Impacts of atmospheric CO_2-enrichment on the functional diversity of collembolans and nematodes in an agroecosystem

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Poster

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Vorwort

Im Rahmen der vorliegenden Dissertation wurde der Einfluss des atmosphärischen CO_2-Anstiegs auf die funktionelle Diversität der Collembolen- und Nematodengemeinschaft eines Agrarökosystems unter Anbau von Zuckerrüben und Winterweizen zu jeweils zwei Pflanzenentwicklungsstadien untersucht. Die stabile C-Isotopenanalyse der verschiedenen Collembolenarten und Ernährungstypen der Nematoden lieferte Einblicke in veränderte Interaktionen und den C-Transport innerhalb des Bodennahrungsnetzes unter zukünftigen Bedingungen. Da die Auswirkungen des atmosphärischen CO_2-Anstiegs auf Agrarökosysteme von maßgeblicher Wichtigkeit für die zukünftige, nachhaltige Ernährungssicherung, und bisher wenig verstanden sind, liefert die vorliegende Arbeit im Kontext des Klimawandels und Biodiversitätsschutzes einen wichtigen Beitrag zur Verbesserung des Kenntnisstandes hinsichtlich von Bodenprozessen.

Die Dissertation gliedert sich in drei Teile. Der erste Teil umfasst die Einleitung, unterteilt in 6 Unterkapitel, in der der Hintergrund der durchgeführten Untersuchungen dargelegt und erläutert wird, sowie eine kurze Darstellung der Ziele der Dissertation.

Den zweiten Teil der Arbeit stellen drei rezensierte Publikationen dar, in denen Teile der durchgeführten Untersuchungen in international renommierten Fachzeitschriften veröffentlicht wurden. Der erste Artikel (2.1) behandelt eine Methodenstudie hinsichtlich der Anwendbarkeit von Aufbereitungs-, Konservierungs- und Fixierungsverfahren für Collembolen und Nematoden im Rahmen der stabilen C-Isotopenanalyse. Der zweite Artikel (2.2) umfasst die Beeinflussung der Collembolenarten und –lebensformtypen, der dritte (2.3) die der Ernährungstypen der Nematoden durch den atmosphärischen CO_2-Anstieg.

Eine integrierende Diskussion der gesamten Ergebnisse sowie Schlussfolgerungen und Ausblicke bilden den dritten Teil der Arbeit.

Anteil der Autoren an der Arbeit und den Artikeln

An der Veröffentlichung der Ergebnisse der Arbeit waren Herr Prof. Dr. Hans-Joachim Weigel als Initiator und Hauptverantwortlicher des FACE-Versuchs und Leiter des Institutes für Biodiversität des Johann Heinrich von Thünen Institutes (vTI); Frau Dr. Anette Giesemann als Leiterin der Arbeitsgruppe für stabile Isotopenanalysen am Institut für Agrarrelevante Klimaforschung des vTI; sowie Prof. Dr. Stefan Schrader als Leiter der Arbeitsgruppe „Funktionelle Bodenzoologie“ des Institutes für Biodiversität des vTI, und Mentor der vorliegenden Dissertation, als Co-Autoren beteiligt. Die Entwicklung des Konzepts, dass im Rahmen der Dissertation verfolgt wurde, alle Ideen und Arbeiten, die Auswertung der Ergebnisse, sowie das Ausarbeiten und Verfassen der Artikel wurden von Frau Dipl. Biol. Christine Sticht durchgeführt.

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Summary

Within the present thesis, impacts of atmospheric CO_2-enrichment on the collembolan and nematode community of an agroecosystem, cultivated in a crop rotation, were analysed. The study was part of a long-term CO_2-enrichment field experiment (FACE: Free Air Carbon Dioxide Enrichment), which was performed at the Johann Heinrich von Thünen Institute (vTI) in Braunschweig. Samples were taken twice each season (during the period of main plant growth and shortly before harvest) under cultivation of sugar beet (2004) and winter wheat (2005). Within the study, CO_2-effects on abundance, diversity and stable C-isotopic signatures ($\delta^{13}C$) of collembolan species and nematode feeding types were analysed. As the stable C-isotopic analysis of taxonomically or functionally classified collembolans and nematodes represents a new research approach, influences of agents generally used during sample preparation on the $\delta^{13}C$ values of animals, and their applicability prior to stable C-isotopic analyses, were analysed.

The results reveal CO_2-enrichment induced changes of food availability and quality in the rhizosphere to affect the soil fauna in arable soil. In this context, modified microbial community compositions and activities, as well as changing quantities and qualities of exudates presumably represent the most important controlling factors. Intensity and manner of impacts, thereby, strongly depend on crop type and plant developmental stage and vary between species and functional groups of the soil fauna according to their food specificity and adaptability. Generally, stronger CO_2-effects under sugar beet compared to winter wheat cultivation, decreasing impacts with increasing trophic distances of organisms to primary producers, and stronger influences on specialists compared to generalists were detected. The results reveal the taxonomical and functional diversity of the soil fauna, and thus nutrient availability and finally soil fertility to change under future atmospheric CO_2-concentrations, depending on cultivated crop and season.

The present study provides insights into CO_2-enrichment induced alterations of C-translocation and interactions within the soil food web and, thus, in the context of climate change and agrobiodiversity conservation, contributes to improving knowledge of changes of below-ground processes, which are to be expected in the future.

Zusammenfassung

Im Rahmen der vorliegenden Dissertation wurde der Einfluss des atmosphärischen CO_2-Anstiegs auf die Collembolen- und Nematodengemeinschaft eines, unter Fruchtwechsel kultivierten, Agrarökosystems untersucht. Die Arbeit war Teil eines Freiland-CO_2-Anreicherungsversuchs nach dem FACE-Prinzip (Free Air Carbon Dioxide Enrichment), der auf dem Gelände des Johann Heinrich von Thünen Institutes (vTI) in Braunschweig durchgeführt wurde. Die Beprobungen erfolgten in den Jahren 2004 und 2005 unter Zuckerrüben- und Winterweizenanbau, jeweils während der Hauptwachstumsphase und kurz vor der Ernte der Kulturpflanzen. Analysiert wurde der CO_2-Effekt auf die Abundanz, die Diversität sowie die stabilen C-Isotopensignaturen ($\delta^{13}C$) der Collembolenarten und Ernährungstypen der Nematoden. Da die stabile C-Isotopenanalyse taxonomisch oder funktionell klassifizierter Collembolen und Nematoden einen neuen Forschungsansatz darstellte, wurden im Vorfeld der Untersuchungen verschiedene, praxisüblich bei der Probenaufbereitung verwendete Agenzien hinsichtlich ihres Einflusses auf die $\delta^{13}C$-Werte der Tiere, und somit hinsichtlich ihrer Anwendbarkeit im Rahmen von C-Isotopenanalysen untersucht.

Die vorliegenden Untersuchungen belegen, dass die Bodenfauna in Agrarökosystemen über CO_2-induzierte Veränderungen der Nahrungsverfügbarkeit und -qualität in der Rhizosphäre beeinflusst wird. Die Ergebnisse weisen darauf hin, dass Modifikationen der Zusammensetzung und Aktivität der mikrobiellen Gemeinschaft, sowie der Quantitäten und Qualitäten der Wurzelexsudate in diesem Zusammenhang die wichtigsten auslösenden Faktoren darstellen. Art und Intensität der Beeinflussung hängen stark von der Kulturpflanze und ihrem Entwicklungsstadium ab und variieren entsprechend der jeweiligen Ernährungsspezifität und Anpassungsfähigkeit zwischen verschiedenen Arten und funktionellen Gruppen der Bodenfauna. So wurde eine stärkere Beeinflussung unter Zuckerrüben-, verglichen mit Winterweizenanbau, eine Abnahme des CO_2-Effektes mit zunehmender trophischer Distanz der Organismen zu den Primärproduzenten und eine stärkere Beeinflussung von Spezialisten im Vergleich zu Generalisten nachgewiesen. Die Ergebnisse belegen, dass sich unter zukünftigen CO_2-Konzentrationen die taxonomische und funktionelle Diversität der Bodenfauna, und als Folge dessen auch die Nährstofffreisetzung und letztlich die Bodenfruchtbarkeit, in Abhängigkeit von der Kulturpflanze und ihrem Entwicklungsstadium ändert.

Die vorliegende Arbeit liefert Einblicke in CO_2-induzierte Veränderungen des C-Transportes und der Interaktionen innerhalb des Bodennahrungsnetzes und leistet somit im Kontext des Klimawandels und Agrobiodiversitätsschutzes einen wichtigen Beitrag zur Verbesserung des Kenntnisstandes hinsichtlich zukünftig zu erwartender Veränderungen von Bodenprozessen.

Part 1

1.1 Introduction

1.1.1 Climate change and atmospheric CO_2-enrichment

Atmospheric greenhouse gases (mainly: carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), sulphur hexafluoride (SF6), hydrofluorocarbons (HFCs), and perfluorocarbons (PFCs)) sustain the average temperature on the earth's surface at a constant level of around 15 degrees Celsius by preventing the heat emitting from the earth from disappearing into space. This effect, referred to as natural greenhouse effect, establishes the basis for the development of all life on earth.

As a consequence of industrialization, the concentration of greenhouse gases, which originally accounted for only 3 ‰ of the total mass of the atmosphere, has risen strongly over the past 150 years. The radiation balance changes due to this rapid increase, whereby the atmosphere is heating up to an unnaturally high level. This non-natural anthropogenic greenhouse effect is continuously supported by industry, traffic, private households, agriculture, and land use changes. Thus, global anthropogenic greenhouse gas emissions increased by about 70 % from 1970 to 2004 (IPCC, 2007b).

Concerning this anthropogenic greenhouse effect, particular attention is directed to the steadily increasing share of carbon dioxide (CO_2), which is unavoidably released during fossil fuel burning. This enrichment of CO_2 in the atmosphere is additionally supported by the decline of the CO_2-sink capacity of terrestrial systems, which originally extracted about one third of combustion-released atmospheric CO_2 (Canadell et al., 2007). Consequently, global CO_2-emissions have risen by about 80 % from 1970 to 2004 (IPCC, 2007b). According to forecasts, if this trend continues atmospheric CO_2-concentrations will reach values of 450-500 μmol mol^{-1} in about 50 years (IPCC, 2001), and of 500-1000 μmol mol^{-1} at the end of the 21st century (Fung et al., 2005).

International measures for climate protection

As progressing climate changes are driven by global causes, a long-term trans-national cooperation and the division of responsibility between states are indispensable fundamentals to implement effective climate protection measures. To ensure internationally co-ordinated approaches, the United Nations Framework Convention on Climate Change (UNFCCC), an international, multilateral environmental protection agreement, was launched in 1992, as part of the "Agenda 21", on the United Nations Conference on Environment and Development (UNCED) in Rio de Janeiro (UNFCCC, 1992). The ultimate objective of this convention is to

achieve the stabilization of greenhouse gas concentrations in the atmosphere at a level that would prevent dangerous anthropogenic interference with the climate system. The time frame set to reach this level, should thereby allow the adaptation of ecosystems to climate change. This way, global warming should slow down, the consequences of climate change should be mitigated, and the maintenance of sufficient food production should be ensured.

The Framework Convention on Climate Change, which entered into force on March 21, 1994, establishes the basis for continuous international negotiation processes concerning climate protection. This political process is accompanied and assisted by the IPCC (Intergovernmental Panel on Climate Change) scientific committee.

A milestone in international climate policies is the Kyoto Protocol (UNFCCC, 1998), which was initially adapted for use on the third Conference of the Parties (COP 3) in 1997, and which entered into force on February 16, 2005 as an internationally binding agreement. The Kyoto Protocol establishes legally binding commitments, especially for the "Annex I" nations (mainly responsible industrialised countries), for the reduction of the emissions of the six most important greenhouse gases (CO_2, CH_4, N_2O, SF_6, HFCs, and PFCs) between 2008 and 2012 by 5.2 % against the 1990 level. Until January 2009, 184 Parties, which in total are responsible for 63.7 % of global greenhouse gas emissions, have ratified, acceded, approved or accepted the Protocol.

National measures for climate protection

Following the international climate protection efforts, Germany committed itself to reduce its CO_2-emissions by 25 % until 2005 compared to 1990 levels at the first conference of the parties in Berlin (1995). To attain this objective, the "National Climate Protection Programme of the Federal Republic of Germany" was adapted by the German government in the year 2000. This programme was elaborated, updated and finally followed by the valid "Climate Protection Programme 2005". As part of the EU burden-sharing under the Kyoto Protocol (UNFCCC, 1998), this programme commits to a 21 % reduction in German greenhouse gas emissions in the period 2008-2012 as compared to 1990 levels. For the same period, a joint reduction target for total CO_2-emissions was set at 844 million tonnes per year (BMU, 2005).

To develop and estimate possible adaptation measures, prospective studies were pursued to assess impacts of climate change on, for example, German agriculture (Schaller & Weigel, 2007), which is of major importance in the context of climate change (see Chapters 1.1.3 and 1.1.4). Since, nonetheless, up to now several uncertainties exist with regard to underlying climate-process relationships, there is still a need for research. In this context, the present

study contributes to improving knowledge of CO_2-enrichment-induced changes of below-ground processes in arable soils.

1.1.2 Biodiversity in the context of climate change

The term "biodiversity" involves the diversity within species (genetic diversity), between species, and of ecosystems (CBD, 1992). Biodiversity is essential for maintaining life-sustaining systems of the biosphere, such as, for instance, the regulation of climate, water balance, or soil formation (BMELV, 2007). Moreover, several ecosystem functions, and hence services, directly depend on the biological diversity within systems (Naeem et al., 2007). Thus, the conservation of biodiversity is of utmost relevance to ensure the provision of numerous ecosystem services required by humans (BMELV, 2007).

Climatic changes, directly or indirectly, influence species distribution (Araújo & Rahbek, 2006), interactions between species (Emmerson et al., 2005, Tylianakis et al., 2008), their genetic constitution, and structures of ecosystems (Blankinship & Hungate, 2007; IPCC, 2007b). Thus, more sensitive species often have no possibility to elude climate-induced habitat changes, disruptions, or other global change-associated stressors, like, for example, land use changes or overexploitation of natural resources. According to the fourth assessment report of the IPCC (IPCC, 2007a), roughly 20-30 % of plant and animal species are expected to be at increased risk of extinction if global temperatures exceed 2 to 3°C above pre-industrial levels. As ecosystems represent functionally complex networks which show a strong interdependence between species, due to multitrophic interactions via food webs or element cycles (Tylianakis et al., 2008), the disappearance of a single species can involve the loss of others. Such substantial species losses have the potential to alter numerous ecosystem processes and functions, at worst resulting in the destabilization or disturbance of whole systems (Balmford & Bond, 2005; Emmerson et al., 2005).

In this context, highly specialized species which generally have more exacting metabolic or ecological requirements are considerably more vulnerable to habitat alterations than generalist species which are able to rapidly adjust themselves to changing conditions. Thus, distribution ranges and abundances of specialists would most probably decline under such scenarios, whereas generalists might profit and expand their ranges (Balmford & Bond, 2005). This selective removal of many sensitive, specialized, and often narrowly distributed species, coupled with increases in a small number of mostly cosmopolitan generalists, will lead to increased homogenization of biota (McKinney & Lockwood, 1999). Communities left behind

will be more resilient and resistant to external anthropogenic impacts (Balmford, 1996), but, however, comprise a decreased functional diversity (McKinney & Lockwood, 1999). Considering that functional diversity refers to the diversity of species traits, and thus to ecosystem functioning and the services a system provides, rather than taxonomic diversity, in particular the conservation of functional biodiversity is crucial to maintain ecosystem processes (Blankinship & Hungate, 2007; Naeem et al., 2007).

Our ability to predict where, when, and by which and how much changes in wild nature and biodiversity human well-being will be affected is limited. Too few data even on current losses exist, our knowledge of the dynamics of future changes is incomplete (Balmford & Bond, 2005), and our understanding of the complex linkages between biodiversity of natural systems, its functions within natural regulation, and its relevance concerning service provision is as yet rudimentary (Tylianakis et al, 2008). Nevertheless, the available evidence clearly indicates that numerous threatened species, regions, and habitats are closely associated to the maintenance of frequent human-required ecosystem services (Tylianakis et al., 2008). Consequently, predicted future losses of natural resources will corrupt human well-being considerably (Balmford & Bond, 2005). Thus, the sustainable conservation of biodiversity is of great importance, particularly in view of progressing climate change.

International measures for biodiversity protection

Two major international initiatives were launched in recent years to detect changing states of various ecosystems and their biodiversity; to assess consequential effects on human society and human welfare which are currently less understood (Balmford & Bond, 2005); to develop appropriate measures, and, thus, to guarantee the world-wide protection of biodiversity. The "Convention on Biological Diversity" (CBD) (CBD, 1992), which was adopted at the United Nations Conference on Environment and Development (Earth Summit) in Rio de Janeiro in 1992, and the "Millennium Ecosystem Assessment" (Millennium Ecosystem Assessment 2004), launched in June 2001, both strive to achieve these goals.

The three central targets of the CBD, which was signed by 190 parties and the European Community, and which entered into force in 1993, are to conserve biodiversity, to enhance its sustainable use, and to ensure an equitable sharing of benefits linked to the exploitation of genetic resources (CBD, 1992). In this context the CBD addresses both in-situ conservation, which focuses on conserving genes, species, and ecosystems in their natural surroundings, and ex-situ conservation, defined as the conservation of components of biological diversity outside their natural habitats, for example by means of gene banks or botanic gardens.

Moreover, existing uses, like agriculture or forestry, must comply with the sustainability principle (CBD, 1992). In September 2002, at the Johannesburg World Summit on Sustainable Development, representatives of 190 countries committed themselves to achieve a significant reduction of the current rate of biodiversity loss at the global, regional and national scale by 2010 ("2010 Biodiversity Target") (CBD, 2007). According to the UNEP (2002), this commitment represents a contribution to poverty alleviation and to the benefit of all life on earth, the central target of the CBD.

The Millennium Ecosystem Assessment (MA) (Millennium Ecosystem Assessment, 2004) bases on the cooperation of a large and diverse suite of natural and social scientists. Like the Intergovernmental Panel on Climate Change (IPCC), the MA assesses current knowledge, scientific literature, and data. It aims to provide decision makers and the public with a broad scientific evaluation of the consequences of current and projected ecosystem changes concerning human society and human well-being (Naeem et al., 2007), and, moreover, gives advice on political approaches for encountering those changes (Millennium Ecosystem Assessment, 2004). The MA, which is working at multiple scales from local to global, was established to meet the needs of already existing international conventions like the CBD, the "United Nations Convention to Combat Desertification" (UNCCD) which entered into force in 1996, and the "Ramsar Convention on Wetlands" (Balmford & Bond, 2005).

For the inner-European implementation of the CBD a "Biodiversity Action Plan" (European Commission, 2001a) was developed in 2001. This plan includes four central targets: (1) to conserve biological diversity within the European Union; (2) to protect global biodiversity; (3) to support the adaptation of biodiversity to climate change; and (4) to improve the knowledge base for conservation and sustainable use of biodiversity in the EU and globally.

During the "EU Summit" in Göteborg 2001, the EU heads of governments, moreover, committed themselves to halting the loss of biodiversity by 2010, according to the "2010 Biodiversity Target" of the CBD.

National measures for biodiversity protection

Concerning the loss of biodiversity in Germany, in particular the threat to species (increased probability of extinction) and the impairment or destruction of habitats represent major problems. In this context, intensive land use in agriculture; the discontinued agricultural use of ecologically valuable marginal land; the direct destruction and dissection of habitats; discharge of pollutants and nutrients; local deficits in forest management; leisure uses which

have an adverse impact on nature; non-sustainable fishing practices; increased proportions of invasive non-native species; and, not least, climate change, represent the main threats to biodiversity in Germany (BMU, 2007). To counteract these stressors, to guarantee the sustainable use of nature, and to protect biodiversity, the Federal Ministry for the Environment, Nature Conservation and Nuclear Safety (BMU) developed the "National Strategy on Biological Diversity" (BMU, 2007), which was adopted by the Federal Cabinet in November 2007. This comprehensive strategy specifies 330 quality targets and action objectives for all biodiversity-related topics and, moreover, involves about 430 measures for biodiversity protection. It fulfils Germany's obligations under Article 6 of the CBD (CBD, 1992), which states that "each contracting party shall develop national strategies, plans or programmes for the conservation and sustainable use of biological diversity or adapt for this purpose its existing strategies, plans or programmes" (BMU, 2007). The strategy serves to implement the CBD at the national level, and is targeted at the mobilization of all social forces with the aim of significantly minimizing and then halting the threat to biological diversity in Germany. Ultimately the current trend should be reversed in favour of an increase in biological diversity, including its typical regional peculiarities. In the overall strategy, equal consideration is given to ecological, economic and social aspects in keeping with the guiding principle of sustainability. Deadlines to achieve all strategies targets range from the immediate term through to the year 2050.

Furthermore, a system of indicators for assessing the impacts of climate change on biological diversity should be formulated and established by 2015 (BMU, 2007).

1.1.3 Key-role of agrobiodiversity in the context of climate change

Globally, approximately 28 % of the total land area (Nösberg & Long, 2006), and nearly 103 million ha land within the European Union (FAO-STAT, 2005; cit. ex Henle et al. (2008)), are covered by agroecosystems. These agricultural landscapes ensure the supply of food, wood, fibre, and renewable energy (Nösberg & Long, 2006), and, therefore, play a major role in human well-being. Moreover, these regions are of importance in the context of climate change as they account for 26-28 % of all terrestrial carbon storage (Nösberg & Long, 2006), contribute to the cycling of carbon, water, and nutrients on a continental scale (Weigel et al., 2006), and feedback on climate by means of land management-induced (fertilization, irrigation, tillage etc.) changes of physical land surfaces and biogeochemical cycles (e.g., Ogle et al., 2005).

Due to this broad range of functions, the protection of the biological diversity of these regions, which is referred to as agrobiodiversity, is relevant to the provision and performance of services essential to human survival. With regard to future food demands, which require increasing agricultural production to prospectively ensure food security for the continuously rising global population (Ingram et al., 2008), this importance is even likely to increase in future. As the adaptation of systems to altering conditions, like those expected, and to some extent already noticeable, under climate change, directly depends on a large species and gene pool (Loreau et al., 2001), the conservation and maintenance of agrobiodiversity is essential to ensure future human welfare.

Agrobiodiversity in this context is defined as the diversity in life forms used or able to be used directly or indirectly by humankind, in efforts to secure the resources vital to survival (BMELV, 2007). According to this definition, agrobiodiversity includes species- and genetic diversity of life forms whose preservation is directly linked to the implementation of basic human needs, like for example crops or livestock. Moreover, the biodiversity associated with these organisms that fulfils various utilization-relevant functions, is involved as well. This associated biodiversity is of major importance since the provision of services by most directly usable organisms strongly depends on their interspecific interactions and on the multifaceted functions of respective ecosystems. Thus, for instance, insects, which play a major role concerning pollination processes, or soil organisms (soil fauna and soil microorganisms), which directly influence soil fertility, represent crucial constituents of agrobiodiversity as well (BMELV, 2007).

The present study, which focuses on CO_2-enrichment effects on the soil fauna in arable soil (Papers 2.1, 2.2 and 2.3), therefore, contributes to supporting efforts on agrobiodiversity conservation by improving the understanding of future below-ground processes, necessary to develop appropriate future management measures.

International measures for agrobiodiversity protection

In the face of climate change and future global food demands, the conservation of agrobiodiversity represents a substantial aim of the CBD and an important aspect of the “2010 Biodiversity Target” (CBD, 2007). Thus, the Conference of the Parties of the CBD adopted a programme of work on agricultural biodiversity in 2000 (CBD, 2000). This programme consists of four elements (assessment, adaptive management, capacity-building, and

mainstreaming) and three cross-cutting initiatives (on pollinators, soil biodiversity and biodiversity for food and nutrition).

European-wide programs have been developed and laws have been passed in order to protect and conserve the biodiversity of arable landscapes (Henle et al., 2008). In this context, the "Biodiversity Action Plan for Agriculture" (European Commission, 2001b) was adopted by the European commission in 2001 under the Common Agricultural Policy of the European Union (CAP). This Action Plan complements the "Agri-Environmental Strategy", which is largely aimed at enhancing the sustainability of agro-ecosystems.

National measures for agrobiodiversity protection

In addition to the "National Strategy on Biological Diversity" (BMU, 2007), the German Federal Ministry of Food, Agriculture and Consumer Protection (BMELV) published the strategy "Conservation of Agricultural Biodiversity, Development and Sustainable Use of its Potentials in Agriculture, Forestry and Fisheries" in December 2007. The main targets of this strategy are to achieve a long-term conservation and a broader-based use of genetic resources; to achieve the sustainable use of agricultural biodiversity while protecting natural ecosystems and threatened species; to strengthen the international cooperation; and to achieve a globally coordinated strategy for the management of global resources (BMELV, 2007). Thereby, this strategy mainly focuses on the conservation of biodiversity which is directly or indirectly used for food, agriculture, forestry and fisheries.

Beside the conservation of these species and their genetic diversity, the strategy pursued the target of ensuring the sustainable use of agricultural systems, thereby contributing to the implementation of "Germany's Sustainability Strategy".

1.1.4 Agroecosystems under atmospheric CO_2-enrichment

Cultivated crops and crop yield

Agroecosystems, cultivated plants, and all agricultural production are directly and indirectly affected by climate change via rising temperatures, changing precipitation regimes, and increasing atmospheric CO_2-levels (IPCC, 2007a). Higher temperatures, for example, induce grain yield losses, mainly by shortening the life-cycle of crops and speeding the rate of development through grain-filling (Porter et al., 2007). Elevated atmospheric CO_2-concentrations, in contrast, stimulate photosynthesis (sugar beet: 45 %; winter wheat: 37 %) (Weigel et al., 2005; Ainsworth & Rogers, 2007), enhance growth and yield ("CO_2

fertilization effect"), and reduce canopy evapotranspiration (sugar beet: 21 %; winter wheat: 6 %) whereby the water-use efficiency is improved (Weigel et al., 2005). Moreover, CO_2-enrichment increases the total biomass production above- and below-ground (Weigel, 2005), changes rhizodeposition processes (Phillips et al., 2006b), and enhances the C-allocation to roots (Weigel, 2005) as well as the use efficiency of light and nitrogen (N) (Dijkstra et al., 2008). The structure of standing crops changes due to altered leaf area indices and accelerated branching (Weigel, 2005).

As our understanding of underlying factors and processes, causing the broad variability of CO_2-enrichment-induced crop responses, is as yet rudimentary, the consequences of climate change on the availability and nutritional quality of numerous foods have been uncertain up to now (Porter, 2007). In order to close existing gaps in knowledge, it is necessary to more strongly include ecosystem components others than cultivated crops, which received the most attention during previous studies. Such constituents might be of importance as they are affected by increasing atmospheric CO_2-concentrations as well and, moreover, have the potential to influence crops and yields via feedback effects and interactions.

In this context, soils and soil food webs, which are actively involved in decomposition processes and closely connected to nutrient availability, are of utmost relevance (Drigo et al., 2008; Pendall et al., 2008). As compositions of soil food webs strongly depend on plant species, the structure of their root systems, and their age (e.g., Yeates & Bongers, 1999), potential soil organism-mediated feedback effects on crops and yields might vary as well, depending on these factors. Accordingly, the inclusion of various crop types and plant growth stages is essential when investigating and assessing CO_2-effects on soil processes in agroecosystems.

Following this requirement, two morphologically and metabolically differing crop types (sugar beet and winter wheat) and, moreover, two plant developmental stages, were included in the present study (Papers 2.2 and 2.3), in order to analyse and consider potential crop-dependences of below-ground CO_2-effects.

Soil and soil food webs

Soils, which store about 70 % of total terrestrial carbon (C), play a major role in the C-cycle of ecosystems and the nutrient supply to plants (Pendall et al., 2008). Due to photosynthetic CO_2-fixation, CO_2-release through respiration, sequestration of C into biomass and soil, and organic matter decomposition by soil organisms, a continuous C-exchange (direct and indirect) exists between soils and atmosphere (Drigo et al., 2008). Globally, the above- to

below-ground C-transport in terrestrial ecosystems is of enormous magnitude and by far exceeds the C-emissions to the atmosphere through combustion of fossil fuels (Litton & Giardina, 2008). Although these top-down C-fluxes influence biological, chemical and physical properties of soils and ecosystems to a vast extent via the regulation of C-storage and decomposition processes, so far they remain minor investigated and understood (Litton & Giardina, 2008). Thus, distinct results and precise details of CO_2-enrichment-induced changes hardly exist (Litton & Giardina, 2008).

However, since 80-90 % of plant-fixed C finally reaches the soil decomposer community (Bardgett et al., 2005), it must be assumed that the C-transport into and within the soil and, thereby, soil habitat properties and conditions change as well under atmospheric CO_2-enrichment. In this context, in particular CO_2-enrichment-induced changes of quantities and qualities of root exudates, along with altered microbial activities (Drigo et al., 2008; Haase et al., 2008), both representing important food sources of the soil fauna (Pollierer et al., 2007; Drigo et al., 2008), might most probably affect the decomposer community via the soil food web (Pendall et al., 2008). Since various soil fauna groups and embedded species differ among each other concerning their adaptation levels to certain habitat conditions, which determine their occurrences and abundances (Balmford & Bond, 2005), such impacts have the potential to alter population densities, species compositions, and interactions within soil food webs, and thus the functional diversity of soils (Tylianakis et al, 2008). This way, nutrient release, soil fertility, and finally, cropping capacity and productivity of soils, might inevitably change as well (Brussaard et al., 2007).

Thus, structural-, trophic- and functional alterations within soil fauna communities provide possible causes of the broad variability of CO_2-induced crop responses (Phillips et al., 2006a). However, globally and regional, at the functional and taxonomical level, up to now, less is known concerning species becoming extinct, population decreases (Balmford & Bond, 2005), and changes of functional networks in soil resulting thereof. A lack of knowledge exists particularly with regard to key-organisms in soil.

Thus, studies of CO_2-enrichment effects focusing on functional aspects of various trophic levels and interactions within soil food webs represent a missing link in the quantification of CO_2-effects on numerous ecosystem services (Tylianakis et al., 2008), like future yield levels. Against the background of increasing food demands, decreasing availability of production areas, the required conservation of natural resources, and, moreover the protection of soil biodiversity which represents a crucial aspect of the programme of work on agricultural biodiversity of the CBD (CBD, 2000), an urgent need for research exists.

Based on this state of knowledge, impacts of atmospheric CO_2-enrichment on the taxonomic or trophic and functional diversity of collembolans and nematodes, which represent key-organisms within the soil fauna of arable soils (Freckman & Ettema, 1993; Petersen, 2000) and thus contribute to agrobiodiversity (CBD, 2000; BMELV, 2007), were investigated in the present study (Papers 2.2 and 2.3). The results should give insights into, up to now, barely understood links and relations within and between modified C-translocation processes and complex interactions within soil food webs under future conditions. Since predictions of future distributions of species from bioclimatic models may fail due to uncertain predictions of local climate changes, inaccurate estimates of the climatic tolerance of species, and unforeseen changes in populations (Araújo & Rahbek, 2006) field experiments were necessary within the scope of the present study.

1.1.5 The FACE field experiment

To meet the need of research concerning impacts of atmospheric CO_2-enrichment on ecosystem processes, FACE (**F**ree **A**ir **C**arbon **D**ioxide **E**nrichment) field experiments have been established in various ecosystems all over the world (FACE Data Management System, 2009). This technology, applied since the late Eighties (Hendrey et al., 1992), allows the study of the impacts of future atmospheric CO_2-concentrations on several components of different ecosystems under natural conditions, with a practical orientation and without chamber effects (Nösberg & Long, 2006). Unique in Europe, the Johann Heinrich von Thünen Institute (vTI), Federal Research Institute for Rural Areas, Forestry and Fisheries (formerly Federal Agricultural Research Centre, FAL) in Braunschweig ran such field experiment from 1999 until 2005 within an agroecosystem managed in a crop rotation (Weigel et al., 2006). The rotation cycle was repeated once during the total duration of the CO_2-exposure experiment. The present study was part of this experiment and was conducted under cultivation of sugar beet as a root crop and winter wheat as a cereal crop in the years 2004 and 2005, during the second crop rotation cycle within the experiment. Both crop types were chosen to investigate CO_2-effects on below-ground processes, as they differ markedly in terms of their root systems, which regulate and influence community compositions within the soil food web, and thus the functional diversity and interactions in soils. Moreover, impacts under sugar beet and winter wheat cultivation are of particular interest since both crops belong to the most common and economically important field crops.

Site description – experimental field

The FACE-equipment was established in a 22-ha field located at the vTI site in Braunschweig, south-east Lower Saxony, Germany (52° 18' N, 10 ° 26' O, 79 m a.s.l.). The local climate is characterized by a mean annual temperature of 8.8°C, a total precipitation of 618 mm $year^{-1}$, 1514 h sunshine $year^{-1}$, and a solar radiation of approximately 350 kJ cm^{-2} $year^{-1}$ (Weigel et al., 2005). The soil at the experimental site is a Luvisol of a loamy sand texture with a pH of 6.5 and a mean organic carbon content of 1.4 %.

The field was managed in a locally typical crop rotation including winter barley (*Hordeum vulgare* cv. Theresa), ryegrass as a cover crop (*Lolium multiflorum* cv. Lippstädter Futtertrio), sugar beet (*Beta vulgaris* cv. Impuls), and winter wheat (*Triticum aestivum* cv. Batis). To avoid water stress, field irrigation was applied during the main growing season (Weigel et al., 2005). Soil-, fertilizer-, and pesticide management measures were carried out according to local farming practices. Crop varieties, CO_2-treatment details, and sampling dates under sugar beet cultivation 2004 and winter wheat cultivation 2005, when samples for the present study were taken, are briefly summarized in Table 1.

Table 1: Crop developmental stages as well as treatment and sampling dates under sugar beet (2004) and winter wheat (2005) cultivation in the FACE experiment in Braunschweig (Table 1, cit ex. Paper 2.2)

Management	**Sugar beet 2004** *Beta vulgaris* cv. "Impuls"	**Winter wheat 2005** *Triticum aestivum* cv. "Batis"
Crop		
Sowing	14 April 2004	26 October 2004
Emergence	26 April 2004	16 November 2004
Final harvest	15 October 2004	27 July 2005
Atmospheric CO_2-enrichment		
Start	14 May 2004	12 January 2005
End	30 September 2004	20 July 2005
Duration	139 days	130 days
Mean CO_2-concentration (Control vs. FACE)	378 vs. 549 ppm	377 vs. 549 ppm
Sampling		
First sampling (t1)	21 June 2004	10 May 2005
plant principal growth stage[1)]	1: Leaf development (BBCH15)	4: Booting (BBCH41)
Second sampling (t2)	21 September 2004	25 July 2005
plant principal growth stage[1)]	3: Rosette growth (BBCH38)	8: Ripening (BBCH89)

[1)] Plant growth stages following the BBCH scale of Meier (2001)

Atmospheric CO_2-enrichment via FACE technique

The FACE equipment consisted of six circular plots (rings) of 20 m diameter, engineered by the Brookhaven National Laboratory New York, USA (Hendrey et al., 1992; Lewin et al., 1992). Each plot was surrounded by 32 vertical vent pipes with several holes facing the inside of the plots. The standing pipes were attached to control valves located at their bases that were all connected to a wide ring-shaped pipe called a plenum. The plenum connected to a blower and instrument shelter. The blower forced air into the plenum, where the air circulated. By opening or closing the computer-regulated pneumatic control valves, quantities and placement of air injection into ring plots via passing the holes of the vent pipes was regulated. To ensure an equal distribution of air across the rings, the supply was regulated depending on wind direction and speed, which were digitally analysed by sensors located in the centres of the rings.

Two of the six rings which were set up in Braunschweig were control rings, wherein unchanged ambient air, with a CO_2-concentration of about 360 ppm, was blown into the standing crop.

Two further rings were treated with air which was enriched in carbon dioxide by adding tank-derived CO_2. The target CO_2-concentration of the air within these FACE rings was about 550 ppm, corresponding to the atmospheric CO_2-level expected to be reached in 2050 (IPCC, 2001). To maintain this CO_2-concentration at a constant level it was regularly monitored by gas analysers located in the middle of both FACE rings, which were directly linked to the computer system regulating the CO_2-supply from the tank. Moreover, the spatial distribution of CO_2 across the area inside the rings was recorded and controlled by a two-level gas sampling system.

Fumigation of FACE and control rings was conducted only during daylight hours and was stopped when wind speeds exceeded 6.5 m s^{-1}. As low air temperatures induce a reduction of plant physiological activities, and thus of the photosynthetic CO_2-fixation, fumigation was also interrupted when air temperatures dropped below 5°C.

The final two rings of the field experiment represented ring dummies which were equipped with the same devices as FACE and control rings, but contained no blowers. These rings were installed at the beginning of the experiment in 1999 to analyse potential equipment effects. As no such effects were detected during the total duration of the FACE experiment, these rings were not considered during the present study. Thus, all presented results refer to control and FACE treatments.

Beside both CO_2-levels, the Braunschweig FACE experiment included two nitrogen (N) fertilization levels (adequate N-fertilization vs. low (50 % of adequate) N-fertilization) in order to simulate future nutrient management scenarios and to analyse potential C-N interaction effects.

Since previous studies reveal the soil fauna to be not affected by CO_2-elevation-N-fertilization interactions (Sticht et al., 2006) only adequately fertilized areas were considered during the present study.

Stable isotopic labelling of surplus CO_2

Within the Braunschweig FACE experiment, isotopically labelled CO_2 was used to simulate atmospheric CO_2-enrichment. Compared to ambient carbon dioxide, the tank-derived CO_2 was depleted in the heavier ^{13}C isotope, resulting in a more negative $^{13}C/^{12}C$ ratio (stable C-isotopic signature expressed as $\delta^{13}C$) of -47 ‰. By mixing this labelled CO_2 with unchanged ambient air, a decrease of $\delta^{13}C$ was induced from an initial value of -9.85 ‰ of the air within the control to about -21 ‰ of the CO_2-enriched air within the FACE rings. This isotopic labelling allowed tracing of the surplus C, added during CO_2-enrichment, in different compartments of the system (crops, soil, nematodes and collembolans) by means of stable C-isotopic analyses. The experimental set-up, therefore, provided a unique opportunity to gain new insights into C-translocation processes, interactions, and trophic shifts within the soil food web under future atmospheric CO_2-conditions.

As the stable C-isotopic analysis of field-sampled nematodes (subdivided into feeding types), and collembolans (classified to species level), represented a new research approach, some preliminary methodical tests were required. In this context an appropriate weight of nematode samples, and therefore a well measurable number of individuals, allowing the precise analyses of animal $\delta^{13}C$ values, had to be determined. Concerning the taxonomical or trophic classification of soil fauna, the preservation and fixation of animals, or, when determined at the species level, moreover, bleaching of organisms is often indispensable. The agents commonly used for this purpose usually represent C-sources which have the potential to modify $\delta^{13}C$ values of species tissues. Generally only less literature was available concerning this methodical problem, and existing publications focussed solely on the preservation of aquatic organisms, various tissues of birds and mammals, or documented investigations of *Drosophila melanogaster* (e.g., Arrington & Winemiller, 2002). Only insufficient knowledge existed on whether and in which way the treatment with such agents alters the $^{13}C/^{12}C$ ratio of soil animals. Thus, prior to field samplings, effects of several chemical agents customarily

used during sample preparation of collembolans and nematodes on the $\delta^{13}C$ values of these organisms were determined, in order to detect under which conditions which agents are suitable prior to stable C-isotopic analysis of soil animals (Paper 2.1).

1.1.6 Soil fauna

According to the body size of organisms, the soil fauna is subdivided into three groups: macrofauna (> 2 mm diameter; e.g., earthworms or myriapodes); mesofauna (100 µm to 2 mm diameter; collembolans, mites etc.); and microfauna (< 100 µm diameter; e.g., nematodes or protozoans) (Swift et al., 1979). Concerning the soil animals analysed during the present study, collembolans represent keystone organisms of the meso- , nematodes of the microfauna.

Collembolans (Arthropoda, Hexapoda)

World wide approximately 7500 collembolan (springtail) species out of 74 genera, whereof about 2000 occur in Central Europe, have been described up to now (Bellinger et al., 1996-2008). Nearly all terrestrial habitats, in the first place the soil, were inhabited by collembolans often in high abundances (Hopkin, 1997).

Despite their small body size of 1-5 mm (in exceptional cases 0.12-17 mm) (Bellinger et al., 1996-2008), collembolans, as important members of the decomposer community, are of major relevance concerning soil nutrient availability as they catalyze nutrient mobilisation and increase microbial activity by grazing on bacteria and fungi (Petersen, 2000; Kaneda & Kaneko, 2008). Particularly with regard to agricultural soils, which often inhabit very high individual densities of collembolans, they play a key role concerning C-cycling and nutrient supply to crops (Hopkin, 1997; Petersen, 2000).

Collembolans are able to use a broad range of various food sources, as for example decomposed plant material, root exudates, fungal spores and hyphae, bacteria, algae, pollen, or diatoms (Berg et al., 2004), but are to a variable extent selective in their food choice (Bracht Jørgensen et al., 2008; Larsen et al., 2008). Preferences for certain food sources thereby differ depending on age, species (Bracht Jørgensen et al., 2008), habitat (Castaño-Meneses et al., 2004), seasonal variations (Berg et al., 2004), quality of food sources (Bracht Jørgensen et al., 2008; Larsen et al., 2008), and inhabited soil layers (Hishi et al., 2007).

According to their vertical stratification, collembolans can be subdivided into three life strategy forms, based on the classification of Gisin (1943). According to this classification,

species that inhabit deeper soil layers are referred to as “euedaphic”, those occurring in the upper soil layer as “hemiedaphic” species. Surface-dwelling “atmobiont” species, living either on the soil surface or on macrophytes, represent the third life form type. As atmobiont species are of subordinate importance with regard to intra-soil processes, they were not considered during analyses of CO_2-enrichment-induced functional changes of soil processes during the present study. Concerning these changes, the attention was directed to euedaphic and hemiedaphic collembolan species (Paper 2.2), which differ markedly in terms of morphological and functional properties (Gisin, 1943). Morphological adaptations, in this context, are the result of a decrease in pore volume and the reduction of light with increasing soil depth. Whereas euedaphic species are generally characterized to be photophobic and drought-sensitive, the hemiedaphic life strategy involves xero-, meso-, and hydrophilous species. As a result of these different adaptabilities and sensitivities, collembolans respond differently to changing habitat conditions depending on respective life strategy and species.
According to the combination of these characteristics, accompanied by high species diversities, short generation times, and a close habitat-linkage due to their low mobility (Ehrnsberger, 1993), collembolans represent valuable biological indicators for assessing anthropogenic impacts on various ecosystems (Ehrnsberger, 1993; Parisi et al., 2005).

Nematodes

Nematodes (roundworms) inhabit all conceivable aquatic and terrestrial habitats, and, according to Bongers & Schouten (1991), account for up to 80 % of all metazoans on earth. Free-living nematodes include about 11,000 described species (Andrassy, 1991), reach body lengths of between 0.3 and 5.0 mm in soil (Yeates & Bongers, 1999), and inhabit most soils (Hoeksema et al., 2000) in often high abundances of up to 10 million individuals m^{-2} (Dunger, 1983). Thus, nematodes, which account for about 5 % of the total soil biomass, behind protozoa, represent the most abundant animals in soils (Gisi, 1997).
Nematodes are mainly characterized by a high trophic diversity between species. Thus, soil nematode communities involve herbivorous, bacterivorous, fungivorous, and predacious as well as omnivorous feeding types, which moreover differ concerning their functional roles in soil systems (Yeates, 2003). With regard to soil decomposition processes, the large group of non-phytopathogenic nematodes is of major importance. Microbial biomass and activity, and thereby C- and N-turnover as well as nutrient availability in soils are directly regulated by the grazing activity of bacterivorous and fungivorous nematodes (e.g., Liang et al., 2005), and indirectly affected by the regulation of root exudation through herbivorous nematodes (e.g.,

Poll et al., 2007). This way, nematodes contribute considerably to fundamental ecological processes in soil, as they regulate both primary production and decomposition processes.
According to these characteristics along with high species numbers, high abundances, short generation times, a high degree of food specialization, and their immediate responses to changing habitat conditions, nematodes species and feeding types offer great potential for use as indicators of biodiversity (Yeates & Bongers, 1999), and for assessing anthropogenic functional changes in various ecosystems (Hoeksema et al., 2000; Yeates, 2003).
Since nematodes, especially herbi-, bacteri- and fungivorous species (Freckman & Ettema, 1993), occur in high individual densities in arable soils (> $100g^{-1}$ soil) (Young et al., 1998), they represent valuable indicator organisms for analysing CO_2-effects on below-ground processes and functional links in agroecosystems as well. The separation of nematode feeding types, in this context, provides insights in impacts on different trophic levels of the soil food web and allows conclusions on changes within the microbial community.

Against this background, collembolans and nematodes were used as indicator-organisms to quantify CO_2-effects on the soil food web and soil processes in an agroecosystem, within the present study (Papers 2.2 and 2.3).

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1.2 Hypothesis and aims of the thesis

Overall hypothesis of the thesis:

Atmospheric CO_2-enrichment induces changes in taxonomic and functional diversity of the soil fauna in agroecosystems through quantitatively and qualitatively changed C-inputs into the soil.

Aims of the thesis:

- to develop sample preparation methods that allow the taxonomic or functional classification of collembolans and nematodes prior to analyses of their stable C-isotopic signatures; to detect impacts of commonly used agents on soil animal $\delta^{13}C$-values; and to assess the expressiveness of animal stable C-isotopic signatures

- to gain insights into currently less understood CO_2-enrichment-induced changes within the soil food web and the C-cycle of an agroecosystem by combining analyses of biodiversity and stable C-isotopic signatures of functionally different groups of collembolans and nematodes; and to assess to which extent this new integrated research approach provides insights into impacts on soil processes

- to trace driving forces regulating CO_2-effects on various functionally and trophically differing soil fauna groups in arable soils, by means of relative abundances and stable C-isotopic signatures of animals (collembolans and nematodes), soil, and selected plant parts; and to analyse whether impacts differ between root and cereal crops

- to detect whether CO_2-effects on soil fauna communities differ depending on crop developmental stage

The present study contributes to improving knowledge of CO_2-enrichment effects on agrobiodiversity and C-turnover processes in arable soils, which are of major importance for the development of appropriate management measures to promote the adaptation of agriculture to climate change.

Part 2

2.1 Influence of chemical agents commonly used for soil fauna investigations on the stable C-isotopic signature of soil animals

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Influence of chemical agents commonly used for soil fauna investigations on the stable C-isotopic signature of soil animals

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Abstract

The use of stable carbon isotope tracers is an approved method in investigating soil carbon cycle and trophic interactions. Within this methodical study collembolans and nematodes were analysed exemplarily. Prior to stable C-isotopic analysis, the animals have to be killed. A preparation of animal tissues (preservation, bleaching etc.) with different customary agents is often indispensable. Substances usually used contain carbon and hence represent potential carbon sources. Therefore, they could modify the C-isotopic signature of animal tissue. This methodical problem is the key point of the present contribution. The influence of commonly used chemical agents on the C-isotopic signature of collembolans was investigated exemplarily for monoethyleneglycol (MEG), ethanol (EtOH) and lactic acid. Furthermore, effects of TAF, formalin and glycerine on the C-isotopic signature of nematodes were analysed. Overall, most investigated agents modified the $\delta^{13}C$ values of animal tissues. Only MEG did not cause any significant alteration in animal C-isotopic signature of both soil fauna groups. In case of collembolans, 96% EtOH also did not change the stable C-isotopic signature significantly. Finally, the use of these agents for soil fauna preparation prior to stable C-isotopic analysis is discussed.

Keywords: C-isotopic analysis; $\delta^{13}C$; Sample preparation; Collembolans; Nematodes; Treatment effects

1. Introduction

Stable isotope mass spectrometry represents a valuable tool in studying biotic interactions in ecosystems providing insights into trophic relationships, origins of nutrients and functional roles of individual species [7]. The basis of this technique is that stable isotope ratios remain unchanged or fractionate in a predictable manner between trophic levels [9,12,14]. The source of nutrients in food webs often has a characteristic isotopic signature, which can be readily differentiated from those of other potential sources [26]. For example, carbon isotopes were used to trace the flow of organic matter within food webs (e.g. [18,25]). The $\delta^{13}C$ value of animal tissue gives insights into diets (e.g. [5,20,27]) and predator–prey relationships [1,16,21]. For this reason, nowadays the use of stable carbon isotope tracers is an approved method for investigating soil carbon cycle and soil trophic relationships (e.g. [17, 24]). Due to the particular procedure of isotope analysis, soil fauna samples collected in the field cannot usually be analysed immediately. Researchers collecting and preparing animal material for stable isotope ratio analysis are frequently faced with the need to preserve tissues prior to isotopic analysis. Therefore, tissues are commonly frozen or dried until further pre-

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paration is conducted [13]. Sometimes preparations within preservatives are indispensable. The composition of such preservatives requires careful consideration because they could affect results of isotopic analysis [3,11]. One important problem is the presence of carbon within the agents, which is expected to alter the $\delta^{13}C$ values of specimens treated therewith. The effects of various treatments on C-isotopic composition are at present in the first place investigated for aquatic (marine and freshwater) organisms (e.g. [2,3,6,8,13,19]) as well as for blood and tissue samples from quail and sheep [11]. Furthermore, $\delta^{13}C$ values of individual compounds, for example amino acids and lipids have been investigated [7].

Less is known about the influence of chemical agents commonly used for soil fauna investigations on the stable C-isotopic signature of these animals. The present study was done to determine the effects of several customarily used agents on the stable carbon isotope ratios of selected soil animals. Therefore, nematodes and collembolans, important decomposers within soil foodwebs [4], were treated with monoethyleneglycol (MEG), 96% ethanol (EtOH), 4% formalin, TAF, glycerine and lactic acid prior to stable C-isotopic analysis, to study treatment effects on stable isotope ratios of carbon. Furthermore, a potential relation between preservation time and $\delta^{13}C$ values of soil animals was investigated.

2. Material and methods

2.1. Sampling

Collembolans were descended from a *Folsomia candida* breeding.

Nematodes were isolated from soil samples taken within an agricultural field (tilled under crop rotation) at the Federal Agricultural Research Centre in Braunschweig, Northern Germany. Forty soil cores of 2 cm diameter and 0–20 cm depth were taken during sugar beet cultivation in March 2004 and combined to one sample. The nematodes were extracted by a modified decanting and sieving method (Cobb, 1918, cit. ex [22]) followed by a cotton-wool filter method (Oostenbrink, 1960, cit. ex [22]).

2.2. Sample processing

Collembolans and nematodes were treated with different chemical agents, commonly used for soil fauna investigations (Table 1). About 200 collembolans and 1000 nematodes were used for each treatment as follows:

Table 1
Customary chemical agents and their functions in collembolan and nematode investigations

Agents	Function	Collembolans	Nematodes
MEG	Killing	X	X
Lactic acid (45%)	Bleaching	X	
EtOH (96%)	Preservation	X	X
Formalin (4%)	Preservation		X
TAF (triethanolamin, aqua dest, 37% formalin)	Preservation		X
TAF + glycerine	Fixation		X

- Collembolans and nematodes were killed by transferring them into MEG for 10 min; thereafter, they were kept in distilled water.
- Collembolans were killed and preserved by transferring them into boiling EtOH (96%).
- Collembolans were killed by keeping them in MEG for 10 min; thereafter, they were transferred to 45% lactic acid for 10 min and then stored in 95% EtOH. Bleaching with lactic acid is often indispensable prior to species determination of collembolans [10].
- Nematodes were killed and preserved by a combined heat and fixation method [23]. Therefore, 4 ml of the preservatives (96% EtOH, 4% formalin or triethanolamin, aqua dest., 37% formalin (TAF)) was poured to the nematodes at a temperature of 90 °C. Then, 4 ml of the corresponding cold solvent was added.
- Nematodes were killed and preserved in TAF. Then, glycerine was added drop by drop until the glycerine/TAF ratio reached 1:3.
- As a control, collembolans and nematodes were killed by transferring them into boiling distilled water.

2.3. Stable C-isotopic analysis

First, 0.03–0.05 mg dry weight of soil animals (equivalent to 15–20 collembolans or about 100 nematodes) were weighed into 3.5 × 9 mm tin containers (IVA, Meerbusch), and oven dried at 60 °C overnight. Then, 0.2 mg vanadium-pentoxide (V_2O_5) was added to each sample as combustion catalyst. The C-isotopic signature of collembolans and nematodes was determined using an elemental analyser (Flash EA, Thermo Finnigan MAT GmbH, Bremen) coupled to an isotope ratio mass spectrometer ("Finnigan Delta ^{plus}", Thermo Finnigan MAT GmbH, Bremen). The measurements of

each treatment for both animal groups were repeated 10 times. The $\delta^{13}C$ value (‰) is defined as:

$\delta^{13}C = [(^{13}C/^{12}C_{sample}/^{13}C/^{12}C_{standard})–1] \times 10^3$ with PDB as international standard.

The reproducibility of the stable isotopic analysis within this study was about 0.03–0.05‰. NBS 22 and acetanilid were used as laboratory standards.

Furthermore, a possible relation between $\delta^{13}C$-values of animal tissue and preservation time was investigated for EtOH and formalin. The animals were exposed to these solvents for 22 days at the most.

2.4. Data analysis

We used paired *t*-test to test for effects of preservation/fixation with the different agents on $\delta^{13}C$-values of collembolans and nematodes. Furthermore, we used analysis of variance (ANOVA) to test for effects of species and preservation time. Data were analysed using SPSS for Windows version 11.0.

3. Results

The use of different preservation and fixation techniques resulted in differences among $\delta^{13}C$-values of collembolans and nematodes. The alterations in carbon isotopic signatures were specific for both soil fauna groups.

Killing soil animals by use of MEG did not alter carbon isotopic signatures of collembolans (mean difference = –0.02‰; $P = 0.965$) and nematodes (mean difference = 0.50‰; $P = 0.121$) significantly (Figs. 1 and 2). The customary preservation in 96% EtOH caused a slight depletion in ^{13}C leading to a shift of about 0.32‰ in C-isotopic signature of collembolan tissue. However, this deviation was not significant ($P = 0.409$) and lay within the range of the standard deviation of control samples (Fig. 1). Bleaching by use of lactic acid significantly affected the C-isotopic signature of collembolan tissue ($P = 0.001$). The $\delta^{13}C$ values of bleached animals deviated from those of the control by a mean difference of about +1.6‰.

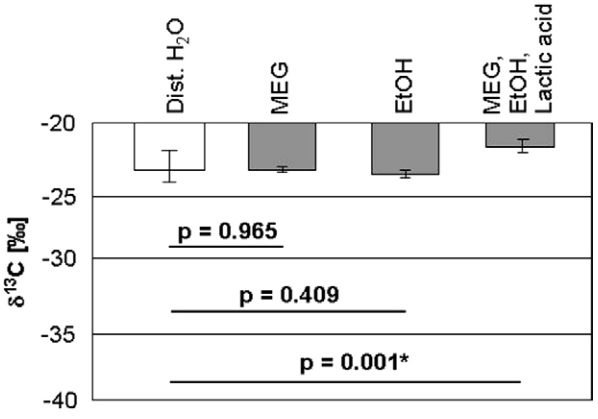

Fig. 1. Stable C-isotopic signature of collembolans after treatment with commonly used agents; white column = control, grey columns = treatments.

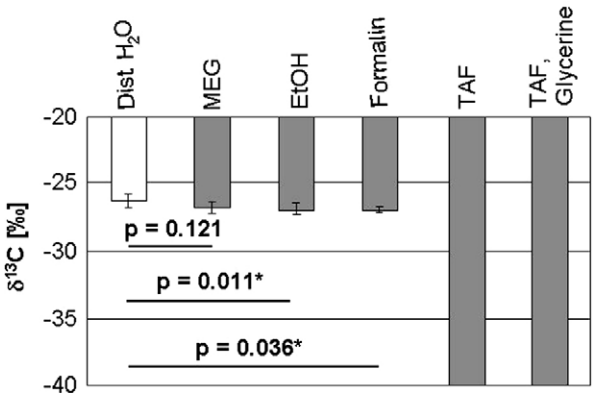

Fig. 2. Stable isotopic signature of nematodes after treatment with commonly used agents; white column = control, grey columns = treatments.

The C-isotopic signature of nematode tissue was significantly ($P = 0.011$) affected when nematodes were treated with 96% EtOH (Fig. 2). We observed a mean shift in $\delta^{13}C$-values of –0.75‰ between preserved and fresh nematode tissue. A similar depletion in ^{13}C was also observed for formalin preserved nematodes. Their $\delta^{13}C$-values deviated significantly ($P = 0.036$) from those of the control by a mean difference of –0.74‰ (Fig. 2). C-isotopic signatures of nematodes treated with TAF, or TAF with glycerine were not measurable because these agents contained such large quantities of carbon which lay beyond the detectable range of the mass spectrometer.

A significant relation between $\delta^{13}C$-values of animal tissue and preservation time could be detected neither for EtOH nor for formalin (Fig. 3).

4. Discussion

The results of our study reveal that customary chemical agents used for sample preparation can modify stable carbon isotope ratios of collembolans and nematodes. As expected the alterations in $\delta^{13}C$-values varied depending on treatment and soil fauna groups analysed. Solely MEG, as a customary agent to kill soil animals, did not significantly affect the $^{13}C/^{12}C$ ratio of animal tissue, neither for collembolans nor for nematodes.

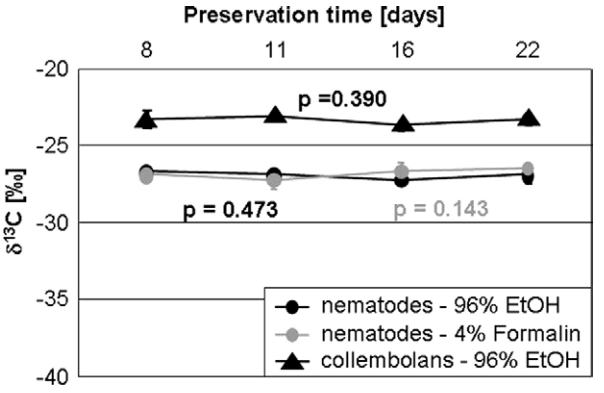

Fig. 3. Relation between δ^{13}C-values of soil animals in different preservatives and preservation time.

Therefore, the use of this agent for sample preparation is obviously unobjectionable and generally applicable within stable C-isotopic analysis of soil animals.

Likewise, the use of 96% EtOH as a preservative did not significantly affect the C-isotopic signature of collembolan tissue. This result confirms investigations of Sarakinos et al. [19] and Hobson et al. [11] who did not found any significant influence of EtOH on the C-isotopic signature of aquatic consumers as well as on blood and muscle tissue of quail and sheep. Contrary to these results, we measured a significant depletion in ^{13}C of nematodes when preserved with 96% EtOH. This finding was in every sense unexpected, as from previous studies EtOH is either known not to modify C-isotopic signatures significantly [11,19], or to deplete in ^{12}C by extraction of isotopically lighter lipids from tissues as described by Kaehler and Pakhomov [13]. Only Mullin et al. [15] proved a slight depletion in ^{13}C leading to more negative δ^{13}C values of zooplankton as a result of preservation within EtOH.

In the present study, the use of 4% formalin as preservative resulted in a mean shift of C-isotopic signatures of nematodes for about –0.7‰. Such decreases in ^{13}C due to formalin treatments have already been described several times [6,8,13,19]. For example, Bosley and Wainright [3] found animal tissue carbon of juvenile winter flounder muscles and tails of mud shrimps preserved with 10% formalin to be depleted in ^{13}C by 0.6–2.3‰ relative to frozen samples. Hobson et al. [11] reported a significant depletion in ^{13}C of quail- (–0.94‰) and sheep-blood (–1.32‰) and of quail muscle (–1.78‰) by preservation within 10% formalin. As supposed by Hobson et al. [11] this modification in δ^{13}C-values is most likely a result of direct incorporation of isotopically light formalin-based carbon. Formalin presumably binds to certain biochemical constituents and contains its own carbon source. Therefore, the alterations of δ^{13}C values can be assumed to depend on the isotopic composition of the preservative itself and the quantity of preservative bound to the tissues [13]. Hence, in the case of formalin our results fit in the range of literature data.

A clear alteration of C-isotopic signature was also observed for collembolan tissues bleached with lactic acid. Due to this treatment δ^{13}C-values were significantly more positive compared with those of the control. This enrichment in ^{13}C is presumably the result of incorporation of heavier C isotopes from the bleaching agent. Unfortunately, lactic acid caused corrosion of the tin containers, which were necessary to introduce samples into the elemental analyser. Therefore, it was not possible to analyse δ^{13}C-values of pure lactic acid.

Completely unsuitable for sample preparation previous to stable C-isotopic analysis are TAF as most common preservative for nematodes as well as glycerine, often added for fixation. These agents added more C to the samples than was actually present in the number of animals usually used for δ^{13}C analysis. The consistence of these agents, which did not evaporate completely during drying at the required temperature range, represented an additional problem.

As the present results illustrate, different methods for treating soil animals have significantly different effects on carbon isotope signatures. Our data and previous studies suggest, that use of EtOH, formalin or lactic acid for sample preparation is limited when absolute data are required. In that case correction factors have to be used to calculate the intrinsic C-isotopic signature of specimens. Because of the high variability of δ^{13}C-values between species and treatments correction factors should be species and agent specific. Additionally, using such correction factors only seem to be sustainable if standard deviations of δ^{13}C alterations are small.

As long as differences between comparative analyses are regarded the use of these agents even without correction factors is sustainable, despite a significant influence. Mean shifts in carbon isotopes did not exceed ±2‰ in any experiment except when TAF was used. If the differences between carbon sources are bigger than the impact through the agents and if the standard deviation is small, as in the present case for EtOH, formalin and lactic acid, slight changes of C-isotopic signatures are acceptable and the agents nonetheless suitable. Additionally, no significant relation between δ^{13}C values and preservation time was found for formalin and EtOH, matching the results of Edwards et al.

[6]. Species preserved 3 weeks prior to stable isotope analysis showed shifts in isotopic composition consistent with those observed for more recently preserved specimens. Nevertheless, we recommend to process samples within 3 weeks as no results exist for longer periods and usually, isotopic analysis of soil animals in field studies could be done after this period of time.

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2.2 Atmospheric CO_2 enrichment induces life strategy- and species-specific responses of collembolans in the rhizosphere of sugar beet and winter wheat

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Atmospheric CO_2 enrichment induces life strategy- and species-specific responses of collembolans in the rhizosphere of sugar beet and winter wheat

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Abstract

We studied atmospheric CO_2 enrichment effects on life form types, species composition, dominance structure and individual density of collembolans under cultivation of sugar beet and winter wheat. The study was part of a long-term CO_2 enrichment field experiment (FACE: Free Air CO_2 Enrichment) at the Federal Agricultural Research Centre (FAL) in Braunschweig (Germany), using isotopically labelled CO_2. The stable C-isotopic signature ($\delta^{13}C$) of collembolan species, plant material, and soil indicated CO_2 impacts on C translocation. The $\delta^{13}C$ values of both crops significantly increased from above-ground to below-ground plant parts and significantly decreased under FACE conditions. The $\delta^{13}C$ values of collembolan species differed significantly depending on CO_2 treatment and crop and showed a distinct tendency depending on plant growth stage. The extent, to which $\delta^{13}C$ values of collembolans decreased under FACE conditions, was species- and life strategy-dependent. The stable C-isotopic signatures of euedaphic and hemiedaphic species were similar in the control, but, depending on crop, differently affected by atmospheric CO_2 enrichment. Under winter wheat cultivation, hemiedaphic species showed more negative $\delta^{13}C$ values than euedaphic ones under FACE conditions. CO_2 enrichment effects on occurrence, density and dominance distribution of the collembolan species differed strongly between crops and their developmental stages, which reveal crop-specific below-ground effects due to different food qualities in the rhizosphere. CO_2 impacts were stronger under sugar beet compared to winter wheat cultivation. Independent of crop, CO_2 enrichment enhanced the diversity of collembolans before harvest and increased the proportion of hemiedaphic in relation to euedaphic species in a community. Our results on collembolan communities imply CO_2-induced changes in the root-derived carbon resources used by the soil food web. The present study reveals atmospheric CO_2 enrichment impacts to specifically affect collembolan species according to their food preferences.

Keywords: FACE; Collembolans; Soil biodiversity; C-turnover; Arable crops; Stable C-isotopic analysis

1. Introduction

Atmospheric CO_2 elevation, one of the most important climate change drivers (IPCC, 2007), is known to enhance plant photosynthetic rates (Körner, 2000) and water use efficiency (e.g., Conley et al., 2001). Elevated CO_2 can also affect the chemical composition of plant tissues, e.g., by changing the carbon (C) to nitrogen (N) ratio. Due to an increased biomass (e.g., Bender et al., 1999; Demmers-Derks et al., 1998) and wider plant C/N ratios, the quality and quantity of litter, and therefore C input into the soil, is also affected by CO_2 elevation (Weigel et al., 2005). Thus, future atmospheric CO_2 conditions will have considerable impacts on ecosystem processes and services.

Studies which deal with impacts of CO_2 elevation on agro-ecosystems are dominated by measurements of plant-mediated processes (Canadell et al., 1996). Less is known about CO_2-induced alterations concerning the soil food web, which is indirectly affected through changes in litter quantity and quality, as well as shifts in root turnover rates and nutrient exudation into the rhizosphere (Coûteaux and Bolger, 2000; Wardle et al., 2004). Recent results of Pollierer et al. (2007) give evidence that root-derived

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carbon resources seem to be much more important for soil animal food webs than previously estimated. Knowledge of this influence is of great importance to fully understand agro-ecosystem responses to atmospheric CO_2 enrichment, since soil biota communities are drivers of the soil C-turnover through the decomposition of organic matter and nutrient mobilisation (for Collembola see Chamberlain et al., 2006; Rusek, 1998). Changes in the diversity structure of the decomposer food web would, therefore, have the potential to alter soil ecological processes and services in agriculture.

Microarthropods, as important members of the decomposer community, are very abundant and their role in soil formation is well recognised (Parisi et al., 2005). They have also been shown to respond sensitively to land use changes (for Collembola see Dittmer and Schrader, 2000; Schrader et al., 2006; Chauvat et al., 2007) and to improve soil functions. Collembolans, as a highly diverse microarthropod group, represent a valuable biological indicator of soil quality, as they include several species representing different soil adaptation levels (Parisi et al., 2005). Furthermore, collembolans show well-differentiated ecomorphological life forms (Gisin, 1943; Rusek, 1998) and feeding guilds (Berg et al., 2004), are selective feeders comprising a wide range of food sources (Bracht Jørgensen et al., 2005; Filser, 2002; Klironomos and Kendrick, 1995), modify soil organic matter at the molecular level, and influence microbial community size through their grazing activity (Chamberlain et al., 2006). These characteristics indicate that collembolans are well suited for analysing impacts of atmospheric CO_2 elevation on the decomposer food web. Previous results range from increasing (Jones et al., 1998) to decreasing densities of collembolans (Klironomos et al., 1997) under atmospheric CO_2 elevation. Within the first crop rotation cycle of the Braunschweig Free Air CO_2 Enrichment (FACE) experiment, collembolan abundances significantly increased under atmospheric CO_2 enrichment when winter wheat was cultivated (Sticht et al., 2006b). These contradictory findings reveal the need for more detailed field studies concerning CO_2 impacts on species level of collembolans and their ecological classification. Furthermore, studies on atmospheric CO_2 impacts on collembolan communities in arable soil, including developmental stages of different crops, have been missing up to now.

To improve knowledge on crop-dependent effects regarding CO_2 impacts, the Braunschweig FACE experiment provided a unique opportunity, since it represented the only European FACE experiment in an agro-ecosystem under crop rotation (Weigel et al., 2006). Future atmospheric CO_2 concentrations were simulated under field conditions by fumigating the standing crop with isotopically labelled CO_2.

The aim of the present study was to investigate CO_2 enrichment effects on individual density and dominance structure of collembolans in arable soil, and to detect whether impacts differ between life strategies and species. Two developmental stages of sugar beet and winter wheat were studied within the second crop rotation cycle to consider crop-dependent nutritional conditions for collembolans below-ground.

The stable C-isotopic composition ($\delta^{13}C$) of animal tissues is correlated with the respective diet (DeNiro and Epstein, 1978). Moreover, recent photosynthate-C is known to be rapidly assimilated by the mesofauna of the rhizosphere, mainly by collembolans (Ostle et al., 2007). Therefore, in the present study, stable C-isotopic analysis was used to trace the C translocation from the CO_2-enriched atmosphere to the plant soil system and to test plant-mediated impacts on life form types and species of collembolans.

2. Materials and methods

2.1. Site description and management

A FACE field experiment (Lewin et al., 1992) was conducted in an agro-ecosystem at the Federal Agricultural Research Centre (FAL) in Braunschweig, Lower Saxony, Germany (10°26′E 52°18′N, 79 m a.s.l.) (details in Hendrey, 1992; Sticht et al., 2006b; Weigel et al., 2006). The local climate is characterized by a mean annual air temperature of 8.8 °C and a total precipitation rate of 618 mm $year^{-1}$. The soil at the site is a luvisol of a loamy sand texture, with a pH of 6.3–6.5 and a mean organic carbon content of 1.4% in the Ap horizon (Weigel et al., 2006). The field (total 22 ha) has been used for agriculture, cultivating only C3 plants, for at least 30 years. During the FACE experiment, the field was managed in a locally typical crop rotation, including winter barley (*Hordeum vulgare*), ryegrass (*Lolium multiflorum*) as a cover crop, sugar beet (*Beta vulgaris*) and winter wheat (*Triticum aestivum*) as sequential crops. The rotation cycle was repeated once, resulting in a total duration of the CO_2 enrichment experiment of 6 years (1999–2005). All soil, fertilizer, irrigation, and pesticide management measures were carried out according to local farming practices (Weigel et al., 2006). Crop management measures and CO_2 treatment details during sugar beet (*B. vulgaris*, spp. Altissima Döll, cv. "Impuls") cultivation in 2004 and winter wheat (*T. aestivum*, cv. "Batis") cultivation in 2005 are briefly summarised in Table 1.

2.2. Experimental design

The FACE system consisted of four circular plots (rings) of 20 m diameter. Each ring was surrounded by vertical vent pipes engineered by the Brookhaven National Laboratory, NY, USA. The pipes extended to the top of the crop canopy and were equipped with blowers. More detailed information about the experimental design, the ventilation system, and the performance of FACE techniques were outlined by Lewin et al. (1992) and Hendrey (1992).

Table 1
Treatment dates, management measures, and plant developmental stages under sugar beet (2004) and winter wheat (2005) cultivation in the FACE experiment in Braunschweig, Germany

Management	Sugar beet (2004)	Winter wheat (2005)
	Beta vulgaris cv. "Impuls"	*Triticum aestivum* cv. "Batis"
Crop		
Sowing	14 April 2004	26 October 2004
Emergence	26 April 2004	16 November 2004
Final harvest	15 October 2004	27 July 2005
Atmospheric CO_2-enrichment		
Start	14 May 2004	12 January 2005
End	30 September 2004	20 July 2005
Duration	139 days	130 days
Mean CO_2-concentration (Control vs. FACE)	378 vs. 549 ppm	377 vs. 549 ppm
Sampling		
First sampling (t1)	21 July 2004	10 May 2005
Plant principal growth stage[a]	1: Leaf development (BBCH15)	4: Booting (BBCH41)
Second sampling (t2)	21 September 2004	25 July 2005
Plant principal growth stage[a]	3: Rosette growth (BBCH38)	8: Ripening (BBCH89)

[a]Plant growth stages following the BBCH scale of Meier (2001).

The FACE experiment included two replicated CO_2 treatments (Sticht et al., 2006b). Two "control rings" were treated with unchanged ambient air, presenting a CO_2 concentration of about 380 ppm (Table 1). In two "FACE rings", the atmospheric CO_2 concentration was enhanced to approximately 550 ppm (Table 1). CO_2 used for enrichment was depleted in ^{13}C ($\delta^{13}C \approx -47‰$). Adding this labelled CO_2, the stable C-isotopic signature of the air decreased from an initial value of about −9.8‰ within the control to about −21.0‰ within the FACE rings.

2.3. Sampling and sample processing

Samples were taken twice each growing season during sugar beet (2004) and winter wheat (2005) cultivation within the second crop rotation cycle after the establishment of the FACE experiment. The first sampling took place during the period of main plant growth (t1), the second one shortly before harvest of both crops (t2) (Table 1). Soil moisture varied between 6% and 14% when samples for the present study were taken.

During each sampling, 10 intact plants, as well as eight undisturbed soil cores of 4 cm diameter and 0–20 cm depth, were taken randomly from the inner part of each control and FACE ring, considering a spatial distance of 4 m between single samples. Collembolans were extracted from soil by use of a MacFadyen high-gradient extractor (MacFadyen, 1961). During the extraction procedure, the collembolans were collected in monoethyleneglycol and preserved afterwards in 96% ethanol for subsequent identification and analysis. Individuals were counted, cleared in 45% lactic acid, and finally identified to species level following the keys of Gisin (1960), Zimdars and Dunger (1994), Bretfeld (1999), Potapow (2001) and Thibaud et al. (2004). The taxonomic classification followed Bellinger et al. (1996–2007). All species were assigned to one of the three different life strategy forms (atmobiont, hemiedaphic, euedaphic) according to Gisin (1943). Preceding studies evidenced monoethyleneglycol, ethanol and lactic acid to be suitable in sample preparation prior to stable C-isotopic analysis, when differences between comparative analyses are regarded (Sticht et al., 2006a).

Subsequent to the extraction of collembolans, remaining dried soil material was sieved using a 2 mm mesh sieve to remove stones and organic residues. Roots, leaves, stems, and beet material were cleaned, oven dried at 110 °C overnight and ground to homogeneous powder. Plant and soil samples as well as collembolan species were subjected to stable C-isotopic analysis as described below.

2.4. Stable C-isotopic analysis

Stable carbon isotope ratios ($^{13}C/^{12}C$ ratio termed as $\delta^{13}C$) are expressed in parts per thousand with $\delta^{13}C(‰) = [(({}^{13}C/{}^{12}C_{sample})/({}^{13}C/{}^{12}C_{standard}))-1] \times 10^3$.

Aliquots of 2 mg soil, 0.07 mg plant material, or 0.005–0.03 mg collembolan tissues (according to body size between 1 and 30 individuals of one species were pooled and dried at 60 °C for at least 24 h) were taken for analysis. The stable C-isotopic signature of specimens was determined (plant and soil samples: $n = 5$; collembolan species: $n = 3$) using a coupled system of an elemental analyser (Flash EA, Thermo Finnigan MAT GmbH, Bremen) and a continuous flow isotope ratio mass spectrometer (Finnigan delta ^{plus}, Thermo Finnigan MAT GmbH, Bremen). The analytical precision of the measurements was 0.03–0.05‰.

2.5. Data processing

CO_2, crop and plant growth stage effects on $\delta^{13}C$ values and collembolan abundances (in total, at life strategy and at species level) were statistically analysed by non-parametric statistics (Kruskal–Wallis H-test; Mann–Whitney U-test) using the program SPSS 13.0 for Windows®. Collembolan diversity was detected by means of Shannon–Wiener index (H') (Shannon, 1949) and Evenness (E) (Pielou, 1975). The dominance structure of the species assemblage was assessed following the classification system of Engelmann (1978). According to this system, species represented by greater than 10% of the total density are classified as dominant, those comprising 3.2–9.9% of the total density are subdominant, 1.0–3.1% recedent, 0.32–0.99% subrecedent and <0.32% sporadic.

3. Results

3.1. Abundance and diversity of collembolans

The total collembolan abundance was significantly ($P<0.001$) higher at Sampling Date 2 (t2) of both crops (late growing season) compared to Sampling Date 1 (t1) (main plant growth) (Fig. 1). From the first to the second sampling, the individual density increased by 200% (FACE) to 300% (control) under sugar beet and 500% (control) to 800% (FACE) under winter wheat cultivation. Neither a significant CO_2 or crop effect, nor any significant interaction effects between the analysed parameters (CO_2, crop and sampling date) on the total collembolan abundance could be detected.

In total, 58 collembolan species out of 11 families were found (Tables 2 and 3). The most frequent life strategy was hemiedaphic (30 species) followed by 19 euedaphic and nine atmobiont species. Under FACE conditions and due to advanced plant growth stages, the ratio between hemiedaphic and euedaphic species (eu/he ratio), the two main life form types analysed, shifted towards hemiedaphic types under both crops (Figs. 2 and 3). The CO_2 effect on this ratio increased from t1 to t2 under winter wheat (Fig. 3), but remained constant under sugar beet cultivation (Fig. 2).

The occurrence, density and the dominance distribution of species differed depending on crop, sampling date and CO_2 treatment. *Mesaphorura krausbaueri* s.l. represented the most abundant species and the only one which was dominant during each sampling and under each treatment. In total, 12 species (10 during each sampling date) were classified as main species (dominant or subdominant) under at least one crop and CO_2 treatment (bold enteries in Tables 2 and 3). With the exception of t2 under cultivation of winter wheat, at least twice as much main species belonged to the euedaphic compared to the hemiedaphic life form type (Table 4). The abundance of the majority of main species (euedaphic and hemiedaphic) in the control, tended to decrease under FACE conditions (e.g., *Willemia anophthalma* (eu), *M. krausbaueri* s.l. (eu), *Isotomodes productus* (eu), *Cryptopygus thermophilus* (he)) (Tables 2 and 3). The densities of the euedaphic species *Folsomia fimetaria* and *Folsomia inoculata* as well as the hemiedaphic species *Sminthurinus aureus*, by contrast, tended to increase under FACE conditions. The abundance of *Isotoma viridis* (he) reflected a crop-specific CO_2 effect and decreased under winter wheat, but increased under sugar beet cultivation due to atmospheric CO_2 enrichment. Plant growth stage-specific CO_2 effects were detected for the abundances of the hemiedaphic species *Isotomurus palustris*, which decreased during t1, but increased during t2.

The total species number was higher under sugar beet compared to winter wheat cultivation, and in both years the number was higher shortly before harvest (t2) compared to the period of main plant growth (t1) (Table 4). A CO_2 enrichment-induced increase in species numbers was found at the end of both growing seasons (t2). During the first sampling date (t1), the number of species was either not affected (winter wheat) or slightly depleted (sugar beet) by atmospheric CO_2 elevation.

The impact of CO_2 and plant growth stage on the number and the life strategy proportion of main species (dominant and subdominant) differed between crops (Table 4). Under sugar beet cultivation, the main species number was higher under FACE (six species) compared to control (three species) conditions, and did not differ between sampling dates. The proportion of life form types was similar during t1 and t2 under control conditions. Due to CO_2 enrichment, the number of euedaphic main species increased from two to five species, while the number of hemiedaphic main species remained unchanged during t1 (Table 4). During t2, the number of main species of both

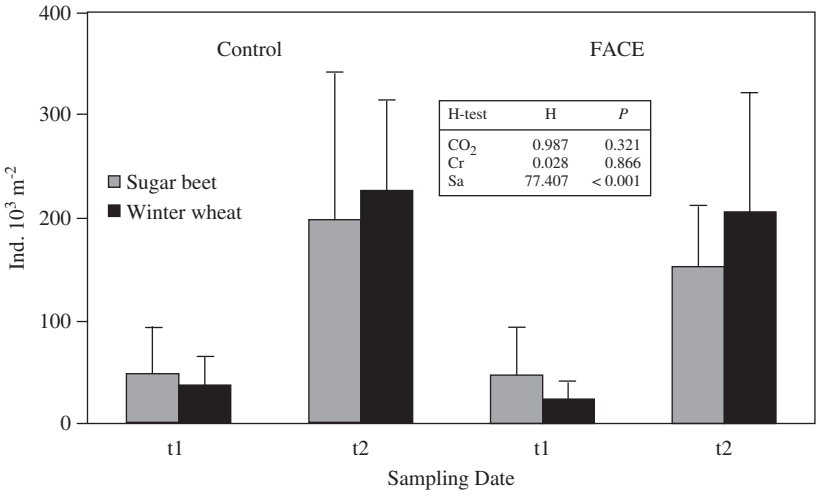

Fig. 1. Arithmetic means of collembolan abundance (Ind. $10^3\,m^{-2} \pm SD$) (0–20 cm soil depth) under sugar beet and winter wheat cultivation of both CO_2 treatments (Control: ambient air; FACE: elevated CO_2) and sampling dates; Kruskal–Wallis *H*-test on effects of CO_2 enrichment, crop (Cr), and sampling date (Sa) on the total collembolan abundance.

Table 2
Sampling Date 1 (t1) during main plant growth: Abundance (Ind. $10^3\,m^{-2}$ and SD) and dominance (dom) (D, dominant; SD, subdominant; R, recedent; SR, subrecedent; S, sporadic) of collembolan species under both crops and CO_2 treatments (0–20 cm soil depth) as well as life strategy of species: eu, euedaphic; he, hemiedaphic; at, atmobiont

Species (after Bellinger et al., 1996–2007)	Life strategy	Control						FACE					
		Sugar beet			Winter wheat			Sugar beet			Winter wheat		
		Ind. $10^3\,m^{-2}$	SD	dom	Ind. $10^3\,m^{-2}$	SD	dom	Ind. $10^3\,m^{-2}$	SD	dom	Ind. $10^3\,m^{-2}$	SD	dom
Neanuridae													
Anurida granaria	he	0.11	0.27	S	0	0		0.15	0.58	SR	0	0	
Frisea mirabilis	he	0	0		0	0		0.10	0.26	S	0.05	0.19	S
Micranurida pygmaea	he	0.11	0.27	S	0	0		0	0		0	0	
Protachorutes pyrenaeus	he	0	0		0.10	0.39	S	0	0		0	0	
Hypogastruridae													
Willemia anophthalma	**eu**	**4.62**	**3.84**	**SD**	**0.75**	**1.11**	**R**	**1.54**	**3.63**	**SD**	**0.70**	**0.55**	**R**
Willemia aspinata	eu	0.42	0.96	SR	0	0		0.35	0.93	SR	0.05	0.19	S
Tullbergiidae													
***Mesaphorura krausbaueri* sl.**	**eu**	**22.34**	**22.23**	**D**	**16.26**	**15.97**	**D**	**23.08**	**24.67**	**D**	**11.19**	**9.36**	**D**
Metaphorura affinis	eu	0	0		0	0		0	0		0.05	0.19	S
Neotullbergia crassicuspis	eu	0	0		0	0		0.05	0.19	S	0	0	
Paratullbergia callipygos	eu	0	0		0	0		0.05	0.19	S	0.05	0.19	S
Stenaphorurella denisi	eu	0.05	0.20	S	0	0		0	0		0	0	
Stenaphorurella quadrispina	eu	0	0		0	0		0	0		0	0	
Entomobryidae													
Entomobrya lanuginosa	at	0.05	0.20	S	0	0		0	0		0	0	
Entomobrya marginata	at	0	0		0.05	0.19	S	0	0		0	0	
Entomobrya multifasciata	at	0	0		0	0		0	0		0	0	
Heteromurus nitidus	he	0	0		0	0		0.05	0.19	S	0	0	
Lepidocyrtus curvicollis	he	0	0		0	0		0	0		0	0	
Lepidocyrtus cyaneus	at	0	0		0	0		0	0		0	0	
Lepidocyrtus lanuginosus	at	0	0		0	0		0	0		0	0	
Pseudosinella immaculata	he	0	0		0	0		0.15	0.42	SR	0	0	
Sinella caeca	he	0	0		0	0		0	0		0	0	
Isotomidae													
Cryptopygus bipunctatus	he	0.05	0.20	S	0	0		0	0		0	0	
Cryptopygus ponticus	he	0	0		0	0		0.15	0.58	SR	0	0	
Cryptopygus scapelliferus	he	0.05	0.20	S	0	0		0.05	0.19	S	0	0	
Cryptopygus sphagneticola	he	0	0		0	0		0	0		0	0	
Cryptopygus thermophilus	**he**	**12.89**	**23.73**	**D**	**0.95**	**1.95**	**R**	**5.42**	**9.36**	**D**	**0.75**	**1.18**	**SD**
Desoria antennalis	he	0	0		0	0		0	0		0	0	
Desoria fennica	he	0	0		0	0		0	0		0	0	
Desoria trispinata	he	0	0		0	0		0	0		0	0	
Desoria violacea	he	0	0		0	0		0	0		0	0	
Folsomia candida	he	0.16	0.43	SR	0	0		1.00	2.24	R	0.20	0.45	SR
Folsomia fimetaria	**eu**	**1.17**	**1.99**	**R**	**0.95**	**1.23**	**R**	**2.19**	**3.32**	**SD**	**1.05**	**0.96**	**SD**
Folsomia fimetarioides	eu	0.21	0.54	SR	0	0		0	0		0	0	
Folsomia inoculata	**eu**	**1.33**	**2.34**	**R**	**5.07**	**5.79**	**D**	**3.88**	**7.30**	**SD**	**4.08**	**5.87**	**D**
Folsomia litsteri	**eu**	**1.11**	**2.01**	**R**	**1.24**	**1.78**	**SD**	**4.78**	**9.51**	**D**	**0.55**	**0.78**	**R**

Folsomia spinosa	he	0	0		0	0		0.05	0.19	S	0.55	2.12	R
Isotoma riparia	he	0	0		0	0		0	0		0	0	
Isotoma viridis	he	0.05	0.20	S	1.09	1.35	R	0.20	0.45	SR	0.60	0.91	R
Isotomiella minor	eu	0.05	0.20	S	0	0		0.10	0.26	S	0	0	
Isotomodes productus	**eu**	**0.42**	**0.76**	**SR**	**1.39**	**1.36**	**SD**	**0.25**	**0.54**	**SR**	**1.34**	**1.56**	**SD**
Isotomurus palustris	**he**	**0**	**0**		**4.53**	**17.33**	**D**	**0**	**0**		**0.05**	**0.19**	**S**
Parisotoma agrelli	he	0	0		0	0		0.05	0.19	S	0	0	
Paranurophorus simplex	eu	0	0		0.05	0.19	S	0	0		0	0	
Parisotoma notabilis	**he**	**0.37**	**0.96**	**SR**	**0.20**	**0.45**	**SR**	**0.05**	**0.19**	**S**	**0.80**	**1.76**	**SD**
Proisotoma coeca	eu	0.05	0.20	S	0	0		0	0		0	0	
Proisotoma minuta	he	0	0		0	0		0	0		0	0	
Proisotoma ripicola	he	0	0		0	0		0	0		0	0	
Arrhopalitidae													
Arrhopalites caecus	he	0.21	0.46	SR	0.30	0.68	SR	0	0		0.20	0.45	SR
Dicyrtomidae													
Ptenothrix leucostrigata	at	0.05	0.20	S	0	0		0.05	0.19	S	0	0	
Bourletiellidae													
Bourletiella hortensis	at	0.27	0.48	SR	0.30	0.68	SR	0.25	0.61	SR	0.55	0.92	R
Deuterosminthurus pallipes	at	0.05	0.20	S	0	0		0	0		0	0	
Heterosminthurus bilineatus	at	0	0		0.10	0.39	S	0	0		0	0	
Neelidae													
Megalothorax incertus	eu	0.05	0.20	S	0.05	0.19	S	0	0		0	0	
Megalothorax minimus	eu	0.32	0.81	SR	0.05	0.19	S	0	0		0	0	
Neelus murinus	eu	0	0		0	0		0	0		0	0	
Sminthurididae													
Sphaeridia pumilis	he	0.16	0.32	SR	0.05	0.19	S	0.40	1.16	SR	0.05	0.19	S
Katiannidae													
Sminthurinus aureus	**he**	**0.37**	**0.82**	**SR**	**3.53**	**7.02**	**SD**	**0.40**	**1.19**	**SR**	**0.15**	**0.31**	**SR**
Sminthurinus elegans	he	0	0		0.15	0.42	SR	0	0		0.05	0.19	S
Not determinable/juvenile species		1.11	1.64		0	0		1.74	2.22		0.40	0.80	

Species representing main species (dominant or subdominant) under at least one treatment are marked in bold.

Table 3
Sampling Date 2 (t2) shortly before harvest: Abundance (Ind. $10^3\,m^{-2}$ and SD) and dominance (dom) (D, dominant; SD, subdominant; R, recedent; SR, subrecedent; S, sporadic) of collembolan species under both crops and CO_2 treatments (0–20 cm soil depth) as well as life strategy of species: eu, euedaphic; he, hemiedaphic; at, atmobiont

Species (after Bellinger et al., 1996–2007)	Life strategy	Control						FACE					
		Sugar beet			Winter wheat			Sugar beet			Winter wheat		
		Ind. $10^3\,m^{-2}$	SD	dom	Ind. $10^3\,m^{-2}$	SD	dom	Ind. $10^3\,m^{-2}$	SD	dom	Ind. $10^3\,m^{-2}$	SD	dom
Neanuridae													
Anurida granaria	he	0	0		0	0		0	0		0	0	
Frisea mirabilis	he	0.20	0.45	S	2.74	9.37	R	0.15	0.31	S	1.29	2.52	SR
Micranurida pygmaea	he	0.10	0.39	S	0	0		0.40	0.93	S	0.25	0.46	S
Protachorutes pyrenaeus	he	0	0		0	0		0.05	0.19	S	0	0	
Hypogastruridae													
Willemia anophthalma	eu	3.88	4.12	R	3.43	3.04	R	1.94	2.61	R	3.28	3.59	R
Willemia aspinata	eu	0.15	0.58	S	0.15	0.58	S	0	0		0	0	
Tullbergiidae													
***Mesaphorura krausbaueri* sl.**	**eu**	**144.28**	**131.19**	**D**	**90.02**	**62.03**	**D**	**94.45**	**51.13**	**D**	**68.79**	**57.92**	**D**
Metaphorura affinis	eu	0	0		0	0		0	0		0	0	
Neotullbergia crassicuspis	eu	0	0		0	0		0	0		0	0	
Paratullbergia callipygos	eu	0.05	0.19	S	0	0		0	0		0	0	
Stenaphorurella denisi	eu	0	0		0	0		0	0		0	0	
Stenaphorurella quadrispina	eu	0	0		0.05	0.19	S	0.10	0.39	S	0	0	
Entomobryidae													
Entomobrya lanuginosa	at	1.34	2.48	SR	0.45	0.74	S	1.74	2.70	R	0.30	0.62	S
Entomobrya marginata	at	2.09	4.31	R	0	0		1.99	2.39	R	0	0	
Entomobrya multifasciata	at	0.05	0.19	S	0	0		0	0		0	0	
Heteromurus nitidus	he	0.05	0.19	S	0.30	0.62	S	0.45	1.09	S	0	0	
Lepidocyrtus curvicollis	he	0.25	0.54	S	0	0		0.20	0.53	S	0.05	0.19	S
Lepidocyrtus cyaneus	at	0.05	0.19	S	0	0		0.10	0.39	S	0.10	0.39	S
Lepidocyrtus lanuginosus	at	0.05	0.19	S	0.35	0.97	S	0.05	0.19	S	0	0	
Pseudosinella immaculata	he	0	0		0.25	0.96	S	0	0		0.15	0.58	S
Sinella caeca	he	0	0		0	0					0.05	0.19	S
Isotomidae													
Cryptopygus bipunctatus	he	0	0		0	0		0	0		0.05	0.19	S
Cryptopygus ponticus	he	0.10	0.39	S	0	0		0	0		0	0	
Cryptopygus scapelliferus	he	0	0		0	0		0.15	0.42	S	1.05	3.85	SR
Cryptopygus sphagneticola	he	0	0		0	0		0	0		0.30	1.16	S
Cryptopygus thermophilus	**he**	**9.90**	**16.10**	**SD**	**13.18**	**17.92**	**SD**	**11.49**	**12.11**	**SD**	**11.64**	**15.36**	**SD**
Desoria antennalis	he	0.05	0.19	S	0	0		0.05	0.19	S	0	0	
Desoria fennica	he	0.05	0.19	S	0	0		0.05	0.19	S	0	0	
Desoria trispinata	he	0	0		2.34	4.51	R	0	0		0.95	2.11	SR
Desoria violacea	he	0	0		5.02	18.03	R	0	0		0.05	0.19	S
Folsomia candida	he	0.65	0.70	SR	0.75	1.24	SR	0.85	1.18	SR	1.49	2.87	SR
Folsomia fimetaria	**eu**	**4.13**	**6.64**	**R**	**4.87**	**7.23**	**R**	**2.59**	**2.49**	**R**	**10.69**	**8.97**	**SD**
Folsomia fimetarioides	eu	0	0		0	0		0	0		0	0	
Folsomia inoculata	**eu**	**5.32**	**5.25**	**R**	**4.58**	**4.69**	**R**	**5.57**	**2.99**	**SD**	**7.96**	**5.03**	**SD**
Folsomia litsteri	**eu**	**8.36**	**8.52**	**SD**	**16.56**	**14.73**	**SD**	**5.52**	**5.22**	**SD**	**31.33**	**39.99**	**D**

Folsomia spinosa	he	0	0		0	0		0.05	0.19	S	0	0	
Isotoma riparia	he	0	0		0	0		0	0		0.40	1.54	S
Isotoma viridis	**he**	**0.30**	**0.48**	**S**	**39.69**	**34.13**	**D**	**0.35**	**0.49**	**S**	**23.97**	**28.76**	**D**
Isotomiella minor	eu	0	0		0	0		0.50	1.93	SR	0.50	1.05	S
Isotomodes productus	**eu**	**4.58**	**9.98**	**R**	**3.18**	**2.81**	**R**	**6.27**	**7.55**	**SD**	**2.44**	**3.23**	**R**
Isotomurus palustris	**he**	**0.20**	**0.45**	**S**	**8.80**	**6.76**	**SD**	**0.80**	**1.62**	**SR**	**21.09**	**19.87**	**D**
Parisotoma agrelli	he	0	0		0	0		0.05	0.19	S	0	0	
Paranurophorus simplex	eu	0	0		0	0		0	0		0	0	
Parisotoma notabilis	**he**	**1.34**	**1.98**	**SR**	**3.53**	**6.07**	**R**	**5.97**	**15.79**	**SD**	**8.06**	**8.61**	**SD**
Proisotoma coeca	eu	0	0		0	0		0	0		0	0	
Proisotoma minuta	he	0	0		1.49	3.52	SR	1.49	5.78	SR	1.64	6.36	SR
Proisotoma ripicola	**he**	**0**	**0**		**21.49**	**41.81**	**SD**	**3.98**	**12.42**	**R**	**4.97**	**8.41**	**R**
Arrhopalitidae													
Arrhopalites caecus	he	0.20	0.35	S	0.80	1.52	SR	0.10	0.26	S	0.40	0.84	S
Dicyrtomidae													
Ptenothrix leucostrigata	at	0	0		0	0		0	0		0	0	
Bourletiellidae													
Bourletiella hortensis	at	0.55	0.67	S	0.30	0.39	S	0.55	0.73	SR	0.30	0.39	S
Deuterosminthurus pallipes	at	0.10	0.39	S	0	0		0.05	0.19	S	0	0	
Heterosminthurus bilineatus	at	0	0		0	0		0	0		0	0	
Neelidae													
Megalothorax incertus	eu	0	0		0.10	0.26	S	0	0		0	0	
Megalothorax minimus	eu	3.83	6.58	R	0.35	0.56	S	2.29	2.90	R	0.40	0.98	S
Neelus murinus	eu	0.40	0.80	S	0	0		0.85	1.33	SR	0.10	0.39	S
Sminthurididae													
Sphaeridia pumilis	he	0.25	0.54	S	0.50	1.01	S	0.05	0.19	S	0.10	0.26	S
Katiannidae													
Sminthurinus aureus	he	0.20	0.35	S	0.15	0.31	S	0.25	0.46	S	0.35	0.49	S
Sminthurinus elegans	he	0.10	0.26	S	0.10	0.26	S	0.25	0.67	S	0.05	0.19	S
Not determinable/juvenile species		3.93	11.22		1.34	1.56		1.14	1.46		1.59	1.59	

Species representing main species (dominant or subdominant) under at least one treatment are marked in bold.

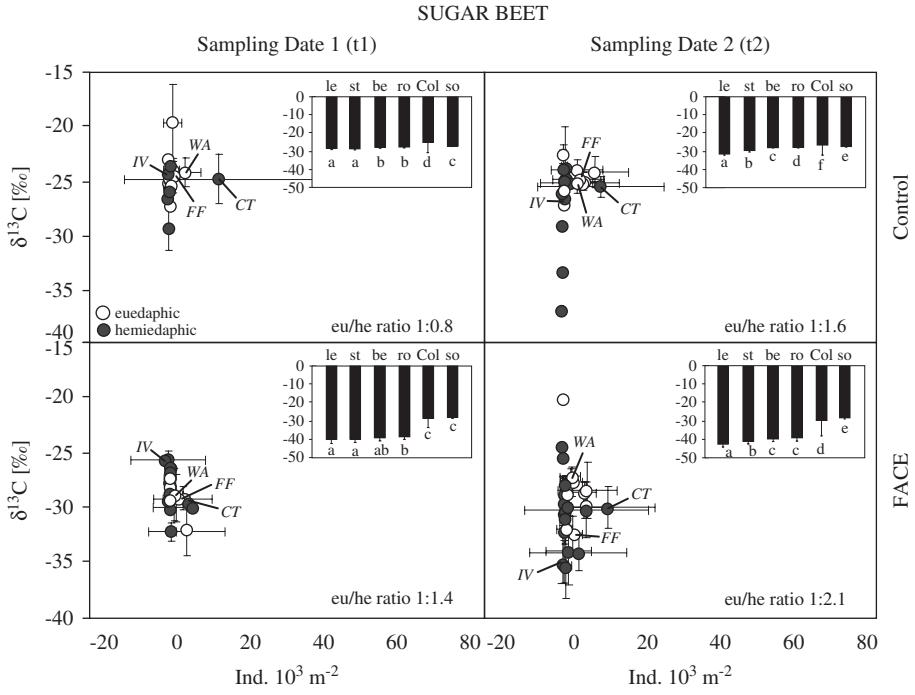

Fig. 2. Sugar beet: $\delta^{13}C$ values (‰ ± SD) vs. abundance (Ind. $10^3\,m^{-2}$ ± SD) of euedaphic (eu) and hemiedaphic (he) collembolan species and $\delta^{13}C$ values (‰ − SD) of all compartments analysed (bar charts) for both sampling dates and CO_2 treatments (0–20 cm soil depth). Means indicated by different letters are significantly different. Exemplarily, two euedaphic and two hemiedaphic representatives of the main species are marked. FF, *Folsomia fimetaria*; WA, *Willemia anophthalma*; CT, *Cryptopygus thermophilus*; IV, *Isotoma viridis*; le, leaf; st, stem; be, beet; ro, root; Col, Collembola; so, soil.

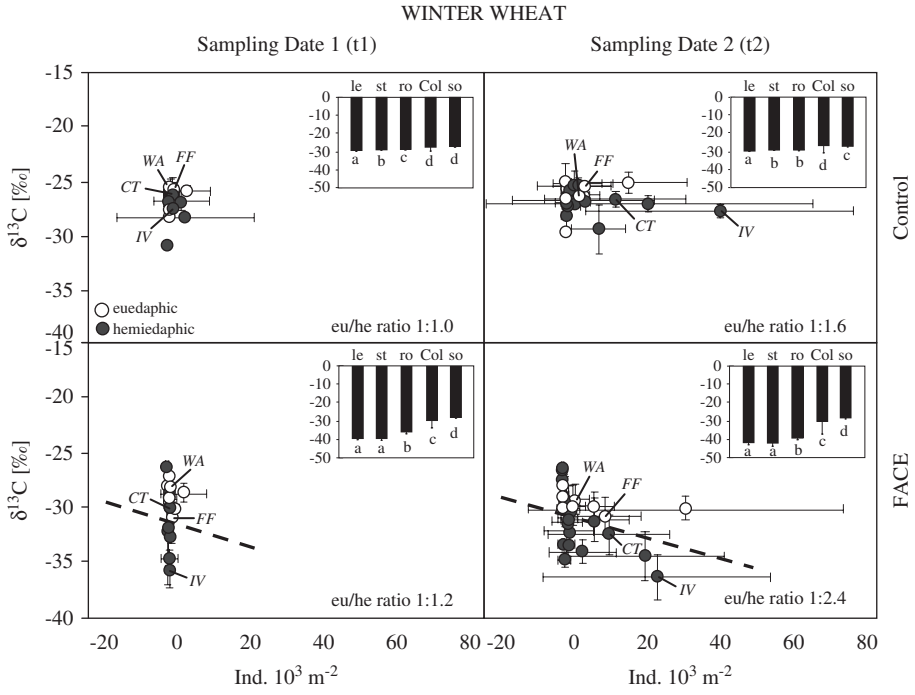

Fig. 3. Winter wheat: $\delta^{13}C$ values (‰ ± SD) vs. abundance (Ind. $10^3\,m^{-2}$ ± SD) of euedaphic (eu) and hemiedaphic (he) collembolan species and $\delta^{13}C$ values (‰ − SD) of all compartments analysed (bar charts) for both sampling dates and CO_2 treatments (0–20 cm soil depth). Means indicated by different letters are significantly different. Exemplarily, two euedaphic and two hemiedaphic representatives of the main species are marked. For abbreviations see Fig. 2.

life form types increased. Under cultivation of winter wheat, twice as many main species (six species) were detected within the control. CO_2 enrichment enhanced this number in the late growing season (t2) (eight species under FACE conditions). The proportion of life form types showed no CO_2 enrichment effect during t1, with four

Table 4
Total and main (dominant and subdominant) species number as well as diversity (Shannon–Wiener index (H') and evenness (E)) of collembolans under sugar beet and winter wheat cultivation for both sampling dates and CO_2 treatments (0–20 cm soil depth)

	Sampling Date 1 (t1)				Sampling Date 2 (t2)			
	Sugar beet		Winter wheat		Sugar beet		Winter wheat	
	Control	FACE	Control	FACE	Control	FACE	Control	FACE
Total species	28	26	21	21	33	37	29	34
H'	0.697	0.758	0.835	0.810	0.517	0.729	0.903	0.957
E	0.482	0.536	0.632	0.613	0.341	0.465	0.617	0.625
Main species	3	6	6	6	3	6	6	8
eu; he	2; 1	5; 1	4; 2	4; 2	2; 1	4; 2	2; 4	4; 4

eu, euedaphic; he, hemiedaphic.

euedaphic and two hemiedaphic main species under both CO_2 treatments. During t2, the number of euedaphic main species increased, while the number of hemiedaphic main species remained constant under FACE conditions.

The diversity of collembolans (expressed by H' and E), was higher under winter wheat ($H'>0.8$, $E>0.6$) compared to sugar beet ($H'<0.8$, $E<0.6$) cultivation. Effects of CO_2 and plant developmental stages were crop-specific. H' and E decreased under sugar beet, but increased under winter wheat cultivation (with the exception of E under FACE conditions) from t1 to t2 (Table 4). CO_2 enrichment effects on the collembolan diversity were stronger under sugar beet as compared to winter wheat cultivation. In 2004 (sugar beet), atmospheric CO_2 elevation enhanced H' and E. This impact was larger at the end of the growing season (t2: $\Delta H' = +0.212$, $\Delta E = +0.124$) compared to the period of main plant growth (t1: $\Delta H' = +0.061$, $\Delta E = +0.054$). Under cultivation of winter wheat, FACE effects changed depending on sampling date. Whereas at the first sampling date (t1) a lower diversity was detected under CO_2-enriched compared to control conditions ($\Delta H' = -0.025$, $\Delta E = -0.019$), in the late growing season (t2) the reverse held true ($\Delta H' = +0.054$, $\Delta E = +0.008$).

3.2. Stable C-isotopic analysis

3.2.1. Stable C-isotopic signatures of plants and soil

The stable C-isotopic signatures of both crops significantly increased ($P<0.05$) from above-ground to below-ground plant parts and significantly decreased ($P<0.001$) under FACE compared to ambient air conditions (presented as bar charts in Figs. 2 and 3).

Regarding below-ground plant parts, winter wheat roots reflected a significantly ($P<0.001$) stronger ^{13}C depletion ($\delta^{13}C_{t1} = -28.5 \pm 0.3‰$, $\delta^{13}C_{t2} = -29.2 \pm 0.5‰$) compared to sugar beet roots ($\delta^{13}C_{t1} = -27.4 \pm 0.5‰$, $\delta^{13}C_{t2} = -27.4 \pm 0.4‰$) within the control. Under FACE conditions, by contrast, sugar beet roots ($\delta^{13}C_{t1} = -38.7 \pm 1.3‰$) showed significantly ($P<0.001$) more negative $\delta^{13}C$ values than winter wheat roots ($\delta^{13}C_{t1} = -36.3 \pm 1.0‰$) during the period of main plant growth. Shortly before harvest, the stable C-isotopic signatures of root material did not differ between crops (sugar beet: $\delta^{13}C_{t2} = -39.3 \pm 1.6‰$, winter wheat: $\delta^{13}C_{t2} = -39.9 \pm 1.0‰$). Independent of CO_2 treatment, $\delta^{13}C$ values of sugar beet roots did not differ between sampling dates, whereas those of winter wheat roots significantly ($P<0.001$) decreased (by about 0.7‰ within the control compared to 3.6‰ under FACE conditions) from t1 to t2.

The $\delta^{13}C$ values of soil material were significantly ($P<0.001$) more negative under FACE (sugar beet: $\delta^{13}C_{t1} = -28.1 \pm 0.5‰$, $\delta^{13}C_{t2} = -28.2 \pm 0.6‰$; winter wheat: $\delta^{13}C_{t1} = -28.2 \pm 0.5‰$, $\delta^{13}C_{t2} = -28.7 \pm 0.6‰$) compared to ambient air conditions (sugar beet: $\delta^{13}C_{t1} = -26.9 \pm 0.2‰$, $\delta^{13}C_{t2} = -26.9 \pm 0.3‰$; winter wheat: $\delta^{13}C_{t1} = -26.9 \pm 0.3‰$, $\delta^{13}C_{t2} = -27.2 \pm 0.3‰$) and significantly ($P<0.001$) decreased under winter wheat cultivation from t1 to t2. Under sugar beets, the stable C-isotopic signatures of soil did not differ between sampling dates. Comparing both crops, the $\delta^{13}C$ values of soil material were significantly ($P<0.001$) more negative under winter wheat compared to sugar beet cultivation at the end of the growing season (t2). During the period of main plant growth (t1), the $\delta^{13}C$ values of soil did not differ between crops.

3.2.2. Stable C-isotopic signatures of collembolans

Figs. 2 and 3 solely focus on hemiedaphic (he) and euedaphic (eu) species. Atmobiont species were excluded here, because only few of them were found due to the sampling method used. *M. krausbaueri* s.l., the most abundant species, was also excluded from Figs. 2 and 3, since densities of individuals of this species were conspicuously higher (minimum: 260.22 Ind. $10^3\,m^{-2}$, maximum: 2308.54 Ind. $10^3\,m^{-2}$) compared to those of other species. Differences between the majority of species would not be presentable when including *M. krausbaueri* s.l. Species-specific results are exemplarily presented for the species *F. fimetaria*, *W. anophthalma*, *C. thermophilus* and *I. viridis*, as these species were found in relatively high abundances during each sampling and represented main species under at least one treatment (Tables 2 and 3).

According to the Kruskal–Wallis H-test, the stable C-isotopic signatures of collembolan species differed significantly depending on CO_2 treatment ($P<0.001$) and cultivated crop ($P<0.01$) and showed a distinct tendency depending on sampling date ($P<0.1$).

The mean stable C-isotopic signatures of collembolans were significantly more negative under winter wheat compared to sugar beet cultivation (Figs. 2 and 3). Impacts of plant developmental stages differed between crops. In the control, collembolan $\delta^{13}C$ values decreased from t1 to t2 under sugar beet, but slightly increased when winter wheat was cultivated. Due to atmospheric CO_2 enrichment, the stable C-isotopic signatures of collembolans significantly decreased (Figs. 2 and 3). Under winter wheat cultivation, this ^{13}C depletion was greater at the end of the growing season (mean $\delta^{13}C$ $shift_{t2}$: −4.0‰), compared to those ascertained during the period of main plant growth (mean $\delta^{13}C$ $shift_{t1}$: −2.9‰). Under sugar beet cultivation, the CO_2-induced $\delta^{13}C$ shift did not differ between sampling dates (mean $\delta^{13}C$ $shift_{t1} = -3.6$‰, mean $\delta^{13}C$ $shift_{t2} = -3.4$‰).

The extent, to which $\delta^{13}C$ values of collembolans decreased under FACE conditions, was species-specific and life strategy-dependent. As can be seen from Figs. 2 and 3, the $\delta^{13}C$-values of euedaphic and hemiedaphic species were similar within the control, but were differently affected by CO_2 enrichment depending on cultivated crop.

Under sugar beet cultivation, euedaphic species (mean $\delta^{13}C_{t1} = -29.2 \pm 4.4$‰) showed more negative $\delta^{13}C$ values compared to hemiedaphic ones (mean $\delta^{13}C_{t1} = -28.0 \pm 1.7$‰) during the period of main plant growth. At the end of the growing season, the reverse held true (euedaphic: mean $\delta^{13}C_{t2} = -28.4 \pm 4.0$‰, hemiedaphic: mean $\delta^{13}C_{t2} = -30.3 \pm 7.1$‰). Since collembolan species of both life form types included a wide spread of $\delta^{13}C$ values (Fig. 2), no specific CO_2-induced influences according to life strategy were detected by means of stable C-isotopic analysis during this year. Compared to the stable C-isotopic signatures of *F. fimetaria*, *W. anophthalma* and *C. thermophilus*, the hemiedaphic species *I. viridis*, for instance, showed significantly more positive $\delta^{13}C$ values during the first sampling (t1) ($P<0.05$), but significantly more negative ones at the end of the growing season (t2) ($P<0.05$) (Fig. 2). The stable C-isotopic signature of *C. thermophilus* (hemiedaphic) was similar to those of *F. fimetaria* and *W. anophthalma* (euedaphic) during the period of main plant growth (t1), and located between them ($P<0.01$) in the late growing season (t2) (Fig. 2).

Under winter wheat cultivation, hemiedaphic species showed more negative $\delta^{13}C$ values (mean $\delta^{13}C_{t1} = -30.6 \pm 3.1$‰, mean $\delta^{13}C_{t2} = -31.0 \pm 5.9$‰) than euedaphic ones ($\delta^{13}C_{t1} = -29.0 \pm 2.8$‰, mean $\delta^{13}C_{t2} = -29.6 \pm 3.7$‰) under FACE conditions at both sampling dates. Therefore, the stable C-isotopic signatures of both life form types split up (illustrated by broken lines in Fig. 3). This finding was significant for the majority of present species, exemplarily demonstrated by the common hemiedaphic species *I. viridis* ($\delta^{13}C_{t1} = -35.6 \pm 1.7$‰, $\delta^{13}C_{t2} = -36.4 \pm 2.1$‰), which showed significantly more negative $\delta^{13}C$ values ($P<0.001$) than the euedaphic species *W. anophthalma* ($\delta^{13}C_{t1} = -28.1 \pm 0.6$‰, $\delta^{13}C_{t2} = -29.4 \pm 1.3$‰) and *F. fimetaria* ($\delta^{13}C_{t1} = -30.9 \pm 2.3$‰, $\delta^{13}C_{t2} = -30.9 \pm 1.7$‰).

Beside impacts of entire crops, the $\delta^{13}C$ values of single collembolan species differed between plant growth stages. Under cultivation of winter wheat, *W. anophthalma*, for example, showed significantly more negative $\delta^{13}C$ values at t2 compared to t1 ($P<0.05$ for control and FACE). The stable C-isotopic signatures of *F. fimetaria*, by contrast, did not differ between sampling dates. Impacts of plant developmental stages on collembolans were hence species-specific.

4. Discussion

4.1. General responses of collembolans to CO_2 and crops

In the present study, atmospheric CO_2 enrichment effects on collembolan species, their life strategies and their stable C-isotopic signatures differed strongly between root and cereal crops. The rhizosphere of the crops seemed to drive and direct CO_2-induced changes within collembolan communities and, hence, plays an essential role concerning soil faunal regulation and C translocation processes belowground. Our results, therefore, agree with the findings of Pollierer et al. (2007) who described root-derived carbon resources to be of greater importance for the majority of soil faunal taxa than assumed before.

Our results indicate rising atmospheric CO_2 concentrations to affect the community composition of collembolans rather than their individual density. Linking this finding to the strongly oppositional results described in studies focussing on CO_2 enrichment effects on soil faunal abundances (e.g., Jones et al., 1998 and Sticht et al., 2006b vs. Klironomos et al., 1997), it gets obvious that factors others than CO_2 enrichment, like, for instance, management type, might predominantly regulate individual densities on higher taxon levels. The collembolan diversity, by contrast, was clearly affected by increasing atmospheric CO_2 concentrations, as indicated by distinct shifts in species numbers, Shannon Wiener Index and Evenness. The functional collembolan diversity, which would be of major interest, however, is difficult to determine. Therefore, species diversity is usually analysed instead (Bengtsson, 1998). As collembolan species differ strongly in their sensitivity to environmental changes, their diversity represents a valuable bio-indicator in field studies focussing on impact assessment (Ponge et al., 2003).

4.2. Crop and plant development-specific CO_2 effects on collembolan diversity

Collembolans are known to be polyphagous feeders (Klironomos and Ursic, 1998), using a great variety of diet

sources (e.g., Moore et al., 1987; Sauer and Ponge, 1988; Rusek, 1998). Several collembolan genera and species show a certain degree of food specialisation (Rusek, 1998; Filser, 2002; Bracht Jørgensen et al., 2005; Berg et al., 2004) and are, depending on habitat, season, substrate, vertical distribution and nutritional status of diet (Sauer and Ponge, 1988; Klironomos and Ursic, 1998; Castaño-Meneses et al., 2004), to a variable extent selective in their food choice. The detected shifts in species composition and diversity due to atmospheric CO_2 elevation base on these complex intra- and interspecific feeding behaviours. Larger amounts of qualitatively minor food (Schädler et al., 2007), resulting from an increased production of above ground and, to a larger extent, belowground plant biomass (Rogers et al., 1996), along with greater quantities of exudates (Phillips et al., 2006) and lower tissue N concentrations (Norby et al., 2001; Philips et al., 2006) may have changed collembolan communities at the species level. Detected shifts in species diversity, thereby, differed markedly between crops since winter wheat and sugar beet strongly differ in their plant physiology and consequently in biochemical processes and metabolism. In this context, the differences below-ground are of particular relevance since recent studies suggest that soil faunal taxa acquire root-derived carbon rather than litter carbon (Pollierer et al., 2007).

Wheat plants generally possess larger and more branched root systems along with greater quantities of exudates and root associated microorganisms compared to sugar beets (e.g., Weigel et al., 2005). Hence, the wheat rhizosphere provides a more diversified food base along with less food competition. According to these basically different nutritional conditions, community composition and dominance distribution of collembolan species differed between crops, which resulted in higher collembolan diversity and main species numbers under winter wheat, reflecting more favourable conditions for a larger number of species.

Moreover, impacts of atmospheric CO_2 enrichment on root growth differed markedly between both crops. Under FACE conditions, the rhizosphere of winter wheat reflected a steady enhancement during the whole growing season, whereas the root production of sugar beets only increased at the end of the growing season (Weigel et al., 2005). Linking this finding to the stronger ^{13}C depletion of sugar beet compared to winter wheat roots during the period of main plant growth (t1), it gets obvious that $\delta^{13}C$ values indicate FACE effects at this time to be stronger under sugar beet compared to winter wheat cultivation, while root growth indicated oppositional impacts. Based on this result, we assume collembolan communities to be primarily affected by changing qualities of plant inputs and exudates during the main growth of sugar beet. In case of winter wheat, changing quantities of exudates, due to the increased amount of fine roots, are of major importance during the period of main plant growth. Since CO_2 enrichment enhanced the diversity and main species number of collembolans only under sugar beet, the results of the present study reflect CO_2 enrichment effects during the period of main plant growth to be beneficial to collembolan communities when sugar beet was cultivated and to exert no impact under cultivation of winter wheat.

The increased rhizosphere along with changed qualities of litter and exudates at the end of the growing season enhanced total and main species numbers as well as diversity of collembolans under both crops. CO_2 impacts at this time were clearly stronger than those ascertained during the periods of main plant growth. Although the stable C-isotopic signatures of root material did not differ between sugar beet and winter wheat in the late season, collembolan diversity was again clearly more strongly enhanced under cultivation of sugar beets. This finding could basically be explained by the more adverse dietary situation of collembolans within sugar beet fields. We assume that communities subjected to unfavourable dietary conditions would be favoured by an increasing quantity and variety of diet, even if it is of lower quality, more heavily than those adapted to more convenient dietary situations. This assumption is supported by the capability of collembolans to adjust lower food quality by compensatory feeding (Lavy and Verhoef, 1996; Haubert et al., 2004).

The CO_2 enrichment-induced occurrence or lack of collembolan species, even if only single species are concerned, is ecologically relevant, since they interact with their environment and perform specific ecological functions. The hemiedaphic species *I. viridis*, for instance, which showed increasing densities under sugar beet, but decreasing densities under winter wheat cultivation due to atmospheric CO_2 enrichment, plays an important role during litter decomposition and in epibiontic communities (Potapow, 2001). *S. aureus*, by contrast, is known to belong to herbofungivorous species, which feed on fungal cell contents and plant cell walls (Berg et al., 2004). Appearance or disappearance of such species would change single processes that, in turn, might affect other organisms or functions in the soil system.

4.3. Crop and plant development-specific CO_2 effects on collembolan $\delta^{13}C$ values

CO_2-induced $\delta^{13}C$ shifts in collembolan life form types and species were crop-specific and again reflect a stronger impact of atmospheric CO_2 elevation under cultivation of sugar beet. During the sugar beet growing season, $\delta^{13}C$ values of collembolan species differed markedly under FACE conditions, without any evidence of a life strategy-specific influence. The latter was to be expected since food preferences change with depth (Ponge, 2000; Hishi et al., 2007), and species reflect specific levels of enzymatic activity according to their vertical stratification (Berg et al., 2004), indicating use of nearly similar diets within one type of life form. Moreover, under sugar beet cultivation, species stable C-isotopic signatures differed markedly

between plant growth stages under FACE conditions. The $\delta^{13}C$ value of *I. viridis*, for example, which represents a very common hemiedaphic species in arable soils, decreased by about 10‰ from the first to the second sampling. Such extreme shifts as well as missing coherences between isotopic signatures and life strategy are most likely the result of distinct diet switching due to markedly changed microbial conditions. The extremely non-uniform shifts of species $\delta^{13}C$ values under sugar beet cultivation, therefore, confirm the conclusions drawn from data on diversity and main species numbers, and reflect the use of several variable food sources by one species. Diet switching and mixing appear to play a major role in sugar beet fields under elevated CO_2.

The difference between stable C-isotopic signatures of hemiedaphic and euedaphic species, detected under FACE conditions when wheat was cultivated, represented the expected dependency of stable C-isotopic signatures on the vertical stratification and thereby on life strategy of species. The closer the dietary relation of a species to plant material and rhizosphere, the stronger the depletion in ^{13}C. This result, therefore, confirms those of Hishi et al. (2007), who described increasing stable C-isotopic signatures in collembolans with decreasing soil depth. Moreover, plant growth stage-dependent $\delta^{13}C$ shifts within species were less distinct under winter wheat compared to sugar beet cultivation. Diet switching due to plant developmental changes seemed, therefore, to be of minor relevance under cultivation of winter wheat.

The mean stable C-isotopic signatures of total collembolans, which basically reflect their crop-dependent dietary distance to plant material, indicate collembolan feeding to be more closely related to root material when wheat was cultivated, since differences between $\delta^{13}C$ values of roots and collembolans were generally smaller under winter wheat compared to sugar beet cultivation.

Comparing CO_2 enrichment effects on types of collembolan life form on the whole, the present results clearly indicate hemiedaphic species to be more strongly affected by CO_2 elevation than euedaphic ones due to their closer relation to the rhizosphere. CO_2-induced, plant-mediated impacts might reach these species sooner and affect them more strongly than species occurring deeper in the soil. In the present study, both, elevated CO_2 and an increased root biomass at the end of the growing season, enhanced the number of hemiedaphic species compared to euedaphic ones under each crop.

5. Conclusions

Impacts on decomposition processes, soil C-cycle and therefore soil fertility, due to changes within collembolan community composition and diversity, are most likely under future atmospheric CO_2 conditions, and might be stronger under sugar beets compared to winter wheat cultivation. The rhizosphere of cultivated plants, as important carbon source of soil animal food webs (Pollierer et al., 2007), plays a key role, since it drives and directs CO_2-induced changes of soil faunal communities. To assess whether changes will be beneficial or unfavourable to arable soils, further investigations are needed to improve knowledge on CO_2 effects on soil bacteria and fungi, which are not yet clearly understood (Phillips et al., 2006) and to find solutions concerning some general limitations along with the application of stable C-isotopic analysis in trophic-oriented field studies.

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2.3 Sensitivity of nematode feeding types in arable soil to free air CO_2 enrichment (FACE) is crop specific

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Sensitivity of nematode feeding types in arable soil to free air CO_2 enrichment (FACE) is crop specific

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Summary

The study was part of a long-term CO_2 enrichment field experiment (FACE: free air CO_2 enrichment), using isotopically labelled CO_2, at the Federal Research Institute for Rural Areas, Forestry and Fisheries (vTI) in Braunschweig (Germany). Impacts of elevated atmospheric CO_2 concentration (550 ppm) on nematode abundance, feeding type composition and the stable C-isotopic signatures ($\delta^{13}C$) of feeding types were analysed in an arable soil of a luvisol soil type and a loamy sand texture under cultivation of sugar beet and winter wheat. Two different crop growth stages were considered. The total nematode abundance was significantly higher under winter wheat compared to sugar beet cultivation and tended to increase under CO_2-enriched conditions. The feeding type composition of nematodes significantly differed between crops and their growth stages. CO_2 enrichment increased the relative abundance of fungivorous nematodes under winter wheat, and the relative abundance of bacterivores under sugar beet cultivation. Accordingly, under FACE conditions, the nematode channel ratio (NCR) indicated a shift towards a more fungal-based food chain under winter wheat, but towards a more bacterial-based energy pathway under sugar beet cultivation. The stable C-isotopic signatures of nematodes confirmed the ingestion of labelled C used for atmospheric CO_2 enrichment. The $\delta^{13}C$ values indicated significant FACE effects on all nematode feeding types. CO_2 impacts depended on either crop type or crop growth stage, or on the combination of both. According to their food specificity, nematode feeding types were specifically affected by atmospheric CO_2 enrichment due to different food qualities and quantities in the rhizosphere. The present results indicate that fungivores benefit from CO_2 enrichment in arable soils under winter wheat, and

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bacterivores under sugar beet cultivation. Due to these changes, the soil C-cycle and decomposition processes might change as well. Generally, FACE effects differed strongly between crops and their growth stages, revealing the need to introduce more than one plant type and sampling date when CO_2 enrichment effects on soil faunal communities are studied.

Introduction

Soils, the major organic carbon (C)-pools in terrestrial ecosystems, are directly and indirectly connected to atmospheric CO_2 through CO_2 fixation during photosynthesis, C sequestration into biomass and soil, and CO_2 release due to respiration and organic matter decomposition (Drigo et al. 2008). Increasing atmospheric CO_2 concentrations (IPCC 2007) are known to affect vegetation through enhanced photosynthetic rates and biomass production above- and below-ground, increased plant water-use efficiency (Ainsworth and Long 2005), changed C/N ratios (e.g., Ehleringer et al. 2002), and modified rhizodeposition (Phillips et al. 2006). According to such plant-mediated impacts, increased atmospheric CO_2 concentrations have the potential to alter organic C dynamics below-ground via changes in quantity and quality of root-derived products. Since life in soils is mainly heterotrophic, and, in total, 80–90% of plant fixed C ultimately enters the decomposer food web (Bardgett et al. 2005), the activity and functioning of soil organisms may similarly be affected by changing atmospheric CO_2 levels (Drigo et al. 2008). As a consequence, interspecific interactions that represent the energy pathway and are, thus intimately associated with the stability of whole soil systems (Emmerson et al. 2005), might change as well. Altered decomposition and mineralization rates (Marhan et al. 2008), as well as modified nutrient fluxes, nutrient retention, and finally, productivity are expected consequences.

Nematodes are very abundant, and represent the major below-ground herbivores in agricultural soils (Yeates and Bongers 1999). Like the majority of soil invertebrates (Pollierer et al. 2007) they directly obtain their carbon from roots. They show a wide range of feeding types which reflect somewhat different functional attributes, and interact functionally with most other groups of soil biota (Yeates et al. 1993; Yeates 2003). They play critical roles in controlling the turnover and the community structure of soil microbial biomass, and are intimately associated with the mineralization, cycling and availability of major nutrients in soil (e.g., Yeates and Bongers 1999; Yeates 2003; Poll et al. 2007). Food specificity is widespread among soil-inhabiting nematodes (Bongers and Bongers 1998; Ruess et al. 2000). According to these characteristics, soil nematode communities represent valuable indicators of the structure of soil food webs (Hoeksema et al. 2000) and, therefore, are well-suited for analysing impacts of atmospheric CO_2 elevation on the decomposer food web.

Studies on CO_2-induced changes of nematode communities reflect highly variable and contradictory results, ranging from increased to decreased individual densities for the total nematode abundance (increased: Klironomos et al. 1996; Yeates et al. 2003; decreased: Niklaus et al. 2003; Neher et al. 2004) and, as well, for all feeding types. Yeates et al. (1997), Hoeksema et al. (2000) and Li et al. (2007), for example, found numbers of carnivorous and omnivorous nematodes to increase, while Niklaus et al. (2003) and Sonnemann and Wolters (2005) observed a decrease due to atmospheric CO_2 enrichment. Similar results, including increased (Yeates and Orchard 1993), decreased (Yeates et al. 1997; Neher et al. 2004) or unchanged (Hungate et al. 2000; Niklaus et al. 2003) populations were detected for bacterivores and fungivores. Herbivorous nematodes increased in number (Hungate et al. 2000) or were shown to be resistant to changing atmospheric CO_2 levels (Sonnemann and Wolters 2005; Ayres et al. 2008).

These contradictory findings, and the fact that, to date, studies dealing with CO_2 enrichment effects on nematodes either based on grassland or forest soils or those that have considered only one crop, illustrate that there is still knowledge gap concerning CO_2-induced below-ground effects in agro-ecosystems. As CO_2 enrichment effects on soil trophic structures and decomposition processes have been shown to be strongly ecosystem specific (Blankinship and Hungate 2007), and plant-type specific (Drigo et al. 2007; Sticht et al. 2008), further investigations in arable soils, especially considering impacts of different types of crops, are needed.

The Braunschweig free air CO_2 enrichment (FACE) experiment, as the only European study in an agro-ecosystem under crop rotation (Weigel et al. 2006),

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provided a unique opportunity to observe such crop-dependent CO_2 enrichment effects on soil faunal communities (Sticht et al. 2006b, 2008). Isotopic labelling of CO_2 used for enrichment enabled tracing of surplus carbon from above- to below-ground.

The aim of the present study was to investigate CO_2 enrichment effects on individual densities of nematodes in an arable soil, and to analyse whether impacts differ between feeding types. To consider crop-specific impacts and seasonal variations, which are known to influence the composition of nematode trophic groups as well (Yeates and Bongers 1999; Hungate et al. 2000; Yeates 2003; Drigo et al. 2007), two crop growth stages of sugar beet and winter wheat were studied within the second crop rotation cycle of the FACE experiment. Since recent photosynthate-C is known to be rapidly assimilated by the rhizosphere fauna (Søe et al. 2004; Ostle et al. 2007; Yeates et al. 1998; Yeates et al. 1999), the stable C-isotopic signatures ($\delta^{13}C$) of nematode feeding types, which are directly correlated with their respective diet as generally found for animal nutrition (DeNiro and Epstein 1978), were measured to gain insights into food-specific impacts.

Materials and methods

Site description and crop management

A free air CO_2 enrichment field experiment (Lewin et al. 1992) was initiated in an agroecosystem in 1999 at the Federal Research Institute for Rural Areas, Forestry and Fisheries (vTI) in Braunschweig, Lower Saxony, Germany (10°26′E 52°18′N, 79 m a.s.l.; total precipitation rate: 618 mm y^{-1}; mean annual air temperature: 8.8 °C) (details in Hendrey 1992; Sticht et al. 2006b; Weigel et al. 2006). The soil at the site where the FACE system was established (total 22 ha) is a luvisol of a loamy sand texture, with a pH of 6.3–6.5 and a mean organic carbon content of 1.4% in the Ap horizon (Weigel et al. 2006). The field has been used to cultivate only C3 agricultural plants for at least 30 years. In the FACE experiment it was managed in a locally typical crop rotation that included winter barley (*Hordeum vulgare*), ryegrass (*Lolium multiflorum*) as a cover crop, sugar beet (*Beta vulgaris*) and winter wheat (*Triticum aestivum*) as sequential crops. The rotation cycle was repeated once during the total FACE experiment (first rotation cycle: 1999–2002; second rotation cycle: 2002–2005). The present study was conducted during the second crop rotation cycle in 2004, when sugar beet (*Beta vulgaris*, ssp. Altissima Döll, cv. ''Impuls''), and in 2005, when winter wheat (*Triticum aestivum*, cv. ''Batis'') were cultivated. All agricultural management were carried out according to local farming practices (Weigel et al. 2006). For more detailed information on treatments and management, see Sticht et al. (2008).

Experimental design

The experimental FACE design included four circular plots (rings) of 20 m diameter each, which were surrounded by air-emitting tubes engineered by the Brookhaven National Laboratory NY/USA. By means of the tubes, which extended to the top of the crop canopy, two levels of atmospheric CO_2 concentration were obtained in the inner parts of the rings. The FACE experiment included two replicated CO_2 treatments (Weigel and Dämmgen 2000; Sticht et al. 2006b). In two ''FACE rings'' the atmospheric CO_2 concentration was enriched by 170 ppm (to 550 ppm) above the CO_2 concentration of unchanged ambient air (about 380 ppm), which was applied to both ''control rings''. The CO_2 used for enrichment purposes was depleted in ^{13}C ($\delta^{13}C \approx -47‰$). By adding this labelled CO_2, the stable C-isotopic signature of the air decreased from an initial value of about −9.8‰ within the control to about −21.0‰ within the FACE rings.

More detailed information about the experimental design, the ventilation system, and the performance of FACE techniques were outlined by Lewin et al. (1992) and Hendrey (1992).

Sampling and sample processing

Samples for the present study were taken twice every growing season during sugar beet (2004) and winter wheat (2005) cultivation. The first sampling took place during the period of main plant growth (Growth Phase) (sugar beet: 21 July 2004, plant principal growth stage 1: leaf development (BBCH15 according to the BBCH scale for describing the phenological growth stages of mono- and dicotyledonous plants (Meier 2001)); winter wheat: 10 May 2005, plant principal growth stage 4: booting (BBCH41) (Meier 2001)) and the second one shortly before harvest of both crops (Mature Crop) (sugar beet: 21 September 2004, plant principal growth stage 3: rosette growth (BBCH38); winter wheat: 25 July 2005, plant principal growth stage 8: ripening (BBCH89) (Meier 2001)). During

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sampling, soil moisture varied between 6% and 14% dry wt.

Each of the two FACE and control rings was subdivided into four quadrants. Twenty soil cores (2 cm diameter; 0–10 cm depth) were collected in each of two quadrants per ring. These twenty are the sum of five from four patches, each patch being separated from other patches by 4 m. Each set of 20 cores was pooled ($n = 4$). Nematodes were extracted from sub-samples (100 g) of these composite samples using a modified decanting and sieving method followed by a cotton-wool filter method (Southey 1986). The extraction lasted 4 days resulting in a yield of 100 ml of clear nematode suspension. Nematodes were counted by means of a counting chamber, using 10 ml aliquots and their abundance was expressed as individuals per 100 g dry soil (ind. $100\,g^{-1}$). Nematodes were killed and preserved using a combined heat and fixation method (Southey 1986). Therefore, excess water was carefully removed and the nematodes transferred into 4 ml of boiling 4% formaldehyde (90 °C). Finally, 4 ml of the corresponding cold solvent were added. The nematodes of one quadrant per ring were microscopically classified to five trophic groups (herbivores, bacterivores, fungivores, omnivores and carnivores), based on their head structures, which are closely related to their feeding habits (Yeates et al. 1993; Yeates and Bongers 1999). The feeding types were subjected to stable C-isotopic analysis as described below. Preceding studies showed 4% formaldehyde to be suitable in sample preparation prior to stable C-isotopic analysis, when no absolute values but differences between comparative analyses are considered (Sticht et al. 2006a).

Stable C-isotopic analysis

Stable carbon isotope ratios ($^{13}C/^{12}C$ ratio termed as $\delta^{13}C$) are expressed in parts per thousand (‰) with $\delta^{13}C$ (‰) $= [(^{13}C/^{12}C_{sample}/^{13}C/^{12}C_{standard}) - 1] \times 10^3$.

Aliquots of 0.005–0.03 mg nematode tissues were taken for analysis. According to body size, these aliquots corresponded to 30–100 individuals of one trophic group which were pooled and dried at 60 °C for at least 24 h. The stable C-isotopic signature of samples was determined ($n = 10$ with the exception of carnivores, which were less abundant) using the coupled system of an elemental analyser (Flash EA, Thermo Finnigan MAT GmbH, Bremen) and a continuous flow isotope ratio mass spectrometer (Finnigan delta^{plus}, Thermo Finnigan MAT GmbH, Bremen). The analytical precision of the measurements was 0.03–0.05‰.

In the interpretation of the stable C-isotopic signatures of nematode feeding types one must consider, that, due to the preparation process, observed $\delta^{13}C$ values do not represent absolute values, and therefore do not reflect the ^{13}C signal of the diet with the normally assumed fractionation deviation (DeNiro and Epstein 1978; Spence and Rosenheim 2005). For that reason, $\delta^{13}C$ values of other ecosystem components (as plant material or soil) are not presented here. The respective ecosystem component samples were, however, analysed and the results are shown in Sticht et al. (2008). The average $\delta^{13}C$ shift caused by the formaldehyde was about −0.74‰ and remained the same in all nematode samples (Sticht et al. 2006a) regardless of feeding type or treatment. Comparison of the stable C-isotopic signatures between nematode feeding types and within feeding types between crops and crop growth stages, nonetheless indicates whether similar or different diets were used and, thereby, provides insights into specific CO_2 enrichment effects.

Data processing

The nematode channel ratio (NCR = B/(B+F), with B and F being the relative contributions of bacterial- and fungal-feeding nematodes to the total nematode abundance) was assessed according to Yeates (2003). The NCR indicates whether soil decomposition processes are driven by either a bacterial-based or a slower fungal-based energy pathway (Moore and Hunt 1988). The NCR is constrained to values between 0 (totally fungal-mediated) and 1 (totally bacterial-mediated) (Yeates 2003).

Due to the division of the rings into quadrants, the use of arithmetic means calculated from each aliquot, and the normal distribution of the data, effects of CO_2, crop type and crop growth stage on the total nematode abundance were statistically analysed by a nested three-way ANOVA (analysis of variance) linear model. Effects on relative abundance, NCR and $\delta^{13}C$ values of feeding types were statistically analysed by use of non-parametric Chi-square and *H*-test using the program SPSS 13.0 for Windows®.

A general statistical problem in FACE experiments is the low number of replicate rings due to high running costs. To avoid pseudo-replication problems, the trade-off between statistical power and experimental expenses has already been discussed in the literature several times (e.g.,

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Filion et al. 2000). However, all the suggestions and proposals for solutions that have been made were either not readily adaptable or undesirable in the Braunschweig FACE experiment. Therefore, we used ordinary statistical analysis on the data, and results should be interpreted in the light of this limitation.

Results

Total nematode abundance

The total nematode abundance varied between 1513 ind. 100 g^{-1} (control under sugar beet, Mature Crop) and 4438 ind. 100 g^{-1} (FACE under winter wheat, Mature Crop), and was significantly ($P = 0.002$) higher under winter wheat compared to sugar beet cultivation (Figure 1). With the exception of the control under sugar beet cultivation, individual densities significantly increased from the Growth Phase to the late growing season (Mature Crop) ($P = 0.005$). Individual numbers increased under FACE conditions, reflecting greater impacts in the late growing season of both crops (Mature Crop) (Figure 1). During this crop growth stage, CO_2 enrichment enhanced the total nematode abundance by 59% under sugar beet compared to 32% under winter wheat. However, this difference was not significant.

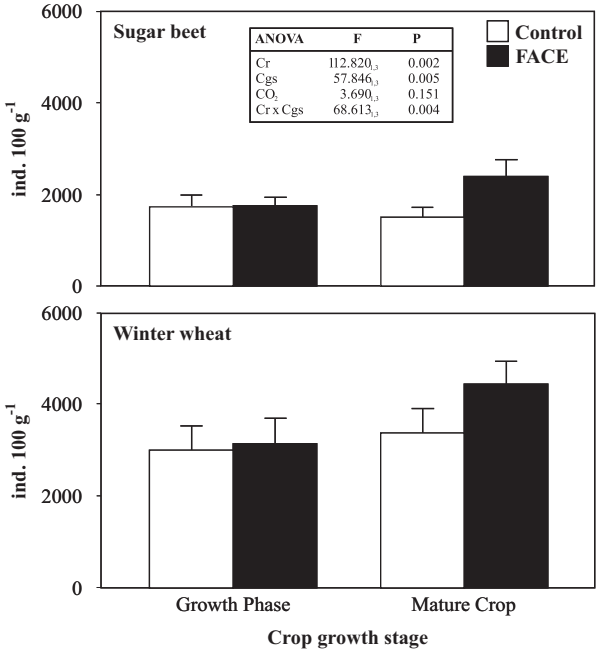

Figure 1. Arithmetic means of total nematode abundance (ind. 100 g^{-1} dry soil+SD) under sugar beet and winter wheat cultivation for both crop growth stages and CO_2 treatments (0–10 cm soil depth); *F*-values (with numerator and denominator degrees of freedom as subscripts) and *P*-values for a nested three-way-ANOVA linear model on effects of CO_2 enrichment, crop (Cr), and sampling date (Sa) on total nematode abundance.

Feeding type composition of nematodes and NCR

The feeding type composition of nematodes significantly differed between crops (Growth Phase, control: $P<0.001$, $\chi^2 = 93.765$, df = 4; Growth Phase, FACE: $P<0.001$, $\chi^2 = 95.226$, df = 4; Mature Crop, control: $P<0.001$, $\chi^2 = 20.657$, df = 4; Mature Crop, FACE: $P = 0.008$, $\chi^2 = 13.668$, df = 4) and, with the exception of those detected under FACE conditions when sugar beet was cultivated, between crop growth stages (sugar beet, control: $P<0.001$, $\chi^2 = 29.615$, df = 4; winter wheat, control: $P = 0.007$, $\chi^2 = 14.169$, df = 4; winter wheat, FACE: $P<0.001$, $\chi^2 = 38.371$, df = 4) (Tables 1 and 2).

Under control conditions, the relative contribution of bacterivorous nematodes to the whole community was significantly higher under winter wheat (Growth Phase: 44%; Mature Crop: 36%) compared to sugar beet cultivation (Growth Phase: 30%; Mature Crop: 25%) during both sampling dates (Growth Phase: $P = 0.025$, $H = 5.026$, df = 1; Mature Crop: $P = 0.037$, $H = 4.333$, df = 1). Relative abundances of herbivores, by contrast, showed the opposite response and were significantly higher (Growth Phase: $P = 0.004$, $H = 8.308$, df = 1; Mature Crop: $P = 0.006$, $H = 7.410$, df = 1) under sugar beet (Growth Phase: 47%; Mature Crop: 51%) than under winter wheat (Growth Phase: 28%; Mature Crop: 37%) (Tables 1 and 2). The contribution of fungivores was significantly higher (+16%; $P = 0.004$, $H = 8.308$, df = 1), while that of omnivores (−9%; $P = 0.004$, $H = 8.308$, df = 1) and carnivores (−2%; $P = 0.004$, $H = 8.308$, df = 1) was significantly lower under wheat compared to beet cultivation during the Growth Phase (Table 1). At the end of the growing season (Mature Crop), the relative proportion of these feeding types did not differ between crops (Table 2).

Under sugar beet, the contribution of fungivores to the whole community significantly increased (+9%; $P = 0.010$, $H = 6.564$, df = 1) while that of omnivores (−6%; $P = 0.025$, $H = 5.026$, df = 1) and carnivores (−2%; $P = 0.004$, $H = 8.308$, df = 1) significantly decreased from the Growth Phase to the stage of Mature Crop. Herbivores (+9%; $P = 0.010$, $H = 6.564$, df = 1) and omnivores (+2%; $P = 0.045$, $H = 4.020$, df = 1) reflected significantly increasing relative abundances from the first (Growth Phase) to the second (Mature

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Table 1. Growth phase: abundance (ind. $100\,g^{-1}$ dry soil $\pm$ SD) and nematode channel ratio (NCR) under both crops and CO_2 treatments (0–10 cm soil depth).

Feeding group	Control				FACE			
	Sugar beet		Winter wheat		Sugar beet		Winter wheat	
	ind. $100\,g^{-1}$	SD	ind. $100\,g^{-1}$	SD	ind. $100\,g^{-1}$	SD	ind. $100\,g^{-1}$	SD
Herbivore	820	52	848	116	802	44	670	74
Bacterivore	515	48	1310	256	564	135	1509	115
Fungivore	184	54	826	186	288	128	1129	57
Omnivore	173	77	23	18	153	25	25	17
Carnivore	39	14	4	3	27	24	5	4
NCR	0.743		0.610		0.661		0.571	

Table 2. Mature crop: abundance (ind. $100\,g^{-1}$ dry soil $\pm$ SD) and nematode channel ratio (NCR) under both crops and CO_2 treatments (0–10 cm soil depth).

Feeding group	Control				FACE			
	Sugar beet		Winter wheat		Sugar beet		Winter wheat	
	ind. $100\,g^{-1}$	SD	ind. $100\,g^{-1}$	SD	ind. $100\,g^{-1}$	SD	ind. $100\,g^{-1}$	SD
Herbivore	769	100	1235	121	1040	193	1667	162
Bacterivore	386	112	1195	205	796	172	1405	115
Fungivore	303	59	819	104	454	74	1294	142
Omnivore	54	30	99	61	86	52	68	38
Carnivore	1	1	4	1	26	26	4	3
NCR	0.551		0.590		0.630		0.521	

Crop) sampling date when winter wheat was cultivated.

CO_2 enrichment effects on the nematode feeding type composition were stronger during the Growth Phase under winter wheat (Growth Phase: $P = 0.099$, $\chi^2 = 7.797$, df $= 4$; Mature Crop: $P = 0.399$, $\chi^2 = 4.055$, df $= 4$), and during the stage of Mature Crop under sugar beet cultivation (Growth Phase: $P = 0.169$, $\chi^2 = 6.439$, df $= 4$; Mature Crop: $P < 0.001$, $\chi^2 = 23.509$, df $= 4$) (Tables 1 and 2). Independent of crop (sugar beet: -3%; $P = 0.055$, $H = 3.692$, df $= 1$; winter wheat: -8%; $P = 0.006$, $H = 7.410$, df $= 1$), the relative proportion of herbivores decreased during the Growth Phase when atmospheric CO_2 concentrations were enriched. At the end of the growing season (Mature Crop) the contribution of this feeding type tended to decrease under sugar beet, and did not change under winter wheat cultivation.

When wheat was cultivated, the relative proportion of fungivores increased under FACE conditions (Growth Phase: +7%; $P = 0.078$, $H = 3.103$, df $= 1$; Mature Crop: +5%; $P = 0.055$, $H = 3.692$, df $= 1$) (Tables 1 and 2). Under sugar beet, by contrast, a clear tendency towards increasing relative abundances of bacterivores, indicating stronger CO_2 effects at the end of the growing season (Mature Crop), was detected. The contribution of omnivorous and carnivorous nematodes was not or only slightly affected by atmospheric CO_2 enrichment.

The nematode channel ratio varied between 0.521 and 0.743. During the Growth Phase, the NCR was higher under sugar beet (NCR > 0.66) compared to winter wheat cultivation (NCR < 0.61), reflecting significant crop effects in the control ($P = 0.025$, $H = 5.026$, df $= 1$). During this period of crop growth, the NCR tended to decrease under FACE conditions independent of crop (sugar beet: -0.082; winter wheat: -0.039) (Table 1). From the Growth Phase to the end of the growing season (Mature Crop) the NCR generally decreased. This finding was significant in the control under sugar beet cultivation ($P = 0.016$, $H = 5.769$, df $= 1$), but showed a very clear trend under FACE conditions when winter wheat was cultivated ($P = 0.055$, $H = 3.692$, df $= 1$). At the stage of Mature Crop, the NCR detected under FACE conditions significantly differed between crops ($P = 0.037$,

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$H = 4.333$, df $= 1$). During this crop growth stage, the NCR increased (+0.079) under sugar beet, but decreased (−0.069) under winter wheat cultivation when atmospheric CO_2 concentrations were enriched (Table 2).

Stable C-isotopic analysis of nematode feeding types

The stable C-isotopic signatures of herbivorous, bacterivorous, fungivorous and omnivorous nematodes are presented in Figures 2 and 3. Carnivorous nematodes were excluded from these analyses due to their low abundance.

The stable C-isotopic signature ($\delta^{13}C$) of nematodes differed among feeding types (Figures 2 and 3) and was specifically affected by the analysed parameters (i.e., CO_2, crop type, crop growth stage) (Table 3). All feeding types, except herbivores and fungivores shortly before harvest of sugar beet (Mature Crop), reflected more negative $\delta^{13}C$ values under FACE compared to control conditions (Figures 2 and 3).

The stable C-isotopic signatures of herbivores significantly depended on plant growth stage ($P<0.005$) and significantly differed between CO_2 treatments independent of crop and sampling date ($P<0.005$) (Table 3). Under control conditions, their $\delta^{13}C$ values decreased from the Growth Phase to the stage of Mature Crop under sugar beet ($P = 0.001$), but increased under winter wheat cultivation ($P = 0.002$) (Figures 2 and 3). Under FACE conditions, $\delta^{13}C$ values of herbivores were significantly more negative ($P<0.001$) during the Growth Phase compared to the Mature Crop, independent of the crop type, and indicated CO_2 enrichment effects to be stronger during the period of main plant growth compared to the end of the growing season.

The stable-C isotopic signatures of bacterivorous nematodes were more negative under winter wheat compared to sugar beet cultivation (Figures 2 and 3). During the Growth Phase, this finding was more significant in the control ($P = 0.002$) than under atmospheric CO_2-enriched conditions ($P = 0.022$). At the end of the growing season significant crop effects were only found under FACE conditions ($P<0.001$) (Table 3). The $\delta^{13}C$ values of bacterivores increased from the Growth Phase to the stage of Mature Crop under sugar beet cultivation (Figure 2), indicating stronger crop growth stage effects under FACE compared to control conditions ($P = 0.092$). Under winter wheat, by contrast, the

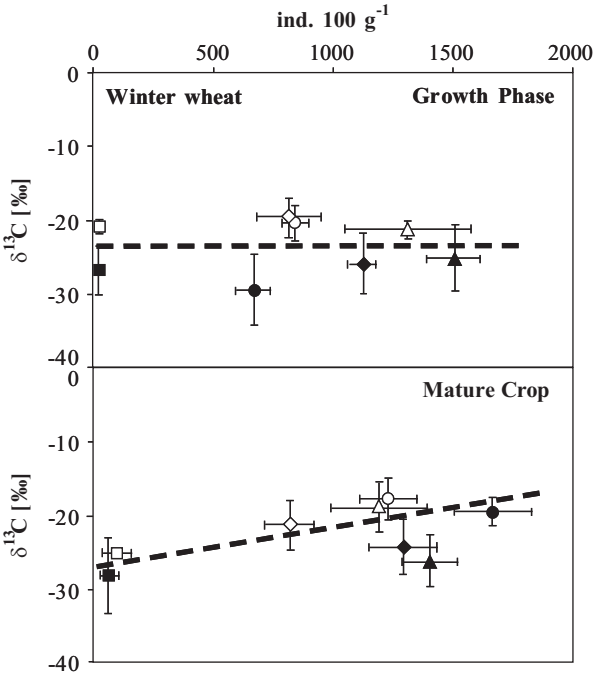

Figure 3. Winter wheat: $\delta^{13}C$ values (‰ ± SD) vs. abundance (ind. 100 g^{-1} dry soil ± SD) of herbivorous, bacterivorous, fungivorous and omnivorous nematodes for both crop growth stages (Growth Phase and Mature Crop) and CO_2 treatments (0–10 cm soil depth); broken lines indicate consistent $\delta^{13}C$ shifts of all feeding types under FACE conditions; legends see Figure 2.

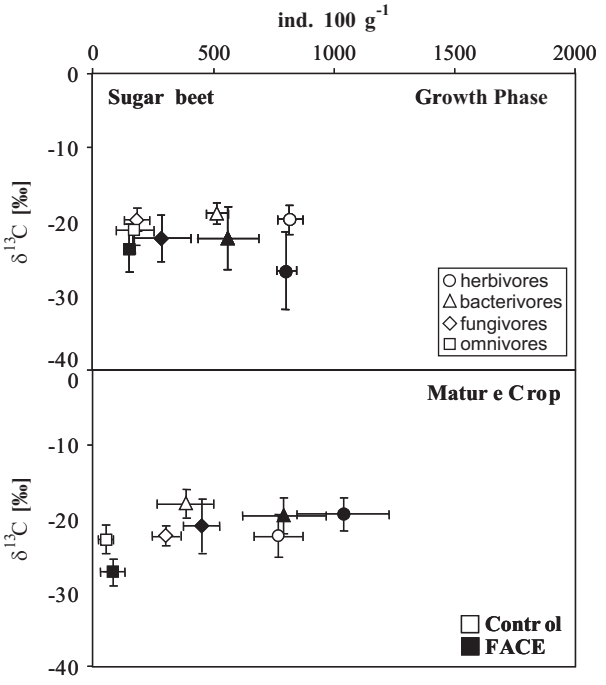

Figure 2. Sugar beet: $\delta^{13}C$ values (‰ ± SD) vs. abundance (ind. 100 g^{-1} dry soil ± SD) of herbivorous, bacterivorous, fungivorous and omnivorous nematodes for both crop growth stages (Growth Phase and Mature Crop) and CO_2 treatments (0–10 cm soil depth).

Table 3. *H*-values of Kruskal–Wallis test on effects of CO_2 enrichment, crop and crop growth stage (df = 1) on the stable C-isotopic signatures of nematode feeding groups (0–10 cm soil depth).

H-test	Herbivore	Bacterivore	Fungivore	Omnivore
CO_2				
Sugar beet				
Growth phase	21.540***	3.448(*)	2.676	2.766(*)
Mature crop	16.485***	4.229*	2.475	10.227***
Winter wheat				
Growth phase	26.314***	3.390(*)	29.153***	7.385*
Mature crop	9.484**	22.402***	2.415	1.222
Crop				
Control				
Growth phase	0.102	9.604**	0.399	0.284
Mature crop	23.511***	0.027	0.600	4.365*
FACE				
Growth phase	1.946	5.217*	4.630*	1.620
Mature crop	0.008	24.949***	6.403*	0.079
Crop growth stage				
Sugar beet				
Control	11.630***	2.072	8.556**	3.227(*)
FACE	28.113***	2.843(*)	0.373	6.613**
Winter wheat				
Control	9.734**	4.820*	1.475	13.200***
FACE	31.482***	1.002	2.307	0.182

Significance of effects: ***$P = 0.001$; **$P < 0.01$; *$P < 0.05$; (*)$P < 0.1$.

stable C-isotopic signatures of bacterivores significantly increased under control ($P = 0.028$), but decreased under FACE conditions from the first (Growth Phase) to the second (Mature Crop) sampling (Figure 3). Generally, the $\delta^{13}C$ values of bacterivores decreased under atmospheric CO_2 enrichment ($P < 0.1$), indicating stronger effects under winter wheat compared to sugar beet cultivation and at the end of the growing season compared to the period of plant growth (Figures 2 and 3).

Fungivores showed significantly more negative stable C-isotopic signatures under winter wheat compared to sugar beet cultivation when atmospheric CO_2 concentrations were enriched (Growth Phase: $P = 0.031$; Mature Crop: $P = 0.011$) (Table 3). Significant CO_2 enrichment effects on the $\delta^{13}C$ values of this feeding type were only detected during the Growth Phase of winter wheat ($P < 0.001$) (Table 3). The results indicate stronger CO_2 impacts on this feeding type under winter wheat compared to sugar beet cultivation and during the Growth Phase compared to the stage of Mature Crop (Figures 2 and 3).

Omnivorous nematodes reflected crop and crop-growth-stage-specific FACE effects (Figures 2 and 3), since their $\delta^{13}C$ values significantly decreased at the end of the growing season (Mature Crop) under sugar beet ($P = 0.001$), and during the period of main plant growth (Growth Phase) under winter wheat cultivation ($P = 0.007$) (Figures 2 and 3). While the stable C-isotopic signatures of omnivores significantly decreased (FACE: $P = 0.010$) or tended to decrease (Control: $P = 0.072$) from the Growth Phase to the stage of Mature Crop under sugar beet independent of CO_2 treatment, such a crop growth stage specific $\delta^{13}C$ shift under cultivation of winter wheat was only detected under control conditions ($P < 0.001$) (Table 3).

Generally, all feeding types showed more negative $\delta^{13}C$ values under FACE conditions when wheat was cultivated (Figure 3). Therefore, the stable C-isotopic signatures of feeding types under both CO_2 treatments diverged (illustrated by broken lines in Figure 3). During the Growth Phase of sugar beet, by contrast, the $\delta^{13}C$ values of herbivores and fungivores increased, while those of all other feeding types decreased due to atmospheric CO_2 enrichment (Figure 2). The extent to which $\delta^{13}C$ values of nematodes shifted due to advanced crop growth and/or CO_2 enrichment, therefore, was feeding type specific and crop-dependent.

Discussion

The present results indicate that nematode communities generally differ between root and cereal crops, in terms of total individual densities and feeding type composition. Under wheat plants, which produce large and highly branched root systems (Dunbabin et al. 2006), thus providing a broad range of food sources for rhizosphere communities, total nematode abundances were nearly twice as high as those detected under sugar beet cultivation. Impacts of crop growth stages on total nematode abundances differed between crops, since nematode densities increased under winter wheat, but decreased slightly under sugar beet cultivation from the period of main plant growth to the end of the growing season. These

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findings indicate strong seasonal trends, which are generally acknowledged for nematode communities in cropped systems (Freckman and Ettema 1993; Yeates and Bongers 1999), and which reveal the need to consider changes over time in any study of nematodes in agro-ecosystems (Yeates and Bongers 1999).

Comparing the feeding type composition of nematodes under both crops, it becomes obvious that wheat plants, which have high root exudation rates, and, consequently, show a broad range of root-associated microorganisms including mycorrhiza (e.g., Wachowska et al. 2006), favour fungivorous and bacterivorous nematodes over other feeding types. According to this finding, the NCR indicated that decomposition pathways are driven by fungi and bacteria, with bacteria accounting for a slightly larger share. Sugar beet plants, which build less extensive root systems along with lower microbial colonization, by contrast, seemed to benefit herbivore and omnivore feeding types, which accounted for over 50% of the total nematode community. Since fungivores were less abundant compared to bacterivores during the period of main sugar beet plant growth, the NCR at this time indicated decomposition processes to clearly follow a bacterial-based pathway (fast cycle), with bacteria as primary decomposers and bacterial-feeding fauna and their predators forming the associated food web. Towards the end of the growing season, the relative proportion of fungivores increased, and the NCR reflected the energy channel to be equally driven by bacteria and fungi. These findings indicate that rhizospheres of both crops provide totally different nutritional conditions, which favour or restrain several feeding types. Crop species and their growth stages, therefore, affect not only specific plant-feeding nematodes, but also the contribution of the microbial-feeding nematodes to the nematode fauna (Yeates and Bongers 1999).

Moreover, root growth of both crops was affected differently under FACE conditions. Results from the first crop rotation cycle in the FACE experiment indicate that the root production of winter wheat was enhanced by elevated CO_2 during the whole growing season, while the sugar beet rhizosphere reflected an increase only at the end of the growing season (Weigel et al. 2005). The fundamentally different conditions under sugar beet compared to winter wheat cultivation, combined with CO_2 enrichment-induced plant-specific shifts in root turnover, exudation rates and quantity and quality of root-derived carbon sources (Norby et al. 2001; Phillips et al. 2006), led to changes in food availability and quality for soil faunal communities. In addition to soil moisture, soil texture, and climate, such nutritional changes are known to be critical in determining the diversity of the nematode fauna (Yeates and Bongers 1999; Ruess 2003). In light of this relationship to food availability and quality, FACE effects on soil nematode communities as shown in the present experiment confirmed the findings of Drigo et al. (2007) and Li et al. (2007), who found CO_2 enrichment effects to differ depending on plant type and crop growth stage.

In the present study, atmospheric CO_2 enrichment did not exert any significant effect on the total nematode abundance, but nonetheless, tended to enhance individual densities at the end of both growing seasons, when plant biomass production was completed. Impacts were stronger under sugar beet compared to winter wheat cultivation. This finding conflicts with the results of Li et al. (2007), who described impacts of elevated CO_2 on nematode abundances in a wheat field under comparable soil conditions (cambisol of a sandy loam texture) to be more pronounced during the early part of the growing season and to disappear later in the season. These contradictory findings again reflect the need to include seasonal changes when investigating CO_2 enrichment effects on soil faunal communities in cropped systems and they also illustrate the broad variety of CO_2 enrichment-induced changes in soil food webs, which might lead to multi-directional effects. Hence, not only abundances but functional levels of soil organisms, such as life strategies (for collembolans during the same growing seasons see Sticht et al. 2008) or in the present case, feeding types of nematodes, need to be analysed to gain insights into below-ground CO_2 enrichment effects.

The remarkably low abundances of carnivorous nematodes detected in the present study could be attributed to the cotton-wool filter technique, which might have excluded these relatively large nematodes. In general, the use of various extraction methods should be taken into account when analysing nematode community composition.

Using the stable C-isotopic signatures, the labelled C derived from that fixed by plants in photosynthesis under FACE conditions could be traced in all nematode feeding types. The $\delta^{13}C$ values indicated all feeding types to be significantly affected by atmospheric CO_2 enrichment under at least one treatment, although significant FACE effects on relative abundance could only be found for herbivorous and clear trends only for fungivorous nematodes. Detected $\delta^{13}C$ shifts and, therefore, impacts of CO_2 enrichment differed significantly between both crops and between crop growth stages, probably indicating the use of

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different food sources due to changed rhizodeposition processes (Phillips et al. 2006). This finding, moreover, confirms the results of Yeates and Bongers (1999), and provides evidence that many nematode species might be able to use various food sources.

Under sugar beet cultivation, herbivorous and bacterivorous nematodes showed the strongest impact from atmospheric CO_2 enrichment, with respect to the relative abundance of feeding types. The contribution of herbivorous nematodes to the whole nematode community decreased, while that of bacterivores generally increased, indicating that the availability of food resources benefited bacterivores under FACE conditions. The $\delta^{13}C$ values of feeding types confirm strong CO_2 enrichment effects on herbivorous and bacterivorous nematodes and, moreover, indicate significant FACE effects on omnivores. Shifts in the $\delta^{13}C$ values of feeding types already reflected significant CO_2 enrichment effects during the period of main plant growth, while the community structure of nematode feeding types indicated significant FACE effects only at the end of the growing season, when root growth was enhanced (Weigel et al. 2005). While these findings might appear to be inconsistent, they may be the result of reproduction adaptation due to qualitatively modified food sources as has been shown for bacterivorous nematodes (Venette and Ferris 1998). Since several nematodes are selective feeders (e.g., Ruess et al. 2000) and differ in terms of food source or prey they are attracted to, shifts in food availability might first alter the stable C-isotopic signatures of affected species, and over time their relative contribution to the whole community. Such diet shifts might, for example, be the result of altered microbial community structure or diversity, as has already been observed under CO_2-enriched conditions (Phillips et al. 2002; Carney et al. 2007; Drigo et al. 2007). According to the CO_2 enrichment-induced increase in the relative abundance of bacterivorous nematodes, the NCR indicated decomposition processes to shift towards a faster, more bacterial-based energy and nutrient flow at the end of the growing season when sugar beet was cultivated.

Under winter wheat cultivation, the stable C-isotopic signatures and the relative abundances indicate that fungivorous nematodes are the primary beneficiaries of atmospheric CO_2 enrichment, independent of crop growth stage. This finding is presumably a result of the CO_2 enrichment-induced stimulation of abundance, hyphal length and root colonization of saprophagous and mycorrhizal fungi (e.g., Klironomos et al. 1996; Rillig and Field 2003; Blankinship and Hungate 2007). FACE effects on fungivores were most probably stronger under winter wheat compared to sugar beet cultivation, since fungi play a more important role in beet compared to wheat fields. Such stimulation in relation to CO2 enrichment-induced fungal composition changes would inevitably affect the next higher trophic level (Blankinship and Hungate 2007), and might have caused the increase in the relative abundances of fungivorous nematodes detected under winter wheat cultivation. According to this beneficial effect of CO_2 enrichment on fungivore nematodes, the NCR indicated a shift towards a slower, more fungal-based decomposition pathway under FACE conditions when wheat was cultivated.

In contrast to the results of Li et al. (2007), the present study indicates that carnivorous and omnivorous nematodes represent the feeding types which were least affected by atmospheric CO_2 enrichment. Neither beneficial effects of elevated CO_2 on carnivores as described by Coll and Hughes (2008), nor a CO_2 enrichment-induced decrease in the abundance of carnivorous nematodes as detected by Sonnemann and Wolters (2005) could generally be found. This finding indicates that CO_2 enrichment effects might decrease with increasing trophic distance from primary producers. In terms of omnivorous nematodes, strong $\delta^{13}C$ shifts but no changes in relative abundances were detected under FACE conditions. This result confirms the assumption of Coll and Hughes (2008) that omnivores are able to mitigate most of the adverse effects of elevated CO_2, such as low plant quality (e.g., Bezemer and Jones 1998), since they represent the only feeding type able to utilize numerous food sources and feed at two trophic levels. Although above- and below-ground primary production increased under both crops due to atmospheric CO_2 enrichment (Weigel et al. 2005), the relative proportion of herbivorous nematodes decreased or remained unchanged under FACE conditions. This finding is most probably a result of declining plant quality, and hence food quality due to increased C/N ratios and decreased plant protein concentration (Bezemer and Jones 1998; Ehleringer et al. 2002; Coll and Hughes 2008). Our results, therefore, conflict with those of Ayres et al. (2008), who showed herbivorous nematodes to be resistant to elevated atmospheric CO_2.

The present results indicate that CO_2 enrichment effects on soil nematodes differ between feeding types. These effects are thus, diet-mediated via plant-specific changes in root-derived carbon resources, representing the main carbon source for soil animal food webs (Pollierer et al. 2007). In the

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context of an arable soil of a luvisol soil type and a loamy sand texture, our study indicated that soil decomposition pathways shifted to a more fungal-based energy channel under winter wheat, but to a more bacterial-based channel under sugar beet cultivation when atmospheric CO_2 concentrations were enriched. The strong crop specificity of below-ground CO_2 effects detected in the present study provides a possible explanation for the diverse results of studies to date, which have revealed either bacteria (Sonnemann and Wolters 2005; Drigo et al. 2007) or fungi (Klironomos et al. 1996; Hu et al. 1999) to be most strongly affected by atmospheric CO_2 enrichment. The present results clearly demonstrate that both conditions are entirely possible, and that crop type and crop growth stage determine whether one or the other might occur. Shifts in individual numbers and structure of nematode communities, as detected in the present study, affect nearly all other soil biota of below-ground food webs via changed inter- and intraspecific interactions (Emmerson et al. 2005). In turn, such changes have the potential to alter decomposition and mineralization processes, thereby, the dynamics of major nutrients (Drigo et al. 2008) and finally, soil fertility or even the stability of the whole system (Emmerson et al. 2005). In terms of CO_2 enrichment effects on below-ground food webs, plant-related nematode–microbe interactions seemed to represent a key factor. Since no significant effect of elevated CO_2 on total microbial biomass could be observed in the Braunschweig FACE experiment (Weigel et al. 2005), the microbial community structure, diversity and activity might have changed under elevated CO_2 (Phillips et al. 2002; Carney et al. 2007; Drigo et al. 2007), thus specifically affecting fungivorous and bacterivorous nematodes. In order to generate the best possible understanding of CO_2 enrichment-induced below-ground changes in arable soils, further investigations on microbial communities, different crop and soil types should be carried out. In addition, larger numbers of sampling dates, distributed over the growing season, are needed.

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Part 3

3.1 Discussion

The results of the present study reveal that atmospheric CO_2-enrichment induces changes in the taxonomic and functional diversity of collembolans and nematodes. Relative abundances and stable C-isotopic signatures of functionally different groups indicate alterations of food availability and supply to drive the modifications of soil fauna community compositions. The results, therefore, confirm the overall hypothesis of the present thesis (Chapter 1.2).

3.1.1 Stable C-isotopic analyses of functionally or taxonomically classified collembolans and nematodes, applicability of chemical agents, and expressiveness of soil animal stable C-isotopic signatures

By means of stable C-isotopic analysis, changing C-translocation processes, feeding behaviours of various organisms, interactions between different trophic levels, and connections and transitions within matter flows and energy fluxes can be analysed and quantified (e.g., Post et al., 2002; Glaser et al., 2006). Due to this broad range of application fields, the use of this technique for analysing biogeochemical processes under changing environmental conditions (Pendall et al., 2008), food web linkages (Post et al., 2002), and wide-ranging climatic impacts (Nösberg & Long, 2006) has increased over the last years.

Whereas applicable methods for analysing stable C-isotopic signatures of earthworms, enchytraeids (Schmidt et al., 2004), mites (Scheu & Falca, 2000), termites (Tayasu et al., 1997), and ladybugs (Ostrom et al., 1997) are described, no studies focussing on stable C-isotopic analysis of functionally classified collembolans and nematodes exist.

Thus, prior to the field study, it was necessary to determine a measurable number of individuals, ensuring a sample dry weight appropriate to mass-spectrometrical analysis of animal stable C-isotopic signatures. This was necessary as the precise determination of the $^{13}C/^{12}C$ ratio of a sample is only possible if the amount of sample material ensures a C-content that, during measurement, engenders peaks with amplitudes of at least 1 V. If amplitudes evince values above or below this range, as respective contents of C-isotopes within samples are too high or low, measurement results do not exactly reflect the stable C-isotopic signatures of specimens.

To detect an appropriate sample size, numbers of 30, 40, 50, 70 and 100 individuals of nematodes were applied for stable C-isotopic analysis. The results indicated dry weights of between 0.005-0.05 mg to represent a well-measurable content of stable C-isotopes, inducing required 1 V amplitudes, and hence to precisely reflect $^{13}C/^{12}C$ ratios of organisms. Thus,

according to body size, individual numbers of between 30 and 100 nematodes were required to perform one stable C-isotopic analysis. With regard to collembolans, representing the second soil fauna group analysed during the present study, previous investigations revealed smaller numbers of between 1 and 30 individuals to provide a well measurable sample dry weight (Sticht et al., 2006).

As the classification of field-sampled collembolans and nematodes to species and feeding type level generally requires the preparation of respective animals, moreover, several methodical tests concerning the applicability of various sample preparation measures prior to stable C-isotopic analyses of organisms were necessary. In this context, preservation, fixation and bleaching measures, which are common practice and often indispensable previous to the taxonomical or trophic classification of soil animals, were of major importance. The chemical agents commonly used for this purpose mostly represent C-sources with the potential to alter the $\delta^{13}C$ values of organisms. Against this background, impacts of various agents on the stable C-isotopic signatures of collembolans and nematodes were analysed before final field samplings were carried out (Paper 2.1). This way, it was assessed under which conditions and limitations these agents are applicable previous to stable C-isotopic analysis of respective soil animals.

The results of these methodical tests revealed monoethyleneglycol, 96 % ethanol and 45 % lactic acid, commonly used to kill, preserve, and bleach collembolans, as well as 4 % formalin, applied to preserve nematodes, to be usable prior to analysis of animal stable C-isotopic signatures (Paper 2.1). Ethanol, formalin and lactic acid admittedly induced small changes of animal $\delta^{13}C$ values, but shifts were < 2 ‰ and standard deviations were low (Paper 2.1). Thus, these agents are nonetheless suitable as long as differences between comparative analyses are regarded, and differences between stable C-isotopic signatures of C-sources are bigger than impacts through the agents. These conditions were given during the present study, as differences between $\delta^{13}C$ values of identical species (Paper 2.2) and feeding types (Paper 2.3), treated in the same manner, should be regarded under two different atmospheric CO_2-concentrations. Hence, stable C-isotopic analyses of collembolans (Paper 2.2) and nematodes (Paper 2.3) were possible within the framework of the FACE experiment. All measurements of soil animals were performed based on these methodical findings (Paper 2.1).

Concerning the interpretation of soil organism and plant $\delta^{13}C$ values, fractionation processes which occur during C-fluxes from the atmosphere via plants to the soil food web have to be

considered. In this context, the photosynthetic discrimination of terrestrial plants against ^{13}C during the CO_2-diffusion along the CO_2-gradient between atmosphere and intercellular spaces of leaves represents one important process. As light isotopes diffuse more quickly than their heavier counterparts, the carbon incorporated by leaves is depleted in ^{13}C compared to that left behind in the atmosphere (Pataki et al., 2007). Moreover, the photosynthetic enzyme RuBisCo (Ribulose-1.5-bisphosphate carboxylase), which catalyzes the first major step of carbon fixation during the Calvin cycle, preferentially converts $^{12}CO_2$-molecules (Griffith, 2006). The ^{13}C depletion within leaf chloroplasts resulting from these discrimination processes affects the stable C-isotopic signature of the respective whole plant. Accordingly, the C3-plant-fixed carbon which is emitted to soil evinced more negative $\delta^{13}C$ values than that of atmospheric CO_2 (Farquar et al., 1988).
Beside this plant-mediated ^{13}C-fractionation, the stable C-isotopic signature, moreover, changes during carbon fluxes through soil food webs. Even though there is only little trophic fractionation of carbon, animals typically become slightly enriched in ^{13}C compared with their diets (Post et al., 2002), due to isotopic discrimination during assimilation (Passey et al., 2005) and metabolic pathways (McCutchan et al., 2003). Thus, animals generally reflect the $\delta^{13}C$ value of their food source with a mean deviation of between 0.4 ‰ (Post et al., 2002) and 1.0 ‰ (Hobbie et al., 2007).

Against this background it becomes obvious that stable C-isotopic signatures of soil animals, as analysed during the present study, do not express absolute values. Thus, no detailed conclusions on exact food sources could be drawn by using this method exclusively. Nevertheless, differences between $\delta^{13}C$ values of identical species, life strategies, or feeding types from both CO_2-treatments (FACE and control) provide insights into specific impacts on various taxonomic and functional groups of the soil food web, and indicate food quality changes and diet switching (Papers 2.2 and 2.3). This way, soil animal stable C-isotopic signatures imply changes within ecosystem processes and interactions, and, thereby can contribute to improving knowledge on, for example, plant type or plant developmental stage dependent CO_2-effects, determining future soil conditions.

Provided the above mentioned requirements are complied with, and use of C-sources during sample preparation could be either entirely avoided, or applied C-sources do not, or only to a low extent, influence stable C-isotopic signatures of organisms, this method could prospectively be used to investigate further functionally different groups of the soil food web

as well. This way, more detailed information on atmospheric CO_2-enrichment-induced functional changes below-ground might be obtained.

3.1.2 Investigating CO_2-enrichment effects on soil processes in agroecosystems by combining biodiversity and stable C-isotopic signatures of functionally different soil fauna groups

In view of future food security, which requires a marked increase in agricultural production, and the essential adjustment of agricultural management to climate change (Ingram et al., 2008; Ortiz et al., 2008), it is of utmost importance to know which crops prospectively enable the production of required yields, and which management measures are necessary to promote their adaptation to future conditions.

Previous investigations of cultivated crops indicated a broad range of different responses to atmospheric CO_2-enrichment, even under otherwise identical conditions and management practices (Bloom, 2006). Most probably soil-driven bottom-up effects (Drigo et al., 2008; Pendall et al., 2008) are responsible for this large variability of plant reactions. These effects in turn are regulated by C-allocation from above- to below-ground. In this context, exudation, secretion, and cell death, permanent processes during root growth, play a major role as they induce a continuous flux of various forms of carbon (C) from roots to the soil (Hill et al., 2006). This top-down C-transport via rhizodeposition regulates microbial communities, and thus organic matter decomposition, N-mineralization, and C-storage in soils (Hill et al., 2006). By controlling below-ground nutrient release, these processes feed back to plant nutrient supply (Tarnawski & Aragno, 2006).

As atmospheric CO_2-enrichment is known to stimulate plant biomass production above- as well as below-ground (Weigel et al., 2005), and to enhance exudation rates via changed rhizodeposition processes (Phillips et al., 2006b), the C-input into the soil increases as well under future atmospheric CO_2-conditions (Hill et al., 2006; Tarnawski & Aragno, 2006). However, up to now our ability to predict the fate of this additional C-input has been limited, and it is so far unknown to which extent it will be present in the form of labile, easily utilisable or stable, accumulating compounds (Hill et al., 2006). Since C-retention represents one of the most important factors when predicting future plant growth and forecasting capacities of agricultural production, knowledge of these processes is of relevance concerning the development of appropriate management measures ensuring future food supply. Beside that, the fate of carbon is still a matter of major interest as an increase of soil C-storage might

offer a short-term solution until long-term measures and policies are developed for reducing CO_2-emissions or increasing CO_2-sequestration (Lal, 2003).

Lacks of knowledge, in this context, mainly base on technical and methodological difficulties in making accurate measurements to quantify below-ground processes (Hill et al., 2006), like for example root secretion (Tarnawski & Aragno, 2006), nutrient cycling, or C-sequestration (Niklaus & Falloon, 2006). Additionally, our understanding of ecosystem-level feedbacks between plants, soil microbes, and soil organic matter is as yet incomplete (Hill et al., 2006).

To gain insights into several, up to now, poorly understood soil C-turnover processes under future atmospheric CO_2-conditions, numerous studies focussing on rhizosphere microbial communities which are directly affected by changing rhizodeposition processes (Haase et al., 2008) and, moreover, stimulate and regulate root exudation (Meharg & Killham, 1995), were carried out over the last years (Tarnawski & Aragno, 2006). The results of these studies reveal strongly differing effects of atmospheric CO_2-enrichment on microbiota, particularly with regard to microbial respiration and activity, and indicate a broad range of potential microbial responses (Phillips et al., 2006a). Concerning this variability of CO_2-effects on microbiota, organisms belonging to higher trophic levels, like for example collembolans and nematodes, might play a major role, as they control microbial growth and community size trough their grazing activity (Tordoff et al., 2008; Larsen et al., 2008; Kaneda & Kaneko, 2008). By regulating microbial communities, soil animals indirectly drive turnover- and exudation rates (Phillips et al., 2006a), and control the flux of nutriments from directly unavailable pools to the plant (Tarnawski & Aragno, 2006). According to these functions, the inclusion of soil fauna is indispensable when quantifying CO_2-effects on below-ground processes (Tarnawski & Aragno, 2006; Blankinship & Hungate, 2007). As soil animals remained unconsidered within most existing studies, there is an urgent need for research concerning CO_2-enrichment-induced, soil fauna-driven, and so far less understood mechanisms of soil C-cycling (Phillips et al., 2006a; Blankinship & Hungate, 2007). Knowledge of these processes is essential in order to develop appropriate measures, promoting the adaptation of ecosystems to climate change, and ensuring biodiversity conservation.

Since such studies represent comparatively new research approaches, their implementation often requires several preliminary analyses, for instance concerning the development, applicability, or modification of methods, as carried out during the present study (Paper 2.1). Also, the detection of suitable indicator organisms, which reflect CO_2-enrichment-induced changes of structures and processes within the soil food web, and which allow conclusions to be drawn on feedback effects on cultivated crops, is currently of major importance.

The results of the present study reveal that the detection of diversity changes within collembolan and nematode communities, linked to the analyses of stable C-isotopic signatures of functionally different species or feeding types, represents a suitable method to gain insights into CO_2-enrichment-induced alterations within soil food web structures, embedded interactions, and C-translocation processes (Papers 2.2 and 2.3). Thus, this combined method contributed during the present study, and might as well prospectively contribute, to closing existing gaps in knowledge.

Diversity changes, in this context, reflect benefits or disadvantages of specific species or feeding types. According to their respective functions within the soil food web and during organic matter decomposition, such alterations allow conclusions to be drawn on changes of fundamental rhizosphere processes. The stable C-isotopic signatures of functional groups additionally reflect food-specific impacts and provide insights into soil food web interactions, thereby indicating dietary interdependences.

Within the present study, use of this combined, integrated approach allowed to reveal quantitative and, in particular, qualitative food changes to drive alterations within soil fauna community compositions under increasing atmospheric CO_2-concentrations (Papers 2.2 and 2.3). Thus, the overall hypothesis of the present study (Chapter 1.2) was confirmed by means of this method.

The detected results agree with the findings of Drigo et al. (2008) and indicate that the food availability and quality of entire soil organisms changes under future atmospheric CO_2-conditions, as influenced by CO_2-enrichment-induced modifications of the biochemical composition of rhizodeposites, for instance through increased C/N ratios (Hu et al., 1999). In this context, root exudates, representing the most readily utilisable substrate for microbial growth (Hill et al., 2006), and the food base of the whole soil food web (Pollierer et al., 2007), play a major role. According to their respective trophic position in the rhizosphere, functionally differing groups of collembolans (Paper 2.2) and nematodes (Paper 2.3), which selectively feed on certain food sources out of a broad dietary range (Yeates & Bongers, 1999; Bracht-Jørgensen et al., 2008; Larsen et al., 2008), clearly differed concerning their food-specific impacts. Intensities of these influences were detected and quantified by means of stable C-isotopic signatures of collembolan species and life strategies (Paper 2.2), as well as of nematode feeding types (Paper 2.3). Since both soil fauna groups are directly involved in C-turnover and nutrient cycling (Kaneda & Kaneko, 2008), and moreover interact with plants and plant-associated microorganisms (Kaneda & Kaneko, 2008), the use of stable C-

isotopic analysis provided insights into C-translocation and functional and trophic interactions which would not have been obtained by means of bulk sizes or diversities only. Moreover, it enabled conclusions to be drawn on potential structural changes of microbial communities (Chapters 3.1.3 and 3.1.4) that direct C-fluxes within the soil system (Jones & Darrah, 1996). Hence, this method, already widely used within food web- (e.g., Hishi et al., 2007; Hobbie et al., 2007), C-allocation- (Pataki et al., 2007; Pendall et al., 2008) and climatic studies (Nösberg & Long, 2006), also represents a valuable tool during integrated approaches such as present investigations (Papers 2.2 and 2.3), which link diversity detections and biochemical dietary analyses.

Further studies combining $\delta^{13}C$ values and diversities of functionally and trophically different soil organisms would contribute to closing remaining gaps of knowledge concerning CO_2-effects on soil food webs, and thus, would help to specify predictions of future soil conditions, plant development and finally yield productivity. The present study, and potentially according further studies, promotes the assessment of climate change effects on agroecosystems, which is of current relevance in order to develop appropriate future management measures and to ensure agrobiodiversity conservation (CBD, 2000).

3.1.3 Interactions between crops and soil fauna regulate CO_2-enrichment effects on rhizosphere processes in agroecosystems

The results of the present study confirm the overall hypothesis of the thesis (Chapter 1.2), and reveal diversities of collembolans and nematodes to change under future atmospheric CO_2-conditions. Interactions between crops and soil fauna, thereby, control CO_2-enrichment-induced changes of C-turnover and –translocation as well as nutrient release in arable soils. The rhizosphere, in this context, represents the key-link of the ecosystem. According to the extent to which root growth is enhanced, the extension of the rhizosphere increases, providing an enlarged habitat for root-associated organisms, particularly for microbial communities (bacteria and fungi). Moreover, rhizodeposition processes, and thereby quantities and qualities of root exudates change as well (Phillips et al., 2006b) as a consequence of altered plant biochemical compounds (Weigel, 2005; Bloom et al., 2006; Taub et al., 2008). As the rhizosphere represents the main-C-source within the soil food web (Pollierer et al., 2007), such CO_2-enrichment-induced alterations of below-ground biomass and associated processes simultaneously cause a modification of food supply and availability (Drigo et al., 2007) for entire rhizosphere communities.

Since several crop types, as in the present case one root- and one cereal-crop, differ strongly concerning the structure and biomass of their root systems (e.g., Weigel et al., 2005), they generally show different compositions of root associated soil organisms.

Whereas cereal crops such as wheat generally possess large and highly branched root systems, which enable high exudation rates and thus allow high densities of root associated microorganisms including mycorrhiza (Smit et al., 1999), the amount of fine roots and associated microbiota of sugar beet plants, which build large sucrose-accumulating tap roots, is only low (Land et al., 1993). Moreover, soil structures might differ depending on cultivated crops, due to the occurrence or absence of certain microorganisms. In this context, mycorrhiza play a major role, as soils containing mycorrhiza are described as showing an increased proportion of small soil aggregates (0.25-1.0 mm) when exposed to increasing atmospheric CO_2-concentrations (Rillig et al., 1999). Altogether, the wheat rhizosphere, aside from differing soil conditions due to a modified soil structure, provides higher food availability and a broader range of food sources along with lower food competition, compared to the rhizosphere of beet plants. Thus, considering that soil conditions and food supply, apart from soil texture and soil moisture, represent key-factors determining soil fauna diversity (e.g., Yeates & Bongers, 1999), the wheat rhizosphere comparatively offers more favourable habitat conditions for the majority of soil organisms.

Collembolan as well as nematode community structures and relative abundances, detected during the present study, confirm and reflect these generally more beneficial dietary conditions under winter wheat cultivation as compared to sugar beets. Thus, collembolans showed an increased species diversity and a higher number of main species (Paper 2.2), nematodes a more equal distribution of typical rhizosphere feeding types (bacterivorous, fungivorous and herbivorous nematodes) and overall higher abundances (Paper 2.3) when wheat was cultivated. Under sugar beet cultivation, by contrast, the proportion of omnivorous nematodes, which are generally abundant in agricultural soils (Coll & Hughes, 2008), and, as generalist feeders, are able to use several food sources independent of a certain trophic level, was clearly higher compared to that detected under winter wheat cultivation (Fig. 1).

These fundamentally different nutritional conditions in the rhizospheres of several crop types represent the initial points that drive impacts of atmospheric CO_2-enrichment on the soil food web, as they determine interactions between trophic levels and species, which in turn regulate changes of soil processes.

The results of the present study indicate that, independent of crop type, impacts of atmospheric CO_2-enrichment on the soil fauna decrease with increasing trophic distance from plants. Thus, hemiedaphic collembolan species as well as bacterivorous, fungivorous and herbivorous nematodes, which inhabit the upper soil layer and mainly obtain their food directly from the rhizosphere (Hishi et al., 2007), reflected stronger FACE-effects than euedaphic collembolans (Paper 2.2) or carnivorous and omnivorous nematodes (Paper 2.3), which show a higher spatial and trophic distance from cultivated crops. Nonetheless, changes of the stable C-isotopic signatures of both investigated soil fauna groups (collembolans and nematodes) verify that the isotopically labelled surplus carbon reaches all species and feeding types, and hence all trophic levels and functional groups within the soil food web (Papers 2.2 and 2.3). The present investigations reveal that the community composition of the rhizosphere fauna changes under atmospheric CO_2-enriched conditions as a result of altered food availability and quality. CO_2-impacts are indicated to spread over the whole soil food web via cascading effects, due to modified trophic and functional interactions. The results, therefore, confirm the overall hypothesis of the thesis (Chapter 1.2), and, moreover, agree with recent studies which suggest modified interactions to be primarily responsible for the most decisive changes of ecosystems, their functions and services under future atmospheric CO_2-conditions (Emmerson et al., 2005; Tylianakis et al., 2008).

As the relative contribution of hemiedaphic species to the whole collembolan community, without exception, increased under atmospheric CO_2-enrichment (Paper 2.2; Fig. 1), a general benefit of this life strategy can be assumed under future conditions. This favourable CO_2-effect on a functional soil fauna group, which shows a close trophic relation to the vegetation (Hishi et al., 2007), and should therefore be most strongly affected by plant biochemical changes like increased C/N ratios or reduced protein contents (Weigel, 2005; Taub et al., 2008), is largely owing to two distinctive collembolan characteristics. Thus, collembolans are, on the one hand, able to use a wide range of various food sources (Berg et al., 2004) and, on the other hand, to adjust adverse dietary situations by compensatory feeding (Haubert et al., 2004). The linkage of both of these feeding capabilities enables collembolans to feed selectively on the most convenient, available food source out of the entire food range (Sabatini & Innocenti, 2000), and, moreover, to compensate decreasing food qualities by increasing food consumption. Therefore collembolans respond positively to an increased food supply, even though the dietary conditions are basically unfavourable (Haubert et al., 2004).

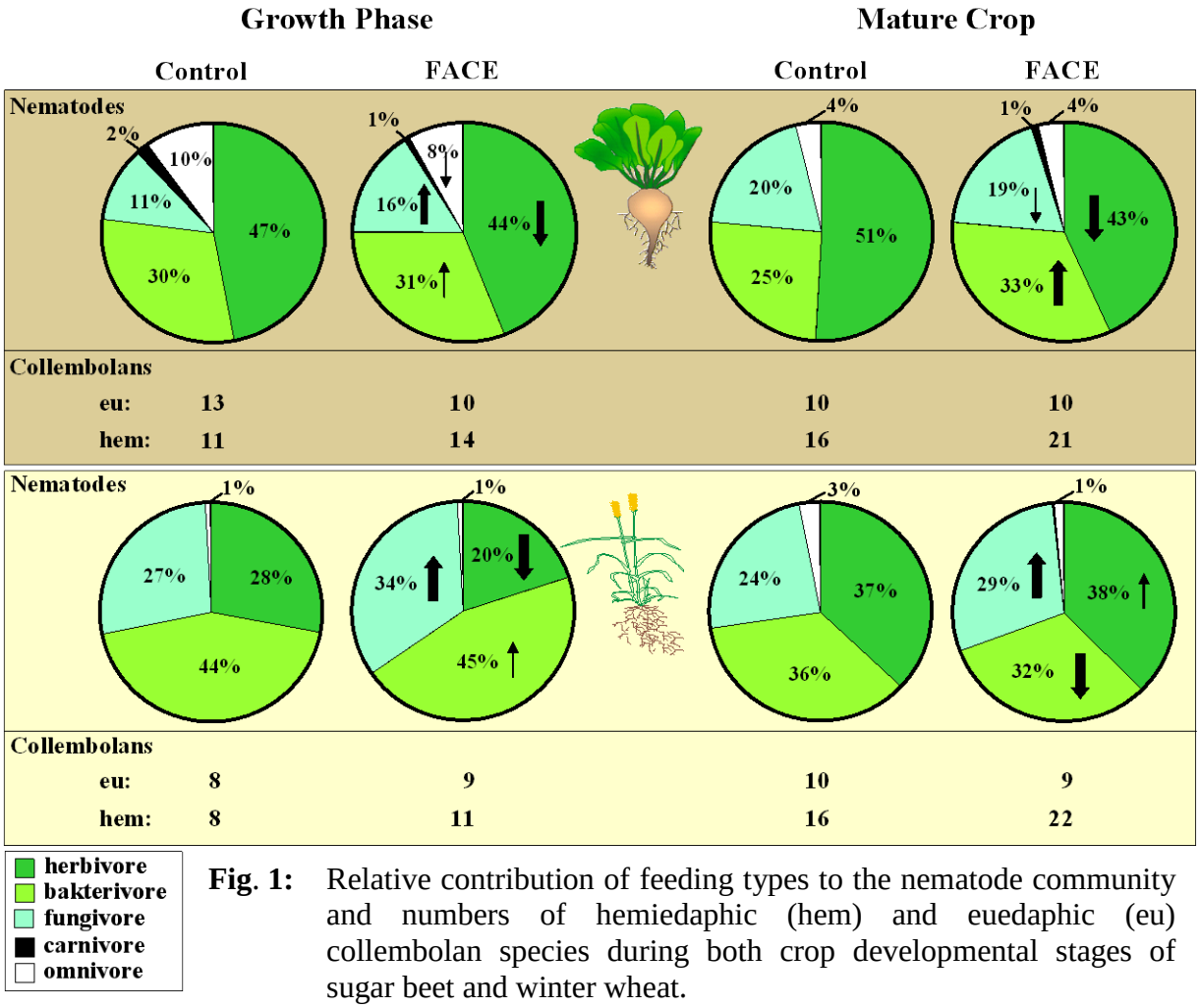

Fig. 1: Relative contribution of feeding types to the nematode community and numbers of hemiedaphic (hem) and euedaphic (eu) collembolan species during both crop developmental stages of sugar beet and winter wheat.

However, atmospheric CO_2-enrichment does not generally benefit organisms which show a close trophic relation to plants, as indicated by the relative abundances of nematode feeding types (Paper 2.3). The present results show decreasing relative proportions of herbivorous nematodes under FACE-conditions during three out of four samplings (Fig. 1). Contrasting to the detected effects on hemiedaphic collembolans (Paper 2.2), this finding clearly reflects impacts of the CO_2-enrichment-induced reduction in food quality (Drigo et al., 2008). This negative influence bases on the nutritional restriction of nematode feeding types (except omnivores) to a certain kind of food source, due to their head structures and mouth parts (Yeates & Bongers, 1999; Blanc et al., 2006). Thus, their capability for diet switching is limited to a change within their respective food sources.

Against this background, reactions of nematode feeding types, which respond more sensitively to changed food availability, indicate impacts on specialized feeders, while collembolans, especially hemiedaphic species (Maraun et al., 2003), rather reflect effects on

food generalists. This diet-specific classification of both soil fauna groups is useful and indicative in the context of the present study as, according to forecasts, specialists are expected to be more strongly affected by increasing atmospheric CO_2-concentrations than generalists (Balmford & Bond, 2005; Ayres et al., 2008; Coll & Hughes, 2008). Based on the intensity of CO_2-enichment effects on collembolan diversities (Paper 2.2) compared to that on the relative abundances of nematode feeding types (Paper 2.3), the present study confirms this assumption.

Concerning the crop-specificity of below-ground CO_2-enrichment effects, the present results reveal that the intensity of impacts on functionally different soil fauna groups varies strongly depending on crop type. Collembolans, as well as herbivorous and bacterivorous nematodes, generally reflected stronger CO_2-effects under sugar beet compared to winter wheat cultivation. Fungivorous nematodes, in contrast, indicated stronger impacts when winter wheat was cultivated (Papers 2.2 and 2.3; Fig. 1).

The modification of relative abundances of bacterivores and fungivores indicates that CO_2-enrichment-induced changes within the microbial community most probably drive impacts of atmospheric CO_2-enrichment on soil fauna communities. As most recent studies describe abundances of root associated microorganisms to be not affected by atmospheric CO_2-enrichment (e.g., Haase et al., 2008), impacts on soil fauna communities are presumably led back to altered microbial enzyme regulations, activities, and productivities (Haase et al., 2008) as well as, especially concerning bacteria, altered species compositions (Drissner et al., 2007; Drigo et al., 2007), by which microorganisms adapt themselves to changed substrate qualities. In this context, investigations of microbial communities indicate fast-growing r-strategists, which mainly feed on easily metabolizable substrates, to benefit under atmospheric CO_2-enriched conditions, to the disadvantage of slow-growing K-strategists, which are more adapted to degrade less labile substrates (Tarnawski & Aragno, 2006). As the structure of fungal and bacterial communities generally depends on plant species composition (Drigo et al., 2007), and, moreover, CO_2-enrichment-induced functional changes vary between microbial communities of differing species composition (Chung et al., 2007), it can be assumed that CO_2-enrichment effects on root-associated microorganisms differ as well, depending on whether sugar beets or winter wheat are cultivated. The following higher trophic levels of the soil food web, exemplarily analysed during the present study, reflect this crop-specificity of below-ground CO_2-enrichment effects (Papers 2.2 and 2.3).

According to the alterations of relative abundances of bacterivores in relation to those of fungivores, the present results indicate a CO_2-enrichment-induced shift of decomposition processes towards a faster, bacterial-based turnover under sugar beet cultivation, but towards a slower (Moore & Hunt, 1988) fungal-based energy channel under cultivation of winter wheat (Paper 2.3). The ratio of fungi to bacteria, which was analysed by a microbiological working group at the Institute of Biodiversity (vTI Braunschweig), however, reflected a shift towards increased relative proportions of bacteria under CO_2-enriched conditions, independent of crop (Anderson et al., in prep.).

Linking these results, an enhanced ingestion of fungi by the soil fauna under winter wheat, counteracting an increase of the relative proportion of fungi concerning the whole microbial biomass can be assumed. The findings imply that bacteria, despite their lower C/N ratio (Paul & Clark, 1996) and therefore higher food quality compared to that of fungi, presumably account for only a minor share of the diet used by the soil fauna under wheat cultivation. Thus the nutritional quality of bacteria as food source of bacterivorous organisms seems to change under CO_2-enriched conditions, depending on cultivated crop. Since nematodes are known to prefer several bacterial species above others (Moens et al., 1999), and moreover, to show varying growth rates depending on food sources used (Venette & Ferris, 1998a; b), the suggested quality changes of bacteria as food source are most probably due to a taxonomically and functionally altered diversity of bacterial communities. These changes could be explained by the stronger impact of atmospheric CO_2-enrichment on bacteria compared to that of fungi (Drigo et al., 2007). Species compositions, as well as the activity of bacterial communities, might therefore change sooner and more strongly than that of fungal communities. In this context the above mentioned shift from K- towards r-strategists within the bacterial community (Tarnawski & Aragno, 2006; Drigo et al., 2008), presumably plays an important role, as the larger root system of wheat, along with higher inputs of root exudates, the main food source of r-strategists (Tarnawski & Aragno, 2006), into the soil, supports bacterial species of this growth strategy more strongly than that of sugar beet plants. Due to the closer trophic relation of r- compared to K-strategists to plants, species of this growth strategy, by comparison, should inevitably reflect more negative stable C-isotopic signatures. Therefore an increased relative proportion of r-strategists most probably led to the higher CO_2-enrichment-induced $\delta^{13}C$-shifts of bacterivores under wheat compared to beet cultivation, detected during the present study (Paper 2.3).

According to these results, it can be assumed that changes within the bacterial community resulted in improved nutritional conditions under sugar beet cultivation, whereas the

nutritional quality of bacteria as soil faunal food source seemed to decrease under cultivation of winter wheat. For that reason, the contribution of bacteria to the nematode diet presumably declined, and the relative proportion of bacterivores, which are reliant upon this food source, decreased due to reproduction adaptation when wheat was cultivated (Paper 2.3; Fig. 1).

The CO_2-enrichment-induced increase of relative abundances of fungal-feeding nematodes under winter wheat cultivation (Fig. 1) is most probably due to increased individual densities, hyphal lengths, and degree of root colonization of sacrophagous fungi and mycorrhiza (Coûteaux & Bolger, 2000; Olsrud et al., 2004; Chung et al., 2007). As the studies of Mougel et al. (2006) and Drigo et al. (2007) reveal that taxonomic compositions of fungal communities respond time-delayed to increasing atmospheric CO_2-concentrations, changes of the fungal species composition are likely to be not of importance until the end of the growing season (Chapter 3.1.4).

Impacts on collembolans, considering their minor food specificity compared to that of nematodes, imply similar conclusions. Thus, they reflected more positive CO_2-enrichment effects, concerning their species diversity and main species number, under sugar beet compared to winter wheat cultivation (Paper 2.2). Taking into account that collembolans prefer certain food sources above others (Bracht-Jørgensen et al., 2008), differ concerning their food preferences depending on species (Scheu & Falca, 2000; Chahartaghi et al., 2005), and show differing survival-, activity-, growth- and reproduction rates depending on food quality (Bracht-Jørgensen et al., 2008; Larsen et al., 2008), this finding again indicates that atmospheric CO_2-enrichment induces a stronger improvement of food supply for a larger number of species under beet compared to wheat cultivation (Paper 2.2).

Since previous studies suggested that collembolans commonly prefer fungi compared to other kinds of food sources (Ponge, 2000), a stronger increase of collembolan diversity parallel to a rise of fungal colonization, as implied by nematodes under cultivation of winter wheat (Paper 2.3), would have been expected. The generally favourable dietary conditions within the wheat rhizosphere, which allow high collembolan species diversities anyway (Paper 2.2), and the increased relative abundances of fungivorous nematodes (Paper 2.3; Fig. 1), which led to enhanced food competition, provide possible explanations for why the present results did not comply with these expectations. Furthermore, collembolans differ in their food preference for certain fungi, depending on individual age and species affiliation (Larsen et al., 2008). They assess the food quality of fungi depending on the fungal substrate (Kaneda & Kaneko, 2004) and respond to modified substrate-N-ratios via altered growth rates and fertility (Hogervorst et al., 2003). Thus, the occurrence of mycorrhiza (Boswell et al., 1998), which act as C-sinks

and modify root exudates, thereby altering substrates of other fungi (Jones et al., 2004), presumably contributes to the minor increase of collembolan diversity under wheat compared to beet cultivation. Moreover, the majority of collembolan species avoids feeding on mycorrhiza as long as appropriate dietary conditions exist and saprophytic or pathogenic fungi are available (Klironomos & Ursic, 1998).

In total, the present results (Papers 2.2 and 2.3) indicate, that beside root turnover, exudation rates, and quantities and qualities of root derived C-sources (Phillips et al., 2006b), the microbial community structure within the rhizosphere of arable crops might change as well, when exposed to atmospheric CO_2-enrichment. Thereby CO_2-effects on microorganisms are directly driven by soil- and crop type (Drigo et al., 2007) and the structure of the rhizosphere, and, moreover, indirectly regulated by the composition of the soil fauna and the food specificity of embedded functional groups and species (Papers 2.2 and 2.3). Based on the present findings, CO_2-enrichment induced a shift of decomposition pathways towards a more bacterial-based energy channel under sugar beet, but towards a more fungal-based energy channel under winter wheat (Paper 2.3) under comparable soil conditions. As, under current environmental conditions, crop rotations are generally assumed to shift the community structure towards a more fungal-dominated community (Six et al., 2006), this result emphasizes that CO_2-enrichment-induced changes of nutrient mineralization strongly depend on crop type. Such changed decomposition processes might have far-reaching consequences for the stability of entire ecosystems, since both energy pathways differ markedly concerning their resistance to external, anthropogenic impacts. In this context, organisms of the bacterial path-way represent trophic links that were generally more resistant to changes, due to their high ability to disperse in time, and passively disperse in space. The fungal-pathway, by contrast, is more susceptible to disturbance as it cannot recover that easily in space and in time (Hedlund et al., 2004).

Beyond that, recent investigations indicate that the fungal energy channel increases the potential for C-storage (Phillips et al., 2006a). Furthermore, when wheat was cultivated decomposition processes and C-mineralization were described to be reduced under CO_2-enriched conditions (Marhan et al., 2008). These findings are confirmed by the stable C-isotopic signatures of soil material detected in the present study, which reflect a decrease in ^{13}C, and therefore an increased input of isotopically labelled surplus carbon, in the course of the wheat growing season (Paper 2.2). Accordingly, it can be assumed that under future atmospheric CO_2-concentrations the cultivation of wheat increases the C-sink-capacity of

soils to a larger extent than the cultivation of sugar beet, provided soil conditions are comparable.

In total, the present results confirm the studies of Emmerson et al. (2005) and Tylianakis et al. (2008), who described that even small changes of diversities and interactions have the potential to sustainably alter stabilities, functions, and services of entire systems. Moreover, the findings reveal that, independent of trophic level or taxon, total abundances do not generally reflect whether or to which extent soil fauna groups are affected by increasing atmospheric CO_2-concentrations (Papers 2.2 and 2.3). Not individual densities, but changes within the functional composition of a community induce alterations of processes that control and drive functions of ecosystems.

3.1.4 Impact of crop developmental stage on CO_2-enrichment-induced changes in soil fauna communities

Beside the clear specificity of CO_2-enrichment effects on crop type, collembolan species (Paper 2.2) as well as nematode feeding types (Paper 2.3), moreover, reflect significantly differing impacts of atmospheric CO_2-enrichment depending on crop developmental stage. The generally important role of this factor concerning the structure of soil fauna communities is reflected by the diversity of collembolans, which decreased under sugar beet, but increased under winter wheat cultivation in the course of the growing season (Paper 2.2). It was assumed that the extent of the CO_2-enrichment-induced increase of root biomass during plant development, which markedly differs between both crops, might be relevant concerning CO_2-effects on below-ground food webs. Whereas the rhizosphere of wheat plants reflects a steady enhancement during the whole growing season, the root production of sugar beets only increases at the end of the season, when atmospheric CO_2-concentrations are enriched (Weigel et al., 2005).

The results of the present study, however, reveal that, due to the use of different food sources and the variable extent of food specialisation (Yeates & Bongers, 1999; Bracht-Jørgensen et al., 2008; Larsen et al., 2008), some collembolan species and nematode feeding types were more strongly affected by atmospheric CO_2-enrichment during the period of main plant growth (“Growth Phase”), others shortly before harvest of crops (“Mature Crop”) (Papers 2.2 and 2.3; Fig. 1). Nevertheless, with regard to the whole community, both soil fauna groups reflected stronger FACE effects at the end of both growing seasons, when plant biomass production was completed (Papers 2.2 and 2.3). This result implies that root elongation in

itself does not permit any conclusions to be drawn on CO_2-enrichment-induced impacts on soil fauna communities. Hence it must be assumed that the biochemical composition of rhizodeposites changes during the season depending on crop type, but independent of biomass production and, confirming the overall hypothesis of the present study (Chapter 1.2), that these changes drive the modifications of the functional and taxonomic structure of soil fauna communities (Papers 2.2 and 2.3).

These crop dependent quality changes of root-derived products in the course of the growing season are reflected by the δ^{13}C-values of respective root materials. The stable C-isotopic signatures of winter wheat roots indicate qualitatively increasing CO_2-effects from the period of main plant growth towards harvest (the difference between δ^{13}C-values of samples taken from the control compared to those taken under FACE-conditions increases). The δ^{13}C-shift of sugar beet roots admittedly reflects a stronger ^{13}C-depletion compared to that of wheat roots, and thus a generally stronger impact of atmospheric CO_2-enrichment, but indicates a steady CO_2-effect, which did not change in the course of the growing season (Paper 2.2). According to these differences, one must assume that the CO_2-enrichment-induced qualitative changes of exudates, and thus of the main food source of rhizosphere communities (Pollierer et al., 2007), varies as well between the period of main plant growth and the end of the growing season, depending on cultivated crop.

Combining stable C-isotopic signatures and species diversities (Paper 2.2) or relative abundances of feeding types (Paper 2.3), it becomes obvious that soil fauna communities adapted themselves to changed habitat conditions and altered food availability via distinctly different diet switching (Papers 2.2 and 2.3).

Collembolans, as well as nematodes, already showed more negative stable C-isotopic signatures under FACE compared to control conditions during the period of main plant growth when sugar beet was cultivated. This finding reflects the uptake of isotopically labelled, plant-fixed carbon early in the season, and indicates the use of qualitatively changed food sources at that time. In the course of the growing season, the stable C-isotopic signatures of rhizosphere nematodes (herbivores, bacterivores and fungivores) (Paper 2.3) as well as of most collembolan species (Paper 2.2) changed distinctly, thereby reflecting diet switching during plant development.

With regard to collembolans, the present results indicate the incorporation of numerous kinds of food sources and the use of mixed diets, independent of life strategy (Paper 2.2). Linking this finding to the CO_2-enrichment-induced increase in species diversity, it becomes obvious

that most collembolan species benefited from altered dietary conditions in the sugar beet rhizosphere.

Concerning the relative abundances of nematode feeding types, an increase in the relative proportion of fungivores was detected during the period of main sugar beet growth (Paper 2.3; Fig. 1). As atmospheric CO_2-enrichment is described to change the community structure of fungi, in contrast to that of bacteria, only in the late season (Drigo et al., 2007), the increased share of fungivores at that time is most probably due to a general rise of the (under sugar beet typically low) fungal colonization, and thus an enhanced availability of the required food source. As the majority of collembolan species present in arable soils prefers fungi compared to other kinds of food sources (Ponge, 2000; Castaño-Meneses et al., 2004), it can be assumed that this increased occurrence of rhizosphere fungi, moreover, induced the increase in collembolan species diversity at this time (Paper 2.2).

Shortly before harvest, by contrast, the nematode feeding type composition reflected an increase of the relative proportion of bacterivores (Paper 2.3; Fig. 1). The decline of the CO_2-enrichment-induced $\delta^{13}C$-shift of this feeding type, as well as of those of fungivores and herbivores from the period of main plant growth to harvest, indicates that the food availability of these functionally different groups changed in the course of the season. One can deduce therefore, that structural changes within the bacterial community enhanced the quality of bacteria as food source, resulting in a rise of the relative contribution of bacterivores to the whole nematode community (Paper 2.3; Fig. 1).

These results clearly show that under CO_2-enriched conditions, the food availability and quality for all soil organisms associated with the sugar beet rhizosphere already change during the period of main plant growth (Papers 2.2 and 2.3). Since no seasonal differences were detected concerning the intensity of CO_2-enrichment effects on the $\delta^{13}C$-values of roots (Paper 2.2), the assumed and nematode-implied plant growth stage specific alterations in the community composition and activity of microorganisms are most probably due to impacts of crop type and plant age (Haase et al., 2008), and the microbial use of changing C-sources over the year (Pelz et al., 2005). This continuous modification of the microbial community presumably induced soil faunal diet switching, which is performed in order to optimize nutrient uptake and energy supply (Sabatini & Innocenti, 2000), and to adapt to altered dietary conditions. According to the quality of the most convenient, available food source, the relative abundances of feeding types and species changed due to reproduction adaptation. This way, the advantages of specific functional groups within the microbial-feeding soil fauna shifted from favouring fungivores in the early season, most probably due to generally

increased food availability, towards benefiting bacterivores at the end of the season (Fig. 1), presumably due to changed bacterial community compositions, as detected by Haase et al. (2008). Hence, CO_2-enrichment-induced changes of rhizosphere- and soil processes during sugar beet development were to a large extent driven by diet switching of the soil fauna, which again controls microbial communities and thereby soil decomposition processes (Knox et al., 2003; Poll et al., 2007) (Papers 2.2 and 2.3).

Contrasting to the findings detected under sugar beet cultivation, a CO_2-enrichment-induced change of diversities and main species numbers of collembolans was only detected at the end of the growing season when winter wheat was cultivated (Paper 2.2). The stable C-isotopic signatures of collembolan species, however, did not differ depending on crop growth stage, and, without exception, reflected the expected life strategy-dependent splitting up of $\delta^{13}C$-values according to the respective trophic distance from primary producers (Paper 2.2), thereby confirming the findings of Hishi et al. (2007). Linking these results, it becomes obvious that during this year similar kinds of food sources were used independent of crop developmental stage, and that enhanced food quantities at the end of the growing season, when plant biomass production was completed and exudation rates were high, allowed an increase in species diversity, for example due to reduced food competition (Paper 2.2).

Concerning the controlling function of microorganisms as an important soil fauna food source, CO_2-enrichment effects on the, compared to collembolans, more food-selective nematodes again allow conclusions to be drawn on alterations within the microbial community structure. In this context, fungivores generally benefited from increasing atmospheric CO_2-concentrations when wheat was cultivated (Paper 2.3; Fig. 1). As the FACE-induced $\delta^{13}C$-shift of fungivores decreased from the period of main plant growth towards the end of the season, and, moreover, the diversity of collembolans, which include numerous species that prefer fungi above other food sources (Ponge, 2000), was enhanced only in the late season, it can be assumed that these results reflect the retarded response of fungi to atmospheric CO_2-enrichment, concerning the modification of their community composition (Drigo et al., 2007). Based on this assumption, the results imply a slow adaptation of fungal species composition in the course of the growing season, presumably due to altered qualities of exudates and substrates. Nematodes as well as collembolans, which, depending on age and species, preferentially feed on certain fungal species (e.g., Larsen et al., 2008), indicate that this restrained adaptation resulted in an increased food quality of fungi at the end of plant development. Beyond that, recent studies (Chung et al., 2007) suggest that increases in biomass production during plant development itself, along with higher cellulose

availabilities, favour heterotrophic fungi, which often represent the major cellulase producers in soil (Lynd et al., 2002)

In total, the present results reveal that, besides general seasonal trends which, especially in agroecosystems, play a major role in collembolan, nematode (Yeates & Bongers, 1999; Petersen, 2000) and microbial communities (Pelz et al., 2005; Haase et al., 2008), CO_2-enrichment alters the availability and quality of foods used by soil organisms depending on crop developmental stage. According to this, impacts of rising atmospheric CO_2-concentrations on functionally different soil fauna groups vary strongly over the season. Structural alterations within the microbial community are assumed to mainly drive these seasonal changes of dietary conditions in the rhizosphere, which were clearly stronger under sugar beet compared to winter wheat cultivation (Papers 2.2 and 2.3). Thus, diet switching played a major role during the adaptation of collembolans and nematodes to changed habitat conditions when sugar beet cultivated, but a minor role under cultivation of winter wheat.
Due to the larger root system, along with higher exudation rates (Phillips et al., 2006b) and presumably due to the retarded reaction of the fungal community to atmospheric CO_2-enrichment (Drigo et al., 2007), impacts on the soil fauna were generally stronger at the end of the growing season compared to the period of main plant growth, independent of crop type.
The results indicate that CO_2-enrichment-induced changes of decomposition processes, and consequently C-transfer and nutrient cycling, are stronger under sugar beet compared to winter wheat cultivation. With regard to plant nutrient supply, the relevance of these changes is likely to increase in the course of the season. Concerning future agricultural productions, the adaptation of management-, fertilizer- and pesticide-measures which is necessary to ensure required crop yields, thus, is more important under cultivation of root-crops compared to cereal-crops, and generally gains in importance with advanced plant development.

3.2 Conclusions and perspectives

The results of the present study confirm the overall hypothesis, and reveal strong impacts of increasing atmospheric CO_2-concentrations on diversity and composition of collembolan and nematode communities in agroecosystems. CO_2-effects on taxonomically and functionally different groups varied, depending on food specificity of organisms, crop type, and developmental stage of plants. It can be concluded that CO_2-enrichment-induced, plant-mediated changes of microbial communities within the rhizosphere presumably drove responses of soil fauna groups, which adjusted themselves to changed nutritional conditions via diet switching, compensatory feeding, and reproduction adaptation. Reversely, the soil fauna in turn controlled the structure and activity of the microbial community by means of selective feeding. Initiated by the rhizosphere, CO_2-enrichment effects spread across the soil food web via cascading effects. Impacts, thereby, decreased in intensity with increasing trophic level and increasing trophic distance between organisms and primary producers. Concerning both analysed crop types, influences of CO_2-enrichment on the soil fauna were clearly stronger under sugar beet than under winter wheat cultivation, and increased under both crops in the course of the growing season. According to changes in diversity and functional community composition of collembolans, nematodes, and, as indicated by both soil fauna groups, microorganisms, decomposition processes shifted towards a faster and more stable (Hedlund et al., 2004) bacterial-based energy pathway under sugar beet, but towards slower, more vulnerable fungal-based energy channels under winter wheat cultivation. Due to these altered turnover processes, the retention of the surplus carbon in agroecosystems differed, depending on type of cultivated crop and plant developmental stage. According to this modification of the C-cycle, soil fertility, and hence, nutrient supply to plants, will change as well under future conditions, depending on microbial and soil faunal responses and impacts on interactions between both groups of soil organisms.

To gain more detailed insights into feedback effects of these changes on crops and future development of crop yields, and, moreover, to develop required measures concerning sustainable future management, further investigations of the soil fauna, including various crop types and crop developmental stages, would be useful in order to quantify CO_2-enrichment effects on agroecosystems.

As demonstrated by the present study, the use of stable C-isotopic signatures, in this context, can provide important insights into trophic interactions and functional linkages, which are not ascertainable by analysing diversities or bulk sizes exclusively.

Concerning the selected soil fauna groups, collembolans, which are able to use a broad range of food sources (Berg et al., 2004), generally reflected more beneficial effects of atmospheric CO_2-enrichment compared to nematodes, which rely on particular kinds of diets (Yeates & Bongers, 1999; Blanc et al., 2006). Against this background, reactions of nematode feeding types (except omnivorous species) which respond more sensitively to changed food supply indicate influences on specialized feeders, whereas collembolans, especially hemiedaphic species (Maraun et al., 2003), which showed the strongest CO_2-effect, rather reflect impacts on food generalists. As, independent of crop, atmospheric CO_2-enrichment induced clear shifts concerning the relative abundances of nematode feeding types, but did not exert any adverse effect on collembolan diversity, the present results confirm the assumption that specialists will be more strongly affected by future climatic conditions compared to generalists (Balmford & Bond, 2005; Ayres et al., 2008; Coll & Hughes, 2008). Since a high functional diversity of systems particularly depends on the occurrence of specialists, and, moreover, these organisms often are essential in order to maintain numerous ecosystem functions and services (Blankinship & Hungate, 2007), the stronger inclusion of food specialists in further studies focussing on the quantification of CO_2-enrichment effects on soil food webs, the soil C-cycle, agrobiodiversity, or future crop yields is of utmost importance.

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