeman et al., 2004) and/or enhanced enzyme activities (Mayr et al., 1999; Ebersberger et al., 2003). Since root exudates are easily available substrate for consumption by soil microorganisms (Kuzyakov, 2002), the availability of such substrates determines the growth strategy of soil microorganisms adapting the microbial community to changes in the environment (Andrews and Harris, 1986). According to their living strategies organisms are classified as *r*- and K-strategists (Pianka, 1970). *r*-strategists show quick growth on easily available substrates, whereas K-strategists use the resources more efficiently and grow slowly even in the absence of limitation. The *r*-strategists could benefit from increased easily available rhizodepositions under elevated atmospheric CO_2 (Blagodatsky et al., 2006). In contrast to the effect of elevated CO_2 on activity and living strategy of soil microbial biomass, the impact of soil structure as a sum of macro- and microaggregates is less clear, since these aggregates create contrasting environment for soil microorganisms (Nishiyama *et al.*, 1992; Wright *et al.*, 1993; Guggenberger *et al.*, 1999).

In the thesis it was attempted to answer two main questions: (i) assuming the increased plant-derived C deposits under elevated CO_2 conditions into the soil, how would these C deposits contribute to the domination of *r*- or K- selected microorganisms and to the activity of enzymes? And (ii) is there a shift in microbial living strategies and in enzyme activities with the decreasing of aggregate size, because of the contrast specific environment existing in micro- and macroaggregates?

5) Objectives

For this thesis, several experiments with soil from agricultural ecosystems subjected to altered environmental conditions (elevated CO_2 in FACE) and land use change (from C_3 grassland to C_4 cultivated bio energy crop *Miscanthus x gigantheus*) were conducted in the laboratory. To follow the transformation of C and N and to evaluate the accessibility of SOM for decomposition by soil microbial biomass the SOM fractionation techniques based on chemical properties of SOM (TG-DSC) and physical properties (occlusion within soil macro- and microaggregates) was used. The objectives of this thesis were subdivided as follows:

I. Experiments with thermal stability of SOM:

- To isolate SOM pools of different thermal stability using TG-DSC approach
- To characterize the SOM quality in terms of thermal properties in soil developed
    a) under 10-year cultivation of *Miscanthus x gigantheus* (Chapter II);
    b) under elevated CO_2 in Mini-FACE-Hohenheim (540 ppm CO_2 vs. 380 ppm ambient). The soil is Gleyic Cambisol (Chapter III);
    c) under elevated CO_2 in FACE-Braunschweig (550 ppm CO_2 vs. 375 ppm ambient) with high and low N fertilization. The soil is Cambisol/loamy sand (Chapter IV).
- To measure $\delta^{13}C$ (all experiments) and $\delta^{15}N$ (experiment with Mini-FACE-Hohenheim) values in isolated SOM pools of different thermal stability.
- To calculate turnover rates and MRTs of C and N in SOM pools of different thermal stability.
- To prove the hypothesis of close relation between thermal degradability and biological stability by comparing $\delta^{13}C$ and $\delta^{15}N$ values of SOM pools decomposed at low and high temperatures in soil after the vegetation changed from former C_3 grassland to C_4 plant *Miscanthus x gigantheus*, and under elevated and ambient CO_2 treatment of two different soil types. If the hypothesis is true, then (i) the thermally labile SOM pools would have $\delta^{13}C$ values closer to the new C_4 vegetation or vegetation grown under elevated atmospheric CO_2 concentrations than thermally stabile SOM pools, (ii) $\delta^{15}N$ values of SOM pools decomposed under lower temperatures would be closer to $\delta^{15}N$ of fertilizers (for experiment with Mini-FACE-

Hohenheim), and (iii) the mean residence time (MRT) of C and N of labile pools would be shorter than those of thermally stable ones.

II. Experiments with the determination of growth strategies of soil microbial biomass and its enzyme activities in differently sized aggregates under elevated atmospheric CO_2:

- To fractionate bulk soil from Mini-FACE-Hohenheim into constituent macro- and microaggregates using modified dry sieving approach.
- To measure microbial growth strategies in bulk soil and isolated aggregates of ambient and elevated CO_2 treatments by means of substrate-induced growth respiration method (Chapter V).
- To measure enzyme activities in bulk soil and isolated aggregates of ambient and elevated CO_2 by a microplate method combined with 4-methylumbelliferone-labelled fluorogenic substrates (Chapter VI).
- To prove the hypothesis of potentially higher microbial activity in soil under elevated vs. ambient atmospheric CO_2 based on activities of enzymes and growth rates of soil microorganisms; to study the effect of soil structure, namely macro- and microaggregates, along with the effect of elevated CO_2 in the atmosphere on the growth strategies of soil microbial biomass and its enzyme activities.

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II) Thermal stability of soil organic matter pools and their $\delta^{13}C$ values after $C_3 - C_4$ vegetation change

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includes two tables and two figures

Abstract

Carbon isotopic composition of soils subjected to C_3-C_4 vegetation change is a suitable tool for the estimation of C turnover in soil organic matter (SOM) pools. We hypothesized that the biological availability of SOM pools is inversely proportional to their thermal stability. Soil samples from a field plot with 10.5 years of cultivation of the C_4 plant *Miscanthus x gigantheus* and from a reference plot under C_3 grassland vegetation were analysed by thermogravimetry coupled with differential scanning calorimetry (TG-DSC). According to differential weight losses (dTG) and energy release or consumption (DSC), five SOM pools with increasing thermal stability were distinguished: I) 20-190 °C, II) 190-310 °C, III) 310-390 °C, IV) 390-480 °C, and V) 480-1000 °C. Their $\delta^{13}C$ values were analysed by EA-IRMS. The weight losses in pool I were connected with water evaporation, since no significant C losses were measured and $\delta^{13}C$ values remained unchanged. The $\delta^{13}C$ of pools II and III in soil samples under *Miscanthus* were closer to the $\delta^{13}C$ of the *Miscanthus* plant tissues (-11.8‰) compared to the thermally stable SOM pool V (-19.5‰). The portion of the *Miscanthus*-derived C_4-C in total SOM in 0-5 cm reached 55.4% in the 10.5 years. The C_4-C contribution in pool II was 60% and decreased down to 6% in pool V. The Mean Residence Times (MRT) of SOM pools II, III, and IV were similar (11.6, 12.2, and 15.4 years, respectively), while pool V had a MRT of 163 years. Therefore, we concluded that the biological availability of thermal labile SOM pools (<480 °C) was higher, than that of the thermal stable pool decomposed above 480 °C. However, the increase of SOM stability with rising temperature was not gradual. Therefore, the applicability of the TG-DSC for the separation of SOM pools with different biological availability is limited.

1. Introduction

Carbon (C) isotopic composition of soil organic matter (SOM) after C_3–C_4 vegetation change (and vice versa) has been frequently used in the last decade to estimate C turnover rates in soil and the incorporation of new C in various SOM pools (Balesdent and Mariotti, 1987; Volkhoff and Cerri, 1987; Ludwig et al., 2003; John et al., 2003, 2005; Kristiansen et al., 2005). This approach is based on the different stable isotope composition (represented as $\delta^{13}C$ value) of residues from plants with C_3 and C_4 photosynthesis (Farquhar et al., 1989; Ehleringer and Cerling, 2002). After C_3 – C_4 vegetation change the $\delta^{13}C$ value of SOM starts to change slowly from the original $\delta^{13}C$ value, which is closer to that of C_3 vegetation, to a new steady state $\delta^{13}C$ value, which is closer to that of C_4 vegetation. When the new steady state is not reached (as in most studies) and the period after the C_3 – C_4 vegetation change is

known, the contribution of the new C_4-derived C to the total SOM can be calculated. Based on this C_4-C contribution and the period after vegetation change, the SOM turnover rates can be roughly estimated (Balesdent and Mariotti, 1996).

Nearly all investigations using ^{13}C natural abundance have been conducted with maize (Balesdent and Balabane, 1996; Ludwig et al., 2003; Kristiansen et al., 2005). In Europe another C_4 plants – *Miscanthus x giganteus* (Greef et Deu.) – is successfully cultivated as a bio energy crop (Beuch, 2000). This perennial C_4 plant can be cultivated from 15 up to 25 years without replanting and is harvested yearly, often in the following spring to reduce ash contents. The first results of C turnover for *Miscanthus*, based on ^{13}C natural abundance showed a higher contribution of *Miscanthus* C to the SOM than maize (Kao, 1997; Hansen et al., 2004).

As the estimation of the $\delta^{13}C$ values of bulk SOM is not very informative, various fractionation techniques including density (Magid et al., 2002; John et al., 2005) and particle size fractionation (Jolivet et al., 2003; Ludvig et al., 2003), as well as sequential extractions (Ellerbrock and Kaiser, 2005) were combined with the $\delta^{13}C$ analyses. One of the other methods suitable for SOM fractionation is based on thermal stability (Leinweber and Schulten, 1992; Siewert, 2001, 2004; Lopez-Capel et al., 2005; Plante et al., 2005). Thermal analysis by thermogravimetry coupled with differential scanning calorimetry (TG-DSC) involves a slow continuous temperature increase that leads to the progressive decomposition (mainly oxidation) of different organic compounds according to their thermal stability. Such a temperature increase coupled with the measuring of weight losses is termed thermogravimetry (TG). Energy released or consumed by the decomposition of organics is measured, simultaneous to the temperature increase, by differential scanning calorimetry (DSC).

Thermal analysis curves and heat fluxes provide important information on the structural composition of SOM (Provenzano and Senezi, 1999; Dell'Abate et al., 2002; Kuzyakov et al., 2006). The exothermic degradation of aliphatic and carboxyl groups at different temperatures was suggested to compare the proportions of labile and more stable components in SOM pools and whole soil (Brown, 1988; Siewert, 2001; Lopez-Capel et al., 2005).

The availability to microbial decomposition is an important characteristic of the SOM quality, which in turn allows to predict the transformation and turnover time of carbon in terrestrial ecosystems. Based on correlations between thermal stability of SOM pools in various temperature ranges and the CO_2 production by classical soil incubations, Siewert

(2001) suggested that the thermal stability of SOM pools can be related to their biological degradability. It assumes that SOM pools decomposable at lower temperatures are more biologically accessible and utilizable compared to organics, which are decomposed at higher temperatures. However, the hypothesis of relation between the thermal stability of SOM pools and their availability to microbial mineralization was mainly supported by correlations between CO_2 evolution and thermogravimetrical weight losses (Siewert, 2001) and, to the best of our knowledge, has never been tested by direct methods.

Thus, the aim of our study was to test the hypothesis of close connection between thermal degradability and biological stability by comparing $\delta^{13}C$ values of SOM pools decomposed at increasing temperature in the soil after the vegetation changed from former C_3 grassland to C_4 plant *Miscanthus x gigantheus*. If the hypothesis is true, then the thermally labile SOM pools would have $\delta^{13}C$ values closer to the new vegetation compared to thermally stabile SOM pools and the mean residence time (MRT) of labile pools would be shorter than those of stable ones.

The preliminary study testing this approach showed weak correlation between thermal and biological degradability when only three SOM pools were separated by TG-DSC (Kuzyakov et al., 2006). However, the preliminary study was insufficient for exact conclusions because of three reasons: (i) the absence of the reference soil developed solely under C_3 vegetation did not allow for estimation of the isotopic fractionation of C by thermal decomposition and by incorporation of C into pools with different thermal stability. So, in the absence of the reference soil the calculation of the MRT of the SOM pools was not possible. (ii) Only three temperature ranges were separated; one of them was responsible for water evaporation and did not affected the $\delta^{13}C$ values. (iii) The fast rate of temperature increase (5 °C min^{-1}) during TG-DSC analysis in preliminary study led to some overlapping of pools with different thermal stability and therefore, did not allow their clear separation.

In the present study the above mentioned deficiencies were considered and therefore, the reference soil under grassland (solely C_3) was included into the experiment and the heating rate (2 °C min^{-1}) was greatly reduced. This allowed for the better separation of the pools, especially those decomposed under high temperature. The inclusion of the reference soil in the experiment design allowed for the calculation of MRT.

2. Materials and Methods

2.1. Soil samples

Soil samples were taken from the long-term experimental field located in Stuttgart-Hohenheim, Baden-Wuerttemberg, Germany (48°43' north latitude, 9°13' east longitude). The soil on the plot with *Miscanthus* and reference plot was a loamy Gleyic Cambisol (WRB, 1998) without carbonates (no reaction with HCl). The soil properties under *Miscanthus* were: pH 5.6, bulk density 1.1 g cm^{-3}, C_{org} 2.13%, N_{tot} 0.18%, C-to-N ratio 12.0. The properties of the reference soil were: pH 6.3, bulk density 1.3 g cm^{-3}, C_{org} 1.62%, N_{tot} 0.18%, C-to-N ratio 9.1. Mean annual temperature is 8.7 °C and average rainfall 680 mm y^{-1} (mean 1961-1990, meteorological station Stuttgart-Hohenheim).

The *Miscanthus x giganteus* (Greef et Deu) was planted on May 24, 1994 on a former grassland plot. The above-ground standing biomass of the *Miscanthus* has been harvested every year and the remaining foliage was mulched annually in March. The *Miscanthus* yields were 0.95 kg C m^{-2} y^{-1} on average. Soil samples for total C and $\delta^{13}C$ analysis were taken on November 10, 2004. Soil samples from the reference plot under grassland were taken on October 27, 2004. The grassland on the reference plot with the domination of perennial ryegrass (*Lolium perenne* L.) has been cultivated for about 20 years with 2 to 3 annual cuttings. The grassland was not grazed and no manure was applied. The *Miscanthus* plot is located about 30 m from the reference plot. The samples were taken from individual points with the distant of 5 to 10 m from each other. The cultivation period at the time of sampling was 10 years and 6 months. Soil samples were taken with a soil corer (inner diameter 6 cm) to a depth of 5 cm. So, only the upper 0-5 cm layer of the Ah horizon was used. The soil samples were air-dried at room temperature and sieved (2 mm mesh size). After that, from a sub-sample of 5 g, all visible roots and plant remains were carefully removed with tweezers and the soil was ball milled (MM2, Fa Retsch) for 15 sec.

2.2. Thermogravimetry – Differential Scanning Calorimetry analysis

Thermogravimetric analysis (TG) and differential scanning calorimetry analysis (DSC) were carried out simultaneously using the NETZSCH STA 409EP instrument, regulated by the TASC 414/3 controller (Fa Netzsch). About 100 mg of soil sample was weighed out on an alumina crucible and then heated from 20 to 1000 °C in air atmosphere (1 ml min^{-1}). The heating rate was 2 °C min^{-1}. The weight of the soil sample (in mg), as well as the heat (in µV mg^{-1}) released or consumed by substance oxidation or water evaporation was continuously scanned. Calcined kaolinite previously heated at 1250 °C was used as the reference material. The weight losses determined by dTG were used to set "threshold" temperature levels (190, 310, 390 and 480 °C) for subsequent $\delta^{13}C$ analysis (see below). Since the DSC curve of soil

samples couldn't be exactly related to individual substances (as for the matrix-free organic substances), we used the DSC only to confirm the differential mass losses. After determination of "threshold" temperature levels by TG-DSC analysis, all soil samples were divided into four groups and combusted in a muffle oven (Heraeus MR-260) under the same conditions (sample weight 100 mg, heating rate 2 °C min^{-1}). Each group of soil samples was combusted to its particular "threshold" temperature. Then, the combustion samples were left to cool to room temperature and prepared for isotopic analyses.

2.3. Sample analyses

10 to 40 mg of soil samples before and after heating in the muffle oven were weighed in tin capsules for total C and $\delta^{13}C$ analyses. The amount of C and its isotopic composition was measured on the Thermo Finnigan isotope ratio mass spectrometer (IRMS) coupled to the Euro EA C/N analyser. Acetanilid was used as the internal standard for $\delta^{13}C$ analyses. The final isotopic composition is expressed as $\delta^{13}C$ units in relation to the international standard Pee Dee Belemnite.

2.4. Calculations

The $\delta^{13}C$ values of individual SOM pools were calculated based on the isotopic mass balance equation (Balesdent and Mariotti, 1996):

$$\delta^{13}C_{b.s.} \times C_{b.s.} = \delta^{13}C_{f.1} \times C_{f.1} + \delta^{13}C_{f.2} \times C_{f.2} + \ldots + \delta^{13}C_{f.n} \times C_{f.n} \quad (1),$$

where $\delta^{13}C_{b.s.}$ is the $\delta^{13}C$ value of the bulk soil without combustion; $C_{b.s.}$ is the amount of C in the bulk soil without combustion; $\delta^{13}C_{f.1}$, $\delta^{13}C_{f.2}$,…, $\delta^{13}C_{f.n}$ are the $\delta^{13}C$ values of the SOM pools after combustion up to the subsequent "threshold" temperature levels; $C_{f.1}$, $C_{f.2}$,…, $C_{f.n}$ are the amounts of C in the SOM pools after combustion.

The portion of *Miscanthus*-derived C_4-C in SOM ($\%C_{\underline{Miscanthus}}$) was calculated according to Balesdent and Mariotti (1996) with the assumption of identical isotopic fractionation of the humification of C_3 and C_4 plant residues:

$$\%C_{Miscanthus} = \frac{\delta^{13}C_t - \delta^{13}C_3}{\delta^{13}C_4 - \delta^{13}C_3} \times 100 \quad (2),$$

where $\delta^{13}C_t$ is the $\delta^{13}C$ value of the soil with *Miscanthus*; $\delta^{13}C_3$ is the $\delta^{13}C$ value of the corresponding SOM fraction of the reference soil with continuous C_3 vegetation; $\delta^{13}C_4$ is the theoretical $\delta^{13}C$ value of a C_4 soil developed solely under *Miscanthus*, calculated based on the $\delta^{13}C$ of the *Miscanthus* plant and corrected for isotopic fractionation during humification by the subtraction of the differences between $\delta^{13}C$ of C_3 vegetation and $\delta^{13}C$ of the corresponding SOM fraction of the C_3 soil.

To calculate the annual contribution of new *Miscanthus*-derived C_4-C in SOM and annual turnover rates (TR) of SOM, a simple exponential approach was selected (Balesdent and Mariotti, 1996):

$$TR = \frac{-\ln\left(1 - \frac{\%C_{Miscanthus}}{100}\right)}{time} \quad (3),$$

where time (years) is the cultivation period of *Miscanthus* and $\%C_{Miscanthus}$ is the portion of C_3-C that was replaced by C_4-C derived from *Miscanthus* (Equation 2).

The mean residence time (MRT) of *Miscanthus*-derived C_4-C in bulk soil and in SOM pools was calculated as a reciprocal to the turnover rates (Gregorich et al., 1995).

The study was conducted with seven replications for *Miscanthus* soil samples and three replications for the reference soil. The significance of differences between $\delta^{13}C$, as well as the C content of different pools was examined using the two-way analysis of variance (ANOVA). The standard errors of means were presented on the figures and in the table as variability parameter.

3. Results

3.1. Thermal stability and differential scanning calorimetry analysis of the soil

The dTG clearly showed 3 maximums of weight losses at temperature increases (Fig. 1): 1) between 20 and 190 °C, 2) between 190 and 310 °C and 3) between 390 and 480 °C. The DSC traces were characterised by three temperature ranges, two with endothermic reactions: 1) from ambient temperature to 210 °C in soil under grassland and to 220 °C in soil under *Miscanthus*; 2) from 335 to 1000 °C in both reference soil and soil under *Miscanthus*; and one range with exothermic reactions between 210 or 220 and 335 °C (Fig. 1). The total weight losses of the soil under *Miscanthus* and the reference plot were 8.45 and 8.27%, respectively (Table 1). Assuming that the amount of SOM is 1.724 times higher than the C

content (Post et al., 2001), then only 34.0 and 43.5% of the total weight losses at up to 1000 °C could be connected with the decomposition of SOM in the reference soil and soil under *Miscanthus*, respectively (Table 1).

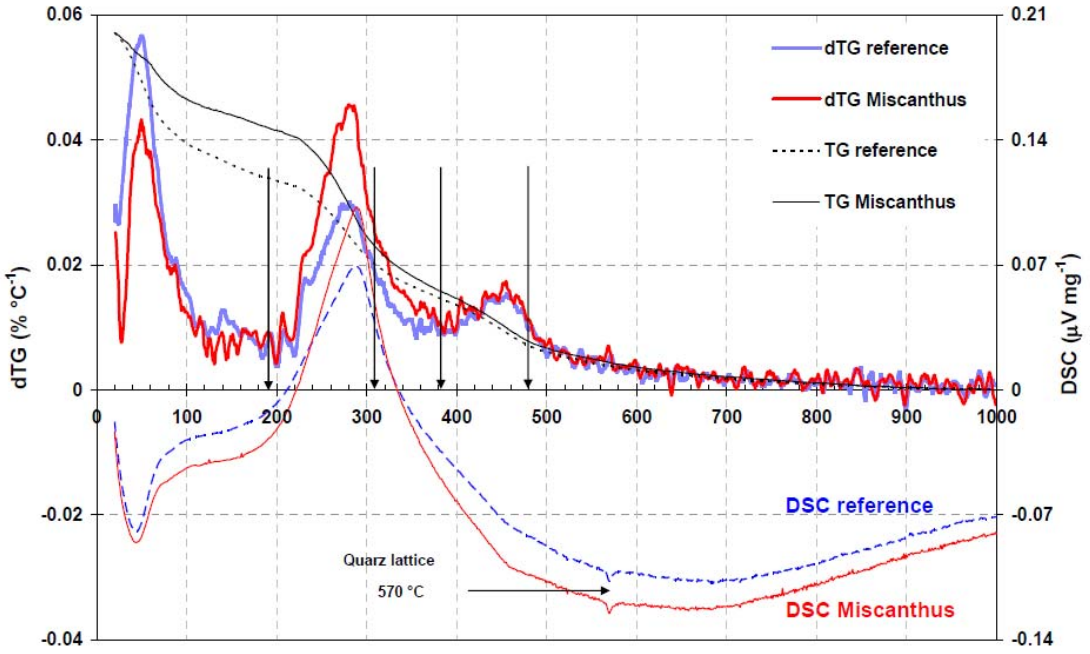

Fig. 1. Differential thermogravimetry (dTG, left Y axis) and Differential Scanning Calorimetry (DSC, right Y axis) of reference soil under grassland and soil under *Miscanthus*. Cumulative losses are scaled to 100% of the left Y axis and total cumulative losses amounted to 8.27 and 8.45% for reference soil and soil under *Miscanthus*, respectively. Negative DSC values represent energy consumption (endothermic reactions) and positive DSC values represent energy release (exothermic reactions). Arrows show the "threshold" temperature chosen for SOM fractionation.

No significant C losses were measured in both soils at the temperature of up to 190 °C, but samples weight decreased by about 0.71% (Table 1). It means that the weight losses in the temperature area up to 190 °C were mainly connected with water evaporation. This was also clearly proven by the negative values of DSC that showed endothermic reactions by water evaporation (Fig. 1). Starting from 190 °C, the weight losses increased simultaneously with strong DSC increases. This was a clear evidence of energy release by the thermal decomposition of organic substances. The maximum weight losses and energy release occurred between 190-310 °C, amounting to 30% of total weight losses (8.27 and 8.45%) for both reference soil under grassland and soil under *Miscanthus*. The maximal DSC values of 0.07 and 0.10 µV mg^{-1} were measured at 290 °C in reference soil and in soil under *Miscanthus*, respectively (Fig. 1). However, the maximum weight losses did not correspond

to the maximum SOM losses. According to the C losses at individual temperature ranges, the maximum SOM decomposition was observed in the temperature range between 310 and 390 °C, where the soil under *Miscanthus* lost 85.3% of SOM from mass losses of fraction and the losses of organic matter from the reference soil amounted to 68.8% (Table 1). The third maximum of mass losses was related to the temperature fraction from 390 to 480 °C (Fig. 1). About 27.4 and 25.7% of total weight losses in this temperature range corresponded to SOM decomposition in soil under grassland and soil under *Miscanthus*, respectively (Table 1). The weight and SOM losses by further temperature increase to above 480 °C were much slower than at below this temperature. At up to 1000 °C, the reference soil and soil under *Miscanthus* lost only 1.12% and 1.30% of weight. SOM losses amounted to 4.7% and 3.3% of the weight losses within the last temperature range for soils under grassland and *Miscanthus*, respectively (Table 1). The DSC curve switched into negative values after the temperature increased to 340 °C and above showing energy consumption.

Table 1. Losses of weight and amount of C (± SE) in SOM pools with different thermal stability for reference soil under grassland (ref) and soil under *Miscanthus* (Misc), and their $\delta^{13}C$ values measured or calculated according to isotopic mass balance

Temperature Fraction (° C)	Weight losses (%)		C content (%)		SOM[a] on total losses (%)		$\delta^{13}C$ (‰ PDB)	
	ref	Misc	ref	Misc	ref	Misc	Ref	Misc
20…1000[b]	8.27	8.45	1.62 ±0.13	2.13 ±0.09	33.7	43.5	–26.47 ±0.26	–17.65 ±0.22
20…190[c]	0.70	0.72	0 ±0.05	0 ±0.09	0	0	ND	ND
190…310[c]	2.62	2.68	0.49 ±0.04	0.81 ±0.05	31.9	51.9	–28.91 ±0.67	–18.80 ±0.33
310…390[c]	2.26	2.31	0.90 ±0.03	1.14 ±0.06	68.8	85.3	–25.91 ±0.25	–16.92 ±0.24
390…480[c]	1.41	1.44	0.22 ±0.02	0.21±0.01	27.4	25.7	–24.95 ±0.29	–17.10 ±0.17
480…1000[b]	1.12	1.30	0.03 ±0.002	0.02±0.001	4.7	3.3	–20.92 ±0.66	–19.54 ±0.48
$LSD_{0.05}$[d]	0.42		0.17				1.14	

[a]SOM (%) was determined as multiplication of the amount of C (%) by 1.724

[b]measured

[c]calculated by isotopic mass balance equation

[d] the least significant differences (P<0.05) were calculated for two-way ANOVA: soil × temperature

3.2. C isotopic composition of SOM pools with different thermal stability

According to the thermal stability of the SOM, five temperature ranges were chosen for soil heating in the muffle oven for the subsequent isotopic analyses: 1) no heating (20 °C) corresponds to the bulk soil, 2) heating up to 190 °C, 3) heating up to 310 °C, 4) heating up to 390 °C, and 5) heating up to 480 °C (Fig. 1). Four SOM pools with increasing thermal stability were obtained after the combustion up to the "threshold" temperatures: pools with peaks between 190 and 310 °C, and between 390 and 480 °C; intermediate pool between 310 and 390 °C; all SOM remaining after 480 °C. The heating up to 310 °C removed the SOM pool with the "threshold" temperatures of between 190 and 310 °C, so only the SOM remaining after 310 °C was analysed. The same approach was used to determine other SOM pools. The temperature range between 310 and 390 °C (intermediate fraction) was selected for better separation of the fractions decomposed below 310 °C and above 390 °C.

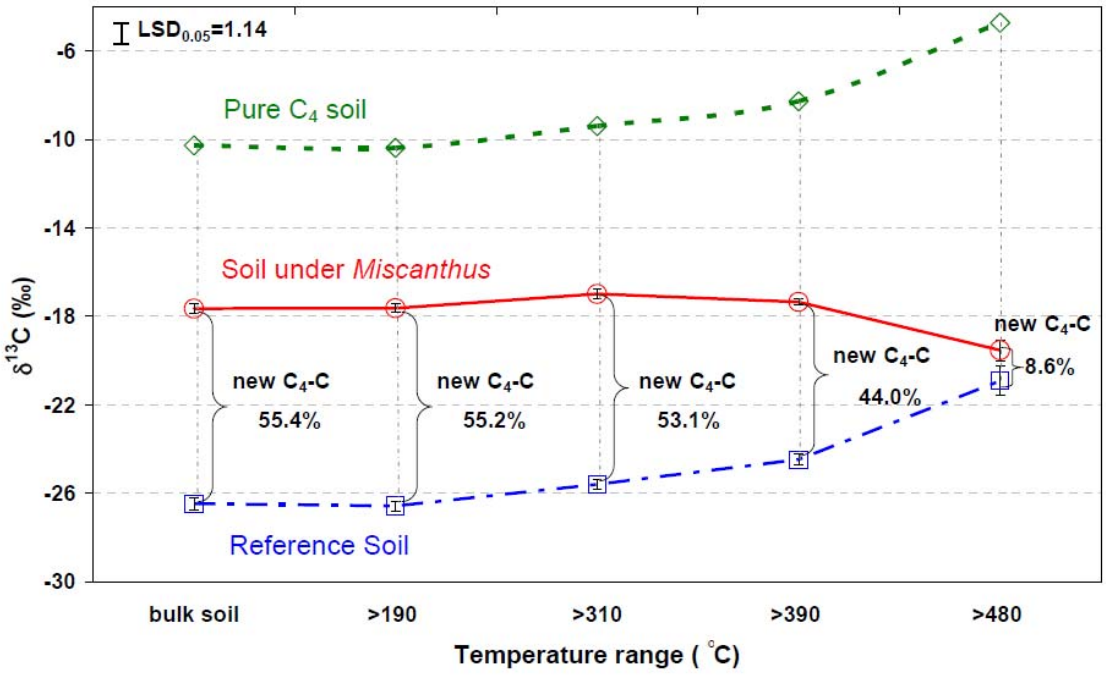

Fig. 2. $\delta^{13}C$ values of bulk reference soil under grassland and soil under *Miscanthus* and four SOM pools remaining after heating up to 190°C (>190°C), up to 310°C (>310°C), up to 390°C (>390°C), and up to 480°C (>480°C). The calculated theoretical $\delta^{13}C$ values of C_4 soil developed solely under C_4 vegetation (pure C_4 soil) were used to estimate the portion of C_4-C in the SOM. Whiskers present standard error (± SE). $LSD_{0.05}$ of means amounting for 1.14‰ shows significant differences between the two soils and temperature ranges.

The analysis of C isotopic composition showed that the heating up to 190 °C does not change $\delta^{13}C$ values of reference soil or soil under *Miscanthus* (Fig. 2). This is in agreement with the absence of total C losses for temperatures up to 190 °C (Table 1). Further heating up to 310 °C increased $\delta^{13}C$ values by 0.99‰ in reference soil, and by 0.64‰ in soil under

Miscanthus (Fig. 2). The $\delta^{13}C$ value of SOM pool remaining after combustion of up to 390 °C showed a significant difference only in the reference soil, where it increased by 1.12‰, compared to $\delta^{13}C$ in the SOM decomposed up to 310 °C. The largest shift in $\delta^{13}C$ value occurred in the SOM remaining after heating up to 480 °C: the $\delta^{13}C$ in the soil under *Miscanthus* decreased by 2.2‰ which implied, that C in the thermal stable SOM pools remained closer to the previous C_3 vegetation ($\delta^{13}C$ = –28.0‰) compared to the C of less stable SOM pools, that decomposed at temperatures of up to 190, 310, and 390 °C. In contrast to the soil under *Miscanthus*, the value of $\delta^{13}C$ in the reference soil in the same fraction (>480 °C) showed an increase of up to 3.55‰ in comparison with the $\delta^{13}C$ value in the SOM fraction decomposed up to 390 °C. The observed ^{13}C enrichment in the SOM pools with increasing thermal stability of the reference soil is mainly connected with isotopic discrimination of ^{13}C by decomposition during slow temperature increase.

3.3. Portion of *Miscanthus*-derived C_4-C and Mean Residence Time of the SOM pools

The portion of *Miscanthus*-derived C_4-C in the SOM was calculated according to the approach by Balesdent and Mariotti (1996) (Fig. 2). During 10.5 years of *Miscanthus* cultivation, the amount of new C_4-C in the SOM of bulk soil reached 55.4% (Table 2). However, the C_4-C contribution in the 190-310 °C pool was 60.4% and decreased down to 6.4% in the pool decomposed above 480 °C. Based on this C_4-C contribution and the period after vegetation change (10.5 years), the SOM turnover rates were calculated (Table 2).

Table 2. The portion of C_4-C, turnover rates, and mean residence time (MRT) of SOM (± SD) accumulated in 10.5 years of *Miscanthus* cultivation.

Temperature Fraction (° C)	*Miscanthus*-derived C_4-C (%)	Turnover rates ($year^{-1}$)	MRT (years)
20…1000	55.4±2.3	0.077±0.005	13.1 ± 0.8
20…190	ND	ND	ND
190…310	60.4±5.2	0.090±0.013	11.6 ± 1.6
310…390	57.9±3.1	0.083±0.007	12.2 ± 1.0
390…480	49.6±2.1	0.065±0.004	15.4 ± 0.9
480…1000	6.4±0.7	0.006±0.001	163 ± 18

The C turnover in the pools decomposed at lower temperatures was much faster than that in the pools decomposed at higher temperatures. The Mean Residence Time (MRT), calculated

as the reciprocal to the turnover rates (Gregorich et al., 1995) of SOM pools 190-310, 310-390, and 390-480 °C were similar: 11.6, 12.2, and 15.4 years, respectively. The pool above 480 °C had a much longer MRT of 162.6 years (Table 2). However, the amount of C_4-C incorporated in the last pool as well as the total amount of C_{org} in this pool was much lower than in the pools decomposed below 480 °C. Therefore, the pool decomposed above 480 °C is of minor ecological importance.

4. Discussion

4.1. Thermal stability

In contrast to our previous study (Kuzyakov et al., 2006), the temperature increase in this study was 2.5 times slower and TG-DSC was used to separate the SOM pools with different thermal stability. Slower temperature increases allowed for much better resolution of individual maximums of mass losses, especially of the maximum above 390 °C. According to the dTG curve, five temperature ranges were grouped (Fig. 1). The first interval up to 190 °C was connected with the losses of hygroscopic water, since no C was lost and no changes of isotopic composition were observed. The consumption of energy by water volatilization was clearly confirmed by negative DSC values up to 210 or 220 °C in reference soil and soil under *Miscanthus*, respectively. The endothermic reactions measured by DSC up to 120-150 °C by TG-DSC studies of SOM were observed by many authors (Dell'Abate et al., 2003; Francioso et al., 2005; Plante et al., 2005). This bound water mainly consisted of water absorbed from air moisture and hygroscopic water of salts (Gaál et al., 1994).

The temperature ranges between 190-310 and 310-390 °C were characterized by the most intensive weight and SOM losses (Table 1). Moreover, the portion of SOM contributing to weight losses in the temperature range 310-390 °C amounted to 85% in soil under *Miscanthus*. The decomposition of organic compounds in these temperature ranges led to strong energy release with maximum at 290 °C. Nearly the same temperature (~300 °C) corresponded to the exothermic maximum observed by decomposition of humic acids extracted from different peats, lignites and leonardites (Francioso et al., 2005). This exothermic reactions reflected thermal decomposition of polysaccharides, decarboxylation of acidic groups and dehydration of hydroxylate aliphatic structures (Dell'Abate et al., 2002).

The third maximum of mass losses was related to the temperature fraction of 390 to 480 °C (Fig. 1). About 26% of the mass losses that occurred in this fraction were from SOM decomposition for both reference soil under grassland and soil under *Miscanthus* (Table 1). The decomposed organics could be referred to as stable constituents with aromatic

compounds, such as lignin dimers (Leinweber et al., 1992; Siewert, 2004; Lopez-Capel et al., 2005), although the mass losses were not supported by the DSC curve, which showed endothermic reactions from 317 and up to 1000 °C. As suggested by Plante et al. (2005), the energy consumption within this temperature range occurred due to the dehydroxylation of the clay minerals, prevailing the decomposition of organics.

In the last temperature range (>480 °C), 1.12 and 1.30% of mass were lost in the reference soil and soil under *Miscanthus*, respectively, whereas only 5.4 and 4.2% of these losses were connected with SOM decomposition in both soils, respectively. The other 95% were referred to the O, S, P, and H losses through mineral changes at temperatures between 480 and 1000 °C (Schultze, 1969). One of such changes could be seen as a small endothermic peak on the DSC curve at 570 °C that indicated the release of constitutional water and the collapse of the lattice of such clay minerals as kaolinite and halloysite (Schultze 1969, p.204).

4.2. Carbon isotopic composition and the portion of the *Miscanthus*-derived C_4-C in SOM

The average shoot and root $\delta^{13}C$ values of *Miscanthus* growing on the soil were –11.8‰ and of grassland plants previously grown on this plot were –28.0‰. Considering the increase of the $\delta^{13}C$ value of the soil under *Miscanthus* up to 8.82‰ comparing to the $\delta^{13}C$ value of bulk soil under continuous C_3 vegetation (–26.47‰) and the isotopic discrimination of 1.0‰ by humification (Agren et al., 1996; Ehleringer and Cerling, 2002), the amount of C exchanged in the total SOM during the last 10.5 years was 55.4% (Table 2). This is much higher than the contribution of new C within 10 years, observed in most studies done on maize (Flessa et al., 2000; Ludwig et al., 2003). This higher contribution of *Miscanthus*-derived C was reflected in its much greater above and below ground biomass and annual mulching of part of the above ground plant residues in comparison to maize. The large amount of *Miscanthus*-derived C in our study contradicted the findings of the study by Foereid et al. (2004), which was conducted with *Miscanthus*, where the portion of new C_4-C reached only 18% after 11 years. The difference is probably connected to the fact that various soil layers had been investigated in their study. In our study, only the upper 0-5 cm was used, where the C turnover is faster compared to the soil depths of 0-10, 0-20 or even 0-30 cm, which were used in other studies (Gregorich et al., 1995; Flessa et al., 2000; Foereid et al., 2004).

The turnover rates (TR) corresponding to the MRT of 13.1 years for bulk soil and of 11.6, 12.2, and 15.4 years for pools decomposed between 190-310, 310-390 and 390-480 °C,

respectively, were faster than the MRTs in the most of other studies with maize (Huggins et al., 1998; Collins et al., 1999). However, Gregorich et al. (1995) obtained a similar MRT of 15 years for soil under maize. These fast turnover rates and short mean residence time were connected to the very high input of *Miscanthus* C in the upper 0-5 cm soil layer and the labile compounds that were accumulated in the SOM during the period of *Miscanthus* cultivation (10.5 years). Foereid and co-workers (2004) showed that the stability of *Miscanthus*-derived SOM was correlated to the time of *Miscanthus* cultivation. The *Miscanthus*-derived C in the 11-year old *Miscanthus* field had a MRT not much longer than the fresh residues (1 year), while the MRT of the older field (18-yearold) was longer (3.5 years).

The MRT of C_4-C in SOM pool decomposed above 480 °C was more than 160 years (Table 2). So due to very low incorporation of *Miscanthus*-derived C into this SOM pool (6.4%), this pool could be considered as an inert or recalcitrant one. Many other studies had looked for appropriate methods to separate the recalcitrant fraction of the SOM and considered this pool by modelling (reviewed by Ludwig et al., 2003). Most methods failed in the experimental estimation of the inert fraction because the recalcitrance may be connected with various chemical, physical, and biological properties (Six et al., 2001; Ludwig et al., 2003; Plante et al., 2005). Foereid with co-workers (2004) hypothesized that the recalcitrance of SOM under *Miscanthus* cropping was connected to the formation of insoluble carbon in the SOM, which increased its stability upon microbial attack.

The slow turnover rate of the pool with the highest thermal stability suggested its recalcitrance. However, the amount of this fraction (14% of SOM) in our study was lower than the results of other studies, where the inert SOM amounted to 30-40% of the total C (John et al., 2005; Kristiansen et al., 2005).

The experimental data showed that the differences in $\delta^{13}C$ values between SOM pools 190-310, 310-390, 390-480 °C were small and therefore, the portion of new Miscanthus-derived C was very similar (Table 1, 2). Consequently, the portion of C subjected to a short-time turnover with rates of years and decades was nearly similar in all SOM pools decomposed up to 480 °C. Only small amounts of C (< 1% of Corg) in the SOM pool decomposed above 480 °C could be referred to as a stable C with MRT of about 160 years.

Conclusions

Thermogravimetry coupled with differential scanning calorimetry was suitable to separate SOM pools based on their stability to thermal decomposition. Despite clear

separation of SOM pools of different thermal stability by TG-DSC, the isotopic analyses showed that the accordance between thermal and biological degradability was not gradual. All SOM pools with low and medium thermal stability decomposed below 480 °C had faster turnover rates than the pool, decomposed above 480 °C. Microbial availability of all pools decomposed below 480 °C was similar (MRT of 12-15 years) suggesting no clear correlation between thermal and microbial decomposability. A long MRT of 160 years was measured for the thermally stable pool decomposed above 480 °C. However, the ecological significance of this pool is of minor importance because of very low C amounts in the pool. Due to the very poor correlation between $\delta^{13}C$ values and thermal stability of SOM pools we conclude that the applicability of the TG-DSC for the separation of SOM pools with different biological availability is limited.

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III) Effects of atmospheric CO_2 enrichment on $\delta^{13}C$, $\delta^{15}N$ values and turnover times of soil organic matter pools isolated by thermal techniques.

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Abstract

CO_2 applied for Free-Air CO_2 Enrichment (FACE) experiments is strongly depleted in ^{13}C and thus provides an opportunity to study C turnover in soil organic matter (SOM) based on its $\delta^{13}C$ value. Simultaneous use of ^{15}N labeled fertilizers allows N turnover to be studied. Various SOM fractionation approaches (fractionation by density, particle size, chemical extractability etc.) have been applied to estimate C and N turnover rates in SOM pools. The thermal stability of SOM coupled with C and N isotopic analyses has never been studied in experiments with FACE. We tested the hypothesis that the mean residence time (MRT) of SOM pools is inversely proportional to its thermal stability.

Soil samples from FACE plots under ambient (380 ppm) and elevated CO_2 (540 ppm; for 3 years) treatments were analyzed by thermogravimetry coupled with differential scanning calorimetry (TG-DSC). Based on differential weight losses (TG) and energy release or consumption (DSC), five SOM pools were distinguished. Soil samples were heated up to the respective temperature and the remaining soil was analyzed for $\delta^{13}C$ and $\delta^{15}N$ by IRMS. Energy consumption and mass losses in the temperature range 20-200 °C were mainly connected with water volatilization. The maximum weight losses occurred from 200-310 °C. This pool contained the largest amount of carbon: 61% of the total soil organic carbon in soil under ambient treatment and 63% in soil under elevated CO_2, respectively. $\delta^{13}C$ values of SOM pools under elevated CO_2 treatment showed an increase from -34.3‰ of the pool decomposed between 20-200 °C to -18.1‰ above 480 °C. The incorporation of new C and N into SOM pools was not inversely proportional to its thermal stability. SOM pools that decomposed between 20-200 and 200-310 °C contained 2 and 3% of the new C, with a MRT of 149 and 92 years, respectively. The pool decomposed between 310-400 °C contained the largest proportion of new C (22%), with a MRT of 12 years. The amount of fertilizer-derived N after 2 years of application in ambient and elevated CO_2 treatments was not significantly different in SOM pools decomposed up to 480 °C having MRT of about 60 years. In contrast, the pool decomposed above 480 °C contained only 0.5% of new N, with a MRT of more than 400 years in soils under both treatments. Thus, the separation of SOM based on its thermal stability was not sufficient to reveal pools with contrasting turnover rates of C and N.

Key words: Thermal stability of SOM; Free Air CO_2 Enrichment; delta ^{13}C; delta ^{15}N; thermogravimetry; differential scanning calorimetry; TG-DSC, SOM turnover times, MRT

1. Introduction

Free-air CO_2 enrichment (FACE) experiments provide an opportunity to examine many aspects of elevated CO_2 effects on ecosystems, and to study the pathways of carbon (C) and nitrogen (N) in soil organic matter (SOM) under realistic field conditions (Leavitt et al., 2001; Miglietta et al., 2001; Kimball et al., 2002). FACE avoids most of the microenvironment effects imposed by the chamber technique and therefore more reliably reproduces soil processes under elevated CO_2 (van Kessel et al., 2000b).

To study the fate of carbon (C) in soils under CO_2 enrichment, most FACE experiments use stable-C isotopic tracers (Hungate et al., 1997; Torbert et al., 1997; van Kessel et al., 2000a, 2000b; Leavitt et al., 2001; Niklaus et al., 2001; Hagedorn et al., 2003). The additional CO_2 is usually derived from fossil fuel, which is depleted in ^{13}C ($\delta^{13}C$ varies from −35‰ to −50‰) compared with atmospheric CO_2 ($\delta^{13}C$ −8‰) (Hungate et al., 1996; Nitschelm et al., 1997; van Kessel et al., 2000b; Jones and Donnelly, 2004). The isotopic composition of CO_2 in the elevated CO_2 treatment is determined by the proportions of atmospheric and fossil fuel-derived CO_2, and provides a continuous C-isotope tracer. Plants grown in the elevated CO_2 atmosphere are depleted in $\delta^{13}C$ and, as the litter and rhizodeposits from these plants decompose and become incorporated into the SOM, the soil $\delta^{13}C$ will decrease. The contribution of the new FACE-derived C to total SOM can be calculated based on the $\delta^{13}C$ of the SOM under ambient conditions and the period after initiating CO_2 enrichment. This new C contribution and the CO_2 treatment duration then allow the SOM turnover rates to be estimated (Balesdent and Mariotti, 1987). Beyond investigating the C pathways in SOM under elevated atmospheric CO_2, the study of N cycling provides insights into the transformation processes of these two elements in soil. Thus, the application of N fertilizers in FACE experiments was used to estimate N flows and plant uptake under elevated CO_2 (Hungate et al., 1997; van Kessel et al., 2006; Zanetti et al., 1997). The use of N fertilizers labeled with ^{15}N provides an opportunity to study N turnover and to separate SOM N from the fertilizer N.

To track the new C and N in SOM pools, fractionation techniques including density (Magid et al., 2002; John et al., 2005) and particle size fractionation (Jolivet et al., 2003; Ludvig et al., 2003) as well as sequential extractions (Ellerbrock and Kaiser 2005) have been combined with $\delta^{13}C$ analyses. Another suitable approach for SOM partitioning is based on its thermal stability (Leinweber and Schulten, 1992; Siewert, 2001, 2004; Lopez-Capel et al., 2005a, 2005b; Plante et al., 2005; Kuzyakov et al., 2006). Thermogravimetry analysis coupled with differential scanning calorimetry involves a continuous, gradual temperature

increase which decomposes (mainly oxidizes) different organic compounds according to their thermal stability. Such a temperature increase coupled with measuring of weight losses is termed thermogravimetry (TG). Simultaneously to temperature increase, energy released or consumed by decomposition of organics is measured by differential scanning calorimetry (DSC).

TG-DSC is extensively applied to various soil science studies. Leinweber and Schulten (1992), Leinweber et al. (1992) and Schulten and Leinweber (1999) used DSC to characterize organo-mineral complexes; Provenzano and Senesi (1999) have used DSC to study humic substances; Lopez-Capel et al. (2005a), Francioso et al. (2005), and Plante et al. (2005) applied the technique to evaluate the humification state of SOM. Studying correlations between thermal stability of SOM pools in various temperature ranges and CO_2 production by classical soil incubations, Siewert (2001) suggested the idea of a relationship between thermal stability of SOM pools and their biological degradability.

Thermogravimetry and differential scanning calorimetry could potentially provide a simple and rapid determination of gross changes in organic matter quality (Plante et al., 2005), but have apparently never been used to examine the quality of SOM under altered environmental conditions such as elevated CO_2 concentration in the atmosphere. The objectives of this study were (i) to characterize the SOM quality in terms of thermal properties under ambient and elevated concentrations of atmospheric CO_2 in a 3-year Free Air Carbon dioxide Enrichment (FACE) experiment and (ii) to test the hypothesis that the mean residence times (MRT) of SOM pools are inversely proportional to their thermal stability by comparing the $\delta^{13}C$ and $\delta^{15}N$ values of the SOM pools decomposed at low and high temperatures in both treatments.

2. Materials and Methods

Soil samples were taken from the Free Air Carbon dioxide Enrichment facility located in Stuttgart-Hohenheim, Baden-Wuerttemberg, Germany (48°43’ north latitude, 9°13’ east longitude). The soil is a Gleyic Cambisol (WRB, 1998) without carbonates (no reaction with HCl). Mean annual temperature is 8.7 °C and average rainfall 680 mm a^{-1} (mean 1961-1990, meteorological station Stuttgart-Hohenheim). Properties of the soil under ambient and elevated CO_2 treatments were similar: pH 6.8, bulk density (0-10 cm) 1.4 g cm^{-3}, C_{org} 1.45 %, N_{tot} 0.16 %, C/N ratio 9.1.

The FACE experiment, starting in 2002, included plots with elevated atmospheric CO_2 level (540 ppm, $\delta^{13}C$ –19‰, because of using CO_2 at –48‰ for CO_2 addition), ambient-plots

(380 ppm, $\delta^{13}C$ –8‰), and control-plots (ambient CO_2 level, no enclosures) (Erbs and Fangmeier, 2006). Each treatment was replicated five times. To represent a typical agricultural ecosystem, spring wheat in combination with several weeds common for arable systems was annually planted on the plots since 2002. Average $\delta^{13}C$ value of plants grown under ambient CO_2 treatment was – 27‰, under elevated CO_2 – 39‰ (Erbs, pers. communication). Soil was tilled in spring before wheat sowing. Beginning in 2003, inorganic NPK fertilizers including KNO_3 labeled with ^{15}N ($\delta^{15}N$ of the fertilizer was 333.75‰) were applied in equal amounts of 140 kg N ha^{-1}, 60 kg K ha^{-1}, 13 kg P ha^{-1} to each plot under ambient and elevated CO_2 treatments. No organic fertilizers were applied.

Soil samples for thermogravimetry and $\delta^{13}C/\delta^{15}N$ analysis were taken in October 2005 from 10 plots (5 elevated treatments, 5 ambient treatments) from the depth of 0-10 cm using soil corers (inner diameters: 5 cm). The soil samples were air-dried at room temperature and sieved through 2 mm mesh. All visible roots and plant remains were carefully removed with tweezers from a sub-sample of 10 g and the soil was ball milled (MM2, Fa Retsch) for 15 sec. Analogous procedure was done for soil sampled before the FACE experiment was started. These soil samples were used to obtain initial properties of soil important for calculation of fertilizer-derived N. No preliminary treatment was done to remove NO_3^- from soil samples prior to thermogravimetry analysis.

2.1 Thermogravimetry – Differential Scanning Calorimetry analysis

Thermogravimetric analysis (TG) and differential scanning calorimetry analysis (DSC) were carried out simultaneously using the NETZSCH STA 409EP device, regulated by the TASC 414/3 controller (Fa Netzsch). One hundred milligrams of soil sample was weighed out on an alumina crucible and then heated from 20 to 1000 °C in air atmosphere (1.0 ml min^{-1}). The heating rate was 2.0 °C min^{-1}. The weight of the soil sample (in mg), as well as the heat (in μV mg^{-1}) released or consumed by substance oxidation or water evaporation, was continuously scanned. Calcined kaolinite previously heated at 1250 °C was used as the reference material. The obtained TG-DSC curves of soil samples from ambient and elevated treatments were used to determine “threshold” temperature levels. These levels were set as temperature values to heat soil samples in a muffle oven (Heraeus MR-260) for subsequent measurements of weight losses, C and N content, as well as $\delta^{13}C$ and $\delta^{15}N$ values of SOM pools. Four groups of soil samples were heated up to 200, 310, 400 and 480 °C. No heating (20 °C) corresponded to the bulk soil. Heating up to 200 °C removed mainly water, but some organic and inorganic substances were decomposed between 20 and 200 °C. Only the SOM

pools remaining after 200 °C were analysed for their C and N content, $\delta^{13}C$ and $\delta^{15}N$ values. The same approach was used to determine the SOM pools at other temperature thresholds. Then, considering the weight, C and N losses after heating up to the threshold temperatures, the corresponding values (weight, C and N) were calculated for the intermediate SOM pools decomposed between 200 and 310, or 310 and 400, or 400 and 480 °C (see below). The temperature range between 310 and 400 °C was selected to better separate the pools decomposed below 310 °C and above 400 °C. The heating rate of the muffle furnace was the same as by TG-DSC analysis: 2.0 °C min^{-1}.

2.2 $\delta^{13}C$ and $\delta^{15}N$ analyses

Ten to forty milligrams of soil samples remaining after heating in the muffle oven up to one of the threshold temperatures were weighed in tin capsules for $\delta^{13}C/\delta^{15}N$ analyses. The C and N isotopic composition was measured with a Delta Plus XP isotope ratio mass spectrometer (Thermo Finnigan, Bremen) coupled to the Euro EA C/N analyser (Eurovector Instruments and Software, Hekatech GmbH, Wegberg). Acetanilide was used as the internal standard for $\delta^{13}C$ and $\delta^{15}N$ analyses. The final isotopic composition is expressed as $\delta^{13}C$ units in relation to Pee Dee Belemnite ($^{13}C/^{12}C$=0.0112372) as the standard for C, and as $\delta^{15}N$ units in relation to atmospheric N_2 ($^{15}N/^{14}N$=0.0036765) as the standard for N.

2.3 Calculations

Weight losses as well as C and N content of SOM pools decomposed between 20-200, 200-310, 310-400 and 400-480 °C were calculated by subtraction of weights, C and N content of soil samples combusted up to the lower temperature threshold and higher temperature threshold:

$$M_{FR}\ (x_n - x_{n+1}) = M_R\ (x_n) - M_R\ (x_{n+1}) \quad (1),$$

where M_{FR} was weight losses, C or N content of a SOM fraction, M_R was weights, C or N content of SOM in residues combusted up to threshold temperatures, x_n was the lower threshold temperature, and x_{n+1} was the higher threshold temperature, respectively. Here, $M_{FR}\ (x_1) = M_R\ (x_1)$ corresponding to weight, C and N of bulk soil, and $M_{FR}\ (x_5) = M_R\ (x_5)$ corresponding to weight, C and N of SOM pool decomposed up to 480 °C. For example, M_{FR} (200-310) was calculated by subtraction of M_R (200) and M_R (310).

The $\delta^{13}C$ and $\delta^{15}N$ values of four individual SOM pools of different thermal stability decomposed between 20-200, 200-310, 310-400 and 400-480 °C were calculated based on the isotopic mass balance equation (Balesdent and Mariotti, 1996):

$$\delta_{FR}\,(x_n - x_{n+1}) = [M_R\,(x_n) \cdot \delta_R\,(x_n) - M_R\,(x_{n+1}) \cdot \delta_R\,(x_{n+1})] \,/\, M_{FR}\,(x_n - x_{n+1}) \qquad (2),$$

where δ_{FR} was $\delta^{13}C$ or $\delta^{15}N$ value of a SOM fraction, M_{FR} was the amount of C or N in a SOM fraction, M_R was C or N content of SOM in residues combusted up to threshold temperatures, δ_R was $\delta^{13}C$ or $\delta^{15}N$ value of SOM in residues combusted up to threshold temperatures, x_n was a lower threshold temperature, and x_{n+1} was a higher threshold temperature. Here, $M_{FR}\,(x_1)$ and $\delta_{FR}\,(x_1)$ corresponded to $M_R\,(x_1)$ and $\delta_R\,(x_1)$ of bulk soil, and $M_{FR}\,(x_5) = M_R\,(x_5)$, $\delta_{FR}\,(x_5) = \delta_R\,(x_5)$ in SOM pool decomposed up to 480 °C.

The portion of FACE-derived C (C_{FACE}) in SOM was calculated for bulk soil and each temperature range according to Balesdent and Mariotti (1996) and adapted for FACE conditions:

$$C_{FACE} = (\delta^{13}C_{elev} - \delta^{13}C_{amb}) \,/\, (\delta^{13}C_{elev.theor.} - \delta^{13}C_{amb}) \cdot 100 \qquad (3),$$

where $\delta^{13}C_{elev}$ was a $\delta^{13}C$ value of the SOM of bulk soil under elevated treatment and $\delta^{13}C$ of SOM pools under elevated treatment calculated according to Eq.2; $\delta^{13}C_{amb}$ was a $\delta^{13}C$ value of the SOM of bulk soil under ambient treatment and $\delta^{13}C$ of SOM pools under ambient treatment calculated according to Eq.2; $\delta^{13}C_{elev.theor.}$ was a theoretical $\delta^{13}C$ value of the bulk SOM and SOM pools decomposed at increasing temperatures developed under vegetation grown in continuously elevated CO_2 conditions. Isotopic signatures of theoretical SOM were calculated based on the difference between the $\delta^{13}C$ of the plants growing under elevated CO_2 and the $\delta^{13}C$ of bulk soil and SOM pools of different thermal stability under ambient CO_2. The values were corrected for isotopic fractionation during humification by subtracting the differences between $\delta^{13}C$ of vegetation growing under ambient conditions and $\delta^{13}C$ of the corresponding SOM pool of the soil developed under ambient CO_2 treatment:

$$\delta^{13}C_{elev.theor.} = \delta^{13}C_{elev.plant} - (\delta^{13}C_{amb.plant} - \delta^{13}C_{amb}) \qquad (4),$$

where $\delta^{13}C_{elev.plant}$ was $\delta^{13}C$ of the plants under elevated CO_2 treatment, $\delta^{13}C_{amb.plant}$ was $\delta^{13}C$ value of the plants under ambient CO_2 treatment, and $\delta^{13}C_{amb}$ was $\delta^{13}C$ of the bulk SOM and corresponding SOM pool of the soil developed under ambient CO_2 treatment.

Similar equation was used to calculate the portion of N derived from labelled fertilizers:

$$N_{fertil} = (\delta^{15}N_{soil\ actual} - \delta^{15}N_{soil\ initial})/(\delta^{15}N_{fertil} - \delta^{15}N_{soil\ initial}) \cdot 100 \quad (5),$$

where $\delta^{15}N_{soil\ actual}$ was a $\delta^{15}N$ value of the bulk SOM and SOM pools decomposed under increasing temperatures after fertilization during two years (Eq. 2), $\delta^{15}N_{soil\ initial}$ was a $\delta^{15}N$ value of the bulk SOM and SOM pools decomposed under increasing temperatures before fertilization, and $\delta^{15}N_{fertil}$ was a $\delta^{15}N$ signature of N fertilizers.

To calculate the contribution of new FACE-derived C and N from fertilizers in SOM for a particular period of the FACE experiment and annual turnover rates (TR) of SOM, a simple exponential approach was selected (Balesdent and Mariotti, 1996):

$$TR = -\ln(1 - M/100)/t \quad (6),$$

where M was the portion of C_{FACE} or N_{fertil} (Eq. 4 and 5) in bulk SOM and SOM pools decomposed under increasing temperatures, and t was the time of soil exposure to elevated CO_2 concentrations (3 years) and the period of N fertilization (2 years).

The mean residence time of FACE-derived C and N from fertilization in bulk soil and in SOM pools was calculated as a reciprocal to the turnover rates (Gregorich et al., 1995):

$$MRT = 1/TR \quad (7)$$

Amounts of C_{FACE} and $N_{fertil.}$ in kg ha^{-1} were calculated using soil bulk density of 1.4 g cm^{-3} and the layer of 10 cm. The study was conducted with five and four replicates under elevated- and ambient CO_2 treatments, respectively, both for TG-DSC and isotopic analysis. The initial soil for no-^{15}N control was replicated three times. The significance of differences between $\delta^{13}C$, $\delta^{15}N$, as well as the C and N content of different pools under ambient and elevated CO_2 treatments was examined using one-way analysis of variance (ANOVA). The standard errors of means are presented on the figures and in the tables as the variability parameter.

3. Results

3.1 TG-DSC analysis of soil samples

Thermal analysis of soil samples provided two data sets: mass losses due to thermal decomposition of soil organic and inorganic constituents, and heat flow defined by exothermic or endothermic reactions while decomposing these constituents. Data for thermogravimetry analysis were represented by two curves: cumulative weight losses (TG, %) and differential weight losses (dTG, % $°C^{-1}$) (Fig. 1). The dTG curve showed three well-defined peaks of differential weight losses from soil samples under elevated and ambient CO_2 treatments during the heating from 20 to 1000 °C: 1) between 20 and 200 °C (temperature range A), 2) between 200 and 310 °C (temperature range B) and 3) between 400 and 480 °C (temperature range D). Minimal weight losses occurred from 310 to 400 °C (temperature range C) and from 480 to 1000 °C (temperature range E) (Fig. 1). The most intensive losses were observed in the temperature range A, with the maximum of 0.035 percent weight loss per degree (% $°C^{-1}$) at 50 °C. The subsequent maxima of differential weight losses were smaller: 0.023 % $°C^{-1}$ in soil under elevated treatment, 0.024 % $°C^{-1}$ in soil under ambient treatment, both at 285 °C in temperature range B, and 0.015 % $°C^{-1}$ at 460 °C in temperature range D for both treatments (Fig. 1). The loss minima were less clearly defined. Within temperature range A the minima were from 0.01 to 0.002 %$°C^{-1}$, in temperature range C from 0.009 to 0.007 % $°C^{-1}$; values in the last temperature range approached zero.

The DSC traces of the samples under both treatments were characterised by three temperature ranges, two with endothermic reaction: 1) 20 - 220 °C, 2) 325 - 1000 °C, and one exothermic reaction between 220 and 325 °C. The first endothermic reaction, occurring within temperature range A, had a maximum energy consumption of -0.06 µV per milligram of a sample weight (µV mg^{-1}) at around 50 °C, corresponding to the maximal weight losses in the same temperature range. Starting from 220 °C the energy release due to decomposition processes of organics (see *Discussion*) switched the DSC curve to positive values. Maximal energy release of 0.052 µV mg^{-1} occurred at 285 °C, corresponding to the peak of differential mass losses within temperature range B (Fig. 1). From 325 °C and up to 1000 °C the heat flow of the soils samples showed a maximum energy consumption of -0.112 µV mg^{-1} between 680 and 715 °C. The distinctive feature of the DSC curves of the soils under both treatments was a small negative peak at 570 °C (Fig. 1). Neither differential weight losses from soil samples nor DSC data showed significant differences between ambient and

elevated CO_2 treatments; this allowed similar temperature levels to be chosen for thermal fractionation of SOM in soil samples under both treatments.

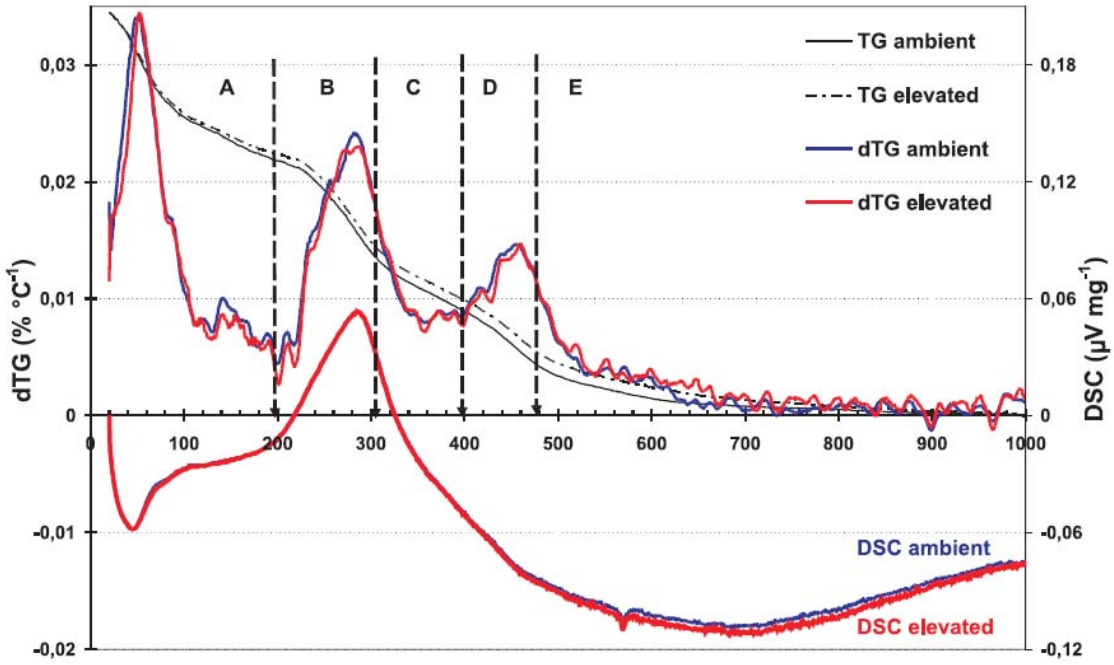

Fig. 1. Differential thermogravimetry (dTG, left Y axis) and Differential Scanning Calorimetry (DSC, right Y axis) of soil under ambient and elevated CO_2 treatments. Cumulative losses (TG) are scaled to 100% of the left Y axis and totalled 6.44% of sample weight for soil under ambient treatment and 6.71% for soil under elevated CO_2 treatment. Negative DSC values represent energy consumption (endothermic reactions), positive DSC values – energy release (exothermic reactions). Arrows show the "threshold" temperature chosen for SOM fractionation

3.2 Weight losses, C and N contents of SOM pools

To fractionate the SOM into individual pools with different thermal stability, the losses of mass for soil samples during heating were chosen as the main criteria. Since the mass losses and energy release are related to various organic and inorganic components, the DSC values cannot be directly linked to the individual substances. Thus, the DSC traces were used here to confirm the temperature thresholds estimated by mass losses.

The total weight losses of the five SOM pools in both ambient and elevated treatments were not significantly different. Losses of weight from soil samples under ambient CO_2 treatment amounted to 6.44 ±0.18% of the total mass of samples and 6.71 ±0.21% - for soil samples under elevated CO_2 treatment (Table 1). Peak weight losses occurred in the SOM pool decomposed between 200 and 310 °C: 2.65 ±0.02% of the initial sample mass was lost

in soil under ambient CO_2 treatment, and 2.61 ±0.03% in soil under elevated CO_2 treatment. The least weight losses were observed at 20-200 °C, where the soil under ambient treatment lost 0.68 ±0.04% and the soil under elevated CO_2 treatment lost 0.69 ±0.02% of total mass losses (Fig. 1).

The maximum C and N losses corresponded to maximal weight losses in SOM pools decomposed at 200-310 °C. The lowest C and N contents were measured in SOM decomposed above 480 °C (Table 1).

Table 1. Measured weight losses, amount of C, N, and $\delta^{13}C$ and $\delta^{15}N$ values in soil under ambient and elevated CO_2 treatments in residue samples after heating up to threshold temperatures. Values are means of four replicates in ambient CO_2 treatment and five replicates in elevated CO_2 treatment ± SE.

Temperature threshold (°C)/ treatment	Weight losses (%)	C content (%)	N content (%)	$\delta^{13}C$ (‰ PDB)	$\delta^{15}N$ (‰ air N_2)
200					
ambient	0.68 ± 0.04	1.287 ± 0.016	0.146 ± 0.0015	–26.19 ± 0.25	18.25 ± 1.12
elevated	0.69 ± 0.02	1.327 ± 0.018	0.150 ± 0.0016	–27.14 ± 0.18*	19.06 ± 0.68
310					
ambient	3.33 ± 0.02	0.394 ± 0.015	0.078 ± 0.0018	–23.37 ± 0.35	19.43 ± 0.70
elevated	3.30 ± 0.03	0.406 ± 0.017	0.082 ± 0.0029	–25.58 ± 0.25**	20.26 ± 0.58
400					
ambient	4.48 ± 0.06	0.110 ± 0.010	0.028 ± 0.0009	–20.79 ± 0.69	15.43 ± 0.81
elevated	4.38 ± 0.04	0.100 ± 0.002	0.029 ± 0.0006	–21.85 ± 0.24	15.82 ± 0.67
480					
ambient	5.56 ± 0.02	0.039 ± 0.003	0.0082 ± 0.0001	–16.86 ± 0.20	8.91 ± 0.37
elevated	5.61 ± 0.08	0.035 ± 0.001	0.0085 ± 0.0002	–18.07 ± 0.35*	9.37 ± 0.57
Bulk soil[a]					
ambient	6.44 ± 0.18	1.451 ± 0.016	0.159 ± 0.0013	–26.90 ± 0.04	18.10 ± 0.93
elevated	6.71 ± 0.21	1.461 ± 0.017	0.160 ± 0.0016	–27.78 ± 0.11**	19.02 ± 0.67

[a]Bulk soil represents not heated soil samples

$^{*}P < 0.05$ and $^{**}P < 0.01$ represent the significance of differences between ambient and elevated CO_2 treatments

3.3 C and N isotopic composition

Due to the available analytical facilities the $\delta^{13}C$ values were measured in soil residues decomposed up to the threshold temperatures rather than directly in the CO_2 released by decomposition at certain temperatures as done by Lopez-Capel et al. (2005a). Then, considering C and N losses, and based on the isotopic mass balance equation (Eq. 1), the

$\delta^{13}C$ and $\delta^{15}N$ values of intermediate SOM pools decomposed from 200-310, 310-400, and 400-480 °C were calculated (Tables 2 and 3).

Table 2. Amount of C, $\delta^{13}C$ values, amount of FACE-derived C and the mean residence time (MRT) of C in bulk soil and SOM pools isolated in five temperature ranges for soils exposed for 3 years to elevated and ambient CO_2 concentrations. Values are means of four replicates in ambient CO_2 treatment and five replicates in elevated CO_2 treatment ± SE.

Temperature interval (°C)/ treatment	C content (%)	$\delta^{13}C$ (‰ PDB)	C_{FACE} (kg ha^{-1})[a]	MRT (years)[a]
20-200				
ambient	0.164 ± 0.016	-32.32 ± 2.15		
elevated	0.134 ± 0.018	-34.32 ± 1.85	37.9 ± 38.82	149 ± 150
200-310				
ambient	0.893 ± 0.015	-27.45 ± 0.18		
elevated	0.921 ± 0.017	-27.82 ± 0.11	419.5 ± 167.9	92 ± 49
310-400				
ambient	0.284 ±0.010	-24.28 ± 0.19		
elevated	0.306 ± 0.002*	-26.81 ± 0.09**	940.5 ± 62.3	12 ± 1
400-480				
ambient	0.071 ± 0.003	-22.97 ± 0.09		
elevated	0.065 ± 0.001	-23.94 ± 0.24	75.2 ± 8.2	35 ± 6
480-1000				
ambient	0.039 ±0.003	–16.86 ±0.20		
elevated	0.035 ±0.001	–18.07 ±0.35*	50.8 ± 8.2	28 ± 5
Bulk soil				
ambient	1.451 ±0.016	–26.90 ±0.04		
elevated	1.461 ±0.017	–27.78 ±0.11**	1523.9 ± 67.6	39 ± 2

[a]FACE-derived C and MRT could only be assessed at the elevated CO_2 treatment. Therefore, the effect of CO_2 was not included for these variables.

$^{*}P < 0.05$ and $^{**}P < 0.01$ represent the significance of differences between ambient and elevated CO_2 treatments.

Fumigation with elevated CO_2 for three years significantly depleted the $\delta^{13}C$ signature of bulk soil (Table 1). In SOM pools decomposed in temperature ranges 310-400 °C and 480-1000 °C the $\delta^{13}C$ values under elevated CO_2 treatment were significantly lower than in the ambient CO_2. However, there were no significant decrease of the $\delta^{13}C$ in temperature ranges 20-200, 200-310 and 400-480 °C the depletion of $\delta^{13}C$ signatures was insignificant (Table

2). The largest ^{13}C depletion occurred at 20-200 °C: the soil under ambient treatment had a $\delta^{13}C$ value of –32.32 ±2.15‰, whereas the soil under elevated treatment had $\delta^{13}C$ of –34.32 ±1.85‰. However, this difference was not statistically significant (Table 2). Large standard errors in the SOM pools decomposed at 20-200 °C compared to other pools were connected with high weight losses by water evaporation which were not accompanied by the respective C and N losses. Therefore, very small changes of total C and N by heating from 20 to 200 °C led to small changes of their $\delta^{13}C$ and $\delta^{15}N$ values resulting in high estimation error. The rise of the $\delta^{13}C$ values under both treatments (Tables 1 and 2) was occurred due to the slow combustion process, when the lighter ^{12}C decomposed faster than the heavier ^{13}C, leading to accumulation of the latter in the residual SOM pools (Lopez-Capel et al., 2005a).

Table 3. Amount of N, $\delta^{15}N$ values, amount of fertilizer-derived N including the percentage from total amount of N in a pool and the mean residence time (MRT) of N after two years of fertilization in bulk soil and SOM pools isolated in five temperature ranges for soils under ambient and elevated CO_2 treatments. Values are means of four replicates in ambient treatment and five replicates in elevated CO_2 treatment ± SE.

Temperature interval (°C)/ treatment	N content (%)	$\delta^{15}N$ (‰ air N_2)	N_{fertil} (kg ha^{-1})/ (% from N content)	MRT (years)
20-200				
ambient	0.0135 ± 0.0015	16.41 ± 1.12	5.2 ± 7.1 (2.69)	73 ± 36
elevated	0.0107 ± 0.0016	18.50 ± 0.68	5.1 ± 4.4 (3.33)	59 ± 43
200-310				
ambient	0.0681 ± 0.0018	16.83 ± 0.86	27.5 ± 2.4 (2.84)	69 ± 7
elevated	0.0676 ± 0.0029	17.53 ± 0.69	29.3 ± 2.1 (3.05)	65 ± 5
310-400				
ambient	0.0493 ± 0.0009	21.69 ± 0.43	30.3 ± 1.0 (4.31)	45 ± 2
elevated	0.0529 ± 0.0006*	22.69 ± 0.30	34.7 ± 0.9 (4.62)*	42 ± 1
400-480				
ambient	0.0201 ± 0.0001	17.99 ± 0.07	9.1 ± 0.1 (3.17)	62 ± 1
elevated	0.0207 ± 0.0002*	18.41 ± 0.21	9.7 ± 0.1 (3.29)**	60 ± 1
480-1000				
ambient	0.0082 ±0.0001	9.16 ± 0.19	0.53 ± 0.07 (0.46)	433 ± 53
elevated	0.0085 ±0.0002	9.57 ± 0.41	0.72 ± 0.15 (0.59)	337 ± 104
Bulk soil				
ambient	0.159 ±0.0013	18.10 ± 0.93	72.5 ± 6.5 (3.20)	61 ± 6
elevated	0.160 ±0.0016	19.02 ± 0.67	79.5 ± 4.7 (3.49)	56 ± 4

*$P < 0.05$ and **$P < 0.01$ represent the significance of differences between ambient and elevated CO_2 treatments.

Statistical analysis showed no significant difference in $\delta^{15}N$ values of bulk soil and SOM pools with different thermal stability between ambient and elevated CO_2 treatments (Table 3). The bulk soil and SOM pools decomposed at 20-200, 200-310 and 400-480 °C had similar $\delta^{15}N$ values, averaging 18.00‰. Higher $\delta^{15}N$ values were observed in the SOM pool decomposed between 310-400 °C: 22.15‰ on average, and the lowest values – 9.37‰ on average – were obtained at 480-1000 °C (Table 3).

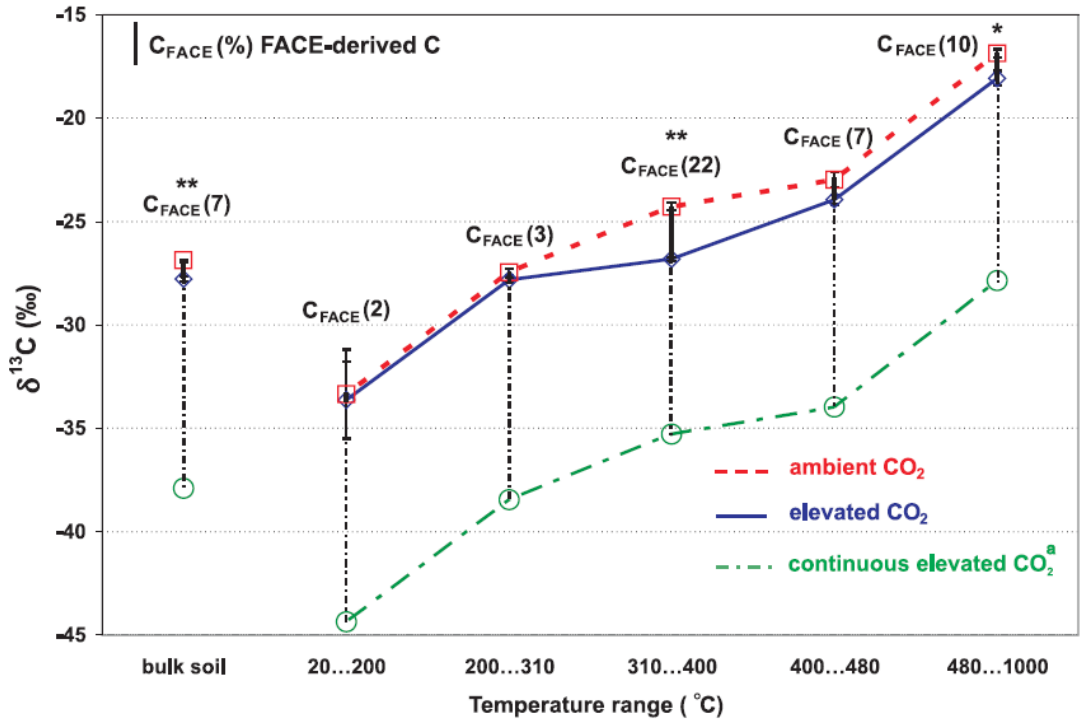

Fig. 2. $\delta^{13}C$ values of bulk soil and five SOM pools with different thermal stability under ambient and elevated CO_2 treatments. The calculated theoretical $\delta^{13}C$ values of soil developed under continuous elevated CO_2 conditions were used to estimate the portion of FACE-derived C (C_{FACE}) in the SOM (vertical bold lines and numbers in brackets). Whiskers present standard error (± SE). $^{*}P < 0.05$ and $^{**}P < 0.01$ represent the significance of differences between ambient and elevated CO_2 treatments

[a] Theoretical $\delta^{13}C$ values under continuous elevated CO_2 were calculated based on Eq. 3:

$$\delta^{13}C_{elev.theor.} = \delta^{13}C_{elev.plant} - (\delta^{13}C_{amb.plant} - \delta^{13}C_{amb})$$

3.4 The portion of FACE-derived C and fertilizer-derived N in SOM pools with different thermal stability

During three years of CO_2 fumigation the amount of the new C reached 7.3% of total C in the upper layer of bulk soil (Fig. 2) – equivalent to 1524 ±68 kg ha^{-1} (Table 2). This amount of FACE-derived C was distributed among SOM pools as follows: 38 ±39 kg ha^{-1} at 20-200 °C, 419 ±168 kg ha^{-1} at 200-310 °C, 941 ±62 kg ha^{-1} at 310-400 °C, 75 ±8 kg ha^{-1} at

400-480 °C and 51 ±8 kg ha^{-1} at 480-1000 °C (Table 2). The calculated turnover rates and MRT of SOM pools showed no relation between the MRT values and temperature increase. Thus, the SOM pools decomposed between 20-200 °C had 2.0% of the new C and between 200-310 °C 3.2%. Their MRT values were 149 ±150 and 92 ±49 years, respectively. The corresponding values at 310-400 °C were 21.6% FACE-derived C (MRT 12 years), at 400-480 °C 8.2% (35 years),and at 480-1000 °C 10.1% (28 years) (Fig. 2, Table 2).

After two years of fertilization, 72.5 ±6.5 kg ha^{-1} of the new N were incorporated in bulk soil under ambient CO_2 treatment and 79.5 ±4.7 kg ha^{-1} – under elevated CO_2 treatment, corresponding on average to 28% of applied mineral N (140 kg ha^{-1} annually) (Table 3). The SOM pools decomposed between 310 and 400 °C had the largest amounts of the fertilizer-derived N: 30.3 ±1.0 kg ha^{-1} for soil under ambient CO_2 treatment and 34.7 ±0.9 kg ha^{-1} for soil under elevated treatment. The lowest quantity (around 1 kg ha^{-1}) was observed in SOM pools decomposed at 480-1000 °C (Table 3). Similar MRT values of approximately 65 years were recorded for the new N of the bulk soil and SOM pools decomposed at 20-200, 200-310 and 400-480 °C. The longest MRT (433 ±53 and 337 ± 104 years) was observed in the SOM pool decomposed between 480-1000 °C in the ambient and elevated treatments, respectively.

4. Discussion

4.1 Thermal properties of SOM under ambient and elevated CO_2 conditions

Unequal weight losses during gradual heating justified a separation into five temperature intervals (Fig. 1). The first interval A (up to 200 °C) reflected losses of bound water and volatile organic substances. As stated earlier (Siewert, 2001; Kuzyakov et al., 2006), weight losses between 20 and 200 °C mainly corresponded to the evaporation of bound water: water absorbed from air moisture, crystalline lattice water and hygroscopic water of salts (Gaal et al., 1994; Zimmermann, 1987). Negative DSC values up to 220 °C (Fig. 1) confirmed the energy consumption by water volatilization. The endothermic reactions measured by DSC up to 120-150 °C were observed by many authors in TG-DSC studies of SOM (Dell'Abate et al., 2003; Francioso et al., 2005; Plante et al., 2005).

Not only water, however, but also organics were decomposed up to 200 °C. According to studies by Johnson (1977), Kristensen (1990) and Zimmermann (1987), these organics are volatile low molecular compounds such as free amino and carboxylic acids, which decomposed at relatively low temperatures (i.e. below 150 °C).

The temperature ranges B (200-310 °C) and C (310-400 °C) yielded the largest losses of C and N (Tables 2 and 3). As observed by Kristensen (1990) the organic substances decomposed up to 310 °C comprise plant material such as cellulose. Herrera et al. (1986) used dTG to determine that the major percentage weight loss of hemicelluloses and celluloses occur at the low temperature range between 200 and 350 °C.

The DSC traces of soil samples under both ambient and elevated CO_2 treatments showed intensive energy release due to decomposition of organic compounds, whereby the maximum at 285 °C exactly coincided with the maximum weight losses (Fig. 1). Nearly the same temperature (~300 °C) corresponded to the exothermic maximum observed by decomposition of humic acids extracted from different peats, lignites and leonardites (Francioso et al., 2005). These exothermic reactions reflected thermal decomposition of polysaccharides, decarboxylation of acidic groups and dehydration of hydroxylate aliphatic structures (Dell'Abate et al., 2002)

C losses in the temperature range D (390 to 480 °C) amounted to 4.5-5% of total C and were lower than the percent N losses (12.5-13%) under both elevated and ambient treatments. Larger losses of N-containing versus C-containing compounds within this temperature range were reported by Schulten and Leinweber (1999). The decomposed organics comprise stable constituents with aromatic compounds such as lignin, humic substances and kerogens (Kristensen, 1990; Leinweber et al., 1992; Siewert, 2004; Lopez-Capel et al., 2005a).

In the last temperature range E (480-1000 °C) the losses of organic matter were very small (Table 1). Most mass losses here reflect clay mineral decomposition (Schultze, 1969). The DSC traces showed strong energy consumption and a small endothermic peak at 570 °C, indicating quartz lattice collapse (Schultze, 1969).

4.2 C isotopic composition and the portion of new FACE-derived C

Three years of elevated CO_2 treatment significantly altered the C isotopic composition of bulk soil, showing the inputs of new C to SOM. However, the C content of the bulk soils under ambient and elevated CO_2 did not differ significantly (Table 1). The lack of total C change under elevated CO_2 was expected because SOM formation and decomposition processes are not strongly affected by elevated CO_2 (Leavitt et al., 2001) and 3 years are insufficient to obtain significant changes in bulk amount. The significant difference in the isotopic signature of the bulk soil under the two treatments was mainly because of the SOM pools decomposed at medium and high temperatures (310 to 1000 °C). The $\delta^{13}C$ of SOM

pools decomposed at 20-200 and 200-310 °C was not significantly altered by elevated CO_2 (Table 2).

The isotopic signatures of the carbon in bulk soils and SOM pools under ambient and elevated CO_2 allowed the amount of new FACE-derived C incorporated into SOM pools over three years to be calculated. After 3 years of CO_2 fumigation, this value in bulk soil under the elevated treatment reached 7.3% (Fig. 2). This agrees with the C inputs by spring wheat in a 2-year enrichment with CO_2 reported by Leavitt et al. (2001). They found the new carbon fraction to be 6.3% in the upper 15 cm of soil. Studies with cotton showed that the average amount of new C ranged from 6 to 12% after three years of elevated CO_2 (Leavitt et al., 1994). Van Kessel et al. (2006) reported higher C inputs of 22-26% under a grassland consisting of *Lolium perenne* and *Trifolium repens* in a FACE experiment after three years of CO_2 enrichment. These larger new C inputs, however, mainly reflect grassland plant species and the absence of tillage (van Kessel et al., 2006).

Calculating the FACE-derived C in SOM pools with different thermal stability as a kg C ha^{-1} provided detailed information on the quantity of the new carbon in a pool. Surprisingly, the SOM pool decomposed at 200-310 °C had the largest C content but accumulated 2.3 times less new C than that decomposed in the next temperature range (310-400 °C) (Table 2). This is indicated by the small differences in $\delta^{13}C$ values between soil samples under ambient and elevated CO_2 decomposed from 200 to 310 °C.

The turnover rate of the FACE-derived C in the bulk soil corresponded to the MRT of 39 ±2 years. The MRTs of the SOM pools decomposed at various temperatures ranged from 12 ±1 years (310-400 °C) to 149 ±150 years (20-200 °C) (Table 2). Results on the MRTs of pools decomposed at 20-200 and 200-310 °C have to be interpreted with care due to the large standard errors appearing from small isotopic differences in corresponding SOM pools between CO_2 treatments. The MRTs of the thermally stable SOM pools showed lower values of 35 ±6 (400-480 °C) and 28 ±5 (480-1000 °C) years compared to thermally labile SOM pools (Table 2). Balesdent (1996) found that chemical separations of SOM failed to isolate the SOM pools of different turnover time. Thus, the SOM pools isolated by acid hydrolysis or wet oxidation and thermal decomposition were poorly correlated with soil C age. In contrast, physical separation of soil into particle-size fractions showed good correlation between the turnover time of SOM and its association with a particle size fraction - the turnover rates decreased with decreasing particle-size (Balesdent, 1996). Studies using density fractionation as another kind of physical separation of SOM showed that light fractions including recent, partially decomposed plant residues have faster turnover rates of

C compared to heavy fractions (organo-mineral complexes) (Pendall et al., 2004; Sleutel et al., 2006; Six et al., 2001). The absence of correlation between the thermally isolated SOM pools and their MRTs in our study suggests that thermal oxidation is more closely analogous to chemical, rather than physical, fractionation of SOM.

It is important to note that the hypothesis of correlation between the thermally isolated SOM pools and their MRTs is complicated by two factors. Firstly, different biochemical plant components (cellulose, lignin) are decomposed in a wide temperature range (Lopez-Capel et al., 2005b). It means that individual components of plant residues may be directly incorporated into or even mixed with the thermal stable SOM fraction and so will mask the low turnover rates of this fraction. Secondly, individual plant compounds such as cellulose and lignin have different isotopic composition (Hobbie and Werner, 2004; Lopez-Capel et al., 2005a). By our hypothesis we assumed that the new C from wheat grown under elevated CO_2 has uniform isotopic compositions. Hence, specific $\delta^{13}C$ values of plant residue compounds decomposed up to threshold temperatures could change the calculated MRTs of SOM pools of different thermal stability and need to be studied in further experiments. However, if we assume that the average difference of $\delta^{13}C$ values between cellulose and lignin is about 4‰ (Hobbie and Werner, 2004), and incorporation of these compounds amounts less than 10% of C of a SOM pool, then the error produced by unequal isotopic composition of plant residues is less than 5% of the calculated contributions of new C. Furthermore, this is the maximal error connected with unequal isotopic composition because the plant residues and rhizodeposits of wheat contain little lignin and because individual compounds are not incorporated into only one specific SOM pool.

4.3 The portion of new fertilizer-derived N

The statistical analysis of $\delta^{15}N$ values performed for the bulk soils and SOM pools decomposed at different temperatures showed no effect of elevated CO_2 treatment on N cycling (Table 3). Other studies yielded contrasting results on N turnover under the elevated CO_2 concentrations. Dijkstra et al. (2005) and van Kessel et al. (2006) reported results similar to ours, showing no significant influence of atmospheric CO_2 on N dynamics. Daepp et al. (2000), Hartwig et al. (2002) and Zanetti et al. (1997) showed lower N turnover under elevated CO_2. Accelerated N turnover under elevated CO_2 was observed for N_2-fixing legumes (Hartwig et al., 2002; Nitschelm et al., 1997; Soussana and Hartwig, 1996; Zanetti et al., 1997).

SOM pools decomposed from 310 to 400 °C under both ambient and elevated CO_2 treatments showed the largest enrichment in ^{15}N. $\delta^{15}N$ values of the latter SOM pools (21.69 ±0.43‰ under ambient treatment and 22.69 ±0.30‰ under elevated CO_2 treatment) were closer to the signature of fertilizers compared to other SOM pools (Table 3). In the most thermally stable SOM pools (480-1000 °C), the $\delta^{15}N$ values (9.16 ±0.19‰ under ambient treatment and 9.57 ±0.41‰ under elevated CO_2 treatment) were similar to that of bulk soil before applying ^{15}N labeled fertilizers (7.2‰). Distribution of the new fertilizer-derived N among the SOM pools of different thermal stability under both treatments showed two pools with the largest amount of N_{fertil}, those decomposed at 200-310 and 310-400 °C. However, MRTs of the pools decomposed in the wide temperature range of 20-480 °C indicated similar values averaging to 60 years (Table 3). Only the most thermally stable SOM pool (480-1000 °C) having the smallest amount of N_{fertil} had MRT of hundred of years indicating very slow N incorporation.

A study conducted by van Groenigen et al. (2002, 2003) on N turnover in SOM pools in grassland by means of density and aggregates size fractionation under elevated CO_2 and N fertilization showed gradual decrease in the N_{fertil}. The largest amount of fertilizer-derived N was detected in free light fraction of SOM, an intermediate amount – in intra-aggregate particular OM, and the lowest amount in mineral associated OM. On the one hand, low incorporation of N_{fertil} into the most thermally stable SOM pool in our experiment coincides with the lowest amount of fertilizer-derived N in mSOM found by van Groenigen et al. (2002, 2003). However, in contrast to the findings of van Groenigen et al. (2002, 2003) our results indicated non-gradual distribution of N_{fertil} among SOM pools with very contrasting values of MRT.

We conclude that differential thermal analysis of soil under three years of elevated CO_2 was not sufficient for clear separation of SOM pools with contrasting turnover rates. However, direct measurements of isotopic signature of CO_2 released by respective temperature may improve the applicability of the approach.

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IV) Thermal stability of soil organic matter pools and their turnover times calculated by $\delta^{13}C$ under elevated CO_2 and two levels of N fertilization

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Abstract

Soil from FACE plots (FAL, Braunschweig) under ambient air (375 ppm; $\delta^{13}C$-CO_2 –9.8‰) and elevated CO_2 (550 ppm; for 6 years; $\delta^{13}C$-CO_2 –23‰), either under 100% N (180 kg ha^{-1}) or 50% N (90 kg ha^{-1}) fertilization treatments was analyzed by thermogravimetry. Soil samples were heated up to the respective temperatures and the remaining soil was analyzed for $\delta^{13}C$ and $\delta^{15}N$ by IRMS. Based on differential weight losses, four temperature intervals were distinguished. Weight losses in the temperature range 20-200 °C were connected mostly with water volatilization. The maximum weight losses and carbon (C) content were measured in the soil organic matter (SOM) pool decomposed at 200-360 °C. The largest amount of N was detected in SOM pools decomposed at 200-360 and 360-500 °C. In all temperature ranges the $\delta^{13}C$ values of SOM pools were significantly more negative under elevated CO_2 vs. ambient CO_2. The incorporation of new C into SOM pools was not inversely proportional to its thermal stability. 50% N fertilization treatment gained higher C exchange under elevated CO_2 in thermally labile SOM pool (200-360 °C), whereas 100% N treatment induced higher C turnover in thermally stable SOM pools (360-500, 500-1000 °C). Mean Residence Time of SOM under 100% N and 50% N fertilization showed no dependence between SOM pools isolated by increasing temperature of heating and the renovation of organic C in those SOM pools. Thus, the separation of SOM based on its thermal stability was not sufficient to reveal pools with contrasting turnover rates of C.

Keywords: Thermogravimetry; IRMS; Soil organic matter; Stable isotopes; $\delta^{13}C$; $\delta^{15}N$

1. Introduction

The atmospheric CO_2 concentrations are predicted to increase from the present 380 ppm to 470 to 560 ppm by the year 2050 [14]. This increase is a result of human activities such as combustion of fossil fuel and land-use changes such as deforestation [28]. Research exploring ecosystem responses to elevated CO_2 has gained widespread attention in the last few decades [21, 23, 31]. Field experiments based on the Free Air Carbon dioxide Enrichment approach (FACE) represent a great opportunity to investigate CO_2 effects on ecosystems under realistic natural conditions [15, 19, 21].

In most FACE experiments, stable-carbon isotopic tracers are used to study the carbon (C) transformations in plants and soils under CO_2 enrichment, since CO_2 used for enrichment is usually depleted in ^{13}C relative to ambient atmospheric CO_2. [19, 29, 32]. Plants grown in the elevated CO_2 atmosphere are therefore further depleted in ^{13}C and, as the

litter and rhizodeposits from these plants decompose and become incorporated into the soil organic matter (SOM), the soil $\delta^{13}C$ will decrease. The contribution of the C metabolized under FACE conditions to total SOM can be calculated based on the $\delta^{13}C$ of the SOM under ambient air and elevated CO_2 conditions. This new C contribution and the CO_2 treatment duration then allow the SOM turnover rates to be estimated [2].

Beyond investigating the C pathways in SOM under elevated atmospheric CO_2, the study of N cycling provides insights into the interaction of these two elements in soil. Thus, the application of N fertilizers in FACE experiments was used to estimate changes of N flows and plant uptake under elevated CO_2 [31, 34].

Different techniques for SOM fractionation that separate labile from recalcitrant soil C pools are useful to determine the dynamics of C and N in SOM [30]. One of the suitable approaches for SOM partitioning is based on its thermal stability [5, 6, 17, 20, 24, 26]. Thermogravimetry analysis involves a continuous, gradual temperature increase which decomposes (mainly oxidizes) different organic compounds according to their thermal stability. Thermogravimetry (TG) could potentially provide a simple and rapid determination of gross changes in organic matter quality [24, 26], but has only once been used to examine the quality of SOM under altered environmental conditions such as elevated CO_2 concentration in the atmosphere [5]. In a previous experiment [5] the approach was tested on soil developed on loess. However, no correlation was detected between the thermal stability of SOM pools and C and N turnover in those SOM pools, indicating the weakness of thermogravimetry to separate SOM pools with different turnover times. In the current study, the soil developed on loamy sand, probably has other interactions of organic and inorganic compounds resulting in different (i) temperature intervals of decomposition for organic and inorganic compounds, (ii) distribution of organic C and mineral N in SOM pools, (iii) rates of C renovation in SOM pools of different thermal stability. Hence, the objective of the present study was to characterize the SOM quality in soils on loamy sand from a FACE experiment (Braunschweig, Germany) conducted with crop rotation according to local farming practice, and two different nitrogen (N) fertilization levels under ambient and elevated concentrations of atmospheric CO_2 in terms of thermal properties.

2. Materials and Methods

2.1. Study site

Soil samples were taken from the Free Air Carbon dioxide Enrichment facility located in the Federal Agricultural Research Centre (FAL) in Braunschweig, Lower Saxony,

Germany (10°26' E 52°18' N, 79 m a.s.l.). The experimental design consisted of four rings, each 20 m diameter, two of which were maintained under ambient air conditions (375 µmol mol^{-1} CO_2). Two rings were conducted under elevated CO_2 conditions (550 µmol mol^{-1} CO_2 during daylight hours). For further details see [33]. The CO_2 supplied to the FACE rings was strongly depleted in ^{13}C. Mixed with ambient air, the $\delta^{13}C$ value of the atmospheric CO_2 within the FACE rings was more negative (approx.-23.‰) than the ambient air CO_2 within the control rings (-9.8‰) [9].

The research site has been used for agricultural purposes for the last 30 years, but only C_3 plants were cultivated [9]. All soil treatments, including irrigation and pesticide management, were carried out according to local farming practices [33]. To study interactions between C and N, N supply was restricted to 50% of adequate N in half of each of the plots [33]. The FACE experiment started in 1999 and was carried out in two crop rotation cycles, consisting of winter barley (*Hordeum vulgare* cv. Theresa) – cover crop: ryegrass (*Lolium multiflorum* cv. Lippstaedter Futtertrio) – sugar beet (*Beta vulgaris* cv. Wiebke) – winter wheat (*Triticum aestivum* cv. Batis) [33]. The soil for the experiment presented here was collected in 2005 when winter wheat was cultivated the second time. Average $\delta^{13}C$ values of crops growing on the experimental site were as follows: winter barley (leaves, stems, roots) -28.7‰ under ambient CO_2, -39.0‰ under elevated CO_2 treatment; ryegrass (whole plants) -29.4‰, -35.3‰; sugar beet (leaves, beet, roots) -28.1‰, -41.9‰; winter wheat (leaves, stems, roots) -29.4‰, -41.9‰, respectively [9, 27].

2.2. Soil sampling and samples processing

The soil at the site is cambisol/loamy sand, with a pH of 6.3-6.5 [33]. The concentrations of soil organic carbon and nitrogen (0–10 cm) are given in Table 1 (see bulk soil values).

Soil samples were taken at the end of July 2005 immediately after the final harvest of the crop six years after the FACE experiment started. Eight soil samples were collected randomly from the FACE rings under elevated CO_2 treatment and the control rings from both N treatments. Soil samples were air-dried at room temperature. All visible roots and plant remains were removed carefully and the soil was ball-milled into powder. Four of eight prepared samples were chosen for thermogravimetry analysis.

Table 1. Measured C, N amount and $\delta^{13}C$, $\delta^{15}N$ values in soil under ambient and elevated CO_2 treatments (CO_2) and under 100% and 50% N fertilization (N) in soil samples after heating up to threshold temperatures. Values are means of eight replicates ± SE

Temperature threshold (°C)	Treatments		C (mg g soil^{-1})	N (mg g soil^{-1})	$\delta^{13}C$ (‰ PDB)	$\delta^{15}N$ (‰ air N_2)
	CO_2	N				
200	ambient	100%	8.86 ± 0.58	1.01 ± 0.07	-28.05 ± 0.01	6.87 ± 0.15
	elevated	100%	9.91 ± 0.35	1.06 ± 0.03	-29.47 ± 0.07	7.07 ± 0.06
	ambient	50%	10.13 ± 0.72	0.95 ± 0.06	-27.88 ± 0.05	6.52 ± 0.09
	elevated	50%	8.25 ± 0.32	0.91 ± 0.03	-29.72 ± 0.05	6.40 ± 0.07
p values[a]	CO_2		0.382	0.893	< 0.01	0.678
	N		0.674	0.061	< 0.01	< 0.01
	CO_2 × N		0.023	0.362	< 0.01	0.140
360	ambient	100%	3.90 ± 0.07	0.53 ± 0.02	-26.55 ± 0.15	9.81 ± 0.13
	elevated	100%	4.24 ± 0.12	0.58 ± 0.02	-27.46 ± 0.14	9.74 ± 0.16
	ambient	50%	3.63 ± 0.13	0.50 ± 0.02	-26.68 ± 0.04	9.35 ± 0.11
	elevated	50%	4.47 ± 0.14	0.57 ± 0.02	-27.85 ± 0.03	9.28 ± 0.11
p values	CO_2		< 0.01	< 0.01	< 0.01	0.595
	N		0.724	0.152	0.031	< 0.01
	CO_2 × N		0.064	0.467	0.239	0.980
500	ambient	100%	0.57 ± 0.02	0.10 ± 0.01	-22.59 ± 0.72	6.01 ± 0.13
	elevated	100%	0.62 ± 0.07	0.11 ± 0.01	-24.57 ± 0.21	6.35 ± 0.21
	ambient	50%	0.74 ± 0.08	0.11 ± 0.01	-21.61 ± 0.20	6.16 ± 0.28
	elevated	50%	0.58 ± 0.05	0.10 ± 0.01	-23.05 ± 0.05	6.17 ± 0.17
p values	CO_2		0.325	0.326	< 0.01	0.440
	N		0.215	0.335	< 0.01	0.939
	CO_2 × N		0.072	0.416	0.417	0.476
Bulk soil[b]	ambient	100%	8.88 ± 0.36	1.00 ± 0.03	-27.97 ± 0.04	7.11 ± 0.16
	elevated	100%	9.94 ± 0.71	1.04 ± 0.05	-29.55 ± 0.09	6.98 ± 0.12
	ambient	50%	10.33 ± 0.97	0.95 ± 0.01	-27.80 ± 0.03	6.53 ± 0.10
	elevated	50%	8.68 ± 0.65	0.94 ± 0.05	-29.73 ±0.07	6.70 ± 0.15
p values	CO_2		0.482	0.640	< 0.01	0.878
	N		0.281	0.099	0.900	< 0.01
	CO_2 × N		0.136	0.579	0.016	0.290

[a]Effects were not significant ($p > 0.05$) or significant at reported *p* values

[b]Bulk soil represented not-heated soil samples

2.3. Thermogravimetry analysis

Thermogravimetric analysis (TG) was carried out using the NETZSCH STA 409EP device, regulated by the TASC 414/3 controller (Fa Netzsch). 55 mg of soil sample was weighed out in an aluminum crucible and then heated from 20 to 1000 °C. During the heating the air flowed into the chamber at a rate of 1.0 ml min^{-1}. The heating rate was 2.0 °C min^{-1}. Calcined kaolinite previously heated at 1250 °C was used as the reference material. The weight of the soil sample (in mg) was continuously scanned. The TG curves obtained for the soil samples from ambient and elevated CO_2, 100% and 50% N fertilization treatments were used to determine "threshold" temperature levels. These levels were set as temperature values to heat soil samples in a muffle oven (Heraeus MR-260) for subsequent measurements of weight losses, C and N content, as well as $\delta^{13}C$ and $\delta^{15}N$ values of SOM pools. Three groups of soil samples were heated up to 200, 360, and 500 °C. No heating (20 °C) corresponded to the bulk soil. Heating up to 200 °C removed mainly water, since no significant C and N contents were measured in residue. Heating up to 360 °C removed organic and inorganic substances already decomposed between 20 and 360 °C. The residual SOM pool remaining after heating to 360 °C was analysed for its C and N content, $\delta^{13}C$ and $\delta^{15}N$ values. The same approach was used to determine the SOM pool at the 500 °C temperature threshold. Then, considering the weight, C and N losses after heating to the threshold temperatures and the corresponding values (weight, C and N) were calculated for the intermediate SOM pools decomposed between 200 and 360, or 360 and 500 °C (see below). The heating rate of the muffle furnace was the same as by TG-DSC analysis: 2.0 °C min^{-1}.

2.4. $\delta^{13}C$ and $\delta^{15}N$ analyses

Isotope C and N ratios, as well as C and N contents, were measured with an elemental analyzer in a dual-element analysis mode (Carlo Erba 1108, Milano, Italy) for Dumas combustion. This was followed by gas chromatographic separation of the gaseous combustion products, which were then fed into a gas-isotope ratio mass spectrometer (delta S Finnigan MAT, Bremen, Germany) via a ConFlo III open-split interface (Finnigan MAT). Relative isotope abundances, denoted as δ-values, were calculated according to the following equation:

$$\delta\text{-value} = (R_{sample} / R_{standard} - 1) \cdot 1000‰ \quad (1),$$

where R_{sample} and $R_{standard}$ are the ratios of heavy isotope to light isotope of the samples and the respective standards. Standard gases were calibrated with respect to international standards (N_2 in the air and CO_2 in PeeDee belemnite) by use of the reference substances N1 and N2 for nitrogen isotope ratios and Australian National University (ANU) sucrose and NBS 19 for the carbon isotopes [3].

2.5. Calculations and statistics

The calculation of weight losses, C and N content as well as the $\delta^{13}C$ and $\delta^{15}N$ values of SOM pools decomposed in different temperature intervals is described in details in a previous study [5].

The portion of FACE-derived C (P_{FACE}) in SOM was calculated according to Balesdent [1] and adapted for FACE conditions:

$$P_{FACE} = (\delta^{13}C_{elev.} - \delta^{13}C_{amb})/(\delta^{13}C_{elev.theor.} - \delta^{13}C_{amb}) \cdot 100 \quad (2),$$

where $\delta^{13}C_{elev.}$ was a $\delta^{13}C$ value of the SOM under elevated CO_2 treatment; $\delta^{13}C_{amb}$ was a $\delta^{13}C$ value of the SOM under ambient air treatment, and $\delta^{13}C_{elev.theor.}$ is a theoretical $\delta^{13}C$ value of the SOM developed under vegetation grown under continuously elevated CO_2 conditions. The isotopic signatures of the theoretical SOM were calculated based on the difference between the $\delta^{13}C$ of the plants growing under elevated CO_2 and the $\delta^{13}C$ of bulk soil and SOM pools decomposed at 200-360, 360-500, 500-1000 °C under ambient CO_2. The values were corrected for isotopic fractionation during humification by subtracting the differences between $\delta^{13}C$ of vegetation growing under ambient conditions and $\delta^{13}C$ of the corresponding SOM pool of the soil developed under ambient CO_2 treatment:

$$\delta^{13}C_{elev.theor.} = \delta^{13}C_{elev.plant} - (\delta^{13}C_{amb.plant} - \delta^{13}C_{amb}) \quad (3),$$

where $\delta^{13}C_{elev.plant}$ was $\delta^{13}C$ of the plants under elevated CO_2 treatment, $\delta^{13}C_{amb.plant}$ was $\delta^{13}C$ value of the plants under ambient CO_2 treatment, and $\delta^{13}C_{amb}$ was $\delta^{13}C$ of the bulk soil and the corresponding SOM pool of the soil developed under ambient CO_2 treatment.

To calculate the contribution of new FACE-derived C in SOM for a particular period of the FACE experiment and annual turnover rates (TR) of SOM, a simple exponential approach was selected [1]:

$$TR = -\ln(1 - M/100)/t \quad (4),$$

where M was the portion of C_{FACE} (Eq. 2) in SOM and t was the time of soil exposition to elevated CO_2 concentrations.

The mean residence time of FACE-derived C in bulk soil and in SOM pools was calculated as a reciprocal to the turnover rates [10]:

$$MRT = 1/TR \quad (5)$$

The study was conducted with four replicates originating from elevated- and ambient CO_2, full- and half-N fertilization treatments for TG and with eight replicates for muffle combustion, C, N measurements and isotopic analysis. The significance of differences between $\delta^{13}C$, $\delta^{15}N$, as well as the C and N content of different pools under ambient and elevated CO_2 treatments was examined using two-way analysis of variance (ANOVA). The standard errors of means are presented on the figures and in the tables as the variability parameter.

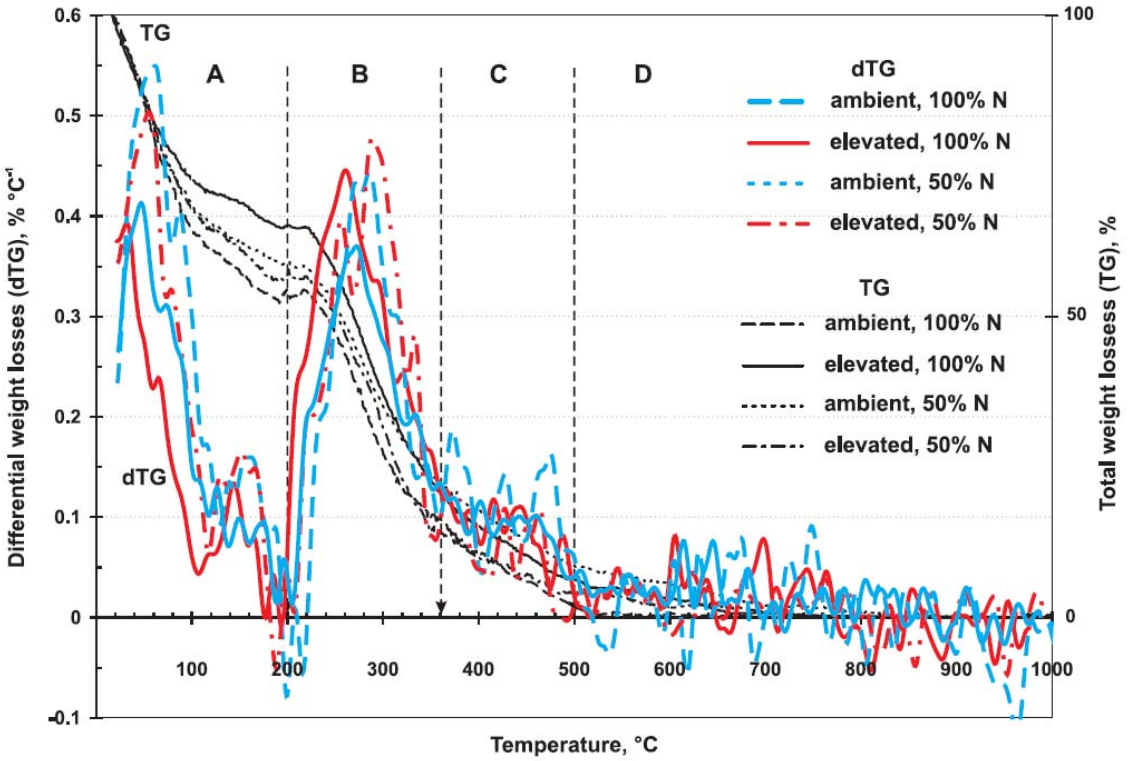

Fig. 1. Differential thermogravimetry (dTG) of soil under ambient/elevated CO_2 and 100/50 % N fertilization treatments. Cumulative losses (TG) are scaled to 100% of the right Y axis. Arrows with dotted lines show the "threshold" temperature chosen for SOM fractionation.

3. Results

3.1. Thermogravimetry, weight losses, C and N content of SOM pools

Data for thermogravimetry analysis were represented by cumulative weight losses (TG, % from initial weight of a soil sample) and differential weight losses (dTG, % loss of total weight per °C heating) (Fig. 1). The dTG curves showed two well-defined peaks of differential weight losses from soil samples under elevated and ambient CO_2 treatments for both 100% and 50% N fertilization during heating from 20 to 1000 °C: 1) between 20 and 200 °C (temperature range A), and 2) between 200 and 360 °C (temperature range B). Less intensive weight losses occurred in temperature ranges C and D.

Weight losses occurred in similar temperature intervals with all samples, allowing similar temperature threshold levels to be chosen for thermal fractionation of SOM. To fractionate the SOM into individual pools with different thermal stability, three threshold temperature levels were chosen: 200, 360 and 500 °C (Fig .1). Three threshold temperatures allowed four SOM pools to be isolated according to the procedure described above (see Materials and Methods). The total weight losses, as well as weight losses of the four individual SOM pools, did not differ significantly between the ambient air and CO_2 enrichment samples. However, N fertilization significantly influenced total weight losses from bulk soil and soil samples decomposed between 20-200, 200-360 and 500-1000 °C (Fig. 2). Peak weight losses were found in the SOM pool decomposed between 200 and 360 °C ranging from 2.23 ±0.12% of the initial weight of soil under elevated CO_2 treatment and 100% N fertilization, to 1.65 ±0.02% of soil under elevated CO_2 treatment and 50% N fertilization. The lowest weight losses were observed at 20-200 and 500-1000 °C, where the soil samples of all treatments lost on average 0.2% of total weight losses (Fig. 2).

For all treatments except one, the maximum C losses corresponded to maximal weight losses in SOM pools decomposed at 200-360 °C. The exception was in the treatment with elevated CO_2 and 50% N, where maximum C losses were detected in SOM pools decomposed at 360-500 °C (Fig. 2, Table 2). Maximum N losses, in contrast, were measured in SOM pools decomposed in two temperature ranges: 200-360 and 360-500 °C. The lowest amounts of C and N were measured in SOM decomposed above 500 °C in soils under all treatments. Minor insignificant C and N losses were detected by decomposition between 20-200 °C because the main weight losses in this temperature interval are connected with water evaporation (Table 2).

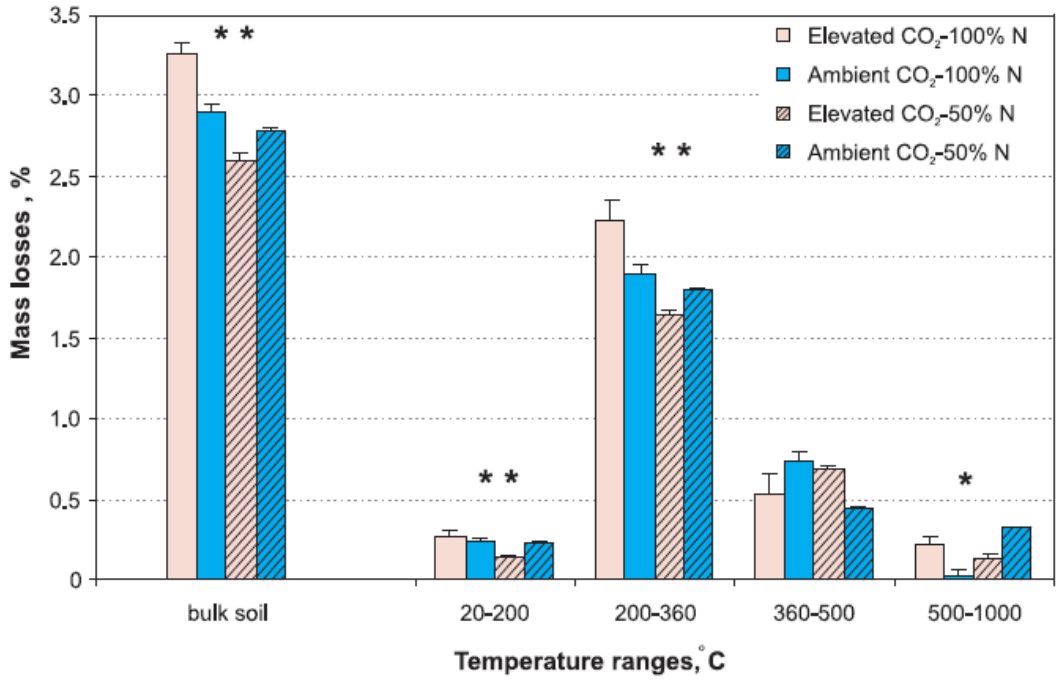

Fig. 2. Weight losses of bulk soil and SOM pools of different thermal stability under ambient/elevated CO_2 and 100/50 % N fertilization treatments after combustion in a muffle oven. Bulk soil represents samples combusted from 20 to 1000 °C. SOM pools showed the distribution of weight losses from bulk soil within four temperature ranges. Weight losses of SOM pools were calculated using Eq. 2. Whiskers present standard error (± SE). $^{*}P < 0.05$ and $^{**}P < 0.01$ represent the significance of differences between 100/50 % N fertilization treatments. The effect of CO_2 treatment was insignificant.

3.2 $\delta^{13}C$, $\delta^{15}N$ and amount of FACE-derived C in SOM pools of different thermal stability

A significant shift in $\delta^{13}C$ values of bulk soil and SOM pools decomposed in temperature ranges was observed after approx. six years of exposition of crops in an agroecosystem to isotopically labelled CO_2 (Tables 1 and 2). The observed average increase of 5.8‰ in $\delta^{13}C$ values between treatments with increasing combustion temperature (Tables 1 and 2) is probably due to the slow combustion process, when the lighter ^{12}C isotope decomposes faster than the heavier ^{13}C, leading to an accumulation of the latter in the residual SOM pools [20].

Statistical analysis showed significant difference in $\delta^{15}N$ values of bulk soil and SOM pools with different thermal stability between ambient and elevated CO_2 treatments by application of 100% and 50% N fertilizers (Tables 1 and 2). $\delta^{15}N$ values of the only SOM pool decomposed above 500 °C were affected neither by N nor by CO_2 treatments.

Table 2. Calculated amounts of C, N and $\delta^{13}C$, $\delta^{15}N$ values in soil under ambient and elevated CO_2 treatments (CO_2) and under 100% and 50% N fertilization (N) in SOM decomposed in different temperature ranges. Values are means of eight replicates ± SE

Temperature ranges (°C)	Treatments		C (mg g soil^{-1})	N (mg g soil^{-1})	$\delta^{13}C$ (‰ PDB)	$\delta^{15}N$ (‰ air N_2)
	CO_2	N				
20-200	ambient	100%	0.02 ± 0.69	N.D.	N.D.	N.D.
	elevated	100%	0.03 ± 0.79	N.D.	N.D.	N.D.
	ambient	50%	0.2 ± 0.72	N.D.	N.D.	N.D.
	elevated	50%	0.43 ± 0.64	0.03 ± 0.06	N.D.	N.D.
p values[a]	CO_2		0.972	N.D.	N.D.	N.D.
	N		0.861	N.D.	N.D.	N.D.
	CO_2 × N		0.489	N.D.	N.D.	N.D.
200-360	ambient	100%	4.96 ± 0.59	0.47 ± 0.07	-29.24 ± 0.57	3.43 ± 0.18
	elevated	100%	5.67 ± 0.37	0.48 ± 0.03	-31.03 ± 0.33	3.70 ± 0.09
	ambient	50%	6.50 ± 0.73	0.45 ± 0.06	-28.57 ± 0.53	3.28 ± 0.14
	elevated	50%	3.78 ± 0.35	0.34 ± 0.04	-32.01 ± 0.45	1.30 ± 0.11
p values	CO_2		0.052	0.473	0.048	< 0.01
	N		0.400	0.253	0.082	< 0.01
	CO_2 × N		0.126	0.083	0.763	< 0.01
360-500	ambient	100%	3.33 ± 0.08	0.44 ± 0.02	-27.21 ± 0.10	10.62 ± 0.09
	elevated	100%	3.62 ± 0.14	0.47 ± 002	-27.94 ± 0.16	10.53 ± 0.12
	ambient	50%	2.90 ± 0.15	0.39 ± 0.02	-27.94 ± 0.27	10.23 ± 0.12
	elevated	50%	3.89 ± 0.15	0.47 ± 0.02	-28.55 ± 0.32	9.93 ± 0.12
p values	CO_2		0.028	0.076	< 0.01	0.095
	N		0.109	0.529	< 0.01	< 0.01
	CO_2 × N		0.046	0.182	0.374	0.231
500-1000	ambient	100%	0.56 ± 0.02	0.096 ± 0.001	-22.59 ± 0.72	6.01 ± 0.13
	elevated	100%	0.62 ± 0.07	0.112 ± 0.003	-24.57 ± 0.21	6.35 ± 0.21
	ambient	50%	0.74 ± 0.08	0.110 ± 0.004	-21.61 ± 0.20	6.16 ± 0.28
	elevated	50%	0.58 ± 0.05	0.102 ± 0.002	-23.05 ± 0.05	6.17 ± 0.17
p values	CO_2		0.343	0.177	< 0.01	0.440
	N		0.277	0.436	< 0.01	0.939
	CO_2 × N		0.097	0.003	0.417	0.476

[a]Effects were not significant ($p > 0.05$) or significant at reported *p* values

The calculated amount of new C in bulk soil calculated based on $\delta^{13}C$ values of SOM under ambient and elevated CO_2 treatments reached 12.6 ± 0.23% of total C under 100% N

fertilization and 15.4 ± 0.16% under 50% N fertilization (Fig. 3). The amount of FACE-derived C was different in the SOM pools. With 100% N fertilization, it amounted to 14.2% at 200-360 °C, 5.8% at 360-500 °C and 15.8% at 500-1000 °C. In the 50% N fertilization samples the amount of FACE-derived C was 27.4, 4.9 and 11.5% at the respective temperature ranges (Fig. 3).

Based on the amount of FACE derived C, the calculated turnover rates and MRTs showed a turnover rate of 42 years under 50% N treatment compared to 52 years under 100% N treatment for bulk soil (Table 3). The fastest SOM turnover rates corresponding to a MRT of 22 years were detected in the SOM pool from the 50% N treatment which decomposed from 200 to 360 °C. The MRT calculated for the same SOM from the 100% N treatment amounted to 46 years, and hence was two times higher .The longest MRTs of 116 and 140 years corresponded to SOM pools decomposed at 360-500 °C under 100% and 50% N treatments, respectively (Table 3).

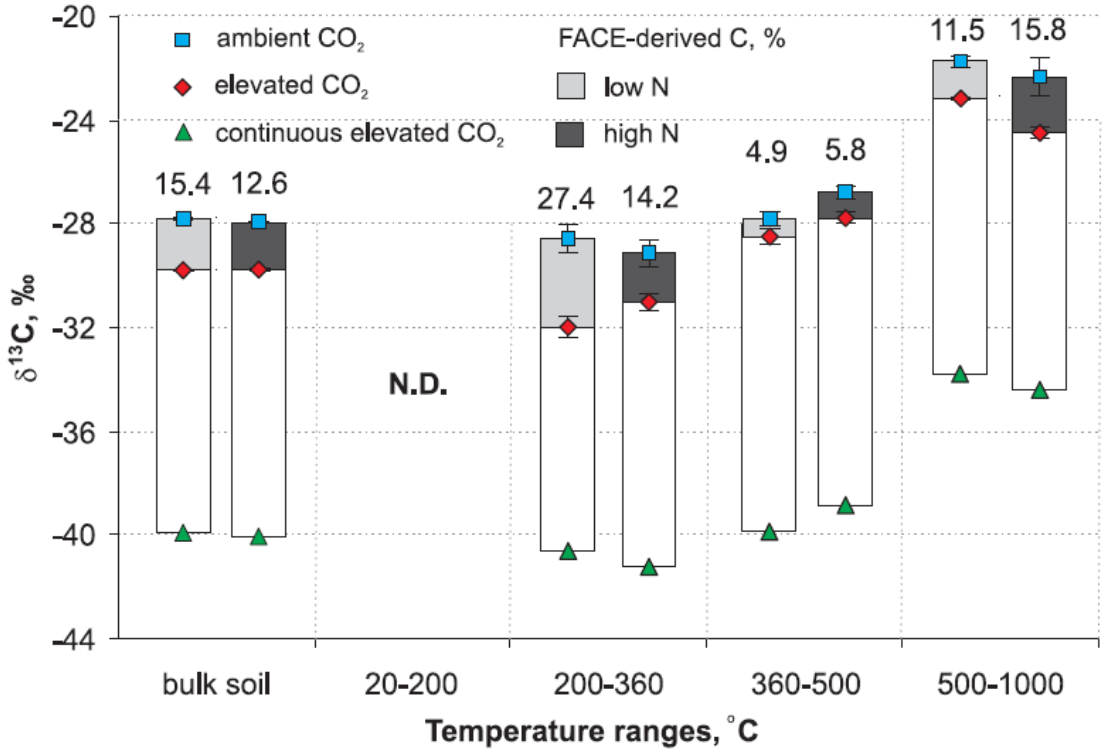

Fig. 3. $\delta^{13}C$ values of bulk soil and three SOM pools with different thermal stability under ambient/elevated CO_2 and 100/50 % N fertilization treatments. The calculated theoretical $\delta^{13}C$ values (Eq. 3) of soil developed under continuous elevated CO_2 conditions were used to estimate the portion of FACE-derived C in the SOM under two N treatments. The portion of FACE-derived C is shown above the columns as a percentage of the total amount of C in bulk soil and SOM pools. Whiskers present standard error (± SE).

4. Discussion

4.1. TG of SOM pools

Weight losses during gradual heating allowed a separation into four conditional temperature intervals (Fig. 1). The first interval A (up to 200 °C) reflected losses of free and bound water [7]. Since the losses of C and N were minor, no change of isotopic composition was observed (Table 2). The temperature ranges B (200-360 °C) and C (360-500 °C) showed the largest losses of C and N. As observed by Kristensen [16], the organic substances decomposed up to 310 °C comprised plant material such as cellulose. Herrera et al. [12] used dTG to determine that the major percentage weight loss of hemicelluloses and celluloses occurs at the temperature range between 200 and 350 °C. At higher temperatures (360-500 °C), the decomposed organics comprise stable constituents with aromatic compounds such as lignin, humic substances and kerogens [16, 20, 26]. In the last temperature range D (500-1000 °C), the losses of organic matter were the smallest compared to other SOM pools (Table 2) as most weight losses here reflect clay mineral decomposition [25].

4.2 Isotopic composition and the portion of FACE-derived C in SOM pools under 100 and 50% N treatments

The results of our experiment showed neither elevated CO_2 treatment nor different N fertilization regimes significantly changed the total amount of C or N in the soil. However, an input of newly synthesized C into the SOM is documented as the $\delta^{13}C$ values of both bulk SOM, and most of the SOM pools shifted, indicating a significant impact of C from the labelled atmospheric CO_2 (Table 1, Table 2).

The lack of changes in total C [19] and N [4] under elevated CO_2 conditions was expected because SOM formation and decomposition processes are not strongly affected by elevated CO_2. N fertilization (both 100% N and 50% N) under an elevated CO_2 environment had no effect on total C and N content in bulk soil. These results go along with results of some other researchers [4, 13, 30, 31]. However, a number of experiments gave contrary results suggesting an increase of total C and N due to elevated CO_2 and high N fertilization rates of more than 30 kg N ha^{-1}. According to de Graaff et al. [4] such observations are so far restricted to soils under herbaceous plant species. In our study, significant effects of CO_2 and fertilisation treatments on C and N content were observed in SOM pools decomposed under intermediate (C) and high (N) temperature ranges of 360-500 and 500-1000 °C (Table 2).

The isotopic signatures of the carbon in bulk soils, and SOM pools under ambient and elevated CO_2, allowed the calculation of the amount of new FACE-derived C incorporated

into SOM pools at both levels of N fertilisation. After six years of CO_2 fumigation, the portion of new FACE-derived C in bulk soil under the 100% N reached 12.6%, and under 50% N treatment, 15.4% of total C (Fig. 3).

Very few studies exist on the effect of elevated CO_2 and N fertilization on SOM turnover under agricultural crops lasting for a period longer than 5 years. Most of the results available are from 2-3 year experiments. Thus, Leavitt et al. [19] reported C inputs by spring wheat in a 2-year CO_2 enrichment experiment to be 6.3% in the upper 15 cm of soil. In another experiment with spring wheat exposed for three years to elevated CO_2 (FACE-Hohenheim) and N fertilization (140 kg ha^{-1} a^{-1}), the amount of newly metabolized C in upper 0-10 cm bulk soil reached 7.3% [5]. Studies with cotton showed that the average amount of new C was 12-13% after three years of elevated CO_2 [18]. However, Van Kessel et al. [31] reported higher C inputs of 23.5% under a high N fertilization rate (540 kg ha^{-1} a^{-1}) and 25.5% under a lower rate (140 kg ha^{-1} a^{-1}) in an experiment with grassland consisting of *Lolium perenne* and *Trifolium repens* under FACE CO_2 enrichment for ten years. These larger new C inputs mainly reflected grassland plant species, a longer CO_2 fumigation time, higher N fertilisation rate and the absence of tillage [31]. Giesemann [9] reported the amount of newly metabolized FACE-derived C in the same soil used in current study to be as much as 16.6% of C in the soil C pool under the 100% N fertilisation plot down to 10 cm depth at the end of the first crop rotation period.

The incorporation of FACE-derived C in the temperature labile SOM pool decomposing at 200-360 °C under 50% N fertilization was two times faster (27.4%) than under 100% N treatment (14.2%) (Fig. 3). However, the C turnover in the more temperature-stable SOM pools decomposed at 360-500 °C and 500-1000 °C, respectively, were higher under the 100% N treatment than under the 50% N treatment (Table 3). MRTs of C in SOM pools were not dependent on increasing thermal stability of those SOM pools (Table 3). MRT of SOM under 100% N increased in temperature intervals 500-1000 °C (41 years) < 200-360 (46) < bulk soil (52) < 360-500 (140). Under 50% N treatment the values were: 200-360 °C (22 years) < bulk soil (42) < 500-1000 (57) < 360-500 (116). Thus, MRTs of SOM under 100% N fertilization were similar at 200-360 and 500-1000 °C (46 and 41 years, respectively). Under 50% N treatment, MRT of SOM pool decomposed at 200-360 °C was 2.5 times shorter (22 years) than at 500-1000 °C (57 years), whereas SOM decomposed at 360-500 °C under both N fertilization treatments had the longest MRT of 116-140 years (Table 3).

Table 3. Amount of FACE-derived C, turnover rates (TR) and mean residence time (MRT) of carbon in bulk soil and SOM pools isolated in three temperature ranges for soils under 100% and 50 % N fertilization. The calculations were made using Eq. 2, 4 and 5. Values are means of eight replicates ± SE

Temperature ranges (°C)	Treatment N	FACE derived C[a] (%)	TR (year^{-1})	MRT (years)
20-200		N.D.	N.D.	N.D.
200-360	100%	14.2 ± 0.5	0.0219	46
	50%	27.4 ± 0.3**	0.0458	22
360-500	100%	5.8 ± 0.7	0.0086	116
	50%	4.9 ± 1.3	0.0071	140
500-1000	100%	15.8 ± 0.5	0.0246	41
	50%	11.5 ± 0.4*	0.0174	57
Bulk soil	100%	12.6 ± 0.3	0.0193	52
	50%	15.4 ± 0.2*	0.0239	42

[a]FACE-derived C, TR and MRT could only be assessed at the elevated CO_2 treatment. Therefore, the effect of CO_2 was not included for these variables. $^{*}P < 0.05$ and $^{**}P < 0.01$ represent the significance of differences between 100% and 50% N treatment.

The data obtained in the current investigation suggests that thermogravimetry is not sufficient as an approach in isolating SOM pools with different turnover times. This conclusion is supported by our previous findings in an experiment with *Miscanthus x giganteus* [6, 17], where, after 10.5 years cultivation of C_4 plant *Miscanthus* on C_3 grassland, the C exchange reached on average 50% in bulk (not heated) soil and SOM pools decomposed up to 190, 310, 390 °C, respectively. The MRT of SOM decomposed up to 190 °C was 13 years, up to 310 °C it was 12 years, and up to 390 °C 15 years. Only the most thermally stable SOM pool, which decomposed above 480 °C and had the lowest amount of *Miscanthus*-derived C (9%) showed a MRT of 163 years [6]. Another attempt to thermal isolation of SOM pools was made on soil from the FACE experiment (Hohenheim, Stuttgart) [5]. This soil had been subjected to elevated CO_2 (+160 ppm compared to ambient air (380 ppm)) and an annual N fertilization (140 kg ha^{-1}) for three years. In contrast to the soil used in the Braunschweig FACE experiment which developed on loamy sand, the soil from the FACE-Hohenheim developed on loess and hence had higher organic C and mineral N contents. However, five separated SOM pools decomposed at 20-200, 200-310, 310-400, 400-480 and 480-1000 °C did not show contrasting turnover rates. Thus, the MRTs of C in SOM pools increased as follows: 12 years (310-400 °C) < 28 (480-1000) < 35 (400-480) <

92 (200-310) < 149 (20-200). Furthermore, there was no dependence between the portion of FACE-derived C in a SOM pool and increasing thermal stability of a SOM pool.

Helfrich et al. [11] supported our impression that thermogravimetry is weak in separating SOM pools with different turnover times. Thus, in their study, the efficiency of thermal oxidation at different temperatures (from 200 to 500 °C) was tested for different soils (loamy sand and silty loam) under different land use regimes (field, grassland, forest). The results showed that the thermal oxidation at different temperatures did not yield residual SOM pools of contrasting MRTs in the silty loam (58 years in non-heated soil and 77 years in soil samples decomposed up to 300 °C). Only the silty loam with large amounts of black carbon, which is relatively stable to thermal decomposition [8], showed an increase of the C-turnover time (940 years compared to the non heated soil with 220 years) [11].

A possible explanation for the inability of thermal oxidation for isolating SOM pools of contrasting turnover times is that the fractionation of SOM pools according to their thermal stability is rather close to chemical separation. In turn, Balesdent [1] found that chemical separations of SOM failed to isolate the SOM pools of different turnover time because different biochemical plant components (cellulose, lignin) are decomposed in a wide temperature range [20]. Individual components of plant residues may be directly incorporated into, or even mixed with, the thermal stable SOM pools and will so mask low turnover rates of these pools.

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V) Microbial growth rate under elevated atmospheric CO_2 depends on soil aggregates size

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Abstract

Increased root exudation under elevated atmospheric CO_2 and contrasting environment in soil macro- and microaggregates may affect the activity of microbial biomass and its composition resulting in change of microbial growth strategy. We investigated the effect of elevated CO_2 on the contribution of fast (*r*-strategists) and slowly growing microorganisms (K-strategists) in microbial communities attributed to soil macro- and microaggregates. We fractionated the bulk soil from ambient and elevated CO_2 treatments performed for 5 years at the FACE-Hohenheim (Stuttgart) into large macro- (>2 mm), small macro- (0.25-2 mm) and microaggregates (<0.25 mm) using an optimal-moist sieving approach. Microbial biomass (C_{mic}) and the kinetic parameters of microbial growth – the maximal specific growth rate (μ) of microorganisms, the amount of active microbial biomass (AMB), the duration of lag-period (t_{lag}) – were estimated by means of the kinetics of the CO_2 emission from bulk soil and aggregates amended with glucose and nutrients. Although C_{org} and C_{mic} were unaffected by elevated CO_2, μ values were significantly higher under elevated than under ambient CO_2 for bulk soil, small macro- and microaggregates. Higher AMB content and shorter lag-period in bulk soil under elevated than under ambient CO_2 confirmed an increase of the contribution of *r*- vs. K-strategists to the total microbial community under elevated CO_2. However, we detected a decrease of AMB content under elevated CO_2 in all separated aggregates. This might be due to the sieving procedure, since the *r*-strategists are very sensitive to any disturbance and might have suffered from sieving. The sharp decrease of AMB under elevated CO_2 resulted in extension of the lag-period. Based on μ values, AMB and duration of the lag-period we conclude that the activity of microorganisms with *r*-strategy is increasing under elevated atmospheric CO_2. This increase could be especially pronounced in soil microaggregates.

1 Introduction

Increased concentrations of atmospheric CO_2 have a pronounced effect on terrestrial plant biomass resulting in enhanced carbon (C) assimilation (Melillo *et al.*, 1993; Schimel, 1995). Along with the higher net primary productivity of plants under elevated CO_2 (Melillo *et al.*, 1993; Schimel, 1995) or changes in plant chemistry (Cotrufo *et al.*, 1994), the stimulation of root growth (Paterson *et al.*, 1997; Rogers *et al.*, 1998) plays an important role for belowground processes. The increased root growth enhances rhizodeposition including root exudation (van Veen *et al.*, 1991; Cheng, 1999; Phillips et al., 2006). In turn, root exudates could stimulate the activity and growth of soil microbial biomass, because of the

high availability of such substrate for decomposition by soil microorganisms (reviewed by Kuzyakov et al., 2002). However, contradictory responses of soil microbial biomass as indirect effects of elevated atmospheric CO_2 have been reported. Diaz *et al*. (1993) proposed a negative feedback mechanism, where increased C input to the soil from increased productivity under elevated CO_2 caused the decrease of C and N mineralization of SOM. On the contrary, Zak *et al*. (1993) postulated a positive feedback, with SOM decomposition rate increasing under elevated CO_2. Later reviews by Zak *et al*. (2000) and Freeman *et al*. (2004) showed that the response of soil microorganisms to elevated CO_2 is highly variable, regardless of whether total biomass, its activity or effects on the N-cycle were studied. Thus, the first aim of this study was to elucidate the effect of elevated CO_2 on the activity of soil microorganisms.

The reported inconsistencies in the effects of elevated atmospheric CO_2 on SOM turnover reflect various factors, one of which – the soil structure represented by aggregates of different size – apparently plays the key role, because the accessibility of plant residues for microbial decomposition can be decreased by occlusion in aggregates (Jastrow *et al*., 2007). Thus, microaggregates (<250 μm diameter) formed by primary particles bound together by plant and microbial debris and by humic substances or polysaccharide polymers protect plant residues better against decomposition than macroaggregates do (>250 μm diameter) (Denef *et al*., 2001; Bossuyt *et al*., 2002; Six and Jastrow, 2002). In turn, microbial community associated with macroaggregates (fungi and bacteria) mineralizes C and N more actively than that in microaggregates (Guggenberger *et al*., 1999). In contrast to macroaggregates, microaggregates having more small pores enhance survival of microorganisms, mostly bacteria, by protecting them from predation by Protozoa and soil animals (Vargas and Hattori, 1986; Postma and Van Veen, 1990; Wright *et al*., 1993) or from desiccation (Nishiyama *et al*., 1992). Therefore, the second process investigated in our study was the effect of soil aggregates on microorganisms and parameters of their activity.

Diverse effects on activity and structure of soil microbial community and soil macro- and microaggregates due to elevated atmospheric CO_2 were investigated just in a couple of studies using incubation technique, phospholipid fatty acids (PLFA) or enzyme activity assays (Eviner and Chapin, 2002; Niklaus et al., 2007). However, all the studies showed no, or very small (not significant) effects of elevated CO_2 and soil aggregation on the composition and activity of soil microbial biomass. The Substrate Induced Growth Respiration (SIGR) method (Panikov, 1995) is another approach which allows to determine the structure of soil microbial community based on growth (living) strategy of soil

microorganisms (Andrews and Harris, 1986). According to their living strategies organisms are classified as r- and K-strategists (Pianka, 1970). *r*-strategists show quick growth on easily available substrates, whereas K-strategists use the resources more efficiently and grow slowly even in the absence of limitation. K- and *r*-strategists can be differentiated by their maximum specific growth rate under conditions with excess substrate (Andrews and Harris, 1986). Since both types of microorganisms – *r*- and K-strategists – are abundant in soil, changes in growth rates of the whole microbial community after amendment of easily available substrate can reflect the shift in domination of *r*- or K types (Blagodatskaya *et al*., 2007). In a previous study the microbial growth strategies were determined for bulk soil under agricultural crops (winter wheat and sugar beet) exposed for 3-4 years to elevated CO_2 using Free Air CO_2 Enrichment (FACE, Braunschweig) (Blagodatsky *et al.*, 2006). It was shown that elevated atmospheric CO_2 affected the formation of microbial community with a higher contribution of *r*-strategists which had higher maximum specific growth rates as compared to the soil under ambient atmospheric CO_2. Because of these promising results, we choose to apply the approach based on microbial growth strategy to investigate the effects of elevated CO_2 on microbial community.

In the current study we investigate the effect of elevated CO_2 on the contribution of *r*- and K-strategists in microbial community attributed to soil macro- and microaggregates. Three main questions were studied: (i) How does elevated CO_2 mediated by increased C rhizodeposition of plants (van Veen *et al*., 1991; Cheng, 1999; Phillips et al., 2006) contribute to a change of *r*- or K- selected microorganisms? (ii) What are the effects of soil aggregate-size on microbial activity and growth strategies? and (iii) Are there any interactions between elevated CO_2 and aggregate-size on the composition and activity of soil microbial biomass? Therefore, we measured microbial growth strategies in bulk soil and isolated aggregates under ambient and elevated CO_2 by means of substrate-induced growth respiration method.

2 Materials and methods

2.1 Study site

Soil samples were taken from the Free Air Carbon dioxide Enrichment (FACE) facility located in Stuttgart-Hohenheim, Baden-Wuerttemberg, Germany (48°43’ north latitude, 9°13’ east longitude). The FACE experiment started in 2002 and included plots with elevated atmospheric CO_2 level (540 ppm, with enclosures), ambient plots (380 ppm, with enclosures), and control plots (ambient CO_2 level, no enclosures) (Erbs & Fangmeier, 2006).

Each treatment was replicated five times. Spring wheat (*Triticum aestivum* cv Triso) was annually planted on the plots from 2002 to 2006. In 2007, oilseed rape (*Brassica napus*) was grown on the plots for the first time (Franzaring *et al*., 2008). Soil was tilled in spring before crop sowing. Beginning in 2003, inorganic NPK fertilizers were applied in equal amounts of 140 kg N ha^{-1}, 60 kg K ha^{-1}, 30 kg P ha^{-1} to each plot under ambient and elevated CO_2 treatments. No organic fertilizers were applied. The soil is a Gleyic Cambisol (WRB, 1998) without $CaCO_3$. Properties of the soil under ambient and elevated CO_2 treatments were identical: 9% sand, 69% silt, 22% clay; pH 6.8; bulk density (0-10 cm) 1.1 g cm^{-3}; C_{org} 1.59 %; N_{tot} 0.17 %; C/N ratio 9.1.

2.2 Aggregate-size fractionation at optimal soil moisture

Soil was sampled from the top 10 cm from each ambient and elevated CO_2 plot using soil corers (inner diameters: 5 cm) in September 2007, three weeks after rape harvest. Soil samples were stored at 7 °C for one week before aggregate-size fractionation. Aggregates were isolated according to Kristiansen *et al.* (2006). Soil samples were placed into a ventilation box (room temperature 22 °C) and spread out into a thin layer. The soil was dried to optimal moisture that would allow limited mechanical stress to induce maximum brittle failure of aggregates along natural planes of weakness. When individual soil clods had reached the desired condition, these were gently manually crumbled to less than 8 mm. The resulting aggregates were size separated by a brief sieving procedure. Portions of 300 g were transferred to a nest of sieves (2 and 0.25 mm) and shaken three times for 2 min. All visible roots and stones were removed and the aggregates >2 mm were collected (large macroaggregates class). The same procedure was done for the material retained on the 0.25 mm sieve, isolating aggregate-size class of 0.25-2 mm (small macroaggregates class). The remaining material passed through the 0.25 mm sieve was identified as aggregate class <0.25 mm (microaggregates class). Preliminary tests showed that the sieving duration was sufficient to quantitatively separate the various aggregate size-classes while minimizing aggregate abrasion during the sieving.

2.3 Microbial biomass and the kinetics of substrate induced respiration

Soil microbial biomass (C_{mic}) and the kinetic parameters of microbial growth response were measured in sub-samples of bulk soil and the three soil aggregate classes which contained 20% of water from the soil dry weight (60% WHC). This value corresponded to an

optimal level for microbial respiration activity determined in preliminary experiments. Soil samples were preincubated for 24 h at room temperature before measurements.

The dynamics of the CO_2 emission from soil amended with glucose and nutrients (Blagodatsky *et al.*, 2000) was used for estimation of C_{mic} and the kinetic parameters of microbial growth. Twelve g of soil (equal to 10 g of dry soil) were amended with a powder-mixture containing glucose (10 mg g^{-1}), talcum (20 mg g^{-1}), and mineral salts: $(NH_4)_2SO_4$– 1.9 mg g^{-1}, K_2HPO_4–2.25 mg g^{-1}, and $MgSO_4 \cdot 7H_2O$– 3.8 mg g^{-1}. Substrate concentrations sufficient for unlimited exponential growth of microorganisms were estimated in preliminary experiments trying different amounts of added glucose. The amount of mineral salts was selected so that the pH changes were less than 0.1 units after substrate addition (Blagodatskaya *et al.,* 2007). After substrate addition, the soil samples were immediately placed into 24 plastic tubes to measure the rate of CO_2 production. Each sample was continuously aerated (100 ml min^{-1}) at 22 °C, and the evolved CO_2 was measured every 1.5 h using an infrared detector (Gas Exchange Measurement System 2250, ADC, UK) connected to the Gas Handling Unit with a flowmeter (ADC, UK).

2.4 Calculations of microbial growth parameters

Soil respiration was used to calculate C_{mic} and kinetic parameters: specific growth rate of microorganisms (μ), the growing (active) microbial biomass (x_0'), physiological state index of microbial biomass before substrate addition (r_0), the total glucose-metabolizing microbial biomass (x_0) and the lag period (t_{lag}).

C_{mic} was calculated using the equation of Anderson and Domsch (1978) and conversion factor 30 suggested by Kaiser *et al*. (1992):

$$C_{mic}\ (\mu g\ g\ soil^{-1}) = (\mu l\ CO_2\ g\ soil^{-1}\ h^{-1}) \cdot 30 \qquad (1)$$

The kinetic parameters were chosen according the theory of the microbial growth kinetics (Panikov, 1995) and were estimated by fitting the parameters of the equation (1) to the measured CO_2 evolution rate ($CO_2(t)$) after addition of substrate:

$$CO_2\ (t) = A + B \exp\ (\mu \cdot t) \qquad (2),$$

where A is the initial respiration rate uncoupled from microbial growth, B is the initial rate of the fraction of total respiration coupled with cell growth, and t is the time (Blagodatsky *et*

al., 2000; Panikov and Sizova, 1996). The parameters of Eq. (1) were fitted by minimizing the least-square sum using Model Maker-3 software (SB Technology Ltd.). The fitting was restricted to the part of the curve that corresponded to unlimited exponential growth, as indicated by maximal values of r, F, and Q statistic criteria (20-22 h).

The total glucose-metabolizing microbial biomass (sustaining + growing; x_0) was calculated as following:

$$x_0 = (B \cdot \lambda \cdot Y_{CO2})/ (r_0 \cdot \mu) \quad (3),$$

where B is derived from the Eq. 1, r_0 – the so-called physiological state index of microbial biomass at time zero (before substrate addition) calculated from the ratio between A and B (Panikov and Sizova, 1996); Y_{CO2} is biomass yield per unit of C–CO_2, assumed to be constant during the experiment and equals 1.1; λ = 0.9 may be accepted as a basic stoichiometric constant (Panikov and Sizova, 1996).

The growing (active) microbial biomass (x_0') was calculated using equation:

$$x_0' = x_0 \cdot r_0 \quad (4),$$

The lag period (t_{lag}) was determined as the time interval from the substrate amendment to the moment when the increasing rate of growth-related respiration [B exp ($\mu \cdot t$)] becomes as high as the rate of respiration uncoupled from the growth of microorganisms [A] (Eq. 1). t_{lag} was calculated using the parameters of the approximated microorganisms' respiration curve by the equation:

$$t_{lag} = \ln (A/B)/ \mu \quad (5)$$

C_{mic} is expressed as amount of C on an oven-dry soil weight basis (105 °C) per gram of bulk soil or isolated aggregates (C_{mic}, mg C g^{-1}). The total amount of C_{mic} in a fraction of aggregates ($C_{mic\ F}$) is shown as a percentage from total C_{mic} in bulk soil. The sum of C_{mic} calculated based on the weight distributions of aggregate-size classes represented the recovery, when compared with the respective values for bulk soil. The study was conducted with soil samples from 8 plots (4 ambient CO_2 plots and 4 of elevated CO_2 plots).

The effects of aggregate sizes and CO_2 treatments were assessed by two-way ANOVA with size of aggregates and CO_2 treatment as independent factors. The Fischer LSD post hoc test was used to evaluate the impact of treatments at the $p<0.05$ significance level.

3 Results

3.1. Distribution of aggregate fractions and C content

The sieving procedure fractionated the bulk soil into aggregate-size classes as follows: large macroaggregates (>2 mm) contributed 31-34 %, small macroaggregates (0.25-2 mm) 58-59% and microaggregates (<0.25 mm) 8-10% of the weight of bulk soil, respectively (Fig. 1, top). The atmospheric CO_2 enrichment did not affect the distribution of aggregates in the different size classes (Fig. 1, top). The C_{org} content in each aggregate class was similar to the C_{org} of the bulk soil. Only the aggregate fraction >2 mm under elevated CO_2 treatment had significantly lower C_{org} content as compared to the bulk soil and to the fraction 0.25-2 mm under the same CO_2 treatment (Fig. 1, center).

3.2. Microbial biomass distribution

Elevated CO_2 tended to increase C_{mic} in bulk soil and in the three aggregate-size classes, but this increase was not significant (Fig. 1, bottom). The amount of C_{mic} significantly increased with decreasing aggregate-size classes. Thus, the smallest value of C_{mic} (mg C g^{-1}) amounted to 0.340 ± 0.025 under ambient and 0.353 ± 0.023 under elevated CO_2 in large macroaggregates. The highest C_{mic} value corresponded to microaggregates and amounted to 0.566 ± 0.031 and 0.585 ± 0.004 mg C g^{-1} under ambient and elevated CO_2, respectively (Fig. 1, bottom).

The total C_{mic} in bulk soil was similar under ambient and elevated CO_2 treatments: 0.44 mg C g^{-1}. C_{mic} in aggregate fractions was distributed similarly to the aggregate weight distribution, i.e. the largest value was found in small macroaggregates and the lowest in microaggregates (Fig. 2). The quality of the fractionation procedure was estimated by the cumulative recovery of C_{mic}. Cumulative C_{mic} in aggregates reached 97.8% of the bulk soil under ambient CO_2 and 100.2% under elevated CO_2 (Fig. 2).

3.3. Microbial growth kinetics

3.3.1. Respiration rate (RR)

The glucose and nutrients amendment exponentially increased the microbial respiration rate (RR) in bulk soil and the three aggregate-size classes under both ambient and

elevated atmospheric CO_2 (Fig. 3), indicating microbial growth after the lag-phase. For the bulk soil, the increase in RR after glucose amendment was expressed more distinctly under elevated than under ambient CO_2 (Fig. 3). There were no significant differences of the substrate-induced RR curves for isolated aggregates under ambient and elevated atmospheric CO_2, except microaggregates, in which the increase in RR was more distinct under ambient than under elevated CO_2 (Fig. 3). However, the RR curve of microaggregates under elevated CO_2 was steeper (leading to higher μ) as compared to the one under ambient CO_2 treatment (Fig. 3). For soil aggregate-size classes under both CO_2 treatments, the RR increased from macro- to microaggregates: large macroaggregates < small macroaggregates < microaggregates (Fig. 3).

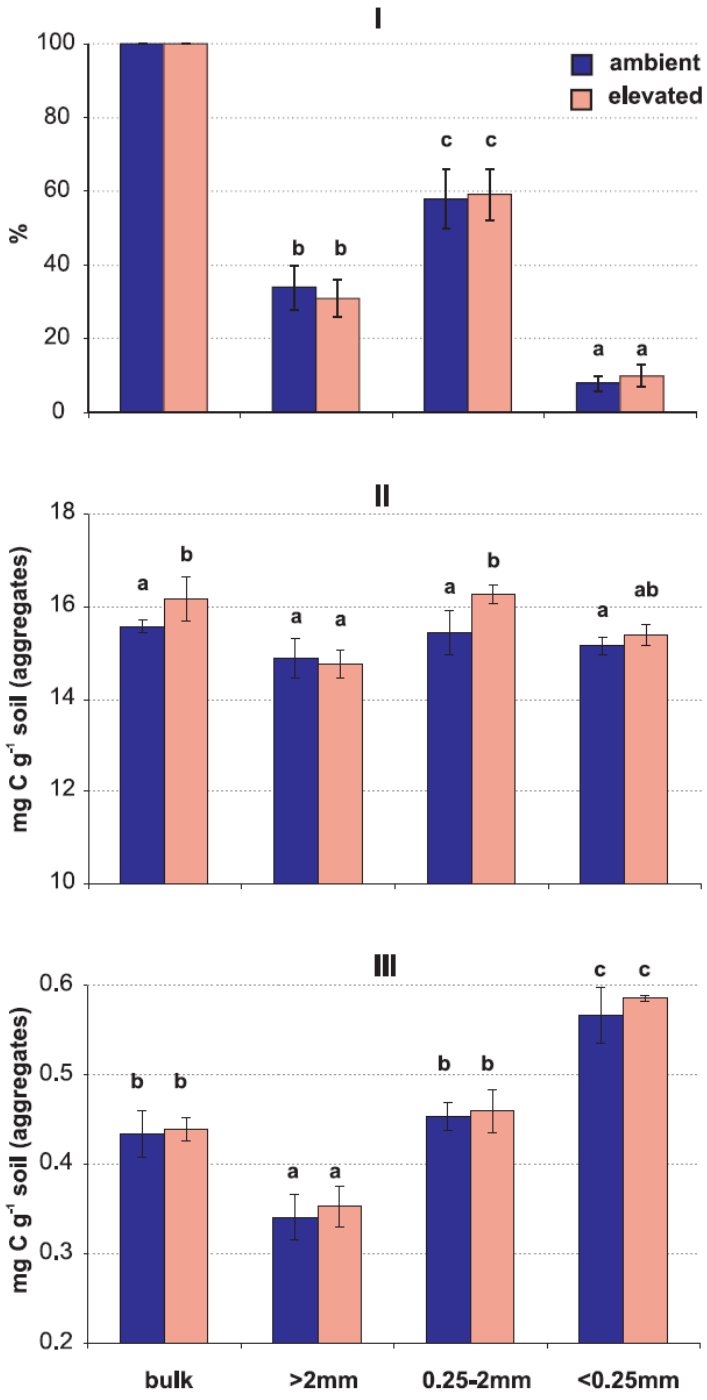

Fig. 1. Distribution of aggregate-size classes by weight from the bulk soil (I), amount of carbon (II) and microbial biomass content (III) in bulk soil and isolated aggregates of different size under ambient and elevated CO_2 treatment. Values are averages of four replicates (± SE). Treatments followed by the same letters are not significantly different between aggregate size classes at $p \leq 0.05$. There were no significant differences between ambient and elevated CO_2 treatments.

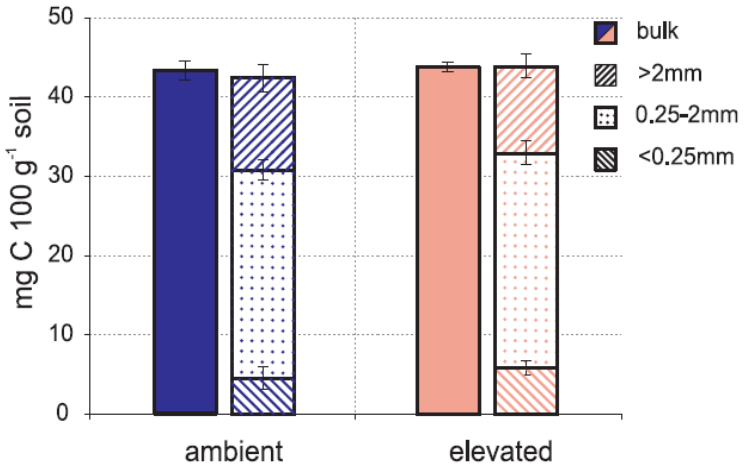

Fig. 2. The recovery of microbial biomass content (C_{mic}) in three aggregate-size classes in comparison with total C_{mic} in bulk soil under ambient and elevated CO_2 treatments.

3.3.2 Specific growth rate (μ)

The specific growth rate (μ) of soil microorganisms was substantially affected by atmospheric CO_2 enrichment: μ values were significantly higher under elevated than under ambient CO_2 for bulk soil, small macro- and microaggregates. Only in large macroaggregates the difference in specific growth rates between elevated and ambient CO_2 was insignificant (Fig. 4). According to the steepness of the respiration curves (Fig. 3), the μ values increased in aggregate-size classes as compared to the bulk soil: bulk soil < large macroaggregates < small macroaggregates < microaggregates (Fig. 4). However, this increase of μ values from macro- to microaggregates was only significant between large macro- and microaggregates under elevated CO_2 and not significant under ambient CO_2 treatment (Fig. 4).

3.3.3 Active microbial biomass (AMB)

Active microbial biomass (AMB) in bulk soil (μg C g^{-1}) was not affected by atmospheric CO_2 enrichment (Fig. 5.I). In isolated soil aggregates AMB was always lower under elevated than under ambient CO_2. However, this pattern was significant only in microaggregates, where the AMB content was 3.4-fold lower under elevated than under ambient CO_2 (Fig. 5.I). The AMB increased with the decreasing of aggregates size from 1.94

± 0.27 to 5.63 ± 1.55 µg C g^{-1} aggregates under ambient CO_2 treatment. In turn, the AMB content under elevated CO_2 was similar in all three aggregate-size classes (Fig. 5.I).

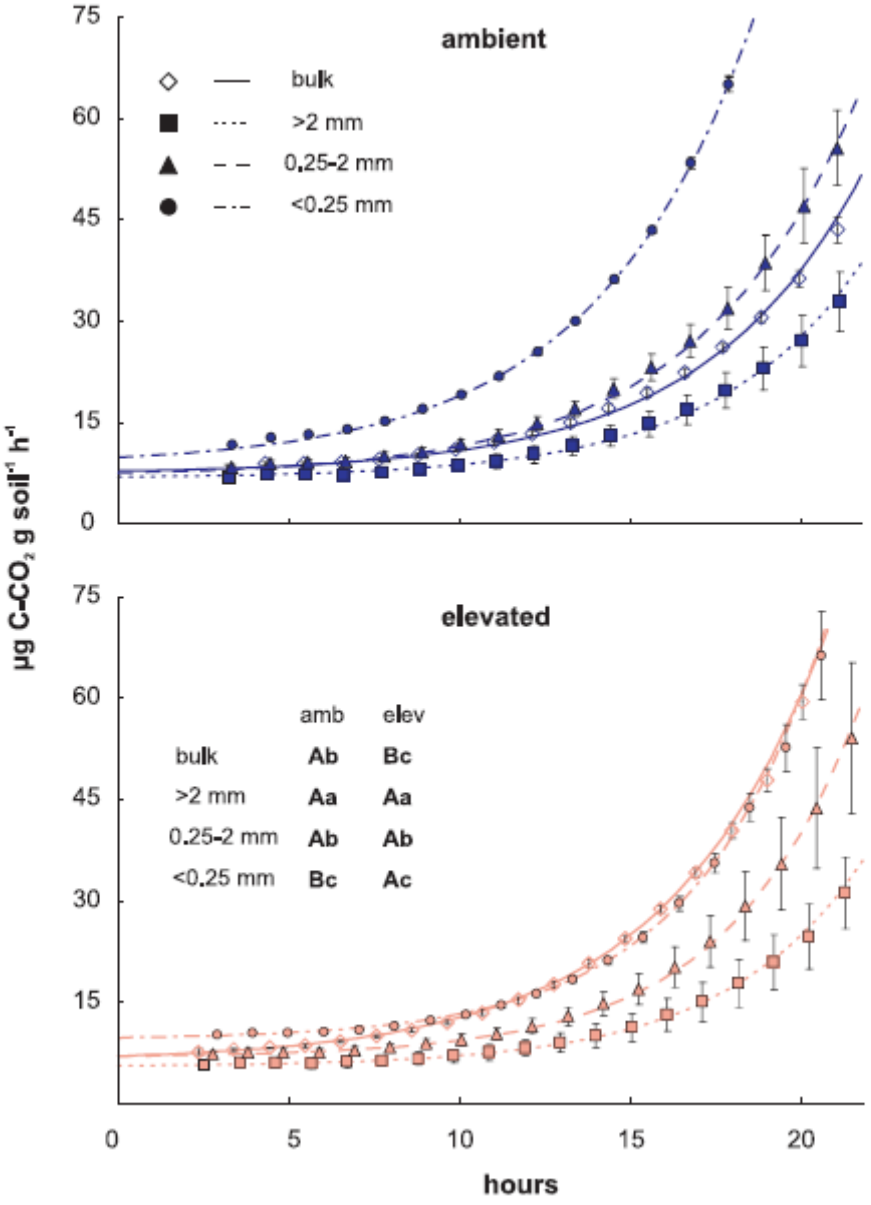

Fig. 3. Measured respiration rates (dots) and approximated values (lines) after glucose and nutrients amendment of the bulk soil and three aggregate-size classes under ambient (top) and elevated (bottom) CO_2 treatments. Values are averages of four replicates (± SE). Significance table in the bottom subfigure: Individual respiration rate followed by the same letters are not significantly different between elevated and ambient CO_2 (uppercase letters) and aggregate size classes (lowercase letters) at $p \leq 0.05$.

3.3.4 Lag-period (t_{lag})

The calculated t_{lag} (Eq. 5) showed that the period of time necessary for microorganisms to start exponential growth after glucose amendment was significantly shorter in bulk soil

under elevated (11 hours) than under ambient (12.5 hours) CO_2 treatment (Fig. 5.III). In contrast to the bulk soil, the fractionation of the latter to the constituent aggregates substantially increased t_{lag} up to 14.5-15 hours under elevated CO_2 as compared to 9-14 hours under ambient CO_2 treatment (Fig. 5.III). t_{lag} in soil aggregates showed different trends when ambient and elevated CO_2 are considered. While t_{lag} was similar in all aggregate-size classes under elevated CO_2 averaging to 14.7 hours, t_{lag} under ambient CO_2 treatment became shorter (from 14 to 9 hours) with decreasing aggregates size. However the difference between macro- and microaggregates under ambient CO_2 was not significant (Fig. 5.III).

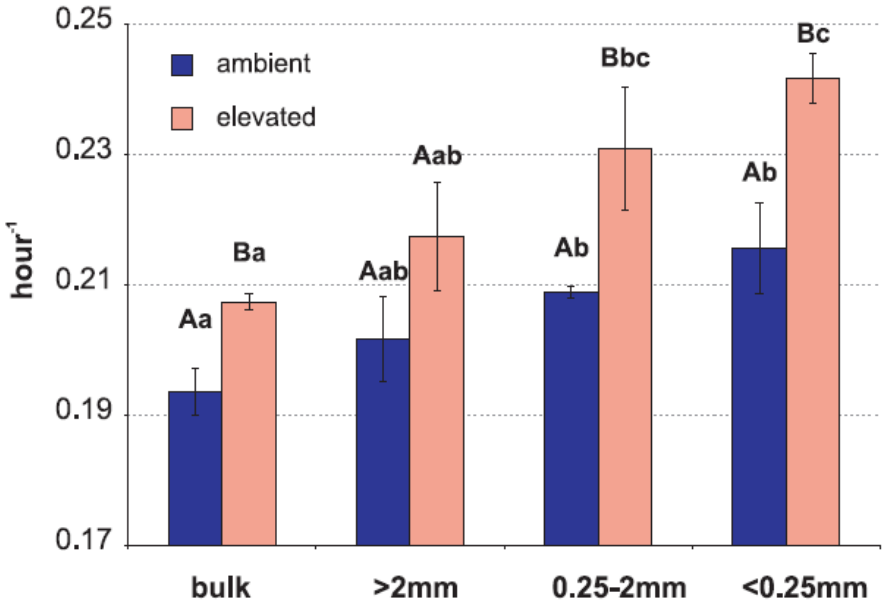

Fig. 4. Specific growth rates of microbial biomass in bulk soil and three aggregate-size classes under ambient and elevated CO_2 treatments. Values are averages of four replicates (± SE). Treatments followed by the same letters are not significantly different between elevated and ambient CO_2 (uppercase letters) and aggregate size classes (lowercase letters) at $p \leq 0.05$.

4 Discussion

4.1. The allocation of microorganisms in soil aggregates

The distribution of microorganisms in soil aggregates is strongly dependent on sieving separation procedure (wet vs. dry). Accordingly, Chotte *et al*. (1998) and Guggenberger *et al*. (1999) found higher microbial biomass in macroaggregates vs. microaggregates separated by wet sieving. Miller and Dick (1995) reported higher microbial biomass in macroaggregates isolated by dry sieving. Askin and Kizilkaya (2006), Santruckova *et al*. (1993) and Schutter and Dick (2002) found higher microbial biomass in microaggregates than in macroaggregates separated by dry sieving. Contrasting literature results could reflect

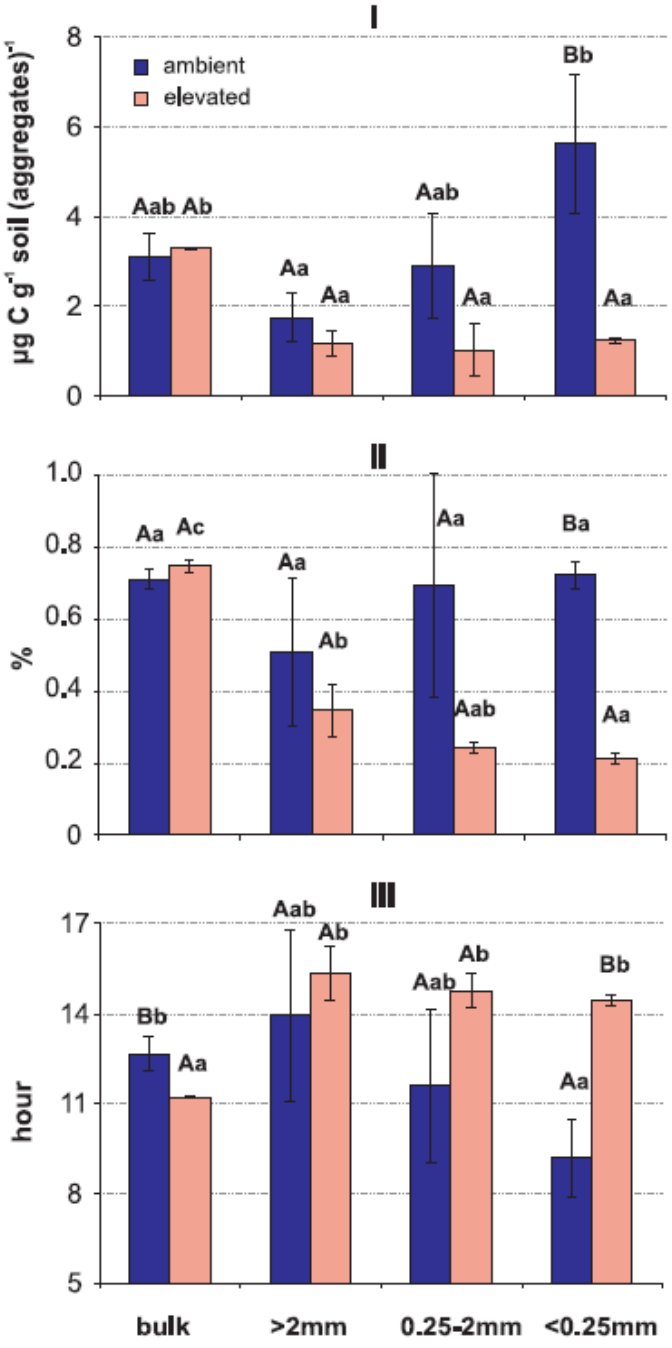

Fig. 5. Amount of active microbial biomass (AMB; I), contribution of AMB to total microbial biomass (II) and duration of the lag-period (III) in bulk soil and three aggregate-size classes under ambient and elevated CO_2 treatments. Values are averages of four replicates (± SE). Treatments followed by the same letters are not significantly different between elevated and ambient CO_2 (uppercase letters) and aggregate size classes (lowercase letters) at $p \leq 0.05$.

the differences in aggregate separation by wet and dry sieving. Fractionation is a destructive procedure and depending on the fractionation method some microorganisms may be transferred with particles and/or microaggregates from the surfaces of larger to smaller

aggregates due to abrasion of the former (dry sieving) or adhere to aggregates during slaking in water (wet sieving) persisting in macroaggregates (Ashman *et al*., 2003). Furthermore, wet-sieving procedure releases water soluble OM and dispersible colloids, causing changes in aggregate composition (Emerson and Greenland, 1990; Watts *et al*., 1996). Strong soil moistening could change the activity of soil microorganisms through anaerobic conditions (Kieft *et al*., 1987; Zhang and Zak, 1998). In turn, prolonged sieving of air-dry soil tends to increase aggregate abrasion rather than fragmentation due to the great tensile strength of dry aggregates (e.g. Munkholm and Kay, 2002). In agreement with Kristiansen *et al*. (2006), we fragmented the soil into constituent aggregates when a water content of individual clods and peds was near the lower plastic limit, corresponding to the optimum water content of medium textured soils at which soil friability is maximal (Dexter and Bird, 2001). Thereby we maximized brittle fracturing along natural planes of weakness while minimizing changes in aggregate-size distribution (Kristiansen *et al*., 2006). Since the approach used is considerably distinguished from conventional wet- and dry sieving procedures, we propose to determine such aggregate-size fractionation approach as **optimal moist sieving.** Such an approach is gentle (Kristiansen *et al*., 2006), hence minimizes microorganism destruction in aggregate separates. Thus, in our study after fractionation of bulk soil by means of the optimal moist sieving approach the cumulative recovery of C_{mic} in aggregates reached 97.8% as compared to C_{mic} in the bulk soil under ambient CO_2 and 100.2% under elevated CO_2 (Fig. 2). Such a high recovery of microbial biomass content in separated soil aggregates indicates the appropriateness of the method for soil microbiological studies. It is also important to underline that microbial biomass content presented in Fig. 2 was estimated by substrate induced respiration. This means that the applied fractionation procedure did not strongly change the respiration parameters of microbial biomass compared to untreated bulk soil. This was a prerequisite for application of the SIGR approach to evaluate the activity parameters of microbial biomass.

Higher microbial biomass content in micro- compared to macroaggregates (Fig. 1.III) found in our study could be explained by the contrasting environment of differently sized aggregates. The location of microorganisms within the soil matrix is a key factor for their survival (Chenu *et al*., 2001). Small-sized pores (micropores, <10 μm) dominate within microaggregates, whereas both small- and large-sized pores (macropores, >10 μm) occur in macroaggregates (Jastrow *et al*., 2007; Paul, 2007). Micropores, in turn, enhance survival by protecting microorganisms from predation by Protozoa and soil animals (Vargas and Hattori, 1986; Postma and van Veen, 1990; Wright *et al*., 1993) or from desiccation (Nishiyama *et*

al., 1992). Furthermore, microaggregates likely have texture (sand-silt-clay ratio) different from macroaggregates (Bronick and Lal, 2005). Clay particles can also directly affect microbial survival in soils (van Gestel *et al*., 1996), since adsorption of microbial cells to the surfaces of clay particles is crucial for microbial biomass accumulation in soils (Amato and Ladd, 1992).

4.2 The effect of elevated CO_2 on the microbial biomass content

The response to CO_2 enrichment of the amount of microbial biomass in soils was reported to vary from positive (elevated CO_2 increased soil microbial biomass) (e.g. Zak *et al*., 1993; Drissner *et al*., 2007) to no effect (e.g. Kampichler *et al*., 1998; Niklaus *et al*., 2003). Our results (Fig. 1.III) support numerous studies which also failed to find a significant response of microbial biomass content to elevated CO_2. Moreover, we observed no changes in total microbial biomass under elevated vs. ambient CO_2 in isolated soil aggregates (Fig. 2). This indicates that the effect of atmospheric CO_2 enrichment on allocation of microorganism into soil macro- and microaggregates is negligible, at least when agroecosystems with high level of manipulation are considered. We therefore conclude that microbial biomass content is an insensitive parameter to evaluate small changes caused by elevated CO_2.

4.3. The effect of aggregates size on microbial growth strategies.

The analysis of microbial respiratory response to the amendment of bulk soil and isolated aggregates with easily available substrate showed the pronounced effect of soil structure on characteristics of the microbial community. The respiration rates increased with the decreasing of soil aggregates size (Fig. 3). Similar, however statistically non-significant patterns were found for specific growth rates (Fig. 4). These results indicate higher contribution of fast vs. slow growing microorganisms to total microbial community in soil microaggregates as compared to macroaggregates. There is evidence in a number of studies that the structure and function of the soil microbial communities vary between different aggregate size classes (Gupta and Germida, 1988; Guggenberger *et al*., 1999; Schutter and Dick, 2002; Vaisanen *et al*., 2005). Correspondingly, Schutter and Dick (2002) showed higher microbial activity in micro- vs. macroaggregates. However, Vaisanen *et al*. (2005) reported contradictory results: the communities associated with large (>2 mm) and small macroaggregates (0.25-2 mm) displayed a significantly higher microbial activity compared to microaggregates (0.053-0.25 mm). Such inconsistencies could be again explained by the

application of contrasting aggregate-size fractionations, e.g. wet- and dry sieving approaches. The studies reporting higher microbial activity in macro- than in microaggregates employed wet sieving techniques (Elliot, 1986; Franzluebbers and Arshad, 1997; Vaisanen *et al*., 2005). In contrast, the studies reporting higher microbial biomass content and activities in microaggregates applied dry sieving techniques (Seech and Beauchamp, 1988; Mendes *et al*.,1999; Schutter and Dick, 2002).

4.4. The effect of elevated CO_2 on microbial specific growth rates.

Although the initial respiration rates after glucose amendment of isolated aggregates did not show significant impacts of atmospheric CO_2 enrichment (Fig. 3), the kinetic parameters of microbial growth revealed the shift in living strategy of soil microorganisms induced by indirect effect of elevated CO_2 as shown by specific growth rates (Fig. 4), active microbial biomass (Fig. 5.I, bulk soil) and duration of lag-period (Fig. 5.III, bulk soil). This shift in growth strategy of the total microbial community to *r*-selected species was most probably mediated by increasing plant rhizodeposition stimulated by elevated CO_2 (van Veen *et al*., 1991; Paterson *et al.*, 1997; Rogers *et al*., 1998; Cheng, 1999).

The results of this study are in a good agreement with a previous experiment, in which the microbial growth strategies were determined for bulk soils under crops (winter wheat and sugar beet) exposed for 3-4 years to elevated CO_2 (FACE, Braunschweig) (Blagodatsky *et al.*, 2006). It was shown that elevated CO_2 affected the formation of a microbial community with a higher contribution of *r*-strategists as compared to the soil under ambient atmospheric CO_2. The observations are in agreement also with findings reported by Hodge *et al*. (1998) who reported an increase of the amount of cultivated bacteria and high rates of substrate mineralization in BIOLOG MicroPlate assays in soil under ryegrass exposed to elevated atmospheric CO_2.

In contrast to the bulk soil, its fractionation into constituent aggregates resulted in a decrease of active microbial biomass (AMB) content and in extension of lag-period, which was especially pronounced under elevated CO_2 (Fig. 5.I, II). We explain such decrease of AMB with the high sensitivity of growing cells to the physical impact of the sieving procedure. However, even after decrease of AMB content in three aggregate-size classes the relative contribution of *r*- vs. K-strategists in active microbial biomass remained higher under elevated CO_2, what was proved by higher specific growth rates.

Conclusions

Aggregate-size fractionation at optimal moisture satisfactorily separated the bulk soil into constituent micro- and macroaggregates. This fractionation technique had minimal mechanical impact on the microorganisms, as demonstrated by a high recovery of microbial biomass and its respiratory activity. Estimation of microbial biomass and kinetics of substrate induced respiration in bulk soil and aggregate fractions after 5 years of CO_2 enrichment lead to the following conclusions:

- The kinetics of microbial respiratory response to the amendment of soil with easily available substrate is an effective tool to describe the features of soil microbial biomass in respect to environmental changes. In contrast to microbial biomass content, all tested kinetic characteristics (the maximal specific growth rate (μ) of soil microorganisms, the amount of active microbial biomass (AMB) and duration of lag-period) were found to be sensitive parameters to characterize the changes in soil microbial community induced by elevated CO_2 in the atmosphere.
- Elevated atmospheric CO_2 stimulated the growth of microorganisms with *r*-strategy due to presumably high amounts of easily decomposable organic substrate in soil as compared to ambient CO_2. The increased activity of microorganisms with *r*-strategy under elevated atmospheric CO_2 was especially pronounced in soil microaggregates (<0.25 mm).
- Soil structure represented by the composition of differently sized aggregates had pronounced effects on amount and characteristics of microbial community: (i) total amount of microbial biomass increased with decreasing aggregates size, (ii) contribution of fast vs. slow growing microorganisms to total microbial community in soil microaggregates was higher as compared to macroaggregates.

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VI) Stimulation of microbial enzyme activities by elevated atmospheric CO_2 depends on soil aggregates size

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Abstract

Increased belowground carbon (C) transfer by plant roots at elevated CO_2 may change properties of the microbial community in the rhizosphere. Previous investigations that focused on total soil organic C or total microbial C showed contrasting results: small increase, small decrease or no changes. We evaluated the effect of five years of elevated CO_2 (540 ppm) on four extracellular enzymes: β-glucosidase, chitinase, phosphatase and sulphatase. We expected microorganisms to be differently localized in aggregates of various sizes and therefore analyzed microbial biomass (C_{mic} by SIR) and enzyme activities in three aggregate-size classes: large macro- (>2 mm), small macro- (0.25-2 mm) and microaggregates (<0.25 mm). To estimate the potential enzyme production, we activated microorganisms by substrate (glucose and nutrients) amendment. Although C_{total} and C_{mic} as well as the activities of β-glucosidase, phosphatase and sulphatase were unaffected in bulk soil and in aggregate-size classes by elevated CO_2, significant changes were observed in potential enzyme production after substrate amendment. After adding glucose, enzyme activities under elevated CO_2 were 1.2-1.9-fold higher than under ambient CO_2. This indicates an increased activity of microorganisms, which leads to accelerated C turnover in soil under elevated CO_2. Significantly higher chitinase activity in bulk soil and in large macroaggregates under elevated CO_2 revealed an increased contribution of fungi to turnover processes. At the same time, less chitinase activity in microaggregates underlined microaggregate stability and the difficulties for fungi hyphae penetrating them. We conclude that quantitative and qualitative changes of C input by plants into the soil at elevated CO_2 affect microbial community functioning, but not its total content. Future studies should therefore focus more on the changes of functions and activities, but less on the pools.

Keywords: Extracellular enzyme activity; Soil microbial biomass; Elevated atmospheric CO_2; Macroaggregates; Microaggregates.

Running title: Extracellular enzymes under elevated CO_2

1. Introduction

During the past two centuries, the CO_2 concentration in the atmosphere has increased by 35%, mainly because of fossil fuel combustion and land-use changes (IPCC, 2007). Studies exploring ecosystem responses to elevated CO_2 have gained widespread attention in the last few decades (Paustian *et al*., 1997; Pendall *et al*., 2004; van Kessel *et al*., 2006). Most investigations, however, have been focusing on plant biomass responses to elevated atmospheric CO_2, while belowground processes in soils have received less attention. The direct effects of increasing CO_2 on plants include (i) higher net primary productivity (Melillo *et al*., 1993; Schimel, 1995) through accelerated photosynthetic rates (Paterson *et al*., 1997), (ii) changes in plant chemistry such as higher C/N ratio, higher concentrations of starch, sugars, and carbohydrates (Cotrufo *et al*., 1994), (iii) reduced stomatal conductance of plants resulting in higher water use efficiency (Körner, 2000), and (iv) stimulated root growth (Paterson *et al*., 1997; Rogers *et al*., 1998), which increases rhizodepositions including root exudates (van Veen *et al*., 1991; Cheng, 1999). In contrast to the response of plant biomass to elevated CO_2, the feedback of belowground components of terrestrial ecosystems, especially soil microorganisms, to atmospheric CO_2 enrichment occurs indirectly through plant-derived deposits; this process is less well studied compared to the aboveground biomass response. For example, the reviews by Zak *et al*. (2000) and Freeman *et al*. (2004) showed that the response of soil microorganisms to elevated CO_2 is highly variable, regardless of whether total biomass, its activity or effects on the N-cycle were studied.

Microorganisms are the main source of crucial enzymes in the cycling of main nutrients (C, N, P, S) in soil (Aon *et al.*, 2001). Moreover, soil enzyme activities are highly sensitive and could serve as indicators of various changes in the plant-soil system: they integrate information on both the microbial status and the physico-chemical soil conditions (Aon *et al.*, 2001). The effect of elevated atmospheric CO_2 on the activity of various soil microbial enzymes has been intensively studied over the last decade (Kandeler *et al*., 1998; Ebersberger *et al*., 2003; Henry *et al*., 2005). For example, it increased the activities of enzymes such as phosphatase and urease that promote P or N cycling (Ebersberger *et al*., 2003). The activity of cellulose-degrading enzymes also tended to increase under elevated CO_2 (Mayr *et al*., 1999), probably due to an increased turnover of fine roots. However, conflicting results have also been reported. Moorhead & Linkins (1997) found that cellulase activity in the surface organic soil horizons of tussock tundra decreased. No effect of elevated atmospheric CO_2 on the soil enzyme activities (phosphatase, α-, β-glucosidase,

leucine aminopeptidase, phenol oxidase, peroxidase) was observed in a northern fen bulk soil (Kang *et al*., 2001) or forest soil under temperate trees (Larson *et al*., 2002).

Such inconsistencies can reflect various factors, one of which – the aggregate structure – apparently plays the key role because the accessibility of soil organic matter for microbial decomposition can be decreased by occlusion in soil aggregates (Jastrow *et al*., 2007). Thus, microaggregates (<250 μm diameter) formed by primary particles coupled together by plant and microbial debris and by humic materials or polysaccharide polymers better protect SOM against decomposition than do macroaggregates (>250 μm diameter) (Denef *et al*., 2001; Bossuyt *et al*., 2002; Six & Jastrow, 2002). The microbial community associated with macroaggregates actively mineralizes C and N; especially fungi play an important role in forming and stabilizing macroaggregates (Guggenberger *et al*., 1999; Vaisanen *et al*., 2005). Although the turnover of C and N in soil aggregates has received considerable attention, this is not the case for the impact of soil structure on microbial enzyme activities. The indirect effect of elevated atmospheric CO_2 on microbial activity in soil aggregates is even more poorly understood. We expect that plant rhizodeposits under elevated CO_2 stimulate enzyme activities differently, depending on aggregate-size classes, because soil microorganisms are unevenly distributed in macro- and microaggregates (Santruckova *et al*., 1993; Miller & Dick, 1995; Chotte *et al*., 1998).

Microbial activity in soil is controlled by the availability of easily decomposable organic substrates (Friedel *et al*. 1996; Klose *et al*. 1999). Glucose is one such substrate; its addition to soil generally stimulates microbial growth as indicated by higher soil respiration, microbial biomass and enzyme activities (Anderson & Domsch, 1973; Lin & Brookes, 2000; Dilly & Nannipieri, 2001). We therefore assume that "activation" of the soil microbial community by adding glucose could stimulate microbial growth and boost enzyme activities. Because microbial activity changes under elevated CO_2 (Blagodatsky *et al*., 2006), we also expect that glucose application will more strongly affect enzyme activities under elevated vs. ambient CO_2 treatment.

This study applies a highly sensitive method, using a microplate system combined with 4-methylumbelliferone-labelled fluorogenic substrates, to measure enzyme activities (Freeman *et al*., 1995; Pritsch *et al*., 2004). We estimated the enzymatic activities representing the main steps of soil biogeochemical nutrient cycles, i.e. C (β-1.4-glucosidase, N-acetyl-β-D-glucosaminidase), N (N-acetyl-β-D-glucosaminidase), P (phosphatase) and S (sulphatase). These enzyme activities were measured in bulk soil and in three aggregate-size classes (large macro-, small macro- and microaggregates) under an agricultural ecosystem

with spring wheat exposed for four years, and oilseed rape exposed for one year to elevated CO_2.

2. Materials and Methods

2.1. Study site

Soil samples were taken from the Free Air Carbon dioxide Enrichment (FACE) facility located in Stuttgart-Hohenheim, Baden-Wuerttemberg, Germany (48°43' north latitude, 9°13' east longitude). The soil is a Gleyic Cambisol (WRB, 1998) without $CaCO_3$. Mean annual temperature is 8.7 °C and average rainfall 680 mm a^{-1} (mean 1961-1990, meteorological station Stuttgart-Hohenheim). Properties of the soil under ambient and elevated CO_2 treatments were identical: 9% sand, 69% silt, 22% clay; pH 6.8; bulk density (0-10 cm) 1.1 g cm^{-3}; C_{org} 1.59 %; N_{tot} 0.17 %; C/N ratio 9.1.

The FACE experiment, starting in 2002, included plots with elevated atmospheric CO_2 level (540 ppm, with enclosures), ambient plots (380 ppm, with enclosures), and control plots (ambient CO_2 level, no enclosures) (Erbs & Fangmeier, 2006). Each treatment was replicated five times. Spring wheat (*Triticum aestivum* cv Triso) was annually planted on the plots from 2002 to 2006. In 2007, oilseed rape (*Brassica napus*) was grown on the plots for the first time. Soil was tilled in spring before crop sowing. Beginning in 2003, inorganic NPK fertilizers were applied in equal amounts of 140 kg N ha^{-1}, 60 kg K ha^{-1}, 30 kg P ha^{-1} to each plot under ambient and elevated CO_2 treatments. No organic fertilizers were applied.

2.2. Aggregate-size fractionation

Soil was sampled from the top 10 cm from each ambient and elevated CO_2 plot using soil corers (inner diameters: 5 cm) in September 2007, three weeks after rape harvest. Soil samples were stored at 7 °C for one week before aggregate-size fractionation. Aggregates were isolated according to Kristiansen *et al*. (2006). Soil samples were placed into a ventilation box (room temperature 22 °C) and spread out into a thin layer. The soil was dried to optimal moisture that would allow limited mechanical stress to induce maximum brittle failure along natural planes of weakness. When individual soil clods had reached the desired condition, these were gently manually crumbled to less than 8 mm. The resulting aggregates were size separated by a brief sieving procedure. Portions of 300 g were transferred to a nest of sieves (2 and 0.25 mm) and shaken three times for 2 min. All visible roots and stones were removed and the aggregates >2 mm were collected (large macroaggregates class). The same procedure was done for the material retained on the 0.25 mm sieve, isolating

aggregate-size class of 0.25-2 mm (small macroaggregates class). The remaining material passed through the 0.25 mm sieve was identified as aggregate class <0.25 mm (microaggregates class). Preliminary tests showed that the sieving duration was sufficient to quantitatively separate the various aggregate size-classes while minimizing aggregate abrasion during the sieving.

2.3. Soil microbial biomass

Subsamples of bulk soil and the three soil aggregate classes were moistened with deionized water to 20 % of the soil dry weight (60 % WHC). This value corresponded to an optimal level for microbial respiration activity determined in preliminary experiments. Soil samples were preincubated for 24 h at room temperature before measurements. Soil microbial biomass (C_{mic}) was measured in bulk soil and isolated soil aggregates using the initial rate of substrate-induced respiration (SIR) (Anderson & Domsch, 1978). Twelve grams of soil (equal to 10 g of dry soil) were amended with glucose (10 mg g^{-1}). After glucose addition, the soil samples were immediately placed into the respiration apparatus, which consisted of 24 plastic tubes, to measure the rate of CO_2 production. Each sample was continuously aerated (100 ml min^{-1}) at 21 °C, and the evolved CO_2 was measured using an infrared detector (Gas Exchange Measurement System 2250, ADC, UK) connected to the Gas Handling Unit with a flowmeter (ADC, UK). C_{mic} was calculated using the equation of Anderson & Domsch (1978) and conversion factor 30 suggested by Kaiser *et al*. (1992):

$$C_{mic}\ (\mu g\ g\ soil^{-1}) = (\mu l\ CO_2\ g\ soil^{-1}\ h^{-1}) \cdot 30 \qquad (1)$$

2.4. Soil "activation" and enzyme assays

To detect the actual (basal) and potential ("activated") enzyme activities under ambient and elevated CO_2 treatments the enzymes activities were measured in bulk soil and isolated soil aggregates before and after addition of glucose and nutrients. For "activation", soil was amended with a powder-mixture containing glucose (10 mg g^{-1}), talcum (20 mg g^{-1}), and mineral salts: $(NH_4)_2SO_4$ – 1.9 mg g^{-1}, K_2HPO_4 – 2.25 mg g^{-1}, and $MgSO_4 \cdot 7H_2O$ – 3.8 mg g^{-1}. After substrate addition, the soil samples were left for 48 hours to induce the microbial growth. The technique of enzyme measurements in substrate-amended and non-amended bulk soil and aggregates was identical.

Extracellular enzyme activities in bulk soil and isolated soil aggregates were measured using fluorogenically labeled substrates according to a modified technique described in

Sowerby *et al*. (2005) and Pritsch *et al*. (2004, 2005). Four fluorogenic enzyme substrates based on 4-methylumbelliferone (MUF) were used: MUF-β-D-glucopyranoside (MUF-G; EC 3.2.1.21, for the detection of β-glucosidase), MUF-N-acetyl-β-D-glucosaminide dihydrate (MUF-NAG; EC 3.2.1.14) for chitinase, MUF-phosphate disodium salt (MUF-P; EC 3.1.3.2) for phosphatase, and MUF-sulfate potassium salt (MUF-S; EC 3.1.6) for sulphatase activity. To dissolve the MUF-substrates, 2 ml of 2-methoxyethanol (Hoppe, 1983) was used. Predissolved MUF-substrates were further diluted with sterile distilled water to give the desired concentrations (see below). All chemicals were purchased from Fluka (Germany).

The soil samples (1 g) were suspended in water (20 ml) and shaken on an overhead shaker for 15 min at room temperature and at maximum speed to ensure thorough mixing. A sub-sample of the soil suspension (1.0 ml) was added to the 3 ml MUF-substrate solution (containing either 400 μmol MUF-G, or 200 μmol MUF-NAG, MUF-P, or MUF-S), already pipetted in Deep Well Plates (24-wells × 10 ml, HJ-Bioanalytik GmbH, Germany), and incubated at 11 °C for 1 h. The calibration solutions were prepared using soil suspension (1 ml) and MUF of different concentrations (0-100 μmol, 3 ml). Deep Well Plates with the soil-MUF-substrates and soil-calibration-MUF concentrations were centrifuged (20×G, 5 min). Thereafter, 0.5 ml of supernatant was pipetted to the 24-well microplates (Becton Dickinson, USA) containing 1.25 ml sterile distilled H_2O and 0.25 ml of 20 mmol glycine-NaOH buffer solution (pH 11) to stop enzyme reactions. Fluorescence was measured in microplates at an excitation wavelength of 355 nm and an emission wavelength of 460 nm, slit width of 25 nm, with a Victor^3 1420-050 Multilabel Counter (PerkinElmer, USA). Calibration curves were included in every series of enzyme measurements. Enzyme activities were expressed as MUF release in nmol per g bulk soil/aggregates dry weight and hour (nmol g^{-1} h^{-1}).

2.5. Calculation of recoveries and statistical analysis

The study was conducted with samples of 8 plots (4 ambient CO_2 plots and 4 of elevated CO_2 plots). Microbial biomass C and enzyme activity (β-glucosidase, chitinase, phosphatase and sulphatase) were calculated on an oven-dry weight (105 °C) basis. The total amount of C_{mic} and enzyme activities calculated based on the weight distributions of aggregate-size classes represented the recovery, when compared with the respective values of bulk soil.

The significance of differences between C_{mic} and enzyme activities in bulk soil and the three aggregate-size classes under ambient and elevated CO_2 treatments was examined using

Table 1

Aggregate-size distribution, their C content (C_{tot}) and amount of microbial biomass (C_{mic}) in bulk soil and soil aggregates in ambient (amb) and elevated (elev) CO_2 concentrations. The recovery of microbial biomass-C in soil aggregates was calculated based on the weights distribution of aggregates in bulk soil. Values are averages of four field replicates. Treatments followed by the same letters are not significantly different between elevated and ambient CO_2 (uppercase letters) and aggregate size classes (lowercase letters) at $p \leq 0.05$ (according to two-way-ANOVA and Fischer LSD test).

Aggregate-size classes/ soil	Weights distribution, %		C content, mg g^{-1} soil (aggregates)		Microbial biomass					
					mg C g^{-1} soil (aggregates)		C_{mic} / C_{tot}		Recovery, %	
	amb	elev	amb	elev	Amb	elev	amb	elev	amb	elev
>2 mm	34 ^{Ab}	31 ^{Ab}	14.89 ± 0.42 ^{Aab}	14.75 ± 0.30 ^{Aa}	0.340 ± 0.025 ^{Aa}	0.353 ± 0.023 ^{Aa}	0.023	0.024	26.7	25.0
0.25-2 mm	58 ^{Ac}	59 ^{Ac}	15.43 ± 0.49 ^{Aab}	16.25 ± 0.20 ^{Ab}	0.453 ± 0.016 ^{Ab}	0.460 ± 0.024 ^{Ab}	0.029	0.028	60.6	61.8
<0.25 mm	8 ^{Aa}	10 ^{Aa}	15.14 ±0.19 ^{Aab}	15.38 ± 0.24 ^{Aab}	0.566 ± 0.031 ^{Ac}	0.585 ± 0.004 ^{Ac}	0.038	0.038	10.5	13.4
bulk	100	100	15.57 ± 0.15 ^{Aab}	16.16 ± 0.48 ^{Ab}	0.434 ± 0.026 ^{Ab}	0.438 ± 0.013 ^{Ab}	0.028	0.027	97.8	100.2

Two-Way ANOVA with two independent factors "CO_2" and "aggregates". The Fischer LSD post hoc test was used to separate treatments at the $p<0.05$ significance level.

3. Results

3.1. Aggregate distribution and C content

The weight distribution among the aggregate-size classes of the bulk soil was as follows: small macroaggregates (0.25-2 mm) contributed 58-59%, large macroaggregates (>2 mm) 31-34 % and microaggregates (<0.25 mm) 8-10% of the weight of bulk soil (Table 1). The atmospheric CO_2 enrichment did not affect the distribution of aggregates in the different size classes (Table 1). The amount of C_{org} in each aggregate class was similar to the C_{org} of the bulk soil. Only the aggregate fraction >2 mm under elevated CO_2 treatment had significantly lower amount of C_{org} as compared to the bulk soil and to the fraction 0.25-2 mm under the same CO_2 treatment (Table 1).

3.2. Microbial biomass distribution

The specific amount of C_{mic} (mg C g^{-1} aggregates) increased in aggregates as follows: large macroaggregates < small macroaggregates < microaggregates. Thus, the smallest class (microaggregates), amounting to about 10% of the total weight of a soil sample and having 12% of the total microbial biomass content, showed the highest specific amount of C_{mic}. The microbial C content (mg C g^{-1} aggregates) was 0.566 ± 0.031 under ambient CO_2 and 0.585 ± 0.004 under elevated CO_2 treatment. The smallest specific C_{mic} amount of 0.340 ± 0.025 and 0.353 ± 0.023 was found in large macroaggregates under ambient and elevated CO_2, respectively. The portion of C_{mic} in the C content of aggregates increased as aggregate size decreased (Table 1). Elevated CO_2 tended to increase C_{mic} both in bulk soil and in the three aggregate-size classes, but this increase was not significant. Cumulative recovery of C_{mic} in aggregates was nearly 100% of the bulk soil under ambient and elevated CO_2 (Table 1).

3.3. Extracellular enzyme activities

Enzyme activities without substrate amendment. The extracellular enzyme activities were differently distributed through aggregate-size classes as well as in the bulk soil under both CO_2 treatments (Fig. 1). Bulk soil activities (864.6 ± 37.6 and 869.6 ± 30.6 $nmol^{-1}$ g^{-1} soil h^{-1}) were the highest for β-glucosidase under ambient and elevated CO_2 treatments, respectively. The lowest activities in bulk soil under both treatments corresponded to sulphatase and amounted to 4.4 ± 0.7 and 4.3 ± 0.7 $nmol^{-1}$ g^{-1} soil h^{-1}, respectively (Fig. 1).

Elevated CO_2 did not significantly change the activities of β-glucosidase, phosphatase and sulphatase in bulk soil, but did significantly increase chitinase activity there.

Activities of extracellular enzymes were lower in micro- compared to macroaggregates, except β-glucosidase, whose activity was the highest in microaggregates under ambient CO_2 treatment (Fig. 1). CO_2 enrichment decreased the enzyme activities in microaggregates. Significantly lower activities in microaggregates under elevated vs. ambient CO_2 were found for β-glucosidase and chitinase. In contrast to microaggregates, CO_2 enrichment tended to increase activities in macroaggregates, although significantly higher values were detected only for chitinase in large macroaggregates (Fig. 1).

Enzyme activities after substrate amendment. Adding glucose significantly increased the activity of all studied enzymes in bulk soil and in the three aggregate-size classes. Moreover, the response of enzymes was always considerably higher under elevated CO_2 (Fig. 1, Fig. 2). Thus, after amendment of bulk soil under ambient CO_2, the activity of β-glucosidase, chitinase, phosphatase and sulphatase increased 1.7, 2.4, 1.8 and 6.0 times, respectively. Simultaneously, the respective increases in bulk soil under elevated CO_2 treatment was 3.2, 3.3, 2.3 and 7.3 times (Fig. 1, Fig. 2). A similar trend of greater enzyme response to glucose addition was observed in soil aggregates under elevated compared to ambient CO_2. Furthermore, microaggregates responded more than macroaggregates under both treatments (Fig. 1).

Recovery of enzyme activities in aggregate-size classes. The total enzyme activities in isolated aggregates were recalculated for bulk soil based on aggregate weight distributions (Fig. 2). The contribution of activities in aggregates to overall activity of bulk soil coincided with the weight distribution of aggregate-size classes: the highest activity of the four enzymes occurred in 0.25-2 mm (the heaviest class by weight), the lowest in <0.25 mm (lightest class). Total activities in each aggregate class without glucose amendment were similar under both treatments. However, adding glucose and nutrients to bulk soil and soil aggregates significantly increased total activities of four enzymes, and the increase was especially pronounced under elevated CO_2 treatment (Fig. 2). The sieving approach we used was chosen as less destructive as compared to other conventional sieving approaches. This was proved by the nearly 100% recoveries for microbial biomass in isolated aggregates (Table 1). The variation of recoveries of enzyme activities in soil aggregates was somewhat larger and varied from 80 to 130%. Because of a high sensitivity to any disturbance the

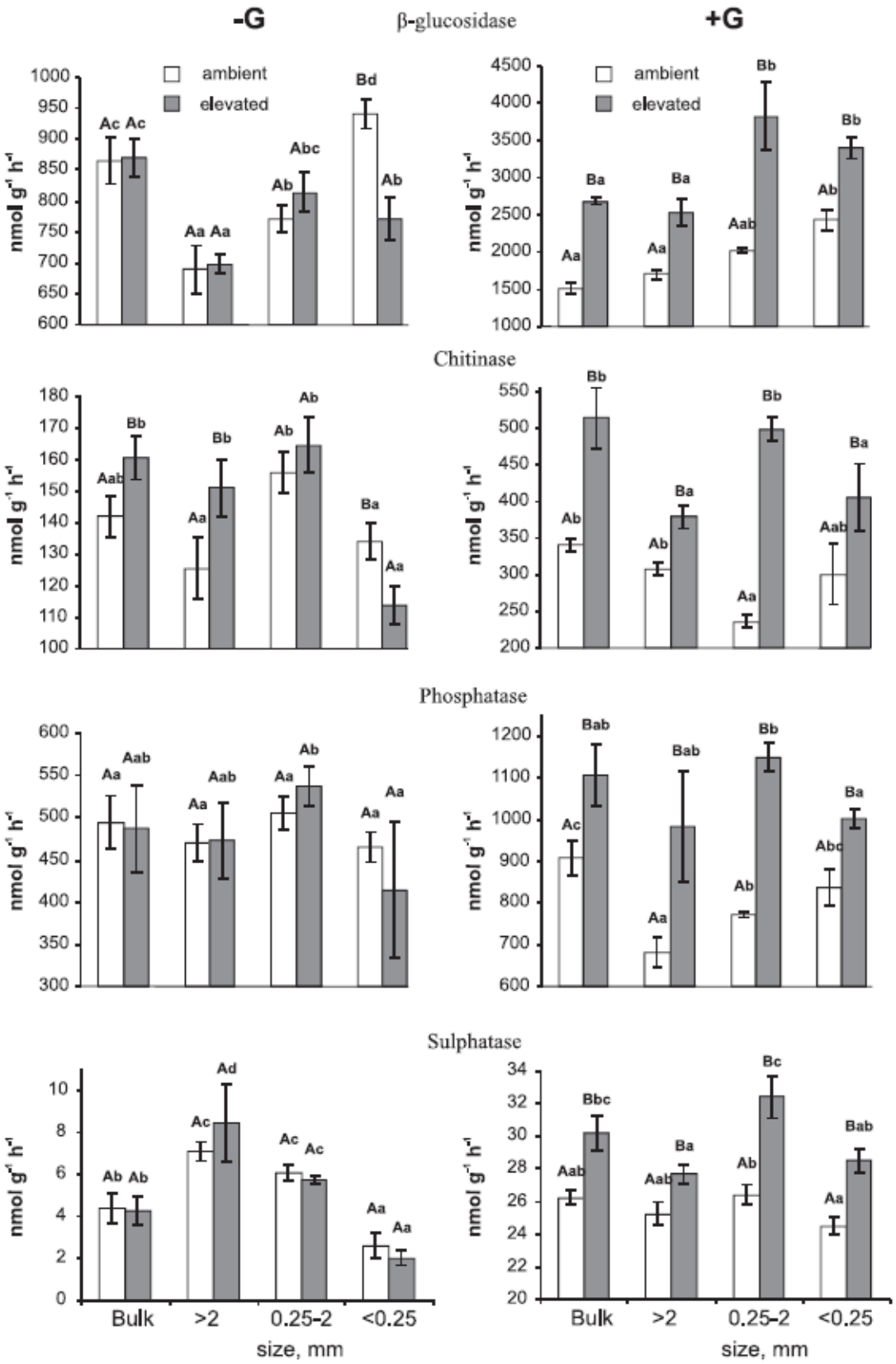

Fig. 1. Activities of β-glucosidase, chitinase, phosphatase and sulphatase in bulk soil and three aggregate-size classes under ambient and elevated CO_2 treatments before (-G) and after (+G) activation with glucose and nutrients. Values are averages of four replicates (± SE). Treatments followed by the same letters are not significantly different between elevated and ambient CO_2 (uppercase letters) and aggregate size classes (lowercase letters) at $p \leq 0.05$.

enzyme activities were overestimated (for β-glucosidase after glucose addition 20%; for sulphatase without glucose addition 30%) or underestimated (for chitinase after glucose addition 20%; for phosphatase after glucose addition under ambient CO_2 15%; for β-glucosidase without glucose addition 10%). The discrepancy was especially pronounced when specific enzyme activities were considered (Fig.1). However, when to be recalculated on the total activities, the discrepancy in recoveries for the most of enzymes did not exceed 10% (Fig. 2).

4. Discussion

4.1. Aggregate-size fractionation

Soil structure controls SOM turnover because it influences gas exchange, water content and solute transport, which in turn determine microbial growth and activity (Stotzky, 1997). Sorption onto mineral surfaces and incorporation into soil aggregates could protect the SOM from microbial decomposition for shorter or longer periods of time (Six *et al*., 2002; Kristiansen *et al*., 2006; Jastrow *et al*., 2007). To study the relationship between soil aggregates and SOM turnover, the bulk soil has to be fractionated into its constituent aggregates using some disruptive technique. The aggregate-size fractionation procedure used in the current study was chosen because it is gentler than conventional wet and dry sieving techniques (Kristiansen *et al*., 2006). Thus, wet-sieving procedure releases water soluble OM and dispersible colloids, causing changes in aggregate composition (Emerson & Greenland, 1990; Watts *et al*., 1996). Furthermore, complete soil watering could change the activity of soil microorganisms through anaerobic conditions (Kieft *et al*., 1987; Zhang & Zak, 1998). In turn, prolonged sieving of air-dry soil tends to increase aggregate abrasion rather than fragmentation due to the great tensile strength of dry aggregates (e.g. Munkholm & Kay, 2002). Similar to Kristiansen *et al*. (2006), we fragmented the soil into constituent aggregates when a water content of individual clods and peds was near the lower plastic limit, corresponding to the optimum water content of medium textured soils at which soil friability is maximal (Dexter & Bird, 2001). Thereby we maximized brittle fracturing along natural planes of weakness while minimizing changes in aggregate-size distribution (Kristiansen *et al*., 2006). Since the approach used is considerably distinguished from conventional wet- and dry sievings, we propose to determine such aggregate-size fractionation approach as **optimal moist sieving.**

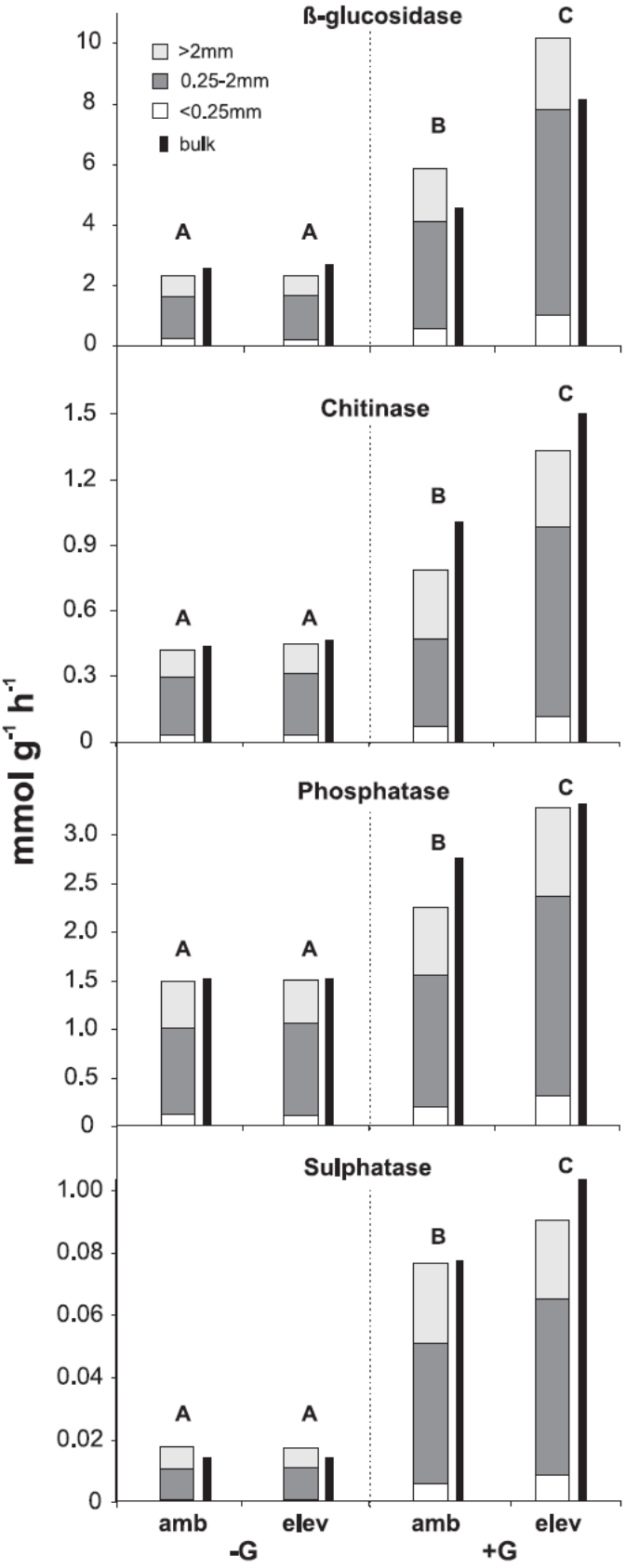

Fig. 2. Cumulated enzyme activities in three aggregate-size classes as compared to enzyme activities of bulk soil under ambient and elevated CO_2. The results of actual (-G, without glucose and nutrients addition) and potential enzyme activities (+G, after glucose and nutrients addition) are presented. Treatments followed by the same letters are not significantly different between elevated and ambient CO_2 (uppercase letters) at $p \leq 0.05$.

The aggregate weight distribution detected here – microaggregates < large macroaggregates < small macroaggregates – coincided with fractionation results obtained for other agricultural soils (Fansler *et al*., 2005; Kristiansen *et al*., 2006). The small macroaggregate class (0.25-2 mm) was found to dominate in arable silty soils mostly due to the effect of tillage (Vaisanen *et al*., 2005).

Five years of elevated CO_2 did not affect the distribution of the aggregate-size classes in our study. The results of similar studies showed both increase (Rillig *et al*., 1999; van Groeningen *et al*., 2002) and decrease (Del Galdo *et al*., 2006; Niklaus *et al*., 2007) of elevated CO_2 on soil aggregation. The lack of the difference in soil aggregation between ambient and elevated CO_2 in our study is explained by the tillage of experimental plots. Tillage had drastically higher direct effect on aggregate-size formation than the possible indirect effects of elevated atmospheric CO_2.

4.2. Microbial biomass in soil aggregates under ambient and elevated CO_2

The allocation of microorganisms in soil aggregates differs when using the wet vs. dry sieving separation procedure. Thus, Chotte *et al*. (1998) and Guggenberger *et al*. (1999) found higher microbial biomass in macroaggregates than in microaggregates separated by wet sieving. Miller & Dick (1995) reported higher values in macroaggregates isolated by dry sieving. Our results coincide with Askin & Kizilkaya (2006) and Santruckova *et al*. (1993), who found more microorganisms in microaggregates than in macroaggregates separated by dry sieving. Contrasting literature results could reflect the differences in aggregate separation by wet and dry sieving. Fractionation is clearly a destructive procedure and some microorganisms are transferred from the surfaces of larger to smaller aggregates (dry sieving) or adhere to aggregates during slaking in water (wet sieving), persisting in macroaggregates (Ashman *et al*., 2003). The optimal moist sieving approach is less destructive (Kristiansen *et al*., 2006), hence minimizes microorganism losses or redistribution between aggregate separates. Thus, cumulative recovery of C_{mic} in aggregates reached 97.8% as compared to C_{mic} in the bulk soil under ambient CO_2 and 100.2% under elevated CO_2 (Table 1).

More microbial biomass in micro- compared to macroaggregates could be explained by the contrasting environment of differently sized aggregates. The location of microorganisms within the soil matrix is a key factor for their survival (Chenu *et al*., 2001). Small pores dominate within microaggregates, whereas both small and large pores occur in macroaggregates (Jastrow *et al*., 2007). Small pores, in turn, enhance survival by protecting

microorganisms from predation by Protozoa (Vargas & Hattori, 1986; Postma & van Veen, 1990; Wright *et al*., 1993) or from desiccation (Nishiyama *et al*., 1992). Furthermore, microaggregates likely have texture different from macroaggregates (Bronick & Lal, 2005). Clay particles can also directly affect microbial survival in soils (van Gestel *et al*., 1996). Amato & Ladd (1992) studied relationships between physical and biological properties for 23 soils, concluding that, along with the location of microorganisms in small pores of aggregates, adsorption of microbial cells to the surfaces of clay particles was crucial for microbial biomass accumulation in soils.

The effect of elevated CO_2 on the microorganism content of soils was reported to vary from positive (elevated CO_2 increased soil microbial biomass) (e.g. Zak *et al*., 1993; Drissner *et al*., 2007) to no effect (e.g. Kampichler *et al*., 1998; Niklaus *et al*., 2003). Our results support numerous studies that also failed to find a significant response of microbial biomass to elevated CO_2. Moreover, we observed no changes in microbial biomass under elevated vs. ambient CO_2 in isolated soil aggregates; this indicates that CO_2 enrichment does not affect soil structure–microorganism interactions. We therefore conclude that total microbial biomass is an insensitive parameter to evaluate small changes caused by elevated CO_2.

4.3. Extracellular enzyme activities in bulk soil and isolated aggregates under ambient and elevated CO_2

4.3.1. Enzyme activities in bulk soil without substrate amendment

The activities of three studied enzymes, β-glucosidase, phosphatase and sulphatase, did not differ between ambient and elevated CO_2 in bulk soil. CO_2 enrichment has been reported to have diverse effects on soil enzyme activities. Studies on grassland ecosystems found both increased (Ebersberger *et al*., 2003; Drissner *et al*., 2007) and decreased activities (Kampichler *et al*., 1998) under elevated CO_2. Lower enzyme activities were found in modeled (artificial) ecosystem of different grass species exposed to elevated CO_2 (Henry *et al*., 2005). As reviewed by Freeman *et al*. (2004), many experiments found no difference in soil enzyme activities. In our experiment, however, N-acetylglucosaminidase (chitinase) was the only enzyme that showed higher activity in bulk, non-amended soil under elevated vs. ambient CO_2 (Fig. 1). The results of another research group working on the same experimental site (FACE-Hohenheim) support our findings. They measured the activities of α- and β-glucosidase, N-acetyl-glucosaminidase, xylosidase, cellobiosidase, phosphatase, L-leucin- und L-tyrosin-peptidase in bulk soil sampled in 2002-2005 from plots under both

treatments. All showed no significant treatment-related differences, except a trend to higher chitinase activity under elevated CO_2 (Marhan, personal communication), which we found to be significant in our measurements. The increased chitinase activity in soil mostly reflects higher fungal abundance (Chung *et al*., 2007). Miller *et al*. (1998) documented a significant positive relationship between chitinase activity and fungal biomass. In their study, N-acetylglucosaminidase was produced by a diverse group of fungi, but not by any of bacterial species. Our findings support those authors, suggesting an increased role of fungi compared to bacteria in SOM turnover under elevated CO_2 (Rillig *et al*., 1999; Treseder & Allen, 2000).

4.3.2. Enzyme activation in bulk soil after substrate amendment

Since microbial activity in soil is controlled by the availability of easily decomposable organic substrates (Friedel *et al*. 1996; Klose *et al*. 1999), glucose amendment activates microbial growth because it is readily degradable (Anderson & Domsch, 1973; Lin & Brookes, 2000). In turn, activation of microbial biomass boosted soil respiration and enzyme activities (Dilly & Nannipieri, 2001). Our addition of glucose and mineral salts to bulk soil significantly increased the activity of four studied enzymes. Renella *et al*. (2007) studied the activities of hydrolases (acid and alkaline phosphomonoesterase, phosphodiesterase, urease, protease, and β-glucosidase) in seven soils with a wide range of properties, in which microbial growth was stimulated by adding glucose and nitrogen. Similar to our study, the authors reported a significantly higher response of enzyme activities to substrate amendment after 60 h of incubation as compared to non-amended soil (Renella *et al*., 2007). This increased enzyme activity after adding glucose and nutrients (N, P) was determined in a number of other studies as well (Dilly & Nannipieri, 2001; Vong *et al*., 2003; Aira *et al*., 2005).

As we expected, glucose amendment revealed the potential differences in SOM turnover under elevated vs. ambient CO_2: activities were higher in the former. This conclusion is novel because little information is available on the effect of atmospheric CO_2 enrichment on enzyme activities in soil amended with easily available substrate and nutrients. The strong increase of enzyme production by activated microbial biomass under elevated CO_2 explains why C stock and microbial biomass content often change only insignificantly in the soil under CO_2 enrichment (Freeman *et al*., 2004; van Kessel *et al*., 2006) despite significant increase of roots (Paterson *et al*., 1997; Rogers *et al*., 1998) and rhizodeposition (van Veen *et al*., 1991; Cheng, 1999). Higher belowground translocation of

C by plants under these conditions (Rogers *et al*., 1994), and especially rhizodeposition (Cheng, 1999), increases microbial activity, which we evaluated based on activity of extracellular enzymes. Accordingly, the contribution of potentially active microorganisms to enzyme production increases under elevated CO_2. This enables the microbial community to utilize and decompose available substrates faster. Thus, the higher input of root-C will be compensated and occasionally overcompensated (Zak *et al*., 1993) by higher decomposition rates and probably lower use efficiency. This fine balance between higher C input and its faster decomposition explains the varying results (no or low changes of C_{org} and C_{mic}) in soil under elevated atmospheric CO_2. At the same time, our results show that the functional rather than the descriptive parameters are more useful to explain sensitive changes in ecosystems under elevated atmospheric CO_2.

4.3.3. Enzyme activities in soil aggregates

Enzyme activities showed different trends depending on aggregate size and atmospheric CO_2 concentration. Despite the observed highest activity of β-glucosidase in microaggregates under ambient CO_2 treatment, the activities of chitinase, phosphatase, sulphatase and β-glucosidase (under elevated CO_2) were higher in macro- than in microaggregates (Fig. 1). This supports the findings suggesting quantitative and qualitative SOM differences between the two sizes classes (reviewed in Jastrow *et al*., 2007). Thus, more labile SOM is concentrated in macroaggregates, more recalcitrant SOM in microaggregates (Jastrow *et al*., 2007). Gupta & Germida (1988) as well as Miller & Dick (1995) concluded that macroaggregate structures provide habitat for soil microorganisms and enzyme activity.

The insignificant increase of enzyme activities in soil aggregates under elevated CO_2 was fully revealed after activation by glucose. Microbial activity in soil aggregates under elevated CO_2 is very poorly studied: no studies are directly comparable with our results. Nonetheless:

(i) Extracellular enzyme activity in **macroaggregates** (0.25-2 mm and >2 mm) was higher under elevated vs. ambient CO_2. As described earlier, elevated CO_2 stimulates root growth (Paterson *et al*., 1997; Rogers *et al*., 1998) and rhizodeposition (van Veen *et al*., 1991; Cheng, 1999). Extra C transferred by plants belowground is preferentially allocated in macroaggregates (Jastrow *et al*., 2007), increasing microbial activity and hence extracellular enzyme activity. Especially pronounced increase was detected for chitinase. A similar increase of chitinase activity in macro- vs. microaggregates was reported in experiments not devoted to elevated CO_2 (Guggenberger *et al*., 1999; Vaisanen *et al*., 2005). The authors

explained the higher chitinase activity by the preference of fungi for macroaggregates as more appropriate living space in comparison to microaggregates. Furthermore, the significantly higher chitinase activity in macroaggregates under elevated CO_2 detected in our study indicates the increasing role of fungi in decomposition of SOM under elevated CO_2 conditions. Since lignin and cellulose content increase in plants grown under elevated compared to ambient CO_2 (Cotrufo *et al*., 1994) and decomposition of those compounds is mediated mainly by fungi (Kirk & Farrel., 1987), the increase of chitinase activity in our study confirms an altered functional structure of the soil microbial community under elevated CO_2.

(ii) In contrast to macroaggregates, substrate amendment changed the activities of enzymes in **microaggregates** (<0.25 mm) in reverse: being higher before substrate amendment, after amendment the activities of four studied enzymes significantly decreased under ambient vs. elevated CO_2 treatment. We explain this by the different biochemical quality of SOM occluded in microaggregates in the two treatments. However, this is true only if the lifespan of microaggregates is considerably shorter than the time from the initialization of the FACE experiment until the FACE-derived plant inputs occlude into microaggregates This requirement is fulfilled in our study because 5 years passed after the FACE experiment was initiated, whereas microaggregate turnover was probably less than 100 days (De Gryze *et al*., 2006). Assuming an altered biochemical quality of SOM occluded in microaggregates under elevated CO_2, we expect a subsequent shift of microbial community structure to fast-growing microorganisms. Such microorganisms benefit from the increased labile rhizodeposits, yet when removed from the ecosystem in a soil sample they lose this supply and become dormant (lower enzyme activities in microaggregates under elevated vs. ambient CO_2 before substrate amendment). Adding glucose and nutrients to microaggregates stimulated fast-growing microorganisms, boosting enzyme activities under elevated vs. ambient CO_2. This hypothesis should be tested using other methods to determine microbial growth strategies.

Conclusions

Estimation of enzyme activities in bulk soil and aggregate fractions after 5 years of elevated CO_2 lead to the following conclusions:

- The pools of C_{org} and microbial biomass in soil were not affected by 5 years of elevated CO_2. However, increased enzyme production after stimulating the

microorganisms confirm accelerated C turnover under elevated CO_2. This effect was pronounced in aggregates of different size.

- Aggregate-size fractionation using optimal moist sieving satisfactorily separated the bulk soil into micro- and macroaggregates with different properties. This fractionation technique had minimal mechanical impact on the biological properties of the separates, as demonstrated by a high recovery of microbial biomass and extracellular enzyme activities.
- Macroaggregates had higher specific and total enzyme activities indicating a potentially more intensive SOM turnover in comparison with microaggregates;
- Elevated atmospheric CO_2 stimulated enzyme activity especially in macroaggregates. The effect was particularly pronounced for chitinase, indicating a higher contribution of fungi to litter decomposition under elevated CO_2.
- Glucose and nutrient amendment to bulk soil and isolated aggregates revealed the potential differences in microbial activity under elevated vs. ambient CO_2. Increased enzyme activities in the former due to substrate amendment represented a shift in microbial community function rather than in its total content.

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VII) Summary

During the past two centuries, the CO_2 concentration in the atmosphere has increased by 35%, mainly because of fossil fuel combustion and land-use changes. The concern over the possible consequences of increasing concentrations of atmospheric CO_2 has gained widespread attention of researchers in the last few decades. Free-air CO_2 enrichment (FACE) experiments provide an opportunity to examine many aspects of elevated CO_2 effects on ecosystems, and to study the pathways of C and N in SOM under realistic field conditions. However, it is not clear whether soil C input and soil C mineralization could lead to soil C accumulation (sequestration), and how N addition could influence C turnover under elevated CO_2. Use of stable-C and N isotopic tracers and SOM fractionation techniques allow us to track and to understand the process of C and N transformations in soil under future altered environmental conditions. Along with stable isotopic techniques, the approaches investigating the activity and composition of microbial biomass promote further understanding of SOM transformations, since the availability of SOM for decomposition by soil microorganisms is a crucial property regarding to SOM turnover in terrestrial ecosystems. Therefore SOM fractions isolated based on their chemical recalcitrance and/or physical protection by means of occlusion in aggregates should be considered in respect of availability for decomposition by the soil microbial biomass.

In the present thesis the C and N transformations in soil as influenced by indirect effects of elevated atmospheric CO_2, soil physical structure and land use change were studied in four laboratory experiments using stable-C and N isotopes, as well as soil microbiological techniques.

To test the interrelations between chemical and biological characteristics of SOM as affected by land use change and elevated atmospheric CO_2 an approach for SOM partitioning based on its thermal stability was chosen. In the first experiment C isotopic composition of soils subjected to C_3-C_4 vegetation change (grassland to *Miscanthus x gigantheus*, respectively) was used for the estimation of C turnover in SOM pools. In the 2nd (FACE Hohenheim) and 3rd (FACE Braunschweig) experiments CO_2 applied for FACE was strongly depleted in ^{13}C and thus provided an opportunity to study C turnover in SOM based on its $\delta^{13}C$ value. Simultaneous use of ^{15}N labeled fertilizers allowed N turnover to be studied (in the 2nd experiment). We hypothesized that the biological availability of SOM pools expressed as the mean residence time (MRT) of C or N is inversely proportional to

their thermal stability. Soil samples were analysed by thermogravimetry coupled with differential scanning calorimetry (TG-DSC). According to differential weight losses between 20 and 1000 °C (dTG) and energy release or consumption (DSC), SOM pools (4 to 5 depending on experiment) with increasing thermal stability were distinguished. Soil samples were heated up to the respective temperature and the remaining soil was analyzed for $\delta^{13}C$ and $\delta^{15}N$ by IRMS. In all experiments the weight losses in the first pool decomposed from 20 to 190-200 °C were connected with water evaporation, since no significant C and N losses were measured and $\delta^{13}C$, $\delta^{15}N$ values remained unchanged. Other findings, depending on the experiment, were:

1. The $\delta^{13}C$ of pools 190-310 °C and 310-390 °C in soil samples under *Miscanthus* were closer to the $\delta^{13}C$ of the *Miscanthus* plant tissues (-11.8‰) compared to the thermally stable SOM pool 480-1000 °C (-19.5‰). The portion of the *Miscanthus*-derived C_4-C in total SOM in 0-5 cm reached 55.4% in the 10.5 years. The C_4-C contribution in pool 190-310 °C was 60% and decreased down to 6% in pool 480-1000 °C. The MRT of SOM pools 190-310, 310-390, and 390-480 °C were similar (11.6, 12.2, and 15.4 years, respectively), while pool 480-1000 °C had a MRT of 163 years.
2. The maximum weight losses occurred from 200-310 °C. This pool contained the largest amount of carbon: 61% of the total organic C in soil under ambient treatment and 63% in soil under elevated CO_2, respectively. $\delta^{13}C$ values of SOM pools under elevated CO_2 treatment showed an increase from -34.3‰ of the pool decomposed between 20-200 °C to -18.1‰ above 480 °C. The incorporation of new C and N into SOM pools was not inversely proportional to its thermal stability. SOM pools that decomposed between 20-200 and 200-310 °C contained 2 and 3% of the new C, with a MRT of 149 and 92 years, respectively. The pool decomposed between 310-400 °C contained the largest proportion of new C (22%), with a MRT of 12 years. The amount of fertilizer-derived N after 2 years of application in ambient and elevated CO_2 treatments was not significantly different in SOM pools decomposed up to 480 °C having MRT of about 60 years. In contrast, the pool decomposed above 480 °C contained only 0.5% of new N, with a MRT of more than 400 years in soils under both treatments.
3. The maximum weight losses and C content were measured in the SOM pool decomposed at 200-360 °C. The largest amount of N was detected in SOM pools decomposed at 200-360 and 360-500 °C. In all temperature ranges the $\delta^{13}C$ values of SOM pools were significantly more negative under elevated CO_2 vs. ambient CO_2. The

incorporation of new C into SOM pools was not inversely proportional to its thermal stability. 50% N fertilization treatment gained higher C exchange under elevated CO_2 in thermally labile SOM pool (200-360 °C), whereas 100% N treatment induced higher C turnover in thermally stable SOM pools (360-500, 500-1000 °C). Mean Residence Time of SOM under 100% N and 50% N fertilization showed no dependence between SOM pools isolated by increasing temperature of heating and the renovation of organic C in those SOM pools.

Thus, for all three experiments the separation of SOM based on its thermal stability was not sufficient to reveal pools with contrasting turnover rates of C and N. A possible explanation for the inability of thermal oxidation for isolating SOM pools of contrasting turnover times is that the fractionation of SOM pools according to their thermal stability is close to chemical separation. In turn, it was found that chemical separations of SOM failed to isolate the SOM pools of different turnover time because different biochemical plant components (cellulose, lignin) are decomposed in a wide temperature range. Individual components of plant residues may be directly incorporated into, or even mixed with, the thermal stable SOM pools and will so mask low turnover rates of these pools.

To evaluate the interactions between availability of SOM for decomposition by soil microbial biomass (biological characteristic) under elevated atmospheric CO_2 and protection of SOM due to the occlusion within aggregates of different sizes (physical property, responsible for SOM sequestration) we measured the activity of microbial biomass (indicated by enzyme activities) and growth strategies of soil microorganisms (fast- vs. slow growing organisms) in isolated macro- and microaggregates. To fractionate the soil to its constituent aggregates the optimal moist sieving was chosen because it was proven to be gentler than conventional wet and dry sieving techniques. We fragmented the soil into constituent aggregates – large macro- (>2 mm), small macro- (0.25-2 mm) and microaggregates (<0.25 mm) – when a water content of individual clods and peds was near the lower plastic limit, corresponding to the optimum water content of medium textured soils at which soil friability was maximal. Thereby we maximized brittle fracturing along natural planes of weakness while minimizing changes in aggregate-size distribution.

The contribution of fast (*r*-strategists) and slowly growing microorganisms (K-strategists) in microbial communities was estimated by the kinetics of the CO_2 emission from bulk soil and aggregates amended with glucose and nutrients (Substrate Induced Growth Respiration method). Using SIGR the kinetic parameters of microbial growth – the maximal specific growth rate (μ) of microorganisms, the amount of active microbial biomass

(AMB), and the duration of lag-period (t_{lag}) – were calculated. Along with microbial growth strategies the microbial enzyme activities representing the main steps of soil biogeochemical nutrient cycles, i.e. C (β-1.4-glucosidase, N-acetyl-β-D-glucosaminidase), N (N-acetyl-β-D-glucosaminidase), P (phosphatase) and S (sulphatase) were measured in the same bulk soil and aggregates under ambient and elevated CO_2 treatments using a microplate system combined with 4-methylumbelliferone-labelled fluorogenic substrates. Since we measured enzyme activities not only before but also after determination of microbial growth strategies, the amendment of glucose and nutrients activated microorganisms and allowed us to estimate the potential enzyme production.

Although C_{org} and C_{mic} were unaffected by elevated CO_2, μ values were significantly higher under elevated vs. ambient CO_2 for bulk soil, small macro- and microaggregates. In soils under both ambient and elevated CO_2, the minimal microbial growth rates were found for macroaggregates and the highest for microaggregates. This indicates more available substrate which was former physically protected in microaggregates. Sharp decrease of AMB under elevated CO_2 resulted in extension of lag-period. Based on μ values, AMB and duration of lag-period, an increased activity of microorganisms with *r*-strategy under elevated atmospheric CO_2 was detected. This increase was especially pronounced in soil microaggregates.

The activities of β-glucosidase, phosphatase and sulphatase were unaffected in bulk soil and in aggregate-size classes by elevated CO_2, however, significant changes were observed in potential enzyme production after substrate amendment. After adding glucose, enzyme activities under elevated CO_2 were 1.2-1.9-fold higher than under ambient CO_2. This indicates an increased activity of microorganisms, which leads to accelerated C turnover in soil under elevated CO_2. Significantly higher chitinase activity in bulk soil and in large macroaggregates under elevated CO_2 revealed an increased contribution of fungi to turnover processes. At the same time, less chitinase activity in microaggregates underlined microaggregate stability and the difficulties for fungi hyphae penetrating them. We conclude that quantitative and qualitative changes of C input by plants into the soil at elevated CO_2 affect microbial community functioning, but not its total content. Future studies should therefore focus more on the changes of functions and activities, but less on the pools.

In conclusion, elevated CO_2 concentrations in the atmosphere along with soil physical structure have a pronounced effect on qualitative but not quantitative changes in C and N transformations in soil under agricultural ecosystem. The physical parameters of soil such as aggregation correlate more with biological availability of SOM than the chemical properties

of soil organic materials. The increase of soil microbial activity under elevated CO_2 detected especially in soil microaggregates, which are supposed to be responsible for SOM preservation, prejudice sequestration of C in agroecosystems affected by elevated atmospheric CO_2.

VIII) Zusammenfassung

Die CO_2-Konzentrationen in der Atmosphäre sind in den vergangenen zwei Jahrhunderten aufgrund der Verbrennung fossiler Brennstoffe und Landnutzungsänderungen um 35% gestiegen. Den möglichen Konsequenzen dieses globalen CO_2-Anstiegs ist in den vergangenen Jahrzehnten viel Aufmerksamkeit geschenkt worden. Die Verwendung von kammerlosen Freilandexpositionssystemem, sog. Free Air CO_2 Enrichment (FACE), stellt eine gute Möglichkeit dar, ökosystemare Effekte steigender CO_2-Konzentrationen zu untersuchen und den Weg von Kohlenstoff und Stickstoff in die organische Bodensubstanz (soil organic matter, SOM) unter realistischen Freilandbedingungen zu verfolgen. Es ist noch ungeklärt, ob der C-Eintrag in den Boden und die C-Mineralisierung zu einer Kohlenstoffanreicherung (C-Sequestrierung) führen und wie die N-Versorgung den Kohlenstoffumsatz unter erhöhtem CO_2 beeinflusst. Die Verwendung stabiler C- und N-Isotope und SOM-Fraktionierungstechniken ermöglichen es, C- und N-Umsätze in Böden zu verfolgen und zu verstehen. Zusätzliche Erkenntnisse zu SOM-Umsätzen lassen sich über die Aktivität und Zusammensetzung der mikrobiellen Biomasse gewinnen. Dabei ist die Verfügbarkeit der SOM für Bodenmikroorganismen von entscheidender Bedeutung für den SOM-Turnover in terrestrischen Ökosystemen. Diese Verfügbarkeit der SOM hängt von chemischen Charakteristika und/oder physikalischer Protektion aufgrund des Einschlusses in Aggregate ab; daher sollten verschiedene SOM-Fraktionen im Hinblick auf ihre Verfügbarkeit für mikrobielle Dekomposition getrennt untersucht werden.

In der vorliegenden Dissertation wurde anhand von vier Laborversuchsreihen untersucht, wie C- und N-Transformationen in Böden indirekt durch erhöhte atmosphärische CO_2-Konzentrationen, durch die physikalische Bodenstruktur und durch Änderungen der Bodennutzung beeinflusst werden. Dabei kamen die Analyse stabiler C- und N-Isotopen sowie bodenmikrobiologische Techniken zum Einsatz.

Um die Relationen chemischer und biologischer Charakteristika von SOM unter dem Einfluss von Bodennutzungsänderungen und erhöhten atmosphärischen CO_2-Konzentrationen zu erfassen, wurde eine SOM-Fraktionierung anhand ihrer thermischen Stabilität durchgeführt. In der ersten Versuchsreihe wurde der C-Umsatz in SOM-Pools anhand der Zusammensetzung der stabilen C-Isotope in Böden nach einem Wechsel des Bestandes von C_3-Arten zu C_4-Arten (temperates Grünland zu *Miscanthus x gigantheus*) analysiert. In der zweiten (FACE Hohenheim) und dritten (FACE Braunschweig)

Versuchsreihe wurde CO_2 zur Konzentrationserhöhung benutzt, das deutlich in der ^{13}C-Signatur abgereichert war und daher als Tracer für den C-Umsatz in den SOM pools genutzt werden konnte. Gleichzeitig wurde (im FACE Hohenheim) der Stickstoffdünger mit ^{15}N markiert und ermöglichte es, den N-Umsatz zu verfolgen. Als Arbeitshypothese sollte getestet werden, ob die biologische Verfügbarkeit von SOM Pools (ausgedrückt als mittlere Verweildauer, mean residence time MRT für C und N) umgekehrt proportional zu ihrer thermischen Stabilität ist. Die Bodenproben wurden mittels Thermogravimetrie, gekoppelt mit differenzieller Scanning Kalorimetrie (TG-DSC), analysiert. Anhand der differenziellen Gewichtsverluste im Temperaturbereich zwischen 20 und 1000 °C und der Energieabgabe bzw. –Aufnahme wurden je nach Versuchsreihe 4 bzw. 5 SOM Pools mit steigender thermischer Stabilität unterschieden. Die Bodenproben wurden bis zur jeweiligen Temperatur aufgeheizt und der verbleibende Rest jeweils auf ^{13}C- und ^{15}N-Gehalte mittels IRMS untersucht. In allen Versuchsreihen waren die Gewichtsverluste im Temperaturbereich zwischen 20 und 190-200 °C durch Wasserevaporation begründet, da keine signifikanten Veränderungen in den C- und N-Gehalten auftraten und die $\delta^{13}C$- und $\delta^{15}N$-Werte unverändert blieben. Andere Ergebnisse, je nach Versuchsreihe, waren:

1. Die $\delta^{13}C$-Wert der C-Pools in den Böden unter *Miscanthus* aus den Temperaturfraktionen 190-310 °C und 310-390 °C lagen näher an den $\delta^{13}C$-Werten in den *Miscanthus*-Geweben (-11.8‰) als derjenige des thermisch stabilen SOM-Pool im Bereich 480-1000 °C (-19.5‰). Der Anteil von C aus *Miscanthus*- C_4-C an der gesamten SOM in der Bodentiefe 0-5 cm erreichte 55.4% in 10,5 Jahren. Der C_4-C-Anteil im Pool 190-310 °C betrug 60% und nur noch 6% Im Pool 480-1000 °C. Die MRT der SOM-Pools 190-310, 310-390 und 390-480 °C waren vergleichbar (11,6, 12,2 und 15,4 Jahre), während der Pool 480-1000 °C eine MRT von 163 Jahren hatte.
2. Der maximale Gewichtsverlust erfolgte im Bereich 200-310 °C. Dieser Pool enthielt den größten C-Anteil: 61% des gesamten organischen Boden-C unter Kontroll-CO_2 und 63% unter erhöhtem CO_2. Die $\delta^{13}C$-Werte der SOM-Pools unter erhöhtem CO_2 stiegen von -34.3‰ im von 20-200 °C zersetzten Pool bis auf -18.1‰ im Pool über 480 °C. Die Inkorporation neuer C- und N-Vorräte in die SOM-Pools war nicht umgekehrt proportional zu deren thermischer Stabilität. Die von 20-200 °C und von 200-310 °C zersetzten SOM-Pools enthielten 2 und 3% neuen Kohlenstoff mit einer MRT von 149 und 92 Jahren. Der von 310-400°C zersetzte Pool enthielt den höchsten Anteil an neuem C (22%) mit einer MRT von 12 Jahren. Der Anteil an Stickstoff aus Düngung nach 2 Jahren unterschied sich in normalem und erhöhtem CO_2 nicht

signifikant in den einzelnen SOM-Pools, die bis 480 °C zersetzt wurden. Die MRT betrug hier ca. 60 Jahre. Demgegenüber enthilet der Pool über 480 °C nur 0.5% neuen Stickstoff mit einer MRT von mehr als 400 Jahren in beiden CO_2-Behandlungen.

3. Der größte Gewichtsverlust und der höchste C-Gehalt wurden im SOM-Pool mit Zersetzung bei 200-360 °C gemessen. Die größten Stickstoffanteile wurden in den SOM-Pools mit Zersetzung bei 200-360 und 360-500 °C gefunden. In allen Temperaturbereichen waren die $\delta^{13}C$-Werte der SOM-Pools unter erhöhtem CO_2 signifikant negativer als unter normalem CO_2. The Inkorporation neuer C-Vorräte in SOM-Pools war nicht umgekehrt proportional zu deren thermischer Stabilität. Eine Reduktion der N-Applikation auf 50% führte zu einem höheren C-Austausch unter erhöhtem CO_2 im SOM-Pool mit Zersetzung bei 200-360 °C, während volle N-Applikation einen höheren C-Umsatz in den thermisch stabilen SOM-Pools (360-500, 500-1000 °C) bewirkte. Die MRT der SOM zeigte sowohl bei 100% als auch bei 50% N-Versorgung keine Abhängigkeit zwischen den thermisch isolierten SOM-Fraktionen und der Erneuerung des organischen Kohlenstoffs in diesen Pools.

Die Fraktionierung von SOM anhand ihrer thermischen Stabilität war somit in allen drei Versuchsreihen nicht ausreichend, um Pools mit unterschiedlichen Umsatzraten von C und N zu differenzieren. Eine mögliche Erklärung dafür kann sein, dass die thermische Stabilität von SOM-Pools eng mit ihrer chemischen Struktur verbunden ist. Entsprechend liegen Befunde vor, dass es anhand einer chemischen Fraktionierung von SOM-Pools nicht möglich war, Pools mit unterschiedlichen Turnover-Raten zu separieren, da die verschiedenen Pflanzenbestandteile (Zellulose, Lignin) in einem weiten Temperaturbereich zersetzt werden. Einzelne Inhaltsstoffe der Pflanzenrückstände können direkt in die thermisch stabilen SOM-Pools inkorporiert oder sogar mit ihnen vermischt werden, so dass potenziell geringere Turnover-Raten maskiert werden.

Um die Interaktionen zwischen der Verfügbarkeit von SOM für mikrobielle Zersetzung (biologische Eigenschaften) und dem Schutz von SOM aufgrund des Einschlusses innerhalb von Bodenaggregaten unterschiedlicher Größe (physikalische Eigenschaften, verantwortlich für die SOM-Sequestrierung) unter erhöhtem atmosphärischem CO_2 zu untersuchen, wurden die Aktivität der mikrobiellen Biomasse anhand von Enzymaktivitäten und die Wachstumsstrategien der Bodenmikroorganismen (schnellwüchsige vs. langsamwüchsige Organismen in isolierten Makro- und Mikroaggregaten gemessen. Zur Fraktionierung der Bodenproben in ihre konstituierenden Bestandteile wurde eine Siebung unter optimaler Feuchtigkeit angewandt, da diese Methode

als schonender als konventionelle Nass- und Trockensiebungstechniken herausstellte. Der Boden wurde in die Aggregatklassen große Makroaggregate (>2 mm), kleine Makroaggreate (0.25-2 mm) und Mikroaggregates (<0.25 mm) bei einem Wassergehalt der einzelnen Schollen am unteren plastischen Grenzbereich fraktioniert, was einem optimalen Wassergehalt von Böden mittlere Textur entspricht, um maximale Bröckeligkeit zu erreichen. Auf diese Weise konnten die Brüchigkeit entlang natürlicher Schwächeebenen maximiert und gleichzeitig Änderungen in der Aggregatgrößenverteilung minimiert werden.

Der Anteil schnellwüchsiger (r-Strategen) und langsamwüchsiger (K-Strategen) Mikroorganismen an der mikrobiellen Gemeinschaft wurde anhand der Kinetik der CO_2-Emission aus dem Gesamtboden und den Bodenaggregaten nach Glukose- und Nährstoff-Applikation bestimmt (Substratinduzierte Wachstumsrespirationsmethode, SIGR). Über SIGR wurde die kinetischen Wachstumsparameter der mikrobiellen Biomasse – die maximale spezifische Wachstumsrate (μ), der Anteil aktiver mikrobieller Biomasse (AMB) und die Dauer der Lag-Periode (t_{lag}) – berechnet. Zusammen mit den mikrobiellen Wachstumsstrategien wurden die Aktivitäten der wichtigsten mikrobiellen Enzyme für die biogeochemischen Stoffkreisläufe bestimmt, d.h. für C die β-1.4-Glucosidase und die N-Acetyl-β-D-Glucosaminidase, für C und N die N-Acetyl-β-D-glucosaminidase), für P die Phosphatase und für S die Sulphatase. Diese Enzyme wurden mittels eines Mikroplattensystems in Kombination mit 4-Methylumbelliferon-markierten fluorogenen Substraten in den gleichen Bodenfraktionen (Gesamtboden und Aggregate) aus normalem und erhöhtem CO_2 bestimmt. Da die Messungen der Enzymaktivitäten nicht nur vor, sondern auch nach der Bestimmung der mikrobiellen Wachstumsstrategien erfolgten, konnte durch die Zugabe von Glukose und Nährelementen auch die potenzielle Enzymaktivität der aktivierten mikrobiellen Biomasse erfasst werden.

Obwohl C_{org} und C_{mic} unter erhöhtem CO_2 unbeeinflusst blieben, waren die μ-Werte durch den CO_2-Anstieg im Gesamtboden, in den kleinen Makroaggregaten und in den Mikroaggregaten signifikant erhöht. Sowohl unter normalem als auch unter erhöhtem CO_2 wurden die geringsten moikrobiellen Wachstumsraten in Makroaggregaten und die höchsten in Mikroaggretagen gemessen. Dies deutet auf vermehrt verfügbares Substrat hin, das vorher physisch geschützt war. Ein steiler Abfall der AMB unter erhöhtem CO_2 führte zu einer Verlängerung der lag-Periode. Anhand der μ-Werte, der AMB und der Dauer der lag-Periode wurde eine erhöhte Aktivität von Mikroorganismen mit r-Strategie unter erhöhtem CO2 festgestellt. Dieser Aktivitätszuwachs war am deutlichsten in den Mikroaggregaten.

Die Aktivitäten von β-Glucosidase, Phosphatase und Sulphatase waren unter erhöhtem CO_2 sowohl im Gesamtboden als auch in den einzelnen Aggregatklassen unverändert. Es traten aber signifikante Veränderungen in den potenziellen Enzymaktivitäten nach Aktivierung durch Glukose- und Nährstoffaddition auf. Nach Glukose-Addition lagen die Enzymaktivitäten unter erhöhtem CO_2 1,2 - 1,9fach höher als in den Kontrollen. Dies deutet auf gesteigerte Aktivität der Mikrorganismen und beschleunigten C-Umsatz in den Böden unter erhöhtem CO_2. Die Chitinase-Aktivität war im Gesamtboden und in den großen Makroaggregaten unter CO_2-Anstieg signifikant höher und belegt einen gestiegenen Anteil von Pilzen an den Umsetzungsprozessen. Gleichzeitig lag die Chitinase-Aktivität in den Mikroaggregaten niedriger, was die Stabilität der Mikroaggregate und ihre schlechte Erreichbarkeit für Pilzhyphen unterstreicht. Zusammenfassend kann postuliert werden, dass quantitative und qualitative Veränderungen im C-Eintrag durch Pflanzen in den Boden unter erhöhtem CO_2 auftreten, welche die Funktion, nicht aber die Gesamtmenge der mikrobiellen Gemeinschaft beeinflussen. Künftige Studien sollten daher vermehrt auf Funktionen und Aktivitäten und weniger auf die Pools fokussiert werden.

Steigende CO_2-Konzentrationen in der Atmosphäre haben deutliche Auswirkungen auf qualitative, weniger aber auf quantitative Aspekte der C- und N-Umsätze in Böden von Agrarökosystemen. Physikalische Bodenparameter wie die Aggregation korrelieren stärker mit der biologischen Verfügbarkeit von SOM als die chemischen Eigenschaften des organischen Materials. Die erhöhte mikrobielle Aktivität unter erhöhtem CO_2, die vor allem in den für die Konservierung von SOM wichtigen Bodenmikroaggregaten gefunden wurde, beeinträchtigt eine potenzielle C-Sequestrierung in Agrarökosystemen unter steigenden atmosphärischen Kohlendioxidkonzentrationen.

IX) Acknowledgements

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I am most grateful to my family and my wife Maria who always gave me motivation to get the work finished.

X) Curriculum Vitae

Maxim Dorodnikov

PERSONAL DATA

Born:	April 2, 1980, Nizhnekamsk, Russia
Marital Status:	married to Maria Serebrennikova, son Roman Dorodnikov, born 18.05.07
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DIPLOMAS and DEGREES

12/08 Anticipated Doctoral Degree (Dr.sc.agr.) in Soil Science and Agroecosystem Research, University of Hohenheim, Stuttgart. Dissertation: "Pathways of C and N turnover in soils under elevated atmospheric CO_2". Supervisors: Prof. Dr. A. Fangmeier (Institute of Landscape and Plant Ecology, University of Hohenheim), Prof. Dr. Y. Kuzyakov (Agroecosystem Research, University of Bayreuth)

9/06 Certificate of successful participation in the ESF Summerschool "Experimental assessment of changes in soil organic matter pools in mountain forests", University Center in Obergurgl, Tyrol, Austria, September 22th-30th 2006

2/06 Certificate of successful participation in the DBG-advanced training "Application of isotopes in the study of soil processes" (Einsatz von Isotopen in der bodenkundlichen Prozessforschung), 40 h, University of Hohenheim, Stuttgart, February 8th-12 2006

9/04 Certificate of successful participation in the ESF Summerschool "Integrated Methodology on soil carbon flux measurements", Centro di Ecologia Alpina, Mt. Bondone, Italy, September 12th-24th 2004

7/04 Postgraduate degree (Soil Science and Agrochemistry). Institute of Physico-Chemical and Biological Problems in Soil Science, Russian Academy of Sciences, Puschino, Russia. Thesis: "*The influence of elevated concentrations of CO_2 in the atmosphere on the active pool of soil organic matter*". Grade: "Excellent "

6/02 M.Sc. in Geography (Sustainable Land Management). Oryol State University, Oryol, Russia. M.Sc. Thesis: "*Evaluation of recreation resources in Bolkhov district of Oryol Region* ". Grade: "Excellent "

6/97 School leaving certificate, Dolinnoye Middle School, Crimea, Ukraine (Grade: 4.9 of maximal 5)

EDUCATION AND PROFESSIONAL EXPERIENCES

4/06-at present	Ph.D.-study on the Faculty of Agricultural Sciences, University of Hohenheim, Stuttgart. A part of work is carrying out at the Department of Agroecosystem Research, University of Bayreuth.
4/06-at present	Temporary work as a scientific assistant (HiWi) in the University of Hohenheim and University of Bayreuth
8/05-4/06	Scholarship holder of the German Academic Exchange Service (DAAD) in the Institute of Soil Science and Land Evaluation, University of Hohenheim, Stuttgart. Project: “The influence of elevated atmospheric CO_2 on the amount and activity of soil microbial biomass”
10/02–7/05	Engineer, Institute of Physico-Chemical and Biological Problems in Soil Science, Puschino, Russia
9/02-7/04	Postgraduate study in the Institute of Physico-Chemical and Biological Problems in Soil Science, Russian Academy of Sciences, Puschino, Russia
9/97-7/02	Study in Oryol State University, Faculty of Biochemistry, Department of Geography
9/86-6/97	Full Middle Education (Abitur) in Dolinnoye Middle School, Crimea, Ukraine

Advanced Courses

2/07 Education Module “Methods of Scientific Work” as a part of Ph.D.-study in the University of Hohenheim by Prof. D.E. Leihner and Prof. G. Cadisch

9/06 ESF Summerschool "Experimental assessment of changes in soil organic matter pools in mountain forests", University Center in Obergurgl, Tyrol, Austria, September 22th-30th 2006

2/06 DBG-advanced training "Application of isotopes in the study of soil processes" (Einsatz von Isotopen in der bodenkundlichen Prozessforschung), 40 h, University of Hohenheim, Stuttgart, February 8th-12 2006

9/04 ESF Summerschool "Integrated Methodology on soil carbon flux measurements", Centro di Ecologia Alpina, Mt. Bondone, Italy, September 12th-24th 2004

SPECIALIZATION in SOIL SCIENCE and ECOLOGY

CO_2 fluxes, C sequestration, nutrient cycling, C and N transformation in soils, soil organic matter, stable and radioactive isotopes, soil microbial biomass, effects of elevated atmospheric CO_2

PUBLICATIONS

18 publications in referred (7) and not referred (11) scientific journals (the list of publications is attached)

AWARDS

4.06/9.08 Ph.D. Fellowship of the Government of Baden-Wuertemberg (Landesgraduierteförderung)

8.05/4.06 German Academic Exchange Service (DAAD) Fellowship

9.03/7.04 Fellowship of the Government of Moscow Region

PROJECTS PARTICIPATION

10.07/at present DFG WI 2810/4-1: "Veränderungen der Lipidzusammensetzung und der Isotopie von Lipiden in Pflanzen und Böden unter erhöhter atmosphärischer CO_2-Konzentration (FACE)". Collaborators: G. Wiesenberg, M. Dorodnikov

4.06/9.08 Land BW: "Pathways of C and N turnover in soils under elevated atmospheric CO_2". Collaborators: M. Dorodnikov, A. Fangmeier, Y. Kuzyakov

8.05/4.06 DAAD: "The influence of elevated atmospheric CO_2 on the amount and activity of soil microbial biomass" (Einfluss erhöhter CO_2-Konzentration auf die Menge und die Aktivität mikrobieller Biomasse im Boden). Collaborators: M. Dorodnikov, Y. Kuzyakov

LANGUAGES

Russian	native
English	good (TOEFL iBT Certificate 90 pt of 120 possible, from 04/06)
German	communicative (Mittelstufe)

LIST OF PUBLICATIONS:

Referred Journals

2008

Dorodnikov M., Blagodatskaya E., Blagodatsky S., Marhan S., Fangmeier A., Kuzyakov Y. Stimulation of microbial extracellular enzyme activities by elevated CO_2 depends on aggregate size. Global Change Biology. accepted doi: 10.1111/j.1365-2486.2009.01844.x.

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2006

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2005

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2000

Trofimets L.N., Dorodnikov M.V. “Highways as a part of ecologically adverse territories”. Proceedings to the Conference “Study of Local Lore and Tourism: Problems, Searching, Perspectives”. Oryol, 57, 2000.