Effects of earthworms on stabilisation and mobilisation of soil organic matter

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It may be doubted whether there are many other animals which have played so important a part in the history of the world, as have these lowly organised creature.

Charles. R. Darwin (1881)

Die vorliegende Arbeit wurde unter Leitung von Prof. Dr. Stefan Scheu am Institut für Zoologie der Technischen Universität Darmstadt durchgeführt und von der Deutschen Forschungsgemeinschaft (DFG) im Rahmen des Schwerpunktprogramms SPP 1090: „Böden als Quelle und Senke für CO_2“ gefördert.

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Eidesstattliche Erklärung

Ich erkläre hiermit an Eides statt, dass ich die vorliegende Dissertation selbständig und nur mit den angegebenen Hilfsmitteln angefertigt habe. Ich habe noch keinen weiteren Promotionsversuch unternommen.

08.08.2004 Sven Marhan

Darmstadt, den (Sven Marhan)

Teile der vorliegenden Arbeit sowie anderer Projekte während der Promotionszeit wurden bisher wie folgt publiziert, bzw. auf Tagungen präsentiert.

MARHAN, S., SCHEU, S. (2001): Stabilisation und Mobilisation organischer Substanz durch Regenwürmer, Poster, Jahrestagung der DBG, Wien.

MARHAN, S., SCHEU, S. (2002): Sand fördert Kohlenstoff-Mineralisation im Kot von *Lumbricus terrestris* L.; Vortrag, Tagung der DBG, AG Bodenbiologie, München - Neuherberg.

MARHAN, S., SCHEU, S. (2002): Sand increases carbon mineralisation in *Lumbricus terrestris* L. casts; Poster, 7th International Symposium on Earthworm Ecology, Cardiff, Wales.

BUTENSCHÖN, O., **MARHAN, S.**, SCHEU, S. (2003): Effect of the availability of sand on carbon and nitrogen mobilization by earthworms; Poster, Int. Konferenz: Mechanisms and Regulation of Organic Matter Stabilisation in Soils, Hohenkammer.

EGERT, M., **MARHAN, S.**, WAGNER, B., SCHEU, S., FRIEDRICH, M.W. (2003): T-RFLP analysis of microbial community structure in food, soil, gut and fresh casts of *Lumbricus terrestris* (Oligochaeta: Lumbricidae) under two different feeding conditions; Poster, Int. Konferenz: Mechanisms and Regulation of Organic Matter Stabilisation in Soils, Hohenkammer.

MARHAN, S., SCHEU, S. (2003): Soil carbon and nitrogen content as limiting factors for the endogeic earthworm *Octolasion tyrtaeum*; Poster, Int. Konferenz: Mechanisms and Regulation of Organic Matter Stabilisation in Soils Hohenkammer.

EGERT, M., **MARHAN, S.**, WAGNER, B., SCHEU, S., FRIEDRICH, M.W. (2004): Molecular profiling of 16S rRNA genes reveals diet-related differences of microbial communities in soil, gut, and casts of *Lumbricus terrestris* L. (Oligochaeta: Lumbricidae); FEMS Microbiology Ecology 48:187-197.

MARHAN, S., SCHEU, S. (2004): Effects of sand and litter availability on organic matter decomposition in soil and in casts of *Lumbricus terrestris* L.; Geoderma, (in press).

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Chapter Five – Lower mineral soil

Chapter Six – Stable soil organic matter

Chapter Seven – Growth of earthworms

Summary

The majority of the soil macrofauna biomass in temperate terrestrial ecosystems comprises earthworms. Mainly anecic earthworm species, such as *Lumbricus terrestris*, contribute significantly to organic matter turnover by ingesting and fragmenting large quantities of litter and incorporating it into the mineral soil. Endogeic species, such as *Octolasion tyrtaeum*, turn over large quantities of mineral soil and induce major structural changes in soil with important consequences for soil development. Earthworms are major drivers of the decomposition of plant litter in many terrestrial ecosystems but knowledge of the factors influencing mobilisation and stabilisation of soil organic matter in soils processed by earthworms is incomplete. This study investigates the role of different factors which influence the effects of earthworms on soil organic matter stability. Six experiments were performed studying the effects of (1) sand on the microbial community structure and (2) the stability of organic matter in casts of *L. terrestris*; (3) the quality of the litter and (4) the availability of deeper mineral on the effects of *O. tyrtaeum* on C and N stabilisation; (5) of *O. tyrtaeum* on the mobilisation of stable carbon pools and (6) of size and quality of the soil organic matter pool on *O. tyrtaeum*.

(1) The effects of the availability of sand particles on changes of the microbial community structure of forest soils processed by the anecic earthworm *L. terrestris* were studied. The analysis of phospholipids fatty acid profiles (PLFAs) was used to differentiate microbial communities in the bulk as well as in particle size fractions of soil and casts. Comminution of litter during the gut passage through earthworms increased the biomass and activity of fungi in the silt fraction. Soil organic matter in the coarse and fine sand fraction primarily is decomposed by fungi. Incorporation of litter into these fractions by the earthworms increased the biomass of fungi. Organic matter of forest soil is stabilised in cast aggregates of *L. terrestris*, predominantly by increasing the soil organic matter content in the clay fraction where it becomes protected against microbial attack. By mechanical disruption of casts physically protected soil organic matter becomes available for microbial decay.

(2) The effects of sand on the decomposition of soil organic matter in arable and forest soils were compared with those in ageing casts of *L. terrestris*. Increasing sand content increased carbon mineralisation in soil but also enhanced the palatability and

comminution of litter by *L. terrestris*. In soils with high clay content sand affected the decomposition of organic matter only little, however, in general sand stimulated carbon mineralisation in soil and earthworm casts. The results suggest that soil characteristics, such as the clay content and nutrient concentrations determine whether organic matter is stabilised or mobilised in casts of earthworms.

(3) The influence of the quality of litter materials on the effects of the endogeic earthworm *O. tyrtaeum* on C and N mineralisation from arable and forest soil was studied. Effects of *O. tyrtaeum* varied with the type and the quality of the litter added; N immobilisation of low quality litter is counteracted by the earthworms. Low quality litter facilitates *O. tyrtaeum* probably by a continuous supply of easily available carbon. Carbon mineralisation was little whereas nitrogen mobilisation and immobilisation was strongly affected by the litter type and interaction with earthworms.

(4) The role of *O. tyrtaeum* and the availability of deeper mineral soil on the process of C, N and lignin mineralisation was analysed. Intimate mixing of organic material with deeper mineral soil, which functioned as a C-unsaturated inorganic matrix, during the gut passage of *O. tyrtaeum* increased the stability of carbon in the casts. In the long-term the pool of stabilised carbon increases slightly until the mineral matrix of the soil is saturated due to mixing of soil and organic matter by endogeic earthworms. This ultimately leads to the formation of organic rich mull soils.

(5) The ability of *O. tyrtaeum* to mobilise stable pools of soil organic matter was investigated. The results suggest that endogeic earthworms contribute to the mobilisation of old carbon pools in soil but further experiments are necessary to finally proof this conclusion.

(6) The effects of the size and quality of the soil organic matter pool on the growth of juvenile *O. tyrtaeum* were studied. The results showed that labile organic matter pools in arable soil are essential for soil microorganisms as well as for endogeic earthworms. Earthworms and soil microorganisms compete for food resources derived from labile organic matter pools. The amount and origin of soil organic matter in arable soils is crucial for the establishment of endogeic earthworm populations and their activity beneficially feeds back to nutrient mineralisation and therefore to soil fertility and plant productivity.

Zusammenfassung

Ein Großteil der Biomasse der Bodenmakrofauna in gemäßigten Regionen der Erde wird von Regenwürmern gebildet. Vor allem Regenwurmarten mit anözischer Lebensweise, wie *Lumbricus terrestris*, tragen durch Fraß und Grabtätigkeit erhebliche Mengen an Laubstreu in den Mineralboden ein, während endogäisch lebende Arten, wie *Octolasion tyrtaeum*, große Mengen an Mineralboden umsetzen. Durch die umfangreiche Durchmischung der oberen Mineralbodenhorizonte spielen vor allem endogäische Regenwürmer eine wichtige Rolle bei der Bodenbildung. In vielen terrestrischen Ökosystemen wird ein Großteil des jährlichen Streuabbaus durch Regenwürmer beeinflusst, bislang ist jedoch unklar inwieweit dies zu einer Mobilisierung oder Stabilisierung der organischen Bodensubstanz führt.

In der vorliegenden Arbeit werden verschiedene Einflussfaktoren untersucht, welche die Rolle von Regenwürmern auf die Stabilität der organischen Bodensubstanz beeinflussen können. Hierzu wurden sechs Experimente durchgeführt. Es wurde der Einfluss von Sand auf die mikrobielle Gemeinschaft (1) und die Stabilität der organischen Substanz (2) in Kotaggregaten *von L. terrestris* untersucht, weiterhin der Einfluss der Qualität der Streu (3) und der Verfügbarkeit von unterem Mineralboden (4) auf die Effekte von *O. tyrtaeum* auf die organische Bodensubstanz, sowie die Fähigkeit von *O. tyrtaeum* stabile C-Pools zu mobilisieren (5), und der Einfluss der Größe und Qualität des Pools an organischer Bodensubstanz auf *O. tyrtaeum* (6).

(1) In diesem Experiment wurde die Auswirkung der Verfügbarkeit von Sand während der Darmpassage auf die mikrobielle Gemeinschaft in Kotaggregaten von *L. terrestris* untersucht. Die Analyse von Phospholipid-Fettsäure-Mustern (PLFA) von intakten Aggregaten und Korngrößenfraktionen diente zur Unterscheidung der mikrobiellen Gemeinschaften des Regenwurmkotes und der des Bodens. Die verstärkte Zerkleinerung der Streu während der Darmpassage führte zu einer Erhöhung der pilzlichen Biomasse in der Schluff-Fraktion. In der Grobsand- und Feinsand-Fraktion enthaltene Streu wird vor allem durch Pilze abgebaut. In diesen Fraktionen war die pilzliche Biomasse durch die verstärkte Inkorporation der Streu durch *L. terrestris* erhöht. Die organische Substanz in Regenwurmkotaggregaten wird überwiegend in der Ton-Fraktion stabilisiert, vermutlich bedingt durch eine

verringerte Zugänglichkeit für Mikroorganismen. Durch eine mechanische Zerstörung der Kotaggregate wird der Abbau der darin eingeschlossenen organischen Substanz wieder erhöht.

(2) In einem Inkubationsexperiment wurden die Effekte von Sand auf die C-Mineralisation in Boden- und Kotaggregaten von *L. terrestris* eines Acker- und eines Waldbodens untersucht. Die Erhöhung der Sandkonzentration führte zu einem Anstieg der C-Mineralisation, jedoch auch zur verstärkten Aufnahme und Zerkleinerung der Streu durch *L. terrestris*. Im Allgemeinen war die C-Mineralisation unter dem Einfluss von Sand erhöht, wobei der mobilisierende Effekt von Sand im tonreicheren Waldboden geringer war. Die Ergebnisse zeigen, dass die Stabilität der organische Bodensubstanz im Regenwurmkot entscheidend von dem Ton-Gehalt des Bodens und der Konzentration an verfügbaren Nährstoffen abhängt.

(3) Die Effekte der Streuqualität in Verbindung mit *O. tyrtaeum* auf die Mineralisierung von C und N wurden in zwei Experimenten mit Acker- bzw. Waldboden untersucht. Der Einfluss von *O. tyrtaeum* auf die N-Mineralisation variierte mit der Art und Qualität der Streu; die Regenwürmer wirkten hierbei einer N-Immobilisierung im Boden durch stickstoffarme Streu entgegen. Die Biomasse von *O. tyrtaeum* wurde durch Streu von geringer Qualität gefördert, was vermutlich auf einer erhöhten Verfügbarkeit von C-Ressourcen beruhte. Im Vergleich zur N-, war die C-Mineralisation nur gering durch das Zusammenwirken von Regenwürmern und Streu unterschiedlicher Qualität beeinflusst.

(4) Der Einfluss von *O. tyrtaeum* auf die Mineralisierung von C, N und Lignin wurde im Hinblick auf die Verfügbarkeit von unterem Mineralboden untersucht. Die enge Verbindung von organischem Material mit der Matrix des unteren Mineralbodens während der Regenwurmdarmpassage erhöhte die Stabilität des Kohlenstoffs im Regenwurmkot. Der untere Mineralboden fungiert hierbei als C-ungesättigte Matrix. Der Gehalt an stabilisierter organischer Substanz dürfte vermutlich solange ansteigen, bis die mineralische Matrix durch die durchmischende Funktion der Regenwürmer gesättigt ist. Dieser Prozess der Durchmischung und Anreicherung wird mit der Zeit zur Entstehung von Mullböden mit hohem organischem Gehalt führen.

(5) In diesem Experiment wurde die Fähigkeit von *O. tyrtaeum* untersucht stabile Pools der organischen Bodensubstanz zu mobilisieren. Die Ergebnisse lassen vermuten, dass engogäische Regenwürmer stabile Kohlenstoff-Pools mobilisieren können; weitere Experiments sind jedoch erforderlich zur Verifizierung dieser Vermutung.

(6) In diesem Experiment wurde der Einfluss der Qualität und Größe des Pools an organischer Bodensubstanz auf das Wachstum von juvenilen *O. tyrtaeum* untersucht. Die Ergebnisse zeigten, dass sowohl für Mikroorganismen als auch für endogäische Regenwürmer das Vorhandensein eines labilen Pools an organischer Bodensubstanz essentiell ist. Regenwürmer und Bodenmikroorganismen konkurrieren um diese Nahrungsquelle. Der organische Gehalt des Bodens und vor allem die Verfügbarkeit von C-Quellen ist entscheidend für die Etablierung endogäischer Regenwurmpopulationen, welche die Nährstoff-Verfügbarkeit erhöhen und sich somit positiv auf Bodenfruchtbarkeit und Pflanzenproduktion auswirken.

1. Introduction

1.1 Earthworms

Earthworms occur in all terrestrial ecosystems of the world, with exception of the driest and coldest land areas (Lee 1985). The majority of the earthworms of Europe belong to the taxon Lumbricidae (Annelida, Oligochaeta). In contrast to the tropics where the diversity of earthworms is high, and also to France where about 180 species have been described (Bouché 1972), only about 38 earthworm species are found in Germany (Schaefer 2003).

Distinct ecological groups can be recognised among the Lumbricidae; Bouché (1972) separated the earthworms into three ecological categories:

(1) Epigeic species are characterised as detritivorous or litter feeding earthworms that live above the mineral soil inside the litter layer (litter dwellers); heavily pigmented species such as *Lumbricus rubellus* and *Dendrobaena octaedra* are typical members of this group. (2) Anecic species: This group comprises the larger mineral soil-dwelling species inhabiting permanent vertical burrows. Anecic earthworms emerge from their burrows to feed on surface litter material. Members of this group include larger species like *Aporrectodea longa* and *Lumbricus terrestris*. (3) Endogeic species live permanently in horizontal burrows in the upper mineral soil layers by consuming large amounts of mineral soil. This is why the term geophagous is often used as synonym for endogeic earthworms (Lee 1985). Typical members of this group are the little pigmented species of the genus *Octolasion* and the species *Aporrectodea caliginosa*.

Environmental conditions constrain the distribution of earthworms in terrestrial ecosystems. Earthworms are rare in soils with pH < 4. Suitable pH for the most lumbricid species ranges from slightly acid to slightly alkaline (Satchell 1955, Piearce 1972). Redox potentials of soils seem also to be important in determining earthworm abundance (Lee 1985). Epigeic earthworms are usually more tolerable to low pH conditions than soil inhabiting species (Edwards & Bohlen 1996).

Earthworm distribution is also dependent by the temperature. In general, lumbricid species can survive long exposure to temperatures only within the range of about 0-35°C. The temperature limits for survival vary between species and intra-specific adaptations exist (Lee 1985).

High soil temperatures are commonly associated with desiccation and water stress for animals. Earthworms have no specialised respiratory organs; instead respiration depends upon diffusion of gases through the body wall, which must be kept moist. Water conservation mechanisms are poorly developed in earthworms and their thin permeable cuticles and epidermis are not able to prevent the animals from continuous water loss when the humidity of the air is below 100%. Much water is also lost by excretion of nitrogen and additionally by casts, which usually have higher water contents than the surrounding soil. Although up to 85% of the fresh weight of earthworms is water, as much as 70-75% of this water content can be lost without killing the animal (Grant 1955, Roots 1956). Some species, such as *A. caliginosa* survive periods of drought in the soil by going into diapause (Baltzer 1956).

The exposure to sun light is harmful for earthworms and may kill the animals. Ultra-violet light in particular is lethal. The different species vary in their tolerance of light, with more pigmented epigeic and anecic species being less sensitive than less pigmented endogeic species.

Earthworm distribution is also determined by the soil texture. Earthworms are generally absent or rare in soils with very coarse texture, probably due to physical abrasion of their body surface by coarse mineral materials and to the susceptibility to drought of such soils (Lee 1985). In regions with high rainfall they are also absent from soils with high clay content because of seasonal or permanent oxygen deficit in such soils. Earthworms, however, are able to tolerate CO_2 concentrations much higher than those characteristic for air in soil.

Life of all animals depends on adequate and suitable food supplies. The primary source of food for the detritivore community including earthworms is dead plant material, especially plant leaf litter. Earthworms are saprophagous animals and prefer to feed on dead and decaying plant remains, which vary greatly in their physical and chemical composition. Because of the limited ability of earthworms to move, they need to live close to food resources (Lee 1985). Earthworm populations are often

food limited and populations increase following organic amendments (Curry 1998, Scheu & Schaefer 1998).

The bulk of the diet of earthworms consists of dead plant tissue and particulate and amorphous soil organic matter, although in part also of living roots (Ferrière 1980, Baylis et al. 1986), seeds of plants (Shumway & Koide 1994) and algae (Atlavinyté & Pociene 1973). By the ingestion of plant remains the associated bacteria and fungi are ingested by the earthworms. There is evidence that earthworms selectively feed on microsites rich in microorganisms (Wolter & Scheu 1999). It is supposed that nutrients derived from ingested soil microorganisms substantially constitute to the earthworm diet (Brown 1995). In particular, essential amino acids may derive more from the digestion of bacterial populations than from a gut associated microflora (Pokarzhevskii et al. 1997). In comparison to many other soil invertebrates the gut of earthworms is relatively simple and does not show any developed indigenous gut microflora (Parle 1963a, Satchell 1967, Jolly et al. 1993, Egert et al. 2004).

It is controversially discussed if the bulk of microorganisms (microbial biomass) is increased or reduced by the earthworm gut passage (Edwards & Bohlen 1996). Some groups of microorganisms, such as ingested N_2O producing bacteria, are favoured in the gut and casts of *L. terrestris* (Horn et al. 2003), whereas others, such as fungi, are reduced during the gut passage (Schönholzer et al. 1999). The number of protozoans also decreases during the gut passage, indicating digestion by earthworms (Edwards & Fletcher 1988, Bonkowski & Schaefer 1997, Cai et al. 2002). There is evidence that in bacteria of the genus *Bacillus* vegetative cells rather than spores are destroyed during gut passage but germination of the spores may also be initiated (Fischer et al. 1997).

By providing favourable habitats earthworms indirectly affect the abundance of soil microorganisms and that of the soil micro- and mesofauna. The walls of the burrows of primarily anecic earthworm species are enriched in nutrients due to the lining with plant debris, earthworm casts and mucus. In comparison to the surrounding soil the abundance of soil microorganisms was shown to be increased in the burrow system of earthworms (Devliegher & Verstraete 1997b, Tiunov & Scheu 1999, Tiunov et al. 2001). By creating burrow systems, earthworms also create new habitats for the soil mesofauna (Marinissen & Bok 1988). Above the soil surface anecic species create

middens consisting of plant remains mixed with earthworm casts, which were shown to form microhabitats with increased microbial and faunal activity (Maraun et al. 1999, Bohlen et al. 2002).

1.2 Soil organic matter

The total pool of soil organic carbon on earth consists of about 1500 $\times 10^{15}$ g C. The soils of the world contain more carbon than the combined total amounts occurring in the vegetation (560 $\times$ 10^{15} g C) and the atmosphere (750 $\times$ 10^{15} g C) (Schlesinger 1997). The product of carbon mineralisation, carbon dioxide (CO_2), is known to be one of the most important climatic relevant gases in the atmosphere and is presumed to be responsible for global warming and global climate change. The CO_2 concentration of the atmosphere has increased during the last century due to increased burning of fossil fuels by humankind. The emission of CO_2 from soils is recognized as one of the largest fluxes in the global carbon cycle and is ten times that caused by burning of fossil fuel (Post et al. 1990). Small changes in the magnitude of soil respiration may have large effects on the concentration of CO_2 in the atmosphere (Schlesinger & Andrews 2000). When soils are brought under cultivation their content of soil organic matter declines and losses of 20 to 30% within the first few decades of cultivation typically occur (Schlesinger 1997). Some of this organic matter from agricultural soils is lost by erosion but most is released as CO_2. The cumulative emission of CO_2 derived from fossil fuels and land use changes of soils is bigger than the sum of oceanic uptake and the atmospheric increase, consequently an up to now unknown or "missing" sink for CO_2 exists (Schimel 1995).

It is known that the decrease of soil organic matter after deforestation or during cultivation can be reversed into an increase during secondary succession on abandoned fields (Richter et al. 1999, Knops & Tilman 2000, Vesterdal et al. 2002). In soils from mature temperate forests, the climax state of vegetation, high amounts of organic matter are stored. This is true for both acidic moder and base rich mull soils (Wolters & Joergensen 1991). Also, with changes in land use practices, e.g. from conventional use of mineral fertilizers and extensive tillage to organic farming,

the content of soil organic matter in cultivated soils increases significantly (Mäder et al. 2002).

In the case of soil organic matter decrease, soils function both as source and, during periods of soil organic matter increase, as sink for CO_2. The potential of soils as source and sink for CO_2 is discussed intensively (Torbert et al. 1997, Swift 2001, Six et al. 2002), but still there is insufficient knowledge of the mechanisms affecting the mobilisation and stabilisation of organic matter in soils.

Carbon mineralisation and decomposition processes typically are coupled to mobilisation or immobilisation of nitrogen due to close matching of the C-to-N ratio in the biomass of organisms. Compared to carbon, smaller amounts of nitrogen are bound in living and dead biomass resulting in C-to-N ratios ranging between 160 and 15 (Schlesinger 1997). The availability of nitrogen and also phosphorus controls many aspects of ecosystem function and often limits the rate of primary production (Schlesinger 1997, Vitousek et al. 2002). Inorganic forms such as ammonium (NH_4^+) and nitrate (NO_3^-) are essential nitrogen resources for plant growth but amino acids also function as nitrogen resource for plants (Lipson & Näsholm 2001).

Not only primary producers are limited by the availability of nitrogen, organisms involved in the decomposition of organic matter may also be limited by nitrogen (Hunt et al. 1988, Joergensen & Scheu 1999, Schaeffer et al. 2003). Decomposer organisms contribute greatly to the mobilisation of nitrogen and the production of ammonium and nitrate soils (Beare et al. 1992, Teuben & Verhoef 1992). Microorganisms such as fungi and bacteria form the most important part of the decomposer community and their metabolisms are responsible for the biggest part of CO_2 emitted during decomposition with their activity strongly affected by the soil micro-, meso- and macrofauna (Swift et al. 1979).

1.3 Effects of earthworms on soil organic matter

The majority of the soil macrofauna biomass in temperate terrestrial ecosystems is formed by earthworms (Lee 1985). The importance of earthworms in affecting soil structure, organic matter processing and nutrient cycling has long been recognised

(Darwin 1881, Edwards & Lofty 1977, Lee 1985). Through their burrowing and casting activities, earthworms turn over tremendous quantities of soil on an annual basis and can induce major structural changes in soil which have important consequences for the development of the soil (Blair et al. 1995). Anecic species which live in vertical burrows predominantly deposit their casts at the soil surface. The horizontal dwelling endogeic species deposit their casts in their own burrows or in other sub-surface spaces. Casts that are deposited below the soil surface contribute to pedogensis whereas those deposited on the surface are more important for soil profile development and soil structure (Kubiena 1948, Bouché 1981, Bal 1982).

The habitats and feeding preferences of different earthworm species relate to their potential effects on biogeochemistry. Mainly anecic species remove partially decomposed plant litter and crop residues from the soil surface, ingest it, fragment it and transport it to subsurface soil layers. The mixing of soil layers with plant residues usually is performed by endogeic species which feed in soil. In general, the ingested organic matter is comminuted, mixed with ingested soil, passed through the gut and deposited as casts.

During the earthworm gut passage only little of the plant litter and other organic material that earthworms remove from the soil surface (anecic) or feed within the soil (endogeic) is digested and assimilated. Most re-enters the soil system being enclosed in earthworm casts. Earthworm casts can be important soil microsites with high microbial abundance and activity, and often a different composition of the microbial community compared to the surrounding soil. Many studies have demonstrated that earthworm casts have different chemical, biological, and physical characteristics compared to the soil in which the casts were produced (Parle 1963b, Dkhar & Mishra 1986). Enhanced microbial activity was found in fresh earthworm casts (Shaw & Pawluk 1986b, Tiwari et al. 1989). The increase of microbial activity in fresh casts at least partly derives from mobilisation of nutrients during the earthworm gut passage which results in an enhanced availability of nutrients (C, N, P, K, Ca) in the casts (Lunt & Jacobson 1944, Blair et al. 1995, Parmelee et al. 1998).

In contrast, lower microbial biomass and decomposition rates have been found in ageing earthworm casts (Scheu 1987a, Scheu & Wolters 1991b, Tiunov & Scheu 2000b). During the earthworm gut passage soil aggregates are destructed and the

organic material therein is intimately mixed with the soil matrix. The association of organic matter with the soil matrix during the gut passage through earthworms may promote stabilisation of carbon by promoting binding with clay minerals (Shaw & Pawluk 1986a, b).

Devliegher and Verstaete (1997a) summarised "gut associated processes" (GAPs) as processes during the earthworm gut passage that mobilise organic matter. In contrast, organic matter may become stabilised in the earthworm casts due to "cast associated processes" (CAPs) (Tiunov & Scheu 1999). The predominance of one of these processes decides if the pool of soil organic matter is enlarged or reduced in the long term.

1.4 Objectives

In times of global change, knowledge of parameters affecting soils in their function as sources and sinks for CO_2 is important. Decomposition of soil organic matter contributes substantial amounts to the CO_2 content of the atmosphere and plays an important role in the global carbon and nitrogen cycle (Schlesinger 1977, Schlesinger & Andrews 2000). Earthworms are major drivers of the decomposition of plant litter in many terrestrial ecosystems (Lee 1985, Edwards & Bohlen 1996), but knowledge of the factors influencing gross mobilisation and stabilisation of soil organic matter in soils processed by earthworm is incomplete.

This study aims to investigate the parameters which are likely to influence the effects of earthworms on soil organic matter stability, such as sand, unsaturated inorganic matrix and litter. Earthworms of two different ecological groups are investigated because of their different effects on soil organic matter. Incubation experiments are conducted in microcosms under controlled conditions to exclude environmental changes. In Chapter One the biology and ecology of the two earthworm species studied and the construction of the microcosms is described.

In Chapter Two the effects of the availability of sand particles on changes of the microbial community structure of forest soils following the gut passage of the anecic earthworm *L. terrestris* are studied. The microbial community structure is hypothesised to be strongly affected during the gut passage due to an increased comminution when sand is present. The analysis of phospholipids fatty acid profiles (PLFAs) is used to differentiate microbial communities in the bulk as well as in particle size fractions of soil and casts.

In Chapter Three the effects of sand on the decomposition of soil organic matter in arable and forest soils are compared with those in ageing casts of *L. terrestris*. The stabilisation of organic matter in the casts is hypothesised to be higher when comminution of litter during earthworm gut passage is increased by the availability of sand. Rates of mineralisation of soils and casts from arable and forest sites, each with and without representative litter additions, are compared during an incubation period of almost ten months.

The influence of the quality of litter materials on the effects of the endogeic earthworm *O. tyrtaeum* on C and N mineralisation from arable and forest soil is studied in Chapter Four. Stabilising effects of earthworms are hypothesised to depend on the decomposition rate of the litter. In addition, microbial activity and biomass is determined after ten months of incubation.

The role of *O. tyrtaeum* together with the availability of deeper mineral soil on the process of C, N and lignin stabilisation is analysed in Chapter Five. Organic matter is hypothesised to be stabilised by a close association with the C-unsaturated matrix from deeper mineral soil during passage through the earthworm gut. Two forest soils of different stages of humus accumulation are compared.

The ability of *O. tyrtaeum* to mobilise stable pools of soil organic matter is investigated in Chapter Six. It is hypothesised that endogeic earthworm are able to mobilise pools of stable organic matter. $\delta^{13}CO_2$ signatures are measured to determine the age of the C-pool which is actually mineralised.

The effects of size and quality of the soil organic matter pool on growth of juvenile *O. tyrtaeum* are studied in Chapter Seven. It is hypothesised that endogeic earthworms in arable fields are limited by the soil organic matter content. Major components of the diet of endogeic earthworms and factors which limit earthworm populations on arable fields are discussed.

Principal effects of earthworms on the stability of soil organic matter, with the focus on cast aggregates as places where organic matter is stabilised are discussed in general in Chapter Eight

1.5 Characterisation of earthworm species

In the present work two earthworm species from two ecological groups were studied. Specimens of the anecic species *Lumbricus terrestris* L. were used because of their importance for litter incorporation into the mineral soil horizons, whereas specimens of the endogeic species *Octolasion tyrtaeum* Savigny are important for the intimately mixing of the organo-mineral soil layers. Both species represent typical members of their ecological groups in Europe.

Lumbricus terrestris (known as lob, dew worm and night crawler)
Animals of this species occur in the temperate regions around the world. *L. terrestris* is abundant in many ecosystems such as grasslands, orchards, public parks and forests, but less common in arable fields and river banks. This species is one of the largest species in central Western Europe reaching up to 350 mm in length and 15 g live weight. The anterior part of the body is pigmented; the colour is brownish to purplish red above and yellow-grey below. Body shape is cylindrical; the posterior region is depressed and strongly paddle-shaped. The prostomium is tanylob and enables the animals to grasp plant leaves and other litter materials which they collected in typical middens above the entrance of their vertical burrows (Maraun et al 1999). Their food consists of fresh and partly decomposed plant residues, i.e. mostly green and brown leaves. The total annual leaf litter entering the soil can be collected and removed from the soil surface by *L. terrestris* in ecosystems with high earthworm densities (Lee 1985). During food collection and also mating procedure the paddle-shaped posterior region is gripped in the burrow in readiness for a rapid retreat into the soil if danger should threaten (Sims & Gerard 1999). The burrow of *L. terrestris* is constructed vertically to a depth of 1-3 m and earthworms stay in the deeper parts of this permanent burrow to resist hot and dry or frozen periods. Burrow walls are stabilised by mucus excretion and cast material generating a rich habitat for soil mesofauna and microorganisms (Tiunov & Scheu 1999, Tiunov et al 2000b). However, the largest part of the cast material is deposited on the soil surface. *L. terrestris* often dominates earthworm populations in terms of biomass because of its large size. Restrictions for the establishment of a stable population of *L. terrestris* are

alkaline soils with pH 6-10 (Sims & Gerard 1999) and the availability of a sufficient amount of palatable litter residues per year. *L. terrestris* is one of the best studied earthworm species in the world. Rates of litter consumption and food preferences of *L. terrestris* (Curry & Bolger 1984, Doube et al. 1997, Cortez & Bouche 1998, Bonkowski et al. 2000) as well as mating behaviour, cocoon production and effects on surface water run-off (Daniel 1995, Michiels 2001, Nuutinen et al. 2001) are known.

Octolasion tyrtaeum

O. tyrtaeum belongs to the group of endogeic earthworms. This species is widespread in pastures, gardens and arable land all over Europe but usually not numerous. In some base-rich forests *O. tyrtaeum* reaches high densities (Scheu 1987b). The prostomium is of the epilob type. Length of the cylindrical body ranges between 35 and 160 mm (Sims & Gerard 1999). Body size and live weight of *O. tyrtaeum* can vary between the specific ecological lineages of this parthenogenetic species (Heethoff et al. 2004). Adult specimens taken from a beech forest on limestone near Göttingen, (Göttinger Wald) are small and reach body masses of only 0.3-0.4 g. In general, the body of *O. tyrtaeum* is unpigmented, the colour ranges from whitish grey to blue, rarely rosy pink or brownish., Animals usually have a characteristic yellow-whitish posterior end. *O. tyrtaeum* lives permanently in the upper horizons of the mineral soil by building non-permanent horizontal burrows. During burrowing the animals consume large amounts of mineral soil (Scheu 1987b). Compared to other endogeic species such as *Aporrectodea caliginosa* little information about food resources and life of *O. tyrtaeum* is available (Lofs-Holmin 1983b, Rozen et al. 1995, Bonkowski et al. 2000). However, some studies investigated the effects of *O. tyrtaeum* on organic matter transformation, soil microorganisms and nitrogen mineralisation (Shaw & Pawluk 1986b, Scheu & Parkinson 1994, Scheu 1994a).

2. Phospholipid fatty acid profiles and xylanase activity in particle size fractions of forest soil and casts of *Lumbricus terrestris* L. (Oligochaeta, Lumbricidae)

Abstract

Earthworms are among the most important decomposer soil invertebrates removing surface plant litter material and mixing it with mineral soil. Thereby earthworms strongly affect decomposition processes of plant residues and the stability of soil organic matter. Plant litter materials are comminuted in the gizzard of anecic earthworms and this is enhanced if sand particles are available. The present study investigates the effects of the presence of sand on litter decomposition and the microbial community in casts of *Lumbricus terrestris* L. Phospholipid fatty acid profiles (PLFA), xylanase activity, basal respiration and microbial biomass was investigated in bulk soil and in particle size fractions with and without addition of beech litter and compared to respective earthworm casts.

Particle size distribution in casts resembled that in the soil; *L. terrestris* did not selectively ingested sand grains. Earthworm casts were enriched with beech litter suggesting that *L. terrestris* selectively ingested litter particles. The C and N content of the fine sand fraction was increased in casts and that of the clay fraction tended to be increased. Also, xylanase activity was increased in the bulk material and the fine sand fraction of casts. Microbial biomass was at a maximum in the fine sand and the clay fraction. Xylanase activity was increased by the addition of litter in the bulk soil, the coarse and fine sand, and the silt fraction. PLFA profiles of the coarse sand were separated from the fine sand fraction and both from the finer size classes but generally they were little affected by the experimental treatments. Only in treatments with litter the clay fraction of soil was separated from that of casts, whereas without litter soil differed from casts in the coarse sand fraction. Fungal-to-bacterial PLFA ratio was highest in the coarse sand and lowest in the silt and clay fraction.

Microorganisms, predominantly fungi, benefited from the addition of litter. Increased fungal PLFA content in the silt fraction indicates that litter fragmentation by earthworms favour the colonisation of fine particle size fractions by fungi at the expense of that of bacteria. The study documents that microbial analyses in particle size fractions improve the understanding of litter decomposition processes in earthworm casts.

2.1 Introduction

Earthworms in combination with other decomposer invertebrates remove litter materials from the soil surface thereby affecting decomposition processes (Edwards & Bohlen 1996). Anecic earthworm species, such as *Lumbricus terrestris* L., may remove most of the litter entering the soil in deciduous forest ecosystems (Satchell 1967, Edwards & Lofty 1977). Since the assimilation efficiency of earthworms is low, most of the litter resources enter the soil enclosed in earthworm casts (Edwards & Bohlen 1996). Decomposition of organic matter enclosed in the casts may be enhanced or reduced depending on the extent the litter is comminuted and the changes in the microbial community structure (Tiunov & Scheu 2000a, b). The soil microbial community together with microorganisms colonising the litter materials are responsible for litter decomposition (Swift et al. 1979). Litter materials are fragmented during the gut passage through *L. terrestris* and the fragmentation process is increased by the availability of sand grains (Schulmann & Tiunov 1999). In general, earthworms preferentially ingest a combination of litter materials and mineral particles (Dickschen & Topp 1987, Hendriksen 1991, Doube et al. 1997). Mineral particles presumably serve as grinding agents supporting the action of the muscular gizzard. Enhanced litter fragmentation likely increases litter digestion (Schulmann & Tiunov 1999). However, litter fragmentation during the gut passage of earthworms not only affects the availability of litter resources to the earthworms but also to the litter and soil microflora in earthworm casts.

Bacterial and fungal biomass, activity and community structures in earthworm casts have been investigated using a number of methods including plating techniques for

bacteria (Atlavinyté & Lugauskas 1971, Shaw & Pawluk 1986b, Daniel & Anderson 1992) and fungi (Moody et al. 1996, Tiunov & Scheu 2000a), as well as direct counting of microorganisms (Parle 1963b, Pedersen & Hendriksen 1993), measuring microbial activity (Scheu 1987a, 1992a, Zhang et al. 2000) and analysing microbial communities by molecular methods (Furlong et al. 2002, Schönholzer et al. 2002, Egert et al. 2004). Also, phospholipid fatty acid profiles (PLFA) have been used to characterise microbial communities in earthworm middens (Bohlen et al. 2002) and casts (Saetre 1998, Clapperton et al. 2001, Enami et al. 2001). PLFA profiles have been considered to be a promising tool for estimating fungal and microbial biomass (Frostegård & Bååth 1996) and microbial community structure (Beese et al. 1994, Zelles & Bai 1994, Hedlund 2002).

The analysis of particle size fractions provides insight into microhabitats of microorganisms and their associations with distinct fractions of the organic matter (Winding et al. 1997, Stemmer et al. 1998, Sessitsch et al. 2001). Particle size fractions in general provide information about changes in the distribution of the organic matter in soil (Christensen 1992, Ladd et al. 1996, Roscoe et al. 2001). Therefore, the combination of PLFA analysis and particle size fractionation is a promising tool to better understand changes in organic matter distribution and microbial community structure occurring during the passage of litter and soil through the gut of earthworms.

The present study investigates effects of the availability of sand and litter on the microbial community structure in casts of *L. terrestris*. PLFA profiles, xylanase activity, basal respiration, microbial biomass C and specific respiration of microorganisms were investigated in bulk samples and in particle size fractions of soil and earthworm casts.

2.2 Material and methods

Sampling

Soil samples and litter material were taken from a 130-year old beech forest on limestone near Göttingen (southern Lower Saxony, Germany), known as Göttinger

Wald. The dominant soil types are *Terra-fusca Rendzina* (59%) and *Rendzina* (28%) (Ulrich et al. 1982). The annual mean temperature is 7.9°C and the mean annual rainfall 720 mm. Soil samples were taken from the upper 5 cm of the mineral soil after removal of litter in October 2000. The soil was passed through a 4 mm sieve to remove stones and plant residues. The soil was then stored at –28°C until the experiment was set up. Prior to placement in experimental containers the soil was kept at 5°C for one week. Beech leaf litter (44.5% C, 1.6% N) was collected from the soil surface in late summer in the forest where the soil had been taken. The litter material was air-dried and mechanically fragmented into pieces < 4 mm. Sand was homogeneously mixed with the soil to a total sand content of 25% dry wt. Quartz sand with a particle size distribution of 40, 40 and 20% in the < 0.5, 0.5-1 and 1-2 mm fraction was used. Adult specimens of *L. terrestris* L. were extracted from an oak-beech forest 20 km south of Darmstadt (Jägersburger Wald) using 0.06% formalin solution in October 2000. The earthworms were washed twice with distilled water and kept for 3 weeks at 5°C in containers with soil of the study site. For acclimation the earthworms were placed at 20°C one week before they were used for the experiment.

For production of cast materials earthworms were incubated in planar cages consisting of two transparent PVC sheets (650 × 310 × 3 mm) separated by PVC strips (10 mm thick) at the two sides and the bottom. The cages were filled with soil to a height of 550 mm; the forest soil was compacted to a bulk density of 0.85 kg dry wt L^{-1}. Each cage was separated vertically in two compartments (650 × 100 × 10 mm) by a plastic-strip. The cages were closed at the top by a fine mesh to prevent the earthworms from escaping. The moisture content of the soils was kept constant throughout the experiment at 60% of the water holding capacity.

In total 6 planar cages were filled with forest soil, to half of them 6 g dry wt beech litter were added on the soil surface. Before placement into the experimental cages the earthworms were kept on wet filter paper to void their gut for three days; the filter paper was changed after two days. Two subadult or adult *L. terrestris* with a mean body weight of 3.53 (±1.72) g (fresh wt) were added to the cages. In addition to cages with earthworms, control cages without earthworms were set up in which 6 g beech litter was homogenously mixed into the soil. The cages were incubated in

darkness at 20°C for 65 days. At the end of the incubation period the cages were opened and earthworm casts were carefully separated from the soil. Earthworm casts were pooled per treatment. Control soil samples were taken from the cages without earthworms.

Particle size fractionation

For physical fractionation soil and cast samples were dispersed by low-energy sonication and the particle size fractions were separated by a combination of wet sieving and centrifugation as described by Stemmer et al. (1998). Fresh soil and casts, equivalent to 20 g dry wt, were dispersed in 100 ml of cooled distilled water by a probe-type ultrasonic disaggregator (50 J s^{-1} for 2 min). The coarse and medium particle size fraction (> 250 µm) and the fine particle size fraction (250-63 µm) were separated by manual wet sieving with 400 ml of cooled distilled water. Silt sized particles (63-2 µm) were separated from the clay fraction (< 2 µm) by five centrifugation steps at approximately 150 g for 2 min at 15°C. Between each centrifugation the pellets were resuspended in water and centrifuged again to purify the silt fraction. The combined supernatants were centrifuged at 3900 g for 30 min at 15°C to obtain clay-sized particles (2-0.1 µm).

Organic carbon and total nitrogen analysis

Oven-dried (65°C for three days) aliquots of the bulk soil and of the particle size fractions of soil and cast samples were pulverised in an agate ball mill. Carbonates in the samples were removed by the dropwise addition of 6 M hydrochloric acid and dried at 65°C for 3 days. Organic carbon and total nitrogen were measured by dry combustion in an elemental analyser (Model 1400, Carlo Erba, Milan, Italy).

Microbial biomass

Soil and cats samples were analysed for microbial biomass (C_{mic}), basal respiration and specific respiration (qO_2) by the substrate-induced respiration method (SIR) (Anderson & Domsch 1978). Measurements were taken using an automated respirometer system based on electrolytic O_2 microcompensation (Scheu 1992a). Control soil and earthworm casts were supplemented with 4 mg glucose g^{-1}.

Glucose was added as an aqueous solution adjusting the water content to 80% of the water holding capacity. Oxygen consumption rates at 22°C were measured every 30 minutes. The mean of the eight lowest measurements during the first 11 h after glucose addition was taken as the maximum initial respiratory response (MIRR). Microbial biomass C (C_{mic}; µg g^{-1} dry wt) was calculated as 38 × MIRR (µl O_2 g^{-1} h^{-1}) (Beck et al. 1997). For basal respiration the average O_2 consumption rate (µl O_2 g^{-1} h^{-1}) of samples not amended with glucose during hours 15-30 after attachment to the respirometer system was used. From data on microbial biomass and basal respiration the specific respiration (qO_2; µl O_2 mg^{-1} C_{mic} h^{-1}) was calculated.

Xylanase activity

Xylanase activity was measured by incubating 0.5-1.0 g (fresh wt) of each of the particle size fractions with a 5.0 ml solution of substrate (1.7% w/v xylan from oat spelts suspended in 2 M acetate buffer, pH 5.5) and 5.0 ml 2 M acetate buffer (pH 5.5) for 24 h at 50°C. Before incubating the clay fraction it was mixed for 1 min with 0.7 g of quartz to improve the dispersion of the suspension. The reducing sugars released during the incubation period reduced potassium hexacyanoferrate (III) in an alkaline solution. Potassium hexacyanoferrate (II) was measured colorimetrically using the Prussian Blue reaction (Schinner et al. 1996).

Phospholipid fatty acid (PLFA) analysis

For analysis of the microbial community structure in soil and casts phospholipid fatty acids (PLFA) were extracted from bulk and particle size fractions of the soil and cast samples. PLFAs were fractionated and quantified using the procedure described in Bardgett et al. (1996) which is based on that of Bligh and Dyer (1959) as modified by White et al. (1979). The separated fatty acid methyl esters were identified by chromatographic retention time and mass spectral comparison using standard qualitative bacterial acid methyl ester mix (Supelco) that ranged from C11 to C24. For each sample, the abundance of individual fatty acid methyl esters was expressed in nmol per unit dry weight. The fatty acid nomenclature as described by Frostegård et al. (1993a, b) was used. The sum of ten fatty acids (i15:0, a15:0, 15:0, i16:0, 16:1ω7, 17:0, i17:0, cy17:0, 18:1ω7 and cy19:0) was used to represent bacterial

PLFAs (bactPLFAs) (Frostegård & Bååth 1996) and 18:2ω6 was used as an indicator of fungal biomass (Federle 1986). The ratio of 18:2ω6-to-bactPLFAs was taken to represent the ratio of fungal-to-bacterial biomass in the soil (Frostegård & Bååth 1996, Bardgett et al. 1996).

Statistical analysis

Data on C and N content, C-to-N ratio, basal respiration, microbial biomass, specific respiration, xylanase activity and the bacteria-to-fungi ratio from PLFA data were analysed by two-factor analysis of variance (ANOVA) with the factors substrate (soil and earthworm casts) and litter (without and with beech litter). ANOVAs data was inspected for homogeneity of variance (Levene test) and log-transformed (if required) in advance. A statistical probability $P < 0.05$ was considered significant. To compare the overall structure of the PLFA profiles between the treatments (soil and cast, with and without beech litter) and the particle size fractions (coarse and fine sand, silt, clay and bulk) multidimensional scaling (MDS) and discriminant function analysis (DFA) were used, based on a scheme proposed by Puzachenko and Kuznetsov (1998), previously applied to analyse microfungal communities by Tiunov and Scheu (2000a). In short, a square matrix of nonparametric Gamma correlation (analogous to Kendall τ) was calculated from the relative frequencies of all PLFAs. This matrix was analysed by multidimensional scaling, i.e. an ordination technique, which "rearranges" objects in a maximally nine-dimensional space, so as to arrive at a configuration that best approximates the observed distances. The number of meaningful dimensions was evaluated by comparing actual stress values with the theoretical exponential function of stress. The coordinates of the samples in the

n-dimensional space were used for discriminant function analysis, with treatment as a grouping variable. Squared Mahalanobis distances between group centroids and reliability of sample classification were determined. Two significant discriminatory roots were derived and the results of DFA were graphically presented in two dimensions. For the interpretation of the discriminant roots with respect to the amount of PLFA, linear correlations were calculated between the discriminant

function scores for each sample and the distribution of single PLFAs. STATISTICA 6.0 software package was used for statistical analyses (Statsoft, Hamburg, Germany).

2.3 Results

Organic C and total N

The weight of the particle size fractions added to 94-106% of the bulk material suggesting complete recovery. The finest fraction (< 0.1 μm) constituted 2-3% of the soil dry weight in each of the treatments and was therefore excluded from further analyses. The quartz sand which was homogenously mixed into the soil added exclusively to the coarse sand fraction (> 250 μm) (Fig. 2.1). Earthworm gut passage did not affect the particle-size distribution whereas addition of fragmented beech litter tended to increase the proportion of the coarse sand fraction ($F_{1,6}$ = 4.34, P = 0.08).

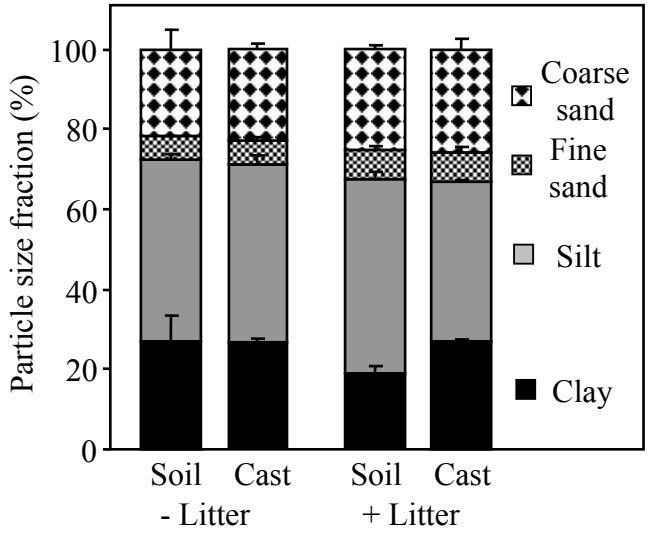

Fig. 2.1
Particle size distribution of soil and casts of *Lumbricus terrestris* without (- Litter) and with addition of beech litter (+ Litter). Means of two or three replicates ±1 S.D.

C_{org} and N_{tot} contents of the bulk soil and the particle size fractions differed between soils and casts and were significantly affected by the addition of litter (Table 2.1). Generally C_{org} was more responsive than N_{tot} to experimental treatments. C_{org} of the bulk soil was significantly increased in earthworm casts ($F_{1,6}$ = 49.37, P < 0.001) and in litter treatments ($F_{1,6}$ = 62.72, P < 0.001), with the increase in earthworm casts being more pronounced in treatments with addition of litter (+36.2%) than in those without litter (+5.8%) (significant substrate × litter interaction; $F_{1,6}$ = 27.17,

$P = 0.002$). N_{tot} content of the bulk soil was also increased in casts ($F_{1,6} = 7.49$, $P = 0.03$), though only in casts with litter (significant substrate × litter interaction; $F_{1,6} = 6.98$, $P = 0.04$).

Recovery of C and N in particle size fractions varied between treatments and differences among treatments were less pronounced than in the bulk soil. C_{org} content was at a maximum in the fine sand fraction followed by the clay, silt and coarse sand fraction (Table 2.1). N_{tot} content declined in the order clay fraction > fine sand > silt > coarse sand fraction. Addition of litter increased the C_{org} content in the coarse sand fraction ($F_{1,6} = 6.65$, $P = 0.04$). In casts the C_{org} content was decreased in the fine sand fraction in treatments without litter (-6.80%) but increased in treatments with litter (+33.23%) (significant substrate × litter interaction; $F_{1,6} = 5.99$, $P = 0.05$). In general, the C_{org} content of the clay sized fraction tended to be higher in the casts ($F_{1,6} = 3.38$, $P = 0.11$). Overall, the N_{tot} content in particle size fractions was not affected by the treatments.

Table 2.1
Organic carbon, total nitrogen content and C-to-N ratio in bulk soil and particle size fractions of soil without and with litter, and earthworm casts without and with litter. Means of two (Soil and Cast + litter) and three replicates (Cast and Soil + litter) and one standard deviation (1 S.D.).

Soil property	Treatment	Bulk	Particle size (μm)				Recovery (%)
			Coarse sand (> 250)	Fine sand (250-63)	Silt (63-2)	Clay (2-0.1)	
C_{org} (mg g^{-1})	Soil	47.68 (1.17)	30.36 (11.28)	109.06 (10.68)	49.35 (2.30)	69.82 (2.55)	103.54 (1.30)
	Cast	50.46 (1.44)	30.72 (3.57)	101.65 (9.91)	50.30 (8.26)	73.32 (3.32)	101.77 (11.64)
	Soil + litter	51.83 (3.41)	40.60 (12.37)	96.22 (4.24)	57.49 (6.05)	65.07 (8.60)	101.39 (12.00)
	Cast + litter	70.58 (2.26)	67.53 (27.20)	128.20 (24.20)	51.78 (8.65)	75.19 (4.56)	89.45 (18.48)
N_{tot} (mg g^{-1})	Soil	3.71 (0.26)	0.98 (0.44)	4.62 (0.29)	3.38 (0.21)	6.59 (0.18)	93.14 (0.70)
	Cast	3.72 (0.17)	0.94 (0.12)	4.16 (0.40)	3.21 (0.52)	6.74 (0.17)	92.63 (10.52)
	Soil + litter	3.69 (0.19)	1.21 (0.37)	4.18 (0.26)	4.12 (0.49)	6.32 (0.65)	93.98 (4.92)
	Cast + litter	4.39 (0.23)	2.15 (1.01)	5.09 (0.93)	3.33 (0.49)	7.00 (0.28)	88.71 (24.41)
C-to-N ratio	Soil	12.87 (0.57)	31.49 (2.53)	23.56 (0.84)	14.60 (0.23)	10.60 (0.09)	
	Cast	13.56 (0.41)	32.85 (3.64)	24.45 (0.10)	15.57 (0.44)	10.87 (0.25)	
	Soil + litter	14.06 (0.58)	33.68 (3.33)	23.06 (0.55)	13.97 (0.64)	10.28 (0.64)	
	Cast + litter	16.09 (0.33)	31.90 (2.37)	25.18 (0.17)	15.50 (0.48)	10.73 (0.23)	

Microbial biomass

Basal respiration, microbial biomass and specific respiration were not affected by the treatments in the bulk soil. Overall, the sum of microbial biomass of the particle size fractions ranged between 198 and 277% of that of the bulk soil. Similarly, the sum of basal respiration of the particle size fractions significantly exceeded that in bulk soil. Basal respiration and microbial biomass calculated on the basis of soil dry weight were highest in the clay fraction followed by the fine sand, silt and coarse sand fraction (Fig. 2.2a, b). Litter only significantly increased microbial biomass in the silt fraction (+55.06%; $F_{1,6}$ = 6.03, P = 0.05). Specific respiration of the bulk soil was similar in each of the treatments (Fig. 2.2c). Compared to the bulk soil the specific respiration was increased in each particle size fraction with a maximum in the silt and clay fraction and a minimum in the fine and coarse sand fraction. In general, specific respiration did neither differ significantly between casts and soil nor between the litter and non-litter treatments. Only in the silt fraction did specific respiration tend to be higher in casts than in soil ($F_{1,6}$ = 3.94, P = 0.09).

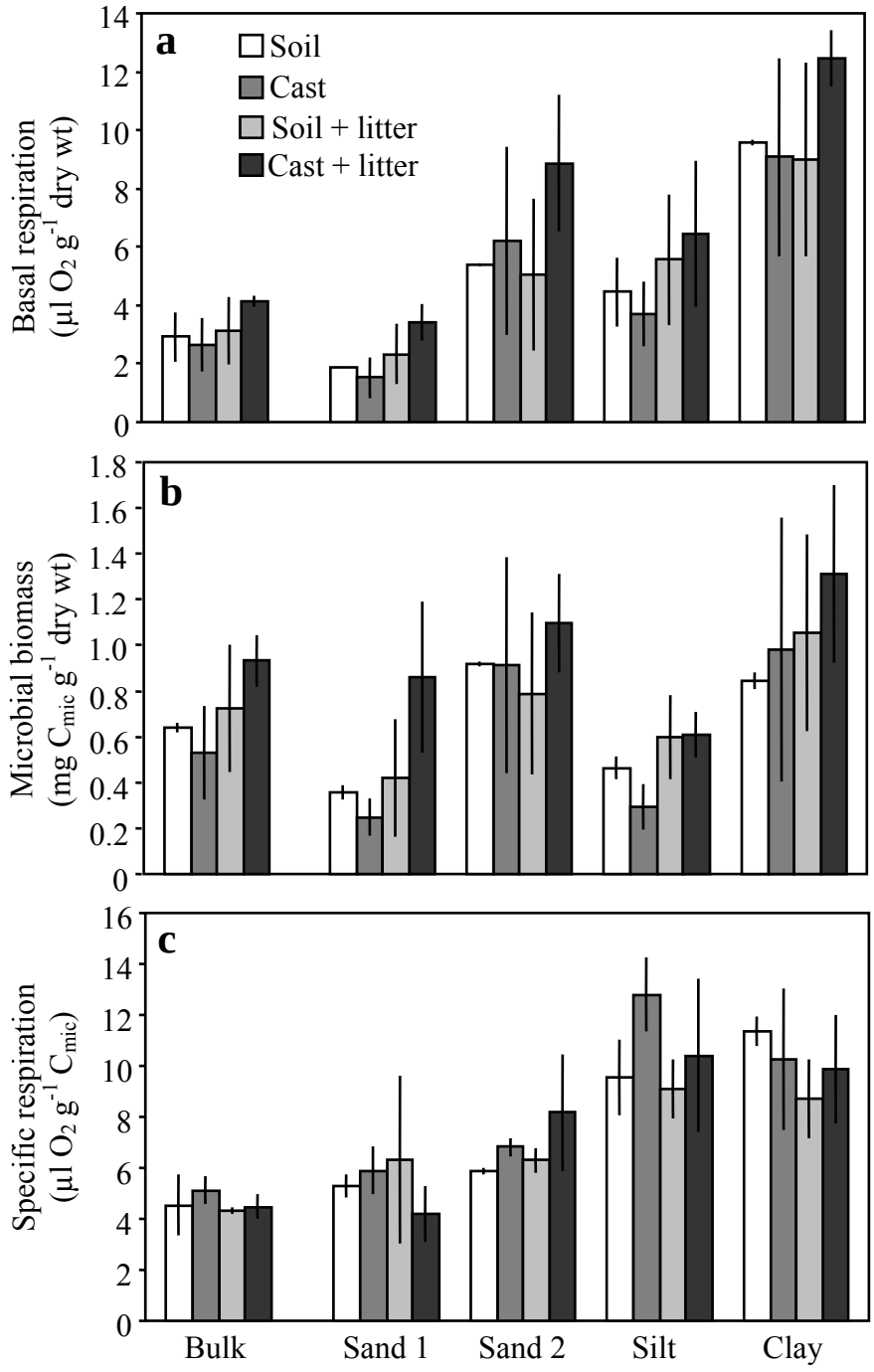

Fig. 2.2
Basal respiration **(a)**, microbial biomass (**b)** and specific respiration (**c)** in bulk soil and particle size fractions of soil and casts of *Lumbricus terrestris* without (- Litter) and with addition of beech litter (+ Litter). Means of two or three replicates ±1 S.D.

Xylanase activity

Xylanase activity was significantly increased by the addition of litter in the bulk soil, on average by 77.44% ($F_{1,6}$ = 20.94, P = 0.004). On average xylanase activity in particle size fractions exceeded that in bulk soil by 5 to 37%. Xylanase activity was at a maximum in the fine sand fraction, lower in the coarse sand fraction and at a minimum in the silt and clay fraction, with the latter two being similar (Fig. 2.3). In the coarse sand fraction of the cast and litter treatments xylanase activity was increased by 39.1% ($F_{1,6}$ = 9.04, P = 0.03) and 124.8% ($F_{1,6}$ = 49.87, P < 0.001), respectively. Also, in the fine sand (+41.4%; $F_{1,6}$ = 7.03, P = 0.04) and silt fraction (+39.9%; $F_{1,6}$ = 98.48, P < 0.001) of casts and soil the addition of litter significantly increased xylanase activity. Furthermore, in the silt fraction of the casts the addition of litter increased xylanase activity (+58.6%) whereas in casts without litter it was decreased (-36.25%; significant substrate × litter interaction; $F_{1,6}$ = 215.52, P < 0.001). In the clay fraction xylanase activity was generally not affected by the experimental treatments.

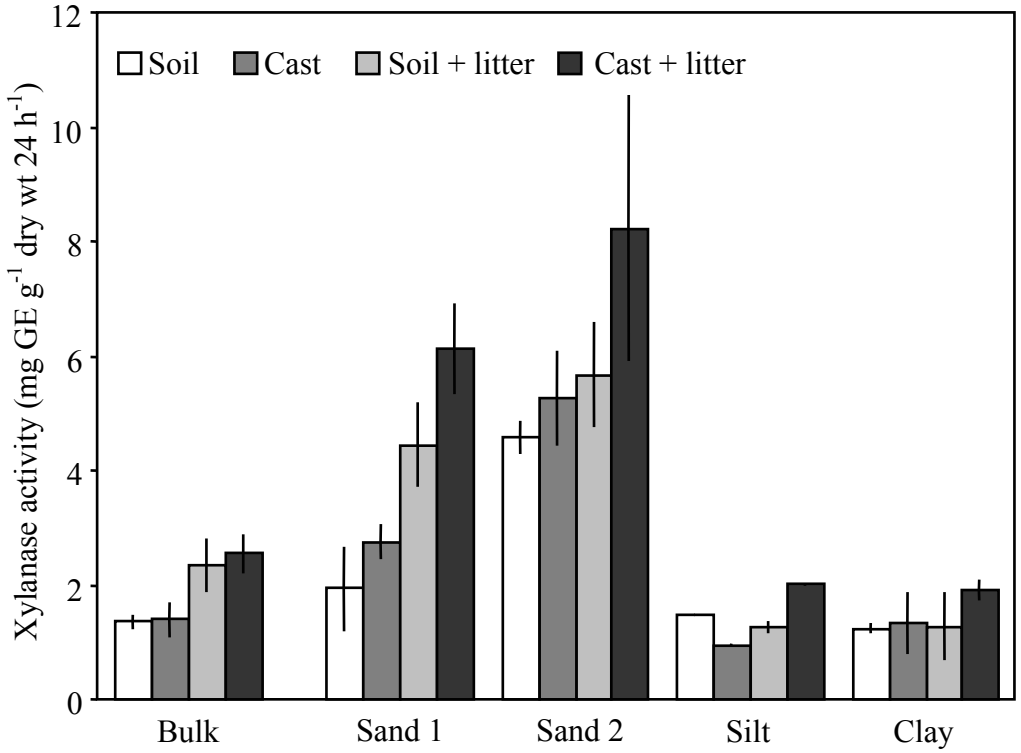

Fig. 2.3

Xylanase activity in bulk soil and particle size fractions of soil and casts of *Lumbricus terrestris* without (- Litter) and with addition of beech litter (+ Litter). Means of two or three replicates ±1 S.D. GE: glucose equivalents.

PLFA

The total amount of PLFAs in the bulk soil ranged between 123.0 and 183.2 nmol g^{-1} dry weight and was not affected by the treatments. The fatty acids 18:1n7, 18:1n9t and 16:0 were the three most abundant PLFAs in each of the treatments (Fig. 2.4). Recovery of PLFAs in particle size fractions ranged between 84 and 101%. The content of PLFAs was highest in the clay and lowest in the coarse sand fraction. Generally, compared to silt fraction of the control soil the content of total PLFAs was reduced in the earthworm casts silt fraction (-28.3%; $F_{1,6} = 6.99$, $P = 0.04$).

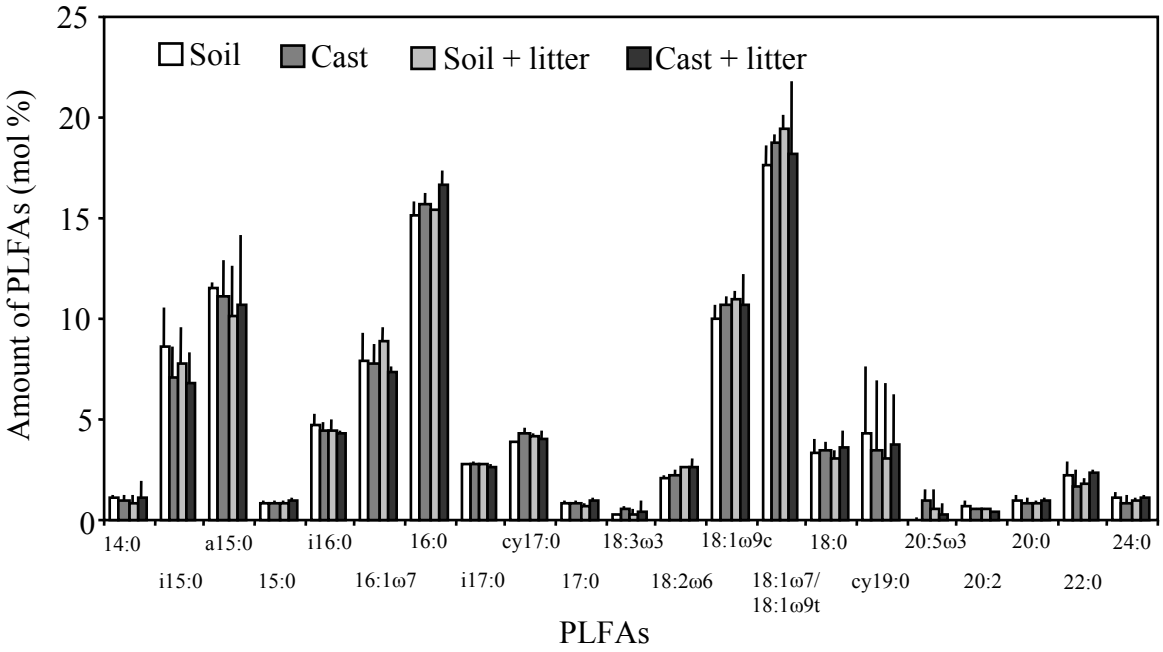

Fig. 2.4

Relative amounts of phospholipid fatty acids (PLFAs) in soil and casts of *Lumbricus terrestris*, without (- Litter) and with addition of beech litter (+ Litter). Means of two or three replicates ±1 S.D.

Overall, the DFA of the PLFAs separated the coarse sand fraction from the fine sand fraction and both of these from the silt and clay fraction (Fig. 2.5). The bulk soil clustered close to the silt and clay fractions and distant to the sand fractions. Within particle size fractions the treatments were little or not separated. In the clay fraction the PLFA profile of casts with litter differed significantly from that of the soil with litter (squared Mahalanobis distances between the treatments of 2.5; $P = 0.04$). In the coarse fraction the PLFA profile of casts without litter differed from that of soil without litter (2.7; $P = 0.03$) and casts with litter (2.4; $P = 0.05$).

The concentrations of the PLFAs 15:0, 17:0 and 20:5ω2 were low and they neither correlated with root 1 nor with root 2 of the discriminant function analysis (Table 2.2). The other PLFAs correlated with root 1 except 18:3ω3, 20:0, 22:0 and 24:0.

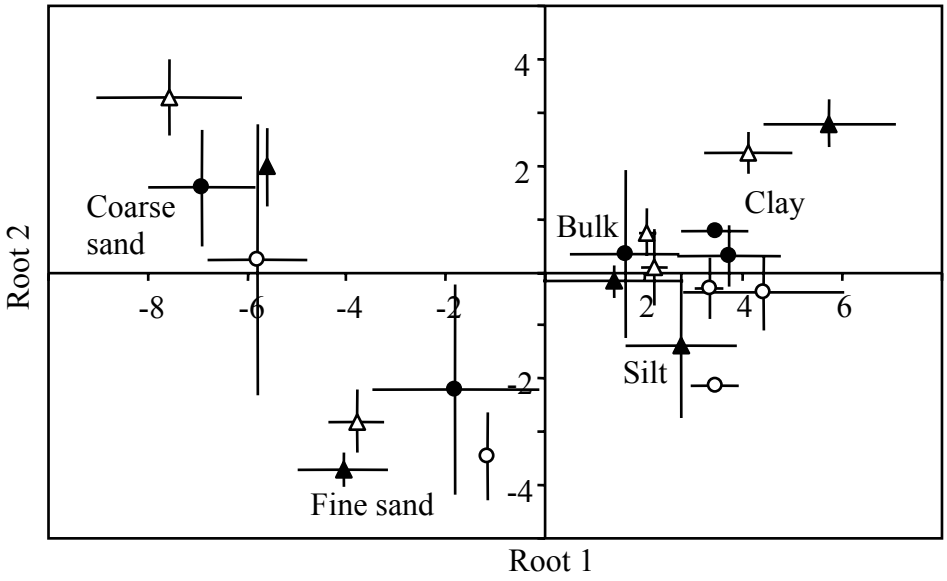

Fig. 2.5
Discriminant function analysis of phospholipid fatty acids (PLFAs) in bulk soil and particle-size fractions of soil (circle) and casts of *Lumbricus terrestris* (triangle) with (closed symbols) and without addition of beech litter (open symbols). Group centroids of treatments with standard deviations for root 1 and root 2. Each centroid represents two or three replicates. (Wilk's lambda = 0.00011, $P < 0.0001$; Root 1 represents 70.4% ($P < 0.0001$) of the variance, root 2 15.1% ($P = 0.002$).

The content of PLFAs specific for bacteria (bactPLFAs) was not affected by the treatments in the bulk soil (Table 2.3). The content of the fungal PLFA 18:2ω6 was therefore significantly increased in bulk soil and casts of treatments with beech litter ($F_{1,6} = 8.92$, $P = 0.02$) (Table 2.3). In particle size fractions of the casts, the content of bactPLFAs was decreased in the fine sand fraction ($F_{1,6} = 7.32$, $P = 0.04$) and tended to be decreased in the clay fraction ($F1_{,6} = 4.22$, $P = 0.08$) compared to the respective fractions in soil. The content of fungal PLFA was increased by the addition of litter in the clay fraction ($F_{1,6} = 6.89$, $P = 0.04$). In the silt fraction of casts it exceeded that in the soil ($F_{1,6} = 6.96$, $P = 0.04$).

The fungal-to-bacterial PLFA ratio in the bulk material was low (0.03-0.05) but was significantly increased by the addition of litter ($F_{1,6} = 6.18$, $P < 0.05$) (Table 2.3). It differed significantly between particle size fractions declining from the coarse sand to the fine sand to the silt and clay fraction. The fungal-to-bacterial PLFA ratio tended to be higher in the fine sand fraction of the earthworm casts than in the respective fraction of the soil ($F_{1,6} = 5.66$, $P = 0.055$). In the silt fraction the fungal-to-bacterial PLFA ratio was higher in casts than in soil ($F_{1,6} = 6.49$, $P = 0.04$). In the clay fraction it tended to be higher in treatments with litter than in those without ($F_{1,6} = 5.2$, $P = 0.06$).

Table 2.2
Linear correlation (r-values) of the total amount of PLFA per gram soil (dry wt) to root 1 and root 2 of the discriminant function analysis (see Fig. 2.5).

PLFA	Root 1	Root 2
14:0	.5034***	.0124ns
i15:1	.5308***	-.0184ns
a15:0	.6633***	.1240ns
15:0	.2300ns	-.1743ns
i16:0	.5793***	-.0748ns
16:1ω7	.5589***	.0271ns
16:0	.3607**	-.1012ns
i17:0	.6564***	.0809ns
cy17:0	.6508***	.0357ns
17:0	.2703ns	-.1778ns
18:3ω3	-.0469ns	-.3467*
18:2ω6	-.6228***	-.0647ns
18:1ω9c	.6189***	.0370ns
18:1ω7 / 18:1ω9t	.6187***	.0601ns
18:0	.4742***	.0354ns
cy19:0	.4959***	.0228ns
20:5ω2	-.0654ns	-.0720ns
20:2	.6426***	.2426ns
20:0	.2538ns	-.3708**
22:0	-.1471ns	-.5233***
24:0	-.2396ns	-.4704***

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ns, not significant

Table 2.3
Fungal (18:2ω6), bacterial (sum of 10) PLFAs and the fungal-to-bacterial PLFA ratio in bulk soil and particle size fractions of soil without and with litter and earthworm casts without and with litter. Means of two (Soil and Cast + litter) and three replicates (Cast and Soil + litter) and one standard deviation (1 S.D.).

	Treatment	Bulk	Particle size (μm)			
			Coarse sand (> 250)	Fine sand (250-63)	Silt (63-2)	Clay (2-0.1)
Fungal PLFA (mol %)	Soil	2.08 (0.08)	20.01 (6.75)	4.92 (0.48)	1.70 (0.10)	1.41 (0.19)
	Cast	2.22 (0.11)	12.89 (0.61)	5.51 (1.20)	1.93 (0.05)	1.29 (0.07)
	Soil + litter	2.58 (0.27)	15.02 (0.61)	3.24 (2.24)	1.79 (0.25)	1.51 (0.04)
	Cast + litter	2.67 (0.43)	14.21 (3.78)	5.58 (0.70)	2.29 (0.36)	1.85 (0.42)
Bacterial PLFA (mol %)	Soil	62.99 (1.58)	32.24 (8.61)	49.63 (3.93)	60.70 (0.88)	64.10 (2.85)
	Cast	61.50 (2.07)	37.84 (5.09)	48.26 (4.57)	58.39 (4.09)	63.96 (2.40)
	Soil + litter	62.37 (2.25)	35.07 (5.16)	54.48 (3.12)	61.32 (4.09)	65.22 (0.70)
	Cast + litter	59.56 (3.65)	31.37 (6.39)	42.11 (4.03)	55.19 (3.44)	58.69 (4.19)
Fungal-to-bacterial ratio	Soil	0.03 (0.01)	0.67 (0.39)	0.10 (0.01)	0.03 (0.01)	0.02 (0.01)
	Cast	0.04 (0.01)	0.34 (0.11)	0.11 (0.03)	0.03 (0.01)	0.02 (0.01)
	Soil + litter	0.04 (0.01)	0.44 (0.10)	0.06 (0.04)	0.03 (0.01)	0.02 (0.01)
	Cast + litter	0.05 (0.01)	0.48 (0.22)	0.13 (0.01)	0.04 (0.01)	0.03 (0.01)

2.4 Discussion

Organic matter

The distribution of particle size fractions did not differ between soil and casts of *L. terrestris*. This indicates that *L. terrestris* did not selectively feed on or excluded sand particles from ingestion. This was true in both treatments with and without beech litter. Schulmann and Tiunov (1999) observed that *L. terrestris* preferentially selects sand particles when feeding on leaf litter material. In contrast to the study of Schulmann and Tiunov (1999) earthworms were kept in mineral soil and therefore ingested not only litter and sand but also mineral soil and this possibly supplemented the demand of the earthworms for mineral particles. Doube et al. (1997) found that ingestion of mineral soil by earthworms facilitates the palatability of organic matter and that *L. terrestris* prefers to feed on mineral soil or a mixture of mineral soil and litter over pure litter. In contrast, Zhang and Schrader (1993) found the concentration of coarse sand to be reduced in casts of *L. terrestris* while the silt sized fraction was increased. However, the higher C and N content in casts of *L. terrestris* could not be explained by selective exclusion of sand particles by the earthworms in the study of Zhang and Schrader (1993). In the present study the C and N content in casts also exceeded that in soil, in particular in treatments with litter. It seems obvious that *L. terrestris* selectively ingested organic particles in the soil and on the soil surface and that these were only partly digested as is typical for anecic earthworms (Judas 1992). However, there is little information on the incorporation of the organic matter ingested into particle size fractions in casts. Scullion and Malik (2000) found carbon associated with the clay fraction to be increased in earthworm casts. The C and N content in the clay fraction of the earthworm casts also tended to be higher in the present study. This was however not significant. Clay associated organic matter is known to be stabilised and the formation of clay associated carbon pools is slow (Thiessen & Stewart 1983, Hassink & Whitmore 1997). Earthworms may accelerate this process by intimately mixing comminuted organic matter with mineral particles of the clay fraction thereby increasing the pool of stabilised organic matter in soil (Oades 1988). This indicates that clay associated organic matter is most affected by the passage through the earthworm gut.

Microbial community

In the present study, the total amount of PLFAs neither differed between casts and soil nor between treatments without or with beech litter. This is in contrast to the higher amount of PLFAs in casts of *Aporrectodea tuberculata* found by Clapperton et al. (2001). In addition, PLFA profiles also differed little between the bulk soil and casts irrespective of the addition of litter. This supports previous findings that the structure of the microbial community in soil is little affected by the gut passage through earthworms even if the earthworms do ingest litter materials (Furlong 2002, Egert et al. 2004). In this study it was hypothesised that the combined ingestion of sand and litter and an associated enhanced comminution of litter materials results in changes in the microbial community structure in earthworm cast. With the exception of three single particle size fractions the PLFA profiles did not differ between the treatments. The differentiation in the clay fraction between soil and cast in treatments with litter supports the results of the C and N content and suggests that the clay fraction in fact was most affected by the gut passage through earthworms. However, in treatments without litter, PLFA profiles of the coarse sand fraction also differed between casts and soil suggesting that even without ingestion of litter materials from the soil surface earthworms do affect the structure of the microbial community even if only to a small extent. It is possible that certain groups of microorganisms of the coarse sand fraction are favoured in earthworm casts. Fungi are known to be the dominant microorganisms of the coarse particle size fraction (Kandeler et al. 2000). In fact, in the present study the fungal PLFA 18:2ω6 was most abundant in the coarse sand fraction. Fungal hyphae are assumed to be disrupted during the gut passage through earthworms and part of the diet of earthworms may constitute of fungal hyphae (Dash et al. 1984, Wolter & Scheu 1999, Bonkowski et al. 2000). However, fungal PLFA were enriched in the silt fraction of the earthworm casts. In addition the fungal-to-bacterial PLFA ratio was similar in the coarse sand fraction of casts and soil, and tended to be higher in the fine sand and silt fraction of the casts. It can be presumed that rather than being detrimentally affected by digestion fungi benefit from favourable conditions in ageing casts which is likely to be connected with increased nutrient availability (Brown et al. 1998, Tiunov & Scheu 2000b). In fact, fungi are known to become more dominant in ageing casts of *L. terrestris*

(Scullion et al. 2003) which is caused by flourishment of certain fungal taxa (Tiunov & Scheu 2000b).

The addition of beech leaf litter also increased the fungal-to-bacterial PLFA ratio in bulk soil which resulted mainly from increased fungal PLFA in the clay fraction. In the coarse and the fine sand fraction the fungal-to-bacterial PLFA ratio was not affected by beech litter. This was unexpected as the litter particles added mainly formed part of the coarse fractions and resulted in an increase in the xylanase activity in these fractions suggesting that fungal activity was increased (Kandeler et al. 1999b).It is possible that fungal hyphae from coarse fractions were transferred into finer fractions by the particle size fractionation procedure.

The addition of beech leaf litter also significantly increased the microbial biomass in the silt fraction. Bacterial PLFAs dominated the PLFA profiles in the silt and clay fractions while fungal PLFA and xylanase activity were low. Bacteria are known to dominate in finer fractions at the expense of fungi (Van Gestel et al. 1996, Kandeler et al. 2000) which is supported by the low fungal-to-bacterial PLFA ratio in the silt and clay fractions. Most of the microbial biomass was located in the fine sand and clay fractions. Similarly, Ladd et al. (1996) found most of the microbial biomass in the fine sand and fine silt fractions. It can be presumed that partly decomposed litter materials aggregate in the fine sand fraction and that this was increased in earthworm casts. Microorganisms in the fine silt fraction are likely to have benefited from soluble components diffusing from decomposing litter particles in coarse fractions; this was also increased in earthworm casts. This scenario is supported by the fact that basal respiration and specific respiration were also at a maximum in the clay fraction. In general, the sum of basal respiration and microbial biomass of the particle size fractions strongly exceeded that of the bulk soil. This was presumably because the microorganisms enclosed in aggregates in bulk soil and casts were liberated and became activated by the ultrasonication and the wet sieving procedure. By the disruption of aggregates physically protected resources are made available and can be used by microorganisms (Shipitalo & Protz 1989, Zhang & Schrader 1993). The physical disruption of aggregates during the fractionation procedure may resemble the destruction of aggregates during the gut passage through earthworms (Devliegher & Verstraete 1995, Hindell et al. 1997) and this may explain the increased basal

respiration and metabolic activity in fresh earthworm casts (Scheu 1987a, Trigo & Lavelle 1993).

In conclusion, earthworms stabilise soil organic matter in cast aggregates predominantly by increasing the soil organic matter content in the clay fraction where it becomes protected against microbial attack. By mechanical disruption of casts, physically protected soil organic matter becomes available for microbial decay. Soil organic matter in the coarse and fine sand fractions is primarily decomposed by fungi. Incorporation of litter into these fractions by the earthworms increases the fungal biomass. Comminution of litter during the gut passage through earthworms increases the biomass and activity of fungi in the silt fraction. Results of this study demonstrate that the analysis of PLFA profiles in combination with other quantitative microbial methods in bulk soil and in particle size fractions can improve the understanding of stabilising and mobilising processes in earthworm casts.

3. Effects of sand and litter availability on organic matter decomposition in soil and in casts of *Lumbricus terrestris* L.

Abstract

The anecic earthworm *Lumbricus terrestris* L. is known to strongly affect organic matter transformation and soil development in temperate ecosystems. Processes in the gut of the earthworm play an important role where sand grains contribute to the grinding of leaf litter in the muscular gizzard. To investigate effects of sand and litter availability and earthworm gut passage on carbon and nitrogen mineralisation two experiments were set up, one with beech forest soil and beech litter, the other with arable soil and rye litter, thereby exploring the effects of agricultural management and litter type on mobilisation and stabilisation of organic matter. Three different sand concentrations were tested in the forest soil: without, with 25% and with 50% sand (dry wt). In the arable soil only treatments without and with 25% sand were established. The soil was taken from a beech forest on limestone (Göttinger Wald) and from a long-term agricultural experiment ("Eternal rye", NPK fertilisation; Halle). Earthworms were fed fragmented beech or rye litter (< 4 mm), or were kept without litter. Earthworm casts produced during 65 days were placed in microcosms and incubated at 20°C for 280 days. CO_2 production and mineral nitrogen in leaching water were measured at regular intervals. Earthworms lost more body mass in arable than in forest soil; even though in arable soil the earthworms produced two times more casts. Litter availability reduced burrow construction. Casts were more enriched in carbon and nitrogen if litter was available. Leaching of N_{min} from casts of *L. terrestris* strongly exceeded that of the corresponding soil treatments in the arable soil but not in the forest soil. In earthworm casts decomposition of rye litter was strongly increased, in particular early in the experiment. In contrast, the decomposition of beech litter was reduced initially and stimulated later. The addition of sand stimulated carbon mineralisation in both organic matter in soil and enclosed

in earthworm casts. In soils with high clay content (forest soil) the stimulating effect of sand was less pronounced than in soils with low clay content (arable soil). Overall, except in earthworm casts of the forest soil without litter, cumulative CO_2 production exceeded that in the corresponding soil suggesting that in earthworm casts mobilisation rather than stabilisation processes prevail.

3.1 Introduction

Earthworms contribute significantly to organic matter turnover by ingesting and fragmenting large quantities of litter and incorporating it into the mineral soil (Darwin 1881, Satchell 1963). Anecic earthworm species, such as *Lumbricus terrestris* L., are among the most important groups of soil animals involved in fragmenting litter, in incorporating plant residues into the soil and in forming soil aggregates. The mechanical fragmentation of organic matter affects decomposition rates due to conditioning of plant remains for microbial decomposition (Satchell 1967, Swift et al. 1979). Devliegher and Verstraete (1995) distinguished between nutrient enrichment processes (NEPs) caused by the incorporation of organic matter and gut associated processes (GAPs) caused by the passage of soil and litter through the earthworm gut. They concluded that increased mineralisation rates in earthworm-worked soil are mainly caused by NEPs. Tiunov and Scheu (2000b) further distinguished cast-associated processes (CAPs) to differentiate between short-term processes during the gut passage through earthworms from long-term processes occurring in ageing earthworm casts.

Processes in the gut of *L. terrestris* play an important role where small stones and particularly sand grains contribute to the grinding of leaf litter in the muscular gizzard (Schulmann & Tiunov 1999). Litter-feeding earthworms prefer to ingest a mixture of soil and litter over pure litter (Doube et al. 1997). The passage of litter materials through earthworms may result in a stimulation or reduction of organic matter decomposition and therefore of carbon mineralisation (Lee 1985, Scheu & Wolters 1991a, Tiunov & Scheu 2000b). Most studies on decomposition processes in ageing casts are restricted to short-term changes; only a few studies have investigated

long-term changes in carbon mineralisation (Martin 1991, Scheu & Wolters 1991a). Therefore, stabilising and mobilising processes of organic matter in earthworm casts in the long-term are little understood.

This study investigates the effect of the availability of sand and litter on carbon mineralisation and nitrogen mobilisation in ageing casts of *L. terrestris* over a medium-term period (280 days). Carbon mineralisation is known to vary with soil conditions, two soils from different ecosystems were therefore included; a mull soil from a beech forest on limestone (*Terra-fusca Rendzina*) and a sandy loess soil from an agricultural field (*Haplic Phaeozem*). The following hypotheses were tested: (1) the stabilisation of organic matter depends on the fragmentation of litter by earthworms; (2) sand facilitates the mechanical comminution of leaf litter during gut passage through earthworms, thereby enhancing its mineralisation; (3) the stabilisation of organic matter is at a maximum at intermediate sand concentrations.

3.2 Materials and Methods

Sampling

Soil samples were taken from two sites representing an arable farming system and a mature beech forest. The arable field site forms part of a long-term fertilisation experiment in Halle (central Germany, Saxony-Anhalt), known as “Eternal Rye”. At the study site winter rye (*Secale cereale* L.) has been planted in permanent monoculture since 1878 with mineral fertilisation (NPK). Mean temperature on site is 9.0°C and annual precipitation 466 mm. The soil is a *Haplic Phaeozem* on sandy loess (pH 6.3) (Merbach et al. 2000). The forest is a 130-year old beech wood on limestone near Göttingen (southern Lower Saxony, Germany), known as Göttinger Wald. The dominant soil types are *Terra-fusca Rendzina* (59%) and *Rendzina* (28%) (pH 6.9) (Ulrich et al. 1982). The annual mean temperature is 7.9°C and the mean annual rainfall 720 mm. Soil textures of the arable soil and forest soil consist of 69% sand, 23% silt and 8% clay (Merbach et al. 2000), and 4% sand, 67% silt and 29% clay, respectively (S. Marhan & S. Scheu, unpubl. data). Soil samples were taken from the upper 10 cm of the soil at the arable site and from the upper 5 cm at the

forest site after removal of litter in October 2000. The soil materials were passed through a 4 mm sieve to remove stones and plant residues. The soil was then stored at -28°C until the experiment was set up. Prior to placement in experimental containers the soil was kept at 5°C for one week.

Rye straw and beech leaf litter were used in the experiments representing the dominant litter types at the arable and forest site, respectively. Rye straw (44.7% C, 0.5% N) was collected after harvest from a rye field close to Darmstadt (Hesse, Germany). Almost one year old, little decomposed beech leaf litter (44.5% C, 1.6% N) was collected from the soil surface in late summer in the forest where the soil had been taken. The litter materials were air-dried and mechanically fragmented into pieces < 4 mm.

Sand was homogeneously mixed with the soil. Pure quartz sand with a particle size distribution of 40, 40 and 20% in the < 0.5, 0.5-1 and 1-2 mm fractions was used. In the forest soil experiment treatments with the addition of 25 and 50% sand of soil dry weight were established. In the arable soil experiment only the addition of 25% sand was studied because of the high sand content of this soil. Adult specimens of *L. terrestris* L. were extracted from an oak-beech forest 20 km south of Darmstadt (Jägersburger Wald) using formalin in October 2000. The earthworms were washed twice with distilled water and kept for three weeks at 5°C in containers containing soil from the respective study sites. For acclimation the earthworms were kept at 20°C one week before they were used for the experiment.

Cast production

For production of cast materials earthworms were incubated in planar cages consisting of two transparent PVC sheets (650 × 310 × 3 mm) separated by PVC strips (10 mm thick) at the two sides and the bottom. Each cage was separated vertically in two compartments (650 × 100 × 10 mm) by a plastic-strip. The cages were filled with soil to a height of 550 mm; the forest soil was compacted to a bulk density of 0.7 (without sand), 0.85 (25% sand) and 1.0 kg dry wt L^{-1} (50% sand). Bulk density of the arable soil was close to 1.2 (without sand) and 1.4 kg dry wt L^{-1} (25% sand). The cages were closed at the top by a fine mesh to prevent the

earthworms from escaping. The moisture content of the soils was kept constant throughout the experiment at 60% of the water holding capacity.

In total 24 planar cages were filled with arable soil, 12 without sand and 12 with 25% sand. To six of each 6 g rye litter was placed on the top of the soil. For the experiment with forest soil 24 planar cages were established, 8 without sand, 8 with 25% sand and 8 with 50% sand. To half of them 6 g beech litter was added on the soil surface. Before being placed into the experimental cages the earthworms were kept on wet filter paper to void their gut for three days; the filter paper was changed after two days. Two adult *L. terrestris* with a mean body mass of 3.06 (±0.67) and 3.53 (±1.72) g (fresh wt) were added to the cages with arable and forest soil, respectively. In addition to cages with earthworms, cages with litter but without earthworms were established in which 6 g rye and beech litter were homogenously mixed into the arable and the forest soil, respectively. One planar cage was established per treatment. The cages were incubated in darkness at 20°C for 65 days. At the end of the incubation period the length of earthworm burrows was measured. The cages were then opened and the faecal material was carefully separated from uningested soil. The earthworms were collected and weighed. Earthworm casts were weighed and pooled per treatment. Corresponding soil samples were taken from the cages without earthworms. The C and N content of the soil and casts was determined using an elemental analyser (Carlo Erba, Milano, Italy)

Incubation experiment

Earthworm casts (0-65 days old) and soil equivalent to 100 g dry wt for the arable soil experiment and 30 g dry wt for the forest soil experiment were transferred into microcosms (Fig 3.1) Casts and corresponding soils from the prior treatments with and without litter were established: without sand, with 25% sand and with 50% sand (forest soil experiment); without sand and with 25% sand (arable soil experiment). Treatments were replicated six times (arable soil experiment) and three times (forest soil experiment). The microcosms consisted of air tight Perspex tubes (Type I: height 150 mm, Ø 45 mm) fixed on ceramic plates. The microcosms allow drainage of soil materials at semi-natural conditions by lowering the atmospheric pressure in a box below the ceramic plates. Leaching water from each of the microcosms was sampled

in vessels placed underneath the microcosms in the box. The microcosms were closed at the top by a lid which had a small vessel attached to the underside. This vessel could be filled with alkali to absorb CO_2 evolved from the soil.

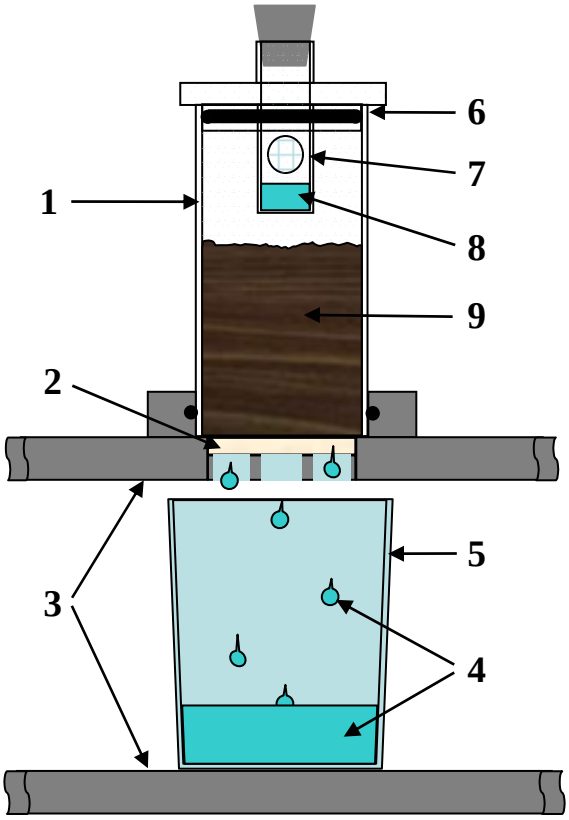

Fig. 1.1
Construction of the microcosms: air tight fixed perspex tube (1) (height 150 mm, Type I: Ø 45 mm; Type II: Ø 60 mm), ceramic plate (2), walls of the box (3), leaching water (4), vessel for collection of leaching water (5), air tight lid (6), small vessel attached to the underside (7), alkali (8) and soil (9).

Microcosms were incubated at 20°C for 280 days and watered weekly with 10 ml distilled H_2O. Leaching water was collected at regular intervals and pooled samples of six weeks were analysed for mineral nitrogen content (N_{min}; NH_4^+ and NO_3^-). CO_2 evolved in the microcosms was absorbed in alkali (1 N KOH). Prior to the addition of alkali used for CO_2 determination, the chambers were pre-incubated with alkali for 24 h. This was done to prevent sampling of CO_2 from dissolved carbonates during the subsequent incubation. For CO_2 determinations the chambers were incubated for 72 h at 2-3 week intervals. Trapped CO_2 was measured titrimetrically with 0.1 N HCl after precipitation of carbonate with saturated $BaCl_2$ solution (Macfadyen 1970).

Openings in the lid ensured free gas exchange between CO_2 determinations. Data on the total amount of nitrogen leached and carbon mineralised are given relative to the initial soil nitrogen (mg N_{min} g^{-1} N 9 $months^{-1}$) and carbon content (mg CO_2-C g^{-1} C 9 $months^{-1}$). This facilitates the comparison of the different sand and litter treatments.

Statistical analysis

Data on earthworm body mass, amount of casts produced and lengths of burrows were analysed by two-factor analysis of variance (ANOVA) with the factors "sand" (without, with 25% and with 50% sand in the forest soil experiment, and without and with 25% sand in the arable soil experiment) and "litter" (without and with beech litter in the forest soil experiment and without and with rye litter in the arable soil experiment). Data on the cumulative CO_2 production and the amount of nitrogen leached were analysed by three-factor ANOVA with the factors "sand" and "litter" (as above) and "substrate" (earthworm casts and soil). Prior to analysis data were inspected for homogeneity of variance (Levene test) and log-transformed if required. Tukey's HSD was used for comparison of means. A statistical probability $P < 0.05$ was considered significant. STATISTICA 6.0 software package was used for statistical analyses (Statsoft, Tulsa, USA).

3.3 Results

Earthworm body mass

Earthworm body mass generally decreased in both arable and forest soil during the incubation period but the decrease was more pronounced in arable soil ($F_{1,86} = 25.72$, $P < 0.0001$; Table 3.1). In arable soil earthworm body mass significantly decreased in the sand treatment ($F_{3,39} = 10.14$, $P < 0.01$). The decrease of earthworm body mass was less pronounced if litter was available ($F_{3,39} = 54.75$, $P < 0.001$). In the forest soil presence of litter also resulted in a lower decline in body mass of *L. terrestris*

($F_{5,39}$ = 12.14, P < 0.01). Sand tended to reduce the earthworm body mass, with the decline in the 50% sand treatment exceeding that in the 25% sand treatment in vessels without litter but tended to increase body mass in the 25% sand treatment if litter was available. By the end of the incubation the earthworms had consumed virtually all the litter provided which is equivalent to litter consumption rates of 15.08 mg rye litter and 13.11 mg beech litter (dry wt) g^{-1} fresh wt d^{-1}.

Table 3.1
Effects of sand (without = 0%, with 25% and with 50% sand) and litter (without and with litter; rye straw and beech litter in arable and forest soil, respectively) on body mass of *Lumbricus terrestris* L., amount of casts produced and length of burrows constructed in arable and forest soil. Values within rows sharing the same letter are not significantly different (Tukey's HSD test, P < 0.05).

	Soil without litter			Soil with litter		
	0% sand	25% sand	50% sand	0% sand	25% sand	50% sand
Arable soil						
Earthworm body mass (fresh wt) % of initial	58.2b	44.7c	-	78.5a	71.6a	-
Amount of casts (g g^{-1} body fresh wt)	43.1b	57.6ab	-	49.5ab	59.1a	-
Length of burrows (mm ind^{-1})	3735a	3723a	-	2815b	2746b	-
Forest soil						
Earthworm body mass (fresh wt) % of initial	79.4ab	73.0ab	67.8b	84.0ab	94.0a	86.6ab
Amount of casts (g g^{-1} body fresh wt)	26.3a	26.3a	25.2a	20.0a	24.8a	31.5a
Length of burrows (mm ind^{-1})	4133ab	5807a	4853ab	3263b	4077ab	4393ab

Casts and lengths of burrows

The addition of sand increased cast production by *L. terrestris* in both the arable and the forest soil but the effect was only significant in the arable soil ($F_{3,41}$ = 9.83, P < 0.01; Table 3.1). The addition of litter reduced the length of burrows constructed in both the arable ($F_{3,41}$ = 17.28, P < 0.001) and forest soil ($F_{5,17}$ = 9.49, P < 0.01).

Overall, the length of the burrows was at a maximum in forest soil with 25% sand and without litter.

C and N content

Total carbon and nitrogen content of the arable soil was considerably lower than that of the forest soil but C-to-N ratio was higher in the arable soil (Table 3.2). As expected the addition of sand reduced the carbon and nitrogen content. Litter addition generally increased C and N contents in soil and casts. In treatments with litter in both the arable and forest soil, casts of *L. terrestris* were more enriched in C and N than the corresponding soil.

Nitrogen leached

Leaching of mineral nitrogen (N_{min}) decreased during the experiment in both the arable and the forest soil. Nitrate concentration in the leachate was about tenfold higher than that of ammonium (data not shown). In the arable soil without litter cumulative N_{min} leaching during the experiment reached 19.81 (±2.49) mg N_{min} g^{-1} N 9 $months^{-1}$ and was not affected by sand (20.27 ±1.23) mg N_{min} g^{-1} N 9 $months^{-1}$) (Table 3.3). The addition of rye straw decreased nitrogen leaching to 13.73 (±2.66) and 15.16 (±2.32) mg N_{min} g^{-1} N 9 $months^{-1}$without and with 25% sand, but in treatments without litter this was only significantly different in the soil without sand (significant substrate × litter interaction) (Table 3.3). Generally, leaching of N_{min} from casts of *L. terrestris* exceeded that from corresponding soil treatments by factors of 2-2.5. There was no significant difference between the cast treatments irrespective of sand or litter availability, but nitrogen leaching from earthworm casts tended to be at a maximum in the combined treatment with sand and litter (47.60 ±7.34 mg N_{min} g^{-1} N). In forest soil cumulative N_{min} leaching ranged between 34 and 52 mg N_{min} g^{-1} N but was not significantly affected by the treatments.

Table 3.2
Carbon and nitrogen content of the soil and cast materials used in the experiment; 0% = without sand, 25% = sand content of 25% dry wt, 50% = sand content of 50% dry wt; without litter and with litter (rye straw and beech litter in arable and forest soil, respectively). Means and (S.D.) of 3 replicates.

	soil						**cast**					
	without litter			with litter			without litter			with litter		
	0% sand	25% sand	50% sand	0% sand	25% sand	50% sand	0% sand	25% sand	50% sand	0% sand	25% sand	50% sand
Arable soil												
C_{org} (%)	1.07 (0.02)	0.80 (0.01)	-	1.18 (0.09)	0.89 (0.04)	-	1.02 (0.01)	0.84 (0.10)	-	1.23 (0.06)	0.99 (0.06)	-
N_{tot} (mg g^{-1} dry wt)	0.67 (0.05)	0.52 (0.00)	-	0.74 (0.04)	0.59 (0.03)	-	0.70 (0.02)	0.58 (0.06)	-	0.77 (0.01)	0.62 (0.02)	-
C/N (ratio)	15.96 (0.89)	15.53 (0.12)	-	16.86 (1.56)	15.15 (0.30)	-	14.44 (0.31)	14.58 (0.28)	-	16.04 (0.74)	15.84 (1.01)	-
Forest soil												
C_{org} (%)	6.42 (0.11)	4.80 (0.05)	3.17 (0.05)	6.97 (0.09)	5.20 (0.04)	3.53 (0.01)	6.73 (0.08)	5.36 (0.04)	3.72 (0.07)	8.76 (0.11)	6.84 (0.06)	4.91 (0.11)
N_{tot} (mg g^{-1} dry wt)	4.62 (0.06)	3.43 (0.05)	2.26 (0.02)	4.89 (0.02)	3.69 (0.01)	2.59 (0.06)	4.75 (0.02)	3.91 (0.05)	2.63 (0.06)	5.55 (0.10)	4.41 (0.02)	3.18 (0.07)
C/N (ratio)	13.89 (0.05)	14.00 (0.07)	14.03 (0.13)	14.24 (0.19)	14.09 (0.03)	13.66 (0.25)	14.19 (0.11)	13.71 (0.08)	14.14 (0.02)	15.80 (0.49)	15.52 (0.05)	15.44 (0.01)

Carbon mineralised

Rates of CO_2 production in the arable soil without litter remained on a similar level throughout the experiment (Fig. 3.2a). The addition of litter increased CO_2 production and rates of CO_2 production in the rye straw treatments were high early during incubation and decreased later. CO_2 production in soil was increased by the addition of sand. In the forest soil CO_2 production generally exceeded that in the arable soil by a factor of about 2. In contrast to the arable soil, rates of CO_2 production in the forest soil increased during incubation reaching a maximum after seven months, and decreased thereafter reaching the initial level by the end of the experiment (Fig. 3.2b).

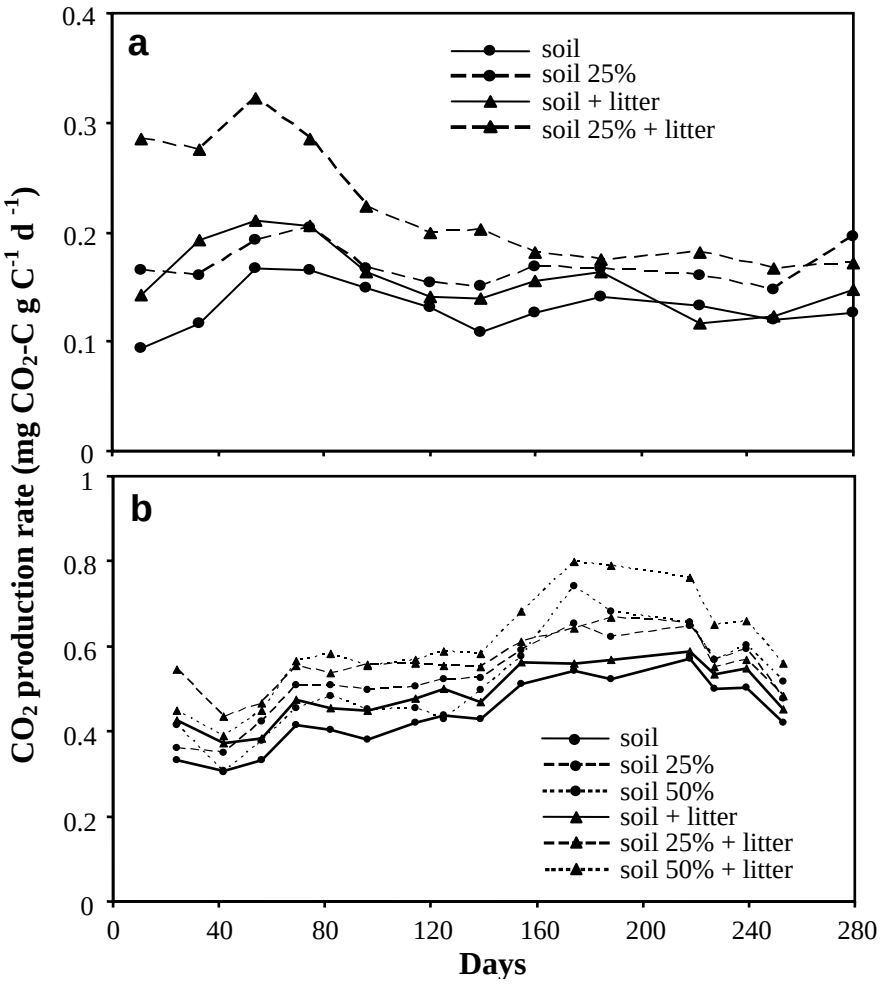

Fig. 3.2
Effects of sand and litter on CO_2 production of **(a)** arable soil (without and with 25% sand and without and with rye litter) and **(b)** forest soil (without, with 25% and with 50% sand and without and with beech litter).

Generally, both sand and litter significantly increased cumulative CO_2 production in the arable and forest soil experiments (Fig. 3.3a and 3.3b; Table 3.3). On average, the addition of sand increased cumulative CO_2 production by 16.1% in the arable soil and by 18.4% (25% sand) and 19.9% (50% sand) in the forest soil. However, in the arable soil the effect of sand was different in casts and soil (significant substrate × sand interaction) (Table 3.3). In soil CO_2 production was increased by 34.3% but only by 6.3% in casts.

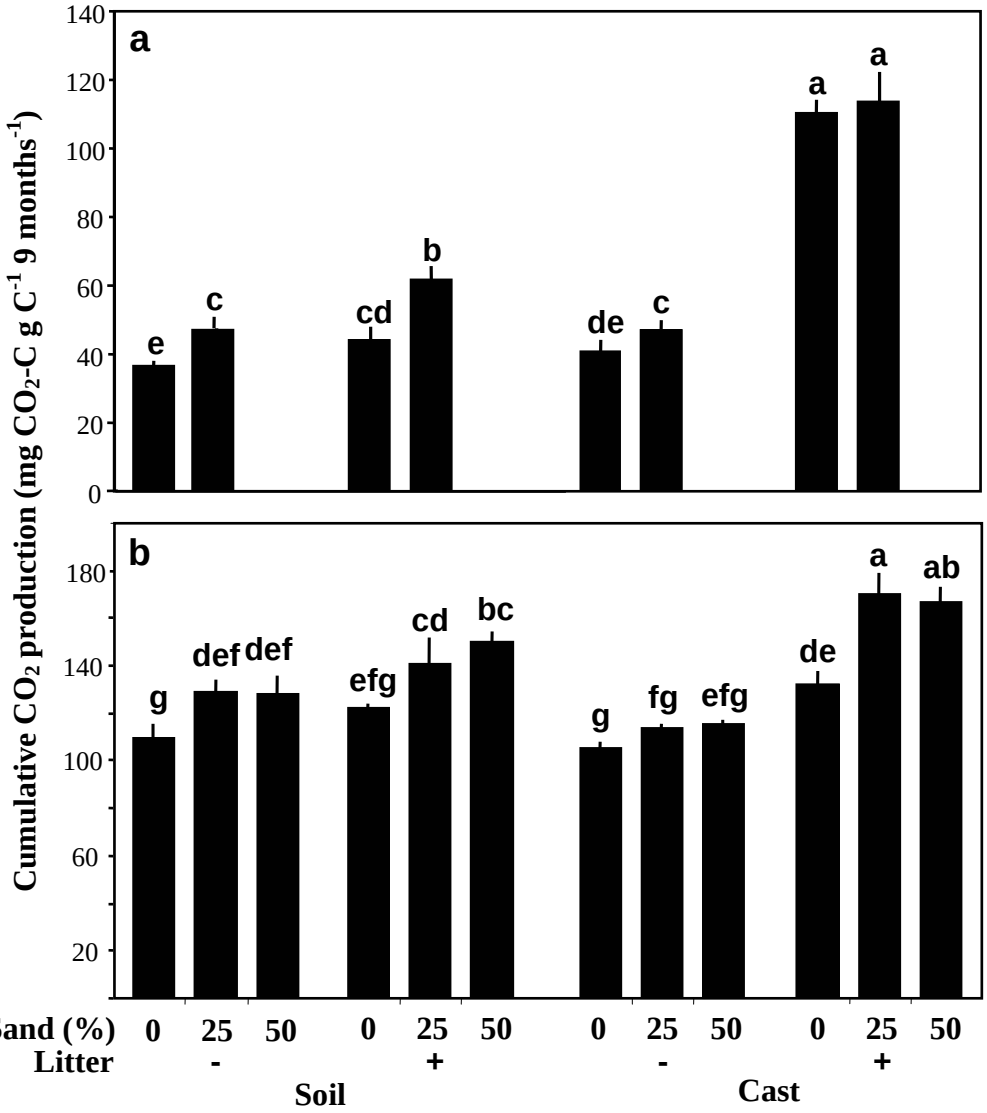

Fig. 3.3

Effect of sand (without = 0%, with 25% and with 50% sand), litter (without = -, and with litter = +) and substrate (soil and cast of *Lumbricus terrestris*) on cumulative carbon mineralisation from microcosms within 9 months. **(a)** arable soil and rye litter addition; **(b)** forest soil and beech litter addition. Cumulative carbon mineralisation is given relative to the initial amount of carbon. Means of 6 (a) and 3 (b) replicates with 1 S.D., bars sharing the same letter are not significantly different ($P < 0.05$, Tukey's HSD test).

Table 3.3
ANOVA table of F-values and the effects of substrate (soil and cast of *Lumbricus terrestris*), sand (arable soil: without and with 25% sand; forest soil: without, with 25% and with 50% sand) and litter (without and with litter) on cumulative N_{min} leaching and cumulative CO_2 production from arable and forest soil incubated for 9 months.

	Arable soil				Forest soil			
	df	Cumulative CO_2 production	df	Cumulative N_{min} leaching	df	Cumulative CO_2 production	df	Cumulative N_{min} leaching
substrate	1,35	491.59***	1,37	409.83***	1,24	3.22ns	1,22	1.66ns
sand	1,35	103.13***	1,37	3.71ns	2,24	234.98***	2,22	0.01ns
litter	1,35	985.22***	1,37	12.04**	1,24	59.23***	1,22	0.34ns
substrate × sand	1,35	31.00***	1,37	0.26ns	2,24	0.56ns	2,22	0.48ns
substrate × litter	1,35	385.97***	1,37	13.79**	1,24	55.49***	1,22	2.34ns
sand × litter	1,35	0.03ns	1,37	0.46ns	2,24	7.19**	2,22	0.57ns
substrate × sand × litter	1,35	8.14**	1,37	0.02ns	2,24	5.26*	2,22	0.56ns

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns = not significant

The addition of litter increased cumulative CO_2 production in the arable and forest soil by 91.6 and 25.5%, respectively, but in both soils the effect varied between casts and litter (significant substrate × litter interaction) (Table 3.3). In the forest soil the addition of beech litter increased cumulative CO_2 production by 12.5%, whereas in casts it was increased by 39.7%. Similarly, in the arable soil the addition of rye straw increased cumulative CO_2 production by 25.1% whereas in casts it was increased by 250%. Furthermore, in the forest soil the effect of litter varied with the availability of sand.

The addition of litter increased cumulative CO_2 production in treatments without sand by 18.2% but in treatments with sand by 27.9% (25% sand) and 29.5% (50% sand). Also, in both the arable and forest soil the effect of sand and litter was different in casts and soil (significant sand × litter × substrate interaction) (Table 3.3). In casts of the arable soil the increase in cumulative CO_2 production in the treatment with rye straw was less pronounced than in that without rye straw. In

contrast, in casts of the forest soil the increase in cumulative CO_2 production in the treatment with beech litter was more pronounced than in that without beech litter. Generally, the effect of earthworms on CO_2 production changed during incubation in both the arable and forest soil experiment (Fig. 3.4a and 3.4b).

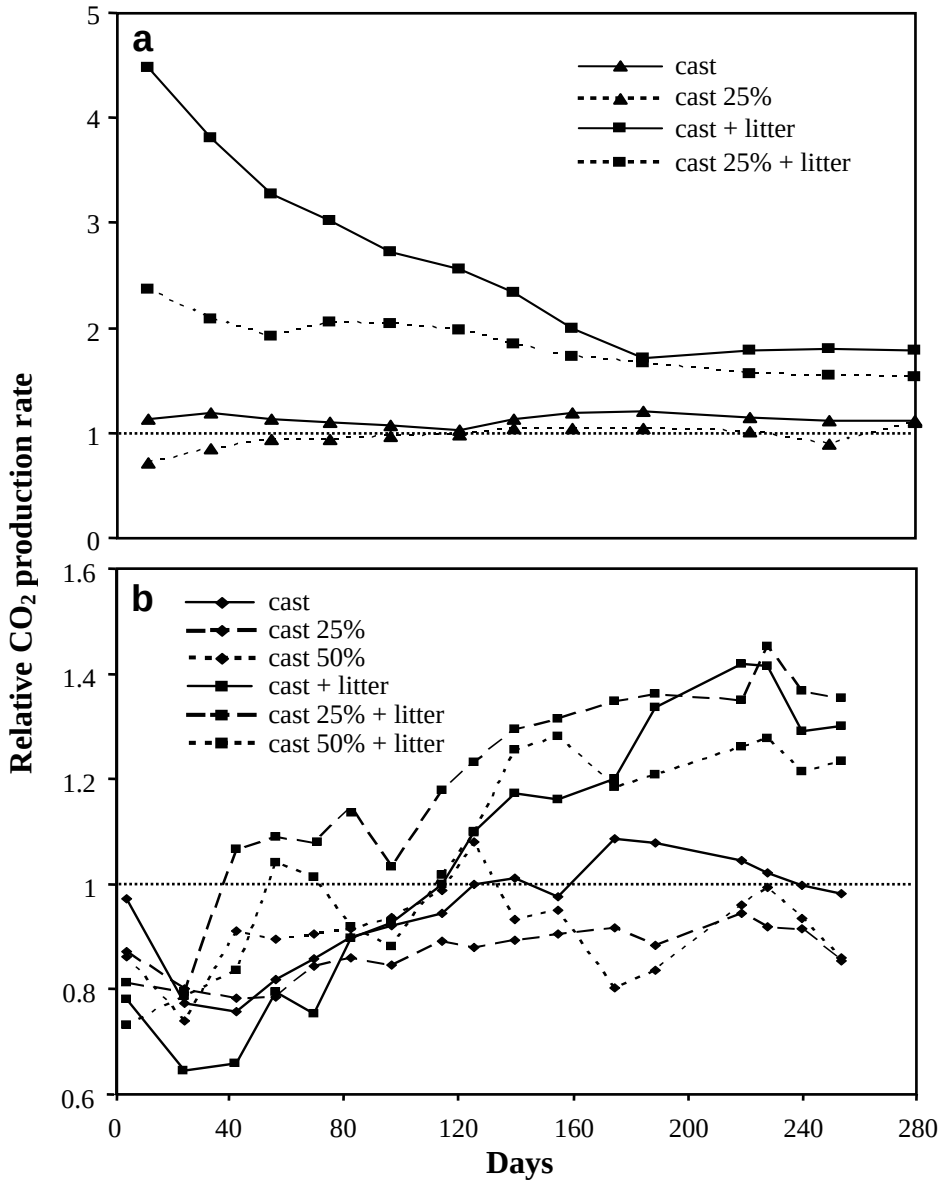

Fig. 3.4
Effect of sand and litter on carbon mineralisation in casts of *Lumbricus terrestris*; **(a)** arable soil (without and with 25% sand) and litter (without and with rye litter) and **(b)** forest soil (without, with 25% and with 50% sand) and litter (without and with beech litter). Data are plotted relative to carbon mineralisation rates of respective control soil treatments.

In the arable soil, CO_2 production rates of casts without litter were similar to the corresponding soil throughout the experiment. In contrast, CO_2 production rates in casts with rye straw strongly exceeded the control early in the experiment and declined later. In the forest soil experiment, changes in CO_2 production were similar in each of the cast treatments; they were lower than in soil early in the experiment but then approached (treatments without litter) or exceeded those in corresponding soil (treatments with litter). The initial decline in CO_2 production was most pronounced in casts with litter but without sand. In the second half of the experiment CO_2 production in casts with litter exceeded that in the corresponding soil by 20-40% irrespective of the sand content.

3.4 Discussion

The role of anecic earthworm species, such as *L. terrestris,* in the removal of litter from the soil surface and in bioturbation has attracted considerable attention (Raw 1962, Satchell 1967, Zicsi 1975, Lamparski & Zöttl 1981). In the present experiments the litter was removed almost entirely from the soil surface by *L. terrestris* within 65 days which corresponds to litter consumption rates of 15.1 and 13.1 mg (dry wt) g^{-1} fresh wt day^{-1} for rye straw and beech litter, respectively. Similar consumption rates for *L. terrestris* of 13.0 and 15 mg litter (dry wt) g^{-1} fresh wt day^{-1} for rye grass (Binet & Trehen 1992) and *Salix aquatica* litter (Curry & Bolger 1984) have been reported previously. In the study of Binet and Trehen (1992), body mass of *L. terrestris* increased slightly during 85 days of incubation at 12°C. In contrast, in the present experiment earthworm biomass generally decreased by ca. 20% during 65 days of incubation at 20°C and the decrease was more pronounced in treatments without litter. It is known that loss of body mass of *L. terrestris* increases at higher temperature (Daniel et al. 1996). Furthermore, the low quality of the rye and beech litter might have contributed to the decrease in earthworm biomass (Satchell & Lowe 1967, Zicsi & Pobozsny 1977, Zicsi 1983). In particular, rye straw with low nitrogen content (0.5% N) may have been of poor food quality (cf. Flegel & Schrader 2000); the loss of body mass of *L. terrestris* was more

pronounced when fed with rye straw than when fed with beech litter (1.6% N). Increased construction of burrows in arable soil without litter suggests that *L. terrestris* counteracted the lack of food by increasing ingestion rates which is consistent with earlier findings for *Dendrobaena octaedra* (Flegel & Schrader 2000). Zhang and Schrader (1993) suggested that the size of particles may limit ingestion by *L. terrestris* but Willems and Huijsmans (1994) found *L. terrestris* to be able to swallow 2 × 2 mm glass beads. Schulmann and Tiunov (1999) showed that *L. terrestris* selectively ingests sand grains of 0.5-1 mm. Present results suggest that ingestion of sand may facilitate assimilation in anecic earthworms; as loss in body mass of *L. terrestris* in forest soil with beech litter was less pronounced when sand was added. This may explain why anecic earthworm species prefer to feed on a mixture of soil and litter rather than on litter only (Doube et al. 1997, Heine & Larink 1993). However, there appears to be an optimum sand concentration for digestion of organic matter; compared to the 25% sand treatment of the forest soil the loss of body mass of *L. terrestris* was more pronounced in the 50% sand treatment. In the arable soil with higher sand content further addition of sand increased the loss of body mass of *L. terrestris*. In the present experiments C and N concentrations in earthworm casts were higher than in soil into which the litter had been mixed homogeneously and this was more pronounced in sand treatments, due to selective litter ingestion by *L. terrestris*.

Nitrogen leaching, mainly in the form of NO_3^-, was higher from earthworm casts in the arable soil experiment. Increased mobilisation of N in earthworm casts and earthworm worked soil has been reported frequently (Aldag & Graff 1975, Scheu 1994a, b, Decaens et al. 1999). In the forest soil leaching of N neither differed between casts and soil nor between treatments without and with litter. Scheu (1995) also found leaching of nitrogen from forest soil with beech litter not to be affected by earthworms, whereas it was increased in the presence of other litter materials. Presumably, additional mineral nitrogen in earthworm worked forest soil was immobilised by microorganisms suggesting that microorganisms were limited by nitrogen. In contrast, in the arable soil nitrogen mobilised by earthworms was leached suggesting that nitrogen did not limit microorganisms in this soil.

The focus of this study was to evaluate whether organic matter enclosed in earthworm casts is stabilised or if mobilisation processes predominate. Since the topsoil materials in many soils consist in large part of earthworm casts it is essential to understand decomposition processes in earthworm casts (Bal 1982, Scheu 1987b, McInerney & Bolger 2000b). There is evidence that the organic matter in earthworm casts may be protected from decomposition (Scheu & Wolters 1991b, Edwards et al. 1995) due to enclosure in stable aggregates (Shipitalo & Protz 1988, Marinissen & Dexter 1990, McInerney et al. 2001). In contrast, it has been documented that CO_2 production in earthworm casts is increased which in part is due to the increased nutrient availability (Scheu 1993c, McInerney & Bolger 2000b, Uyl et al. 2002). As stressed previously (McInerney & Bolger 2000b) the overall effect of earthworms on carbon mineralisation depends on the temporal scale since carbon mineralisation may be stimulated initially but reduced later. Unfortunately, few medium- and long-term studies on the decomposition of organic matter in earthworm casts have been carried out (Shaw & Pawluk 1986a, b, Martin 1991, McInerney et al. 2001). In the present study carbon mineralisation of organic matter enclosed in earthworm casts in most treatments exceeded that of the corresponding soil treatments suggesting that during the period studied (280 days) mobilisation processes prevailed. Only in casts produced from the forest soil without litter was less carbon mineralised than in the corresponding soil, thus indicating that the organic matter enclosed in casts was stabilised. Scullion and Malik (2000) stated that organic matter is more intimately mixed with clay in the presence of earthworms and Shaw and Pawluk (1986a) suggested that this contributes to the stabilisation of organic matter. In our study the clay content of the forest soil was three times higher than in the arable soil and therefore, the higher clay content may have contributed to the stabilisation of organic matter in earthworm casts of the forest soil without litter. However, stabilisation of organic matter with reduced CO_2 production was restricted to the first half of the experiment. Later on in the experiment the CO_2 production rates of casts were similar to that in soil. Shipitalo and Protz (1989) stressed that the stabilisation of organic matter in earthworm casts is increased by drying. Shrinkage during drying enhances physical protection of the organic matter in casts by fostering the association with mineral components (Martin 1991). In the present experiment casts

and soil were kept moist throughout the incubation and this may have contributed to low stabilisation of organic matter enclosed in earthworm casts.

In the arable soil without addition of rye straw cumulative carbon mineralisation in casts and in soil was similar. In contrast, it was strongly increased in earthworm casts in treatments with rye straw. The amount of plant residues in earthworm casts are considerably higher compared to the corresponding soil with litter where litter was manually mixed into the soil. As a result, the fraction of partly decomposed plant residues which had a rapid turnover was higher in earthworm casts. This might be one reason for the enhanced cumulative CO_2 production in the cast with litter treatment. The increase in CO_2 production was most pronounced early in the experiment and decreased later. Obviously, decomposition of rye straw enclosed in earthworm casts was stimulated strongly suggesting that the enclosure in earthworm casts did not protect organic matter from microbial attack. Rather, the microbial attack of the litter material was stimulated, possibly by increased availability of phosphorus (cf. Syers et al. 1979, Scheu 1987a, Haynes & Fraser 1998). Similar to rye straw in the arable soil carbon mineralisation of beech litter enclosed in earthworm casts was stimulated. However, in contrast to the arable soil decomposition of beech litter was reduced initially and stimulated later. Hence, the initial stabilisation of organic matter enclosed in earthworm casts was counteracted later by increased carbon mineralisation in beech leaf litter. Since microorganisms of the forest soil studied are known to be limited by phosphorus (Scheu 1987a, Scheu & Schaefer 1998) and leaching of nitrogen from casts and forest soil was similar, the increased mineralisation of beech litter was probably caused by mobilisation of phosphorus (see above).

Sand generally increased carbon mineralisation even when it was only manually mixed into the soil. Decomposition and mineralisation of organic matter is known to be faster in coarse-textured soils than in fine-textured soils (Van Veen & Kuikman 1990) and litter materials are decomposed more rapidly in sandy soils than in clay soils (Sörensen 1981, Ladd et al. 1985). Hassink (1995) further suggested that microbes are more active in sandy soils than in loams and clays. In casts the effect of sand was less pronounced in the arable soil. Sand is known to facilitate litter comminution in the gizzard of earthworms (Schulmann & Tiunov 1999) and this

likely was responsible for the increased carbon mineralisation in earthworm casts of the sand treatments. The less pronounced effect of sand in casts of the arable soil may have been due to the sand content of the arable soil being high (70%) and additional sand therefore increased litter comminution only little.

In conclusion, this study indicates that an increasing sand content generally increases carbon mineralisation in soil. The increased content of sand and the higher litter content in casts of earthworms alter the decomposition of organic matter enclosed in casts. In soils with high clay content the addition of sand may affect the decomposition of organic matter little but in general the addition of sand stimulates carbon mineralisation and this applies to both organic matter in soil and enclosed in earthworm casts. Overall, soil characteristics, such as the clay content and nutrient concentrations and also soil moisture, determine whether the enclosure of organic matter in casts of earthworms results in stabilisation or mobilisation of organic matter.

4. Effects of endogeic earthworms (*Octolasion tyrtaeum*, Lumbricidae) on decomposition of plant residues differing in litter quality in an arable and forest soil

Abstract

Litter decomposition is influenced by internal factors, such as nitrogen and lignin content, and external factors including soil type and soil biota. We studied the effects of the endogeic earthworm species *Octolasion tyrtaeum* on the decomposition of litter materials of different quality, i.e. forming a gradient from high to low C-to-N ratio, in an arable and forest soil. Fragmented rye, maize and rape litter were separately mixed into arable soil, and maple, beech and woodgarlic litter into forest soil. Soil with and without addition of litter was incubated in microcosms at 20°C for 282 days. Rates of carbon mineralisation of high quality litter (low C-to-N ratio) were high in the first months but decreased rapidly to low levels later in the experiment. Decomposition of low quality litter started slower but decomposition rates remained high throughout the experiment. Carbon mineralisation was increased by earthworms in the first half but decreased in the second half of the experiment. Stabilisation of soil organic matter by *O. tyrtaeum* later in the experiment was more pronounced in the forest soil than in the arable soil, possibly due to higher content of silt and clay. Leaching of mineral nitrogen was reduced by low quality litter. Earthworms counteracted the immobilisation of N by low quality litter, possibly by interrupting hyphal connections between litter and soil or by competing with microorganisms for easily available carbon resources. Earthworms lost body mass (treatments with litter) or died (treatments without litter) in the arable soil whereas they did not grow (treatments without litter) or increased in biomass (treatments with litter) in the forest soil. Microbial biomass was increased by the addition of litter, in particular by high quality litter. Earthworms in general slightly reduced microbial biomass. The results suggest that soil organic matter may be stabilised in casts of

endogeic earthworms in the long term but the stabilisation depends on the soil material in which the litter is incorporated. Early during litter decomposition earthworms uniformly increase litter decomposition irrespective of its quality. Litter quality strongly affects the growth of endogeic earthworms and this likely feeds back to earthworm-mediated changes in litter decomposition and nitrogen mobilisation.

4.1 Introduction

In most terrestrial ecosystems the majority of the plant biomass enters the decomposition pathway as plant litter. Decomposition processes govern nutrient and carbon cycling and therefore the global nitrogen and carbon cycle. Decomposers mineralise nutrients bound in plant litter thereby replenishing the pool of plant available nutrients (Swift et al. 1979). The rate of plant litter decomposition depends strongly on the quality of the litter materials (Cadisch & Giller 1997). Nitrogen is a key element driving decomposition processes and therefore, the C-to-N ratio of litter is frequently taken as a measure of litter quality (Swift et al. 1979). Other factors influencing decomposition processes include environmental variables, such as temperature and humidity (Jensen et al. 1997), and decomposer organisms, such as bacteria, fungi and soil invertebrates (Wardle & Lavelle 1997). The uncertainty in current estimates of the global carbon balance results in large part from the poor understanding of decomposition processes as mediated by soil organisms (Schröter et al. 2003).

Soil macro-invertebrates, such as earthworms, are important components of the soil fauna and play a major role in soil nutrient and carbon cycling (Lee 1985). In contrast to anecic earthworms, such as *Lumbricus terrestris* L., endogeic earthworm species are less important for the incorporation of litter materials into the mineral soil. However, endogeic earthworms ingest large amounts of soil and the intimate mixing of plant residues with the soil matrix affects litter decomposition (Scheu 1995). Despite the fact that about 90% of the litter in a beech forest on limestone is mineralised by bacteria and fungi (Schaefer 1990), endogeic earthworms may alter the microbial decomposition processes by changing nutrient mineralisation,

especially that of nitrogen (Anderson et al. 1983, Scheu 1987c). In arable ecosystems knowledge of nitrogen cycling is of crucial importance for crop yield but also for contamination of ground water through the leaching of nitrate. In addition, the contribution of arable ecosystems to the global carbon cycle has increased strongly with the increase in human population (Schlesinger 1991). Understanding of driving forces of decomposition processes in arable ecosystems is therefore of substantial importance. Endogeic earthworm species often dominate earthworm populations in density and biomass in both forest (Scheu 1992b) and arable ecosystems (Curry & Byrne 1992, Fraser & Piercy 1998). It has been hypothesised that endogeic earthworms stabilise the organic matter in the litter by intimate mixing with the soil matrix and that this stabilising effect depends on the quality of litter and on soil conditions (Marinissen et al. 1996, Haynes & Fraser 1998). To prove these hypotheses two experiments were set up investigating the effects of endogeic earthworms on the decomposition of plant residues in an arable and a forest soil. In each experiment three litter types differing in litter quality (C-to-N ratio) were used to investigate the interdependence of external and internal driving factors of litter decomposition.

4.2 Material and Methods

Sampling

Soil samples were taken from an arable field and a deciduous forest. The arable field formed part of the long-term fertilisation experiment "Ewiger Roggenbau" in Halle (Saxony-Anhalt, Germany). Rye (*Secale cereale* L.) had been planted continuously on this field and only mineral fertilisers (NPK) had been added since 1878. The soil is a *Luvic Phaeozem* (FAO) on sandy loess. Mean annual rainfall is 466 mm, and the mean annual temperature is 9.0°C (Garz et al. 1999). The site is located 113 m a.s.l. and represents a typical arable cropping system in the dry region of eastern Germany. The forest known as "Göttinger Wald" is situated on a limestone plateau 8 km east of Göttingen (Lower-Saxony,Germany). The canopy layer is formed by beech trees of an age of approximately 130 years (*Fagus sylvatica* L.). The soil is shallow and

consists mainly of *Rendzina* with patches of *Terra fusca* (Schaefer 1991). The mean annual rainfall is 720 mm and the mean annual temperature is 7.9°C. Soil samples from both sites were taken from the upper 10 cm of the mineral soil in October 2001. Plant residues and stones were excluded by passing the soil trough a 4 mm sieve. To kill earthworms the soil was frozen at -28°C and stored frozen for 11 months until the experiment was set up. The litter materials used strongly differed in C-to-N ratio as a major component of litter quality. Litter materials originated from plants which typically grow at the respective study sites. For the arable soil experiment rye straw (*Secale cereale* L.), maize (*Zea mays* L.) and rape leaves (*Brassica napus* L.) were used. For the forest soil experiment maple (*Acer platanoides* L.), beech (*Fagus sylvatica* L.) and woodgarlic leaves (*Allium ursinum* L.) were used. Rye, maize and rape litter were taken from plants immediately after harvesting of the crops from fields close to Darmstadt (Hesse, Germany) during the summer of 2002. Freshly fallen maple leaves were taken from a forest in Darmstadt. One year old beech leaves were taken from the litter layer in the "Göttinger Wald". Leaves of withered woodgarlic plants were also taken at this beech forest. Woodgarlic leaves were sampled in spring and tree litter in autumn 2002. Litter materials were dried at 60°C and broken into pieces of about 4 mm. Earthworms were sampled by hand from the beech forest in October 2002 and stored in buckets filled with soil of the beech forest at 5°C until the experiments were set up.

Total carbon and nitrogen contents of the materials used in the experiment were determined by an elemental analyser (Model 1400, Carlo Erba Milan, Italy). Data of initial carbon (C_{org}) and nitrogen (N_{tot}) content, and C-to-N ratio of the materials are listed in Table 4.1.

Table 4.1
Initial carbon (C_{org}), nitrogen (N_{tot}) content and C-to-N ratio of the soil and litter materials which were used in the experiments. Means and (1 S.D.) of 3 replicates.

	C_{org} (%)	N_{tot} (%)	C-to-N ratio
Arable soil	1.06 (0.05)	0.06 (0.01)	16.35 (0.69)
Rye	45.94 (0.71)	0.52 (0.01)	88.20 (2.29)
Maize	41.17 (0.03)	1.84 (0.01)	22.40 (0.02)
Rape	42.57 (0.19)	3.88 (0.11)	10.98 (0.27)
Forest soil	10.12 (0.19)	0.80 (0.02)	12.65 (0.09)
Maple	45.05 (0.07)	0.77 (0.01)	58.19 (0.35)
Beech	44.49 (0.42)	1.58 (0.01)	28.19 (0.46)
Woodgarlic	44.66 (0.04)	6.14 (0.03)	7.27 (0.03)

The experiment

Before the experiment was set up, soil samples were placed at 5°C for one week and for acclimation for another week at 20°C. The following treatments were studied in the arable soil experiment: (1) arable soil only (Ctrl), (2) with rye straw (Rye), (3) with maize litter (Maize) and (4) with rape litter (Rape). The forest soil experiment was set up with the following treatments: (1) forest soil only (Ctrl), (2) with maple (Maple), (3) with beech (Beech) and (4) with woodgarlic litter (Woodgarlic). Ten microcosms were established per treatment and to six of them one subadult *O. tyrtaeum* was added. The remaining four microcosms served as control. The experiment was set up in microcosms consisting of perspex tubes (height 150 mm, Ø 60 mm) fixed air tight on ceramic plates. They allowed drainage of soil materials at semi-natural conditions by lowering the atmospheric pressure in a box below the ceramic plates. Leaching water from each of the microcosms was sampled in vessels placed underneath the microcosms in the box. The microcosms were closed at the top by a lid which had a small vessel attached to the underside. This vessel could be filled with alkali to absorb CO_2 evolved from the soil.

Microcosms were filled with arable and forest soil equivalent to 300 and 150 g dry wt, respectively, filling the microcosms to a similar volume. To litter treatments 2 g dry litter were homogenously mixed into the soil. Before the earthworms were placed into the microcosms they were kept on wet filter paper to void their gut for

two days. One specimen of *O. tyrtaeum* of a mean body mass of 200 mg (fresh wt), ranging from 94 to 319 mg, was added per chamber. Smaller and larger specimens were added to each treatment.

The microcosms were placed in a climate chamber at 20°C and were incubated in darkness for 282 days. Microcosms were irrigated weekly with 10 ml distilled H_2O. Leaching water was collected at regular intervals and pooled samples of 6 weeks were analysed for mineral nitrogen content (N_{min}: $NH_4^+ + NO_3^-$). Ammonium (NH_4^+) and nitrate (NO_3^-) concentrations in the leachate were measured by ion-selective ISE-electrodes (Winlab, Windaus, Germany). Prior to addition of alkali (1 N KOH) used for CO_2 determination, the chambers were pre-incubated with alkali for 12 h. This was done to prevent sampling of CO_2 from dissolved carbonates during the subsequent incubation. For CO_2 determination the chambers were incubated at two week intervals for 24 h. Trapped CO_2 was measured titrimetrically with 0.1 N HCl after precipitation of carbonate with saturated $BaCl_2$ solution (Macfadyen 1970). Openings in the lid ensured free gas exchange between CO_2 determinations.

At the end of the experiment earthworms were sampled and individual body mass was measured.

Microbial biomass

Soil samples taken at the end of the experiment were analysed for basal respiration, microbial biomass (C_{mic}) and specific respiration (qO_2). C_{mic} was determined by the substrate-induced respiration method (SIR) (Anderson & Domsch 1978). Measurements were done using an automated respirometer system based on electrolytic O_2-microcompensation (Scheu 1992a). For each treatment four replicates were analysed. SIR was measured after addition of 8 mg glucose g^{-1} soil (dry wt). Glucose was added as an aqueous solution adjusting the water content to about 80% of the water holding capacity. Oxygen consumption rates were measured at 22°C every 0.5 h. The mean of the eight lowest measurements during the first 11 hours after glucose addition was taken as the maximum initial respiratory response (MIRR). Microbial biomass C (C_{mic}; µg g^{-1} dry wt) was calculated as 38 × MIRR (µl O_2 g^{-1} h^{-1}) (Beck et al. 1997). For basal respiration, the average O_2 consumption rate (µl O_2 g^{-1} h^{-1}) of samples not amended with glucose during hours 15-30 after

attachment to the respirometer system was used. From data on microbial biomass and basal respiration the specific respiration (qO_2; µl O_2 mg^{-1} C_{mic} h^{-1}) was calculated.

Statistical analysis

Data on water content, cumulative mineral nitrogen leaching, cumulative CO_2 production, basal respiration, microbial biomass and specific respiration were analysed separately from the experiment with arable and forest soil by a two-factorial analysis of variance (ANOVA). Factors were earthworms (with and without *O. tyrtaeum*), and litter (without litter, with low quality litter, with intermediate quality litter and with high quality litter). Changes in earthworm body mass were analysed by ANCOVA using the initial earthworm body mass as covariable. Prior to ANOVAs data were inspected for homogeneity of variance (Levene test) and log-transformed if required. Results of the ANOVAs are presented as F- and *P*-values and as the proportion of the total variation (sum of squares, SS) accounted for by a particular factor. Tukey's HSD test for unequal N was used for comparison of means. A statistical probability $P < 0.05$ was considered significant. Correlations between parameters were tested by multiple regression analysis. STATISTICA 6.0 software package was used for statistical analyses (Statsoft, Hamburg, Germany).

4.3 Results

Water content

At the end of the experiment water content in the arable soil (overall mean 12.3 ±0.7% of dry wt) was not affected by the experimental treatments (Table 4.2). In contrast, in the forest soil experiment earthworms significantly increased the water content from 83.7 to 87.1%, thereby accounting for 33.2% of the total variation. Also, addition of litter significantly increased the water content and litter accounted for 29.6% of the total variation. Water content increased in the order Ctrl (82.8%), Woodgarlic (84.3%), Maple (85.9%) and Beech treatment (87.1%).

Table 4.2
ANOVA table of F-values on the effects of earthworms (without and with, EW) and litter (without and with rye, maize, rape, maple, beech and woodgarlic litter in the arable and forest experiment, respectively) on water content, nitrate-to-ammonium nitrogen ratio, cumulative N_{min} leaching, cumulative CO_2 production, basal respiration, microbial biomass and specific respiration incubated for 282 days.

	df	Water content (% dry wt^{-1})	Nitrate-to-ammonium N ratio #	N_{min} (µg N_{min} g^{-1} dry wt) #	df	CO_2 (mg CO_2-C g^{-1} dry wt)	df	Basal respiration	Microbial biomass C_{mic} (SIR)	Specific respiration
Arable										
EW	1	2.35ns	0.02ns	7.94**	1	0.97ns	1	80.44***	9.97**	107.81***
Litter	3	0.51ns	78.38***	418.99***	3	919.11***	3	9.37***	152.94***	18.36***
EW × Litter	3	1.95ns	0.43ns	5.36**	3	0.05ns	3	4.68*	13.28***	7.33**
Total	32				32		26			
Forest										
EW	1	32.40***	0.20ns	0.23ns	1	16.88***	1	47.57***	5.46*	22.16***
Litter	3	9.64***	106.28***	330.19***	3	301.47***	3	5.52**	61.59***	9.84***
EW × Litter	3	1.40ns	0.47ns	1.37ns	3	0.49ns	3	1.84ns	7.79**	2.07ns
Total	32				32		29			

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns = not significant; #, log-transformed data

Earthworms

In the arable soil experiment *O. tyrtaeum* had processed 10-40% of the soil in Ctrl treatments without litter but about 80% in litter treatments by the end of the experiment. In the forest soil experiment 90-100% of the soil was processed by earthworms.

Body mass of *O. tyrtaeum* was significantly affected by the addition of litter in the arable soil experiment ($F_{3,19} = 17.66$, $P < 0.001$). Earthworm body weight generally decreased in each of the treatments (Fig. 4.1a); in the arable soil without litter none of the earthworms survived. The decline in body weight was most pronounced in the rape (-49.1%), low in the rye (-17.0%) and intermediate in the maize litter treatment (-38.5%); litter accounted for 67.0% of the total variation.

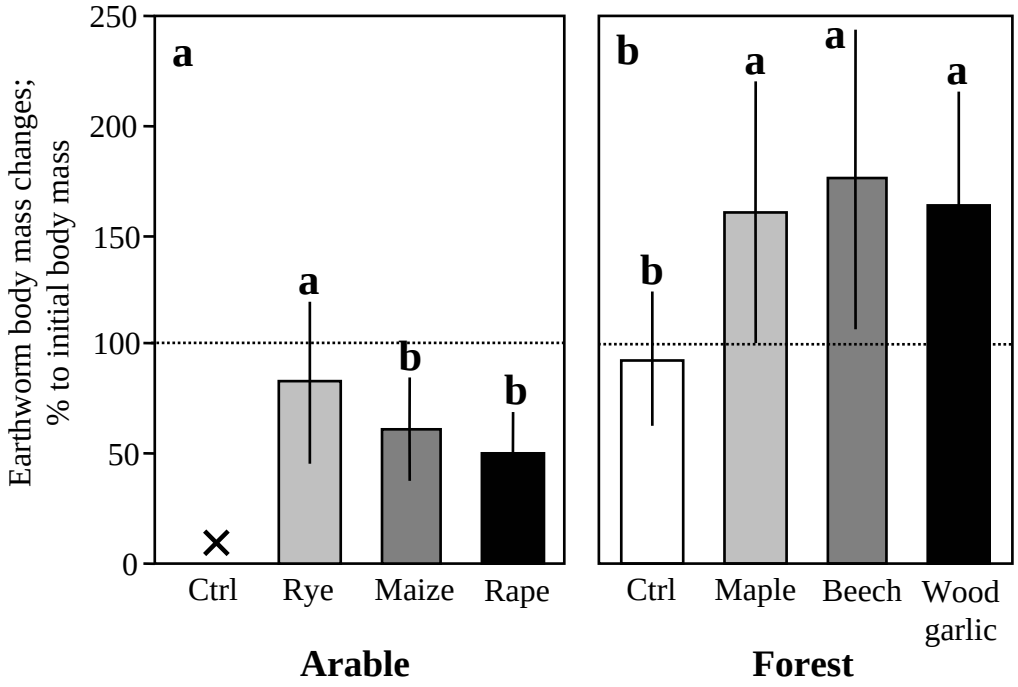

Fig. 4.1

Body mass of adult *Octolasion tyrtaeum* as affected by the availability and quality of litter in **(a)** arable soil without (Ctrl) and with rye, maize and rape litter, and **(b)** forest soil without (Ctrl) and with maple, beech and woodgarlic litter after 282 days of incubation. Means of 6 replicates with 1 S.D.; bars sharing the same letters are not significantly different ($P < 0.05$, Tukey's HSD test); ×, specimens deceased.

In the forest soil experiment earthworm body weight increased in the litter treatments but decreased in the control treatment without litter ($F_{3,19} = 25.96$, $P < 0.001$) (Fig. 4.1b). However, the decline in body weight in the control treatment was low (-6.6%)

and the increase was similar in each of the litter treatments (+60.3, +63.9% and +75.5% for the Maple, Woodgarlic and Beech treatments, respectively). Litter accounted for 77.2% of the total variation.

Nitrogen leaching

Rates of mineral nitrogen (N_{min}) leached per day decreased slightly during the incubation period in both experiments (Fig. 4.2). In the arable soil *O. tyrtaeum* increased leaching of N_{min} in the Ctrl, Maize and particular in the Rye treatment at most of the sampling dates whereas in the Rape treatment it was increased only a little (Fig. 4.3a). In the forest soil experiment earthworms decreased leaching of N_{min} initially in each of the treatments but later it was increased in the Ctrl, Maple and Beech treatments, however, in the Ctrl treatment only slightly (Fig. 4.3b). In contrast, in the Woodgarlic treatment, earthworms reduced leaching of N_{min} throughout the experiment.

Cumulative N_{min} leaching was significantly affected by the presence of litter in both experiments (Table 4.2). In the arable soil experiment, cumulative N_{min} leaching was highest in the Rape and lowest in the Rye treatment, with the Ctrl and Maize treatments being intermediate. Similarly, in the forest soil experiment, cumulative N_{min} leaching was highest in the high nitrogen litter treatment (Woodgarlic) and lowest in the low nitrogen litter treatment (Maple), with the Ctrl and medium nitrogen litter (Beech) being intermediate. Litter accounted for 97.9% and 96.7% of the total variation in the arable and forest soil experiment, respectively. Although earthworms affected the time course of leaching of $N_{min,}$ they neither significantly affected cumulative N_{min} leaching in the arable experiment, nor in the forest soil experiment.

In both experiments nitrogen leached almost exclusively as nitrate; leaching of ammonium was at least two magnitudes lower. Due to increased leaching of ammonium, the nitrate-to-ammonium nitrogen ratio was lowest in high quality litter treatments (data not shown). Litter significantly affected the nitrate-to-ammonium nitrogen ratio in the arable and forest soil experiment and accounted for 87.6 and 91.0% of the total variation (Table 4.2).

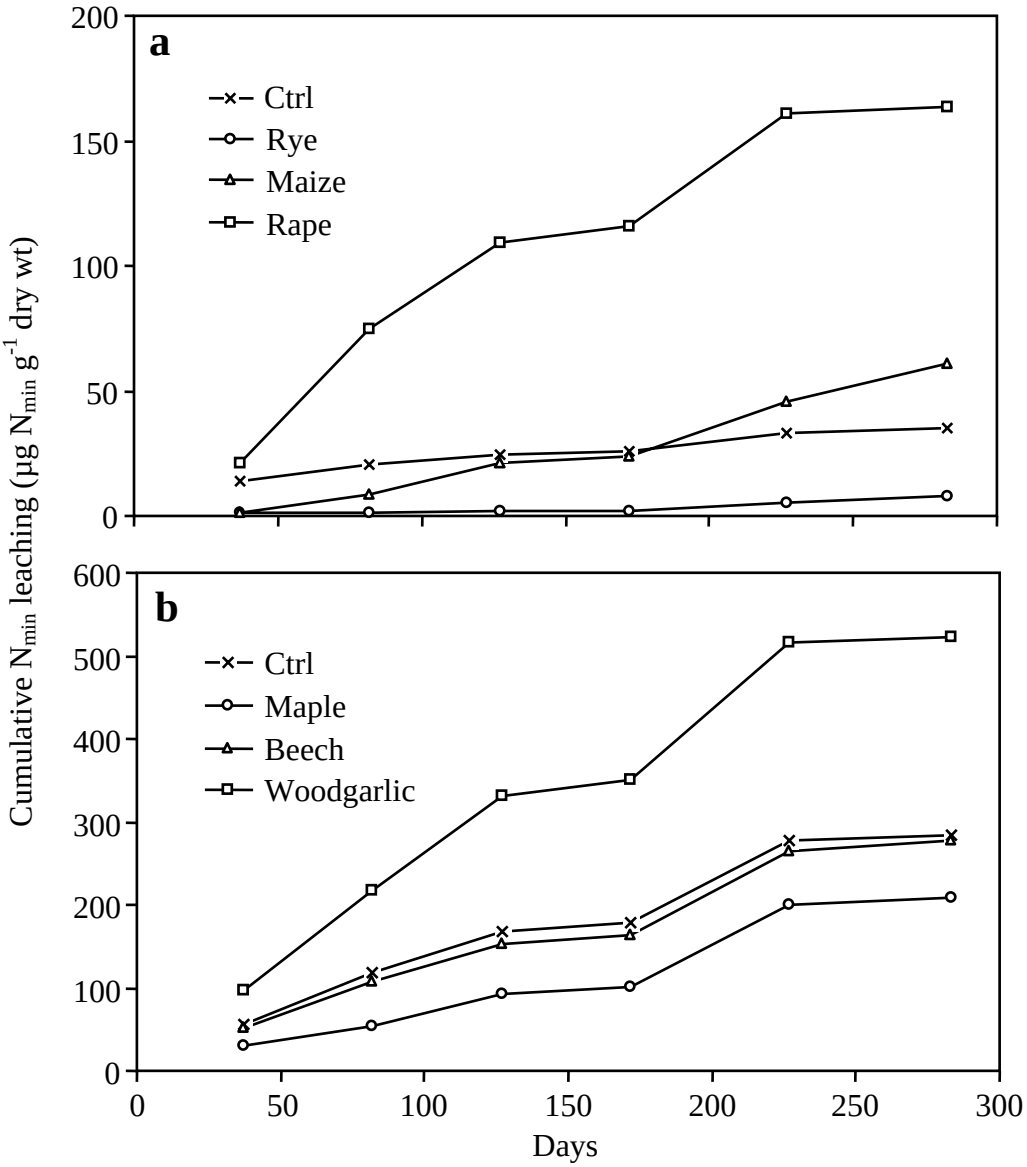

Fig. 4.2
Cumulative leaching of mineral nitrogen (N_{min}) from treatments without *Octolasion tyrtaeum*, as affected by the presence and quality of litter in **(a)** arable soil without (Ctrl) and with rye, maize and rape litter, and **(b)** forest soil without (Ctrl) and with maple, beech and woodgarlic litter during 282 days of incubation.

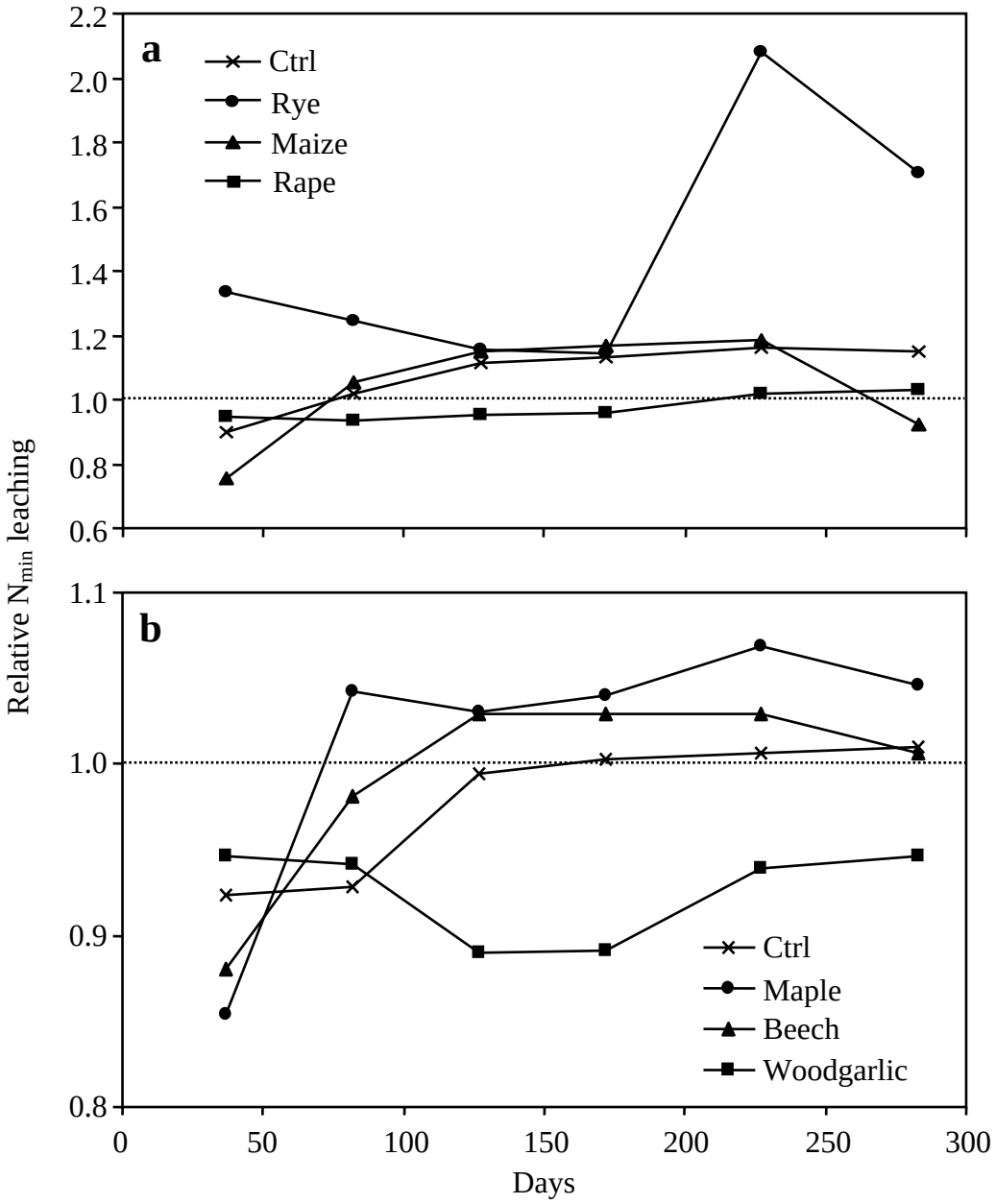

Fig. 4.3
Leaching of mineral nitrogen (N_{min}) from treatments with *Octolasion tyrtaeum* as affected by the presence and quality of litter in **(a)** arable soil without (Ctrl) and with rye, maize and rape litter, and **(b)** forest soil without (Ctrl) and with maple, beech and woodgarlic litter during 282 days of incubation. Data is plotted relative to N_{min} leaching of the respective treatments without earthworms.

CO_2 production

Carbon mineralisation rates of Ctrl treatments remained almost constant throughout the incubation period in both the arable and forest soil experiment (Fig. 4.4). In both experiments CO_2 production was increased by addition of litter; rates of CO_2

production were at a maximum initially and then decreased steeply for ca. 100 days in most treatments. The decline was steeper in the medium (Maize and Beech) and high quality litter treatments (Rape and Woodgarlic) and less pronounced in the low quality litter treatments (Rye and Maple). In the latter, rates of CO_2 production continued to decline throughout the experiment, whereas in the former they reached a constant level after about 100 days.

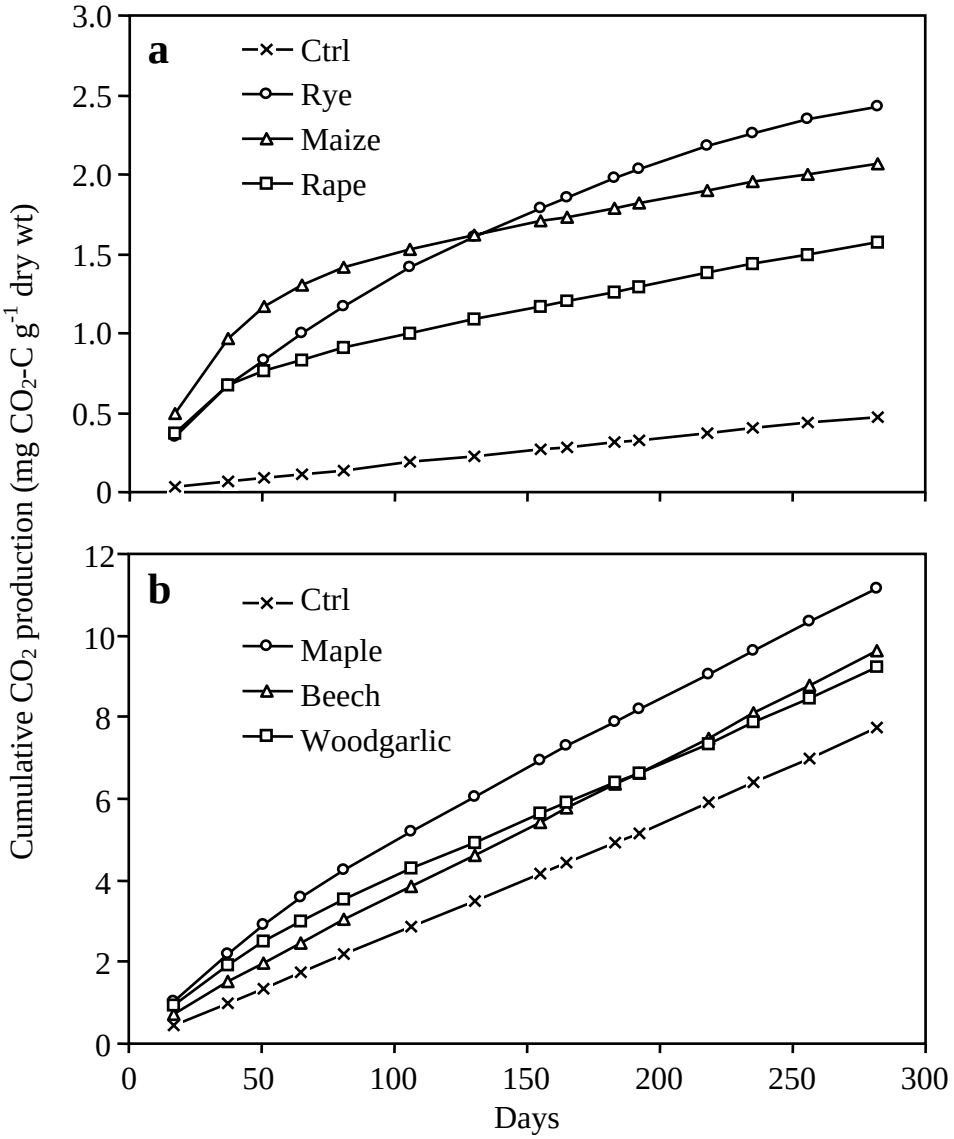

Fig. 4.4
Cumulative CO_2-C production from treatments without *Octolasion tyrtaeum* as affected by the presence and quality of litter in **(a)** arable soil without (Ctrl) and with rye, maize and rape litter, and **(b)** forest soil without (Ctrl) and with maple, beech and woodgarlic litter during 282 days of incubation.

In the arable soil experiment, mineralisation rates of the Ctrl and Maize treatments with earthworms were similar to the respective treatments without earthworms throughout the experiment (Fig. 4.5a). In contrast, earthworms reduced mineralisation rates in the Rape and Rye treatment in the second half of the experiment. In the forest soil experiment, mineralisation rates in treatments with *O. tyrtaeum* were similar to or slightly higher than in the respective treatments without earthworms during the first half of the experiment, but later earthworms uniformly reduced CO_2 production in each of the treatments (Fig. 4.5b).

In both experiments, cumulative carbon mineralisation was lowest in the Ctrl treatments. Addition of litter significantly increased cumulative CO_2 production in both experiments (Fig. 4.4, Table 4.2). In the arable soil experiment, cumulative CO_2 production of litter treatments was highest in the Rye and lowest in the Rape treatment, with the Maize treatment being intermediate. In the forest soil experiment, addition of litter affected cumulative CO_2 production less than in the arable soil experiment. CO_2 production was highest in the Maple, and lowest in the Woodgarlic treatments with the Beech treatment being intermediate. In both the arable ($r = 0.97$, $F_{1,28} = 434.61$, $P < 0.001$) and forest soil experiment ($r = 0.77$, $F_{1,28} = 40.93$, $P < 0.001$) cumulative CO_2 production was positively correlated to the initial N content of the litter materials. Earthworms did not significantly affect the cumulative CO_2 production in the arable soil experiment. In the forest soil experiment, CO_2 production was slightly but significantly decreased by *O. tyrtaeum*, on average by 3.3% (Table 4.2).

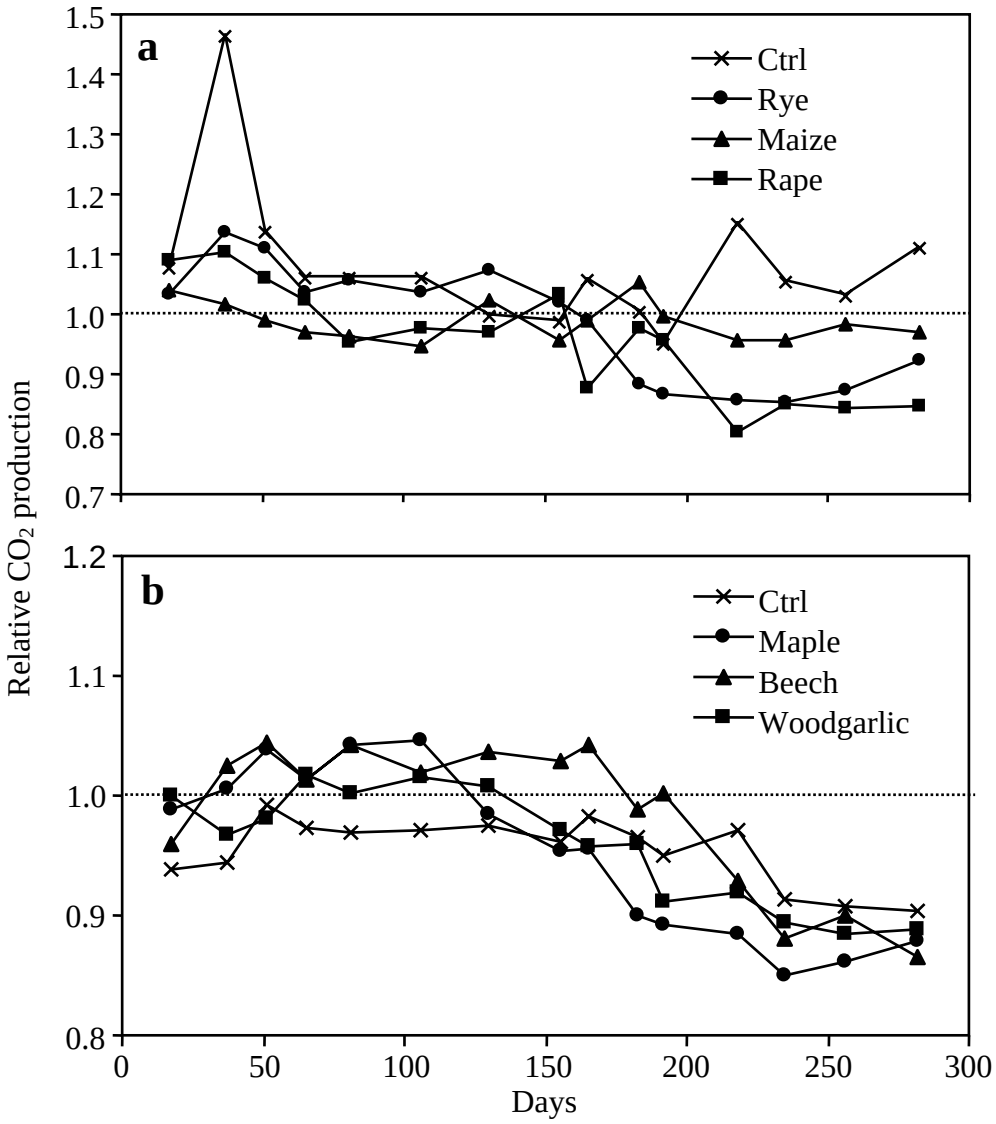

Fig. 4.5

CO_2-C production from treatments with *Octolasion tyrtaeum* as affected by the presence and quality of litter in **(a)** arable soil without (Ctrl) and with rye, maize and rape litter, and **(b)** forest soil without (Ctrl) and with maple, beech and woodgarlic litter during 282 days of incubation. Data is plotted relative to N_{min} leaching of the respective treatments without earthworms.

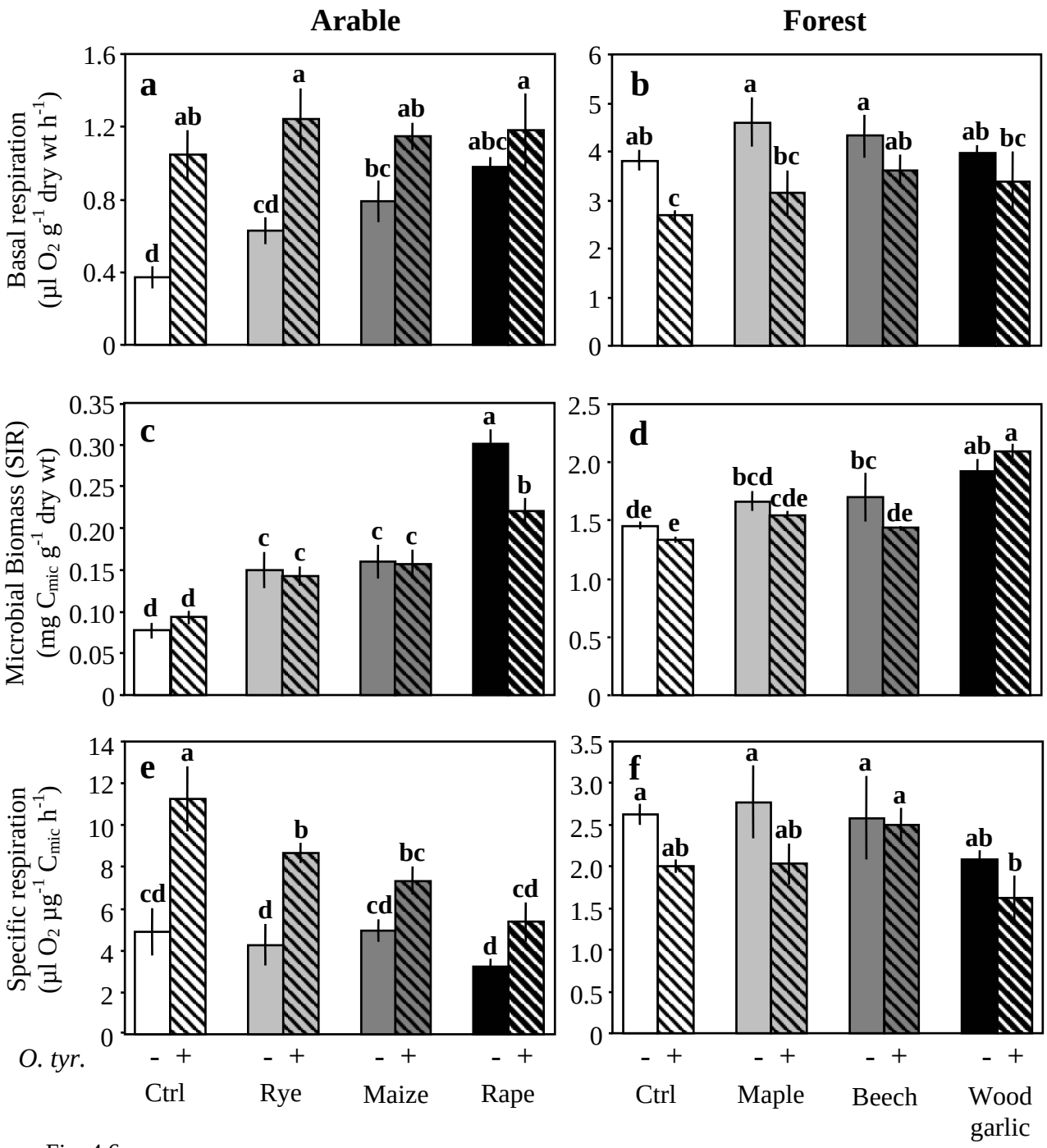

Fig. 4.6

Effects of *Octolasion tyrtaeum* (*O. tyr.*) and the availability and quality of litter on basal respiration, microbial biomass and specific respiration in **(a, c, e)** arable soil without (Ctrl) and with rye, maize and rape litter, and **(b, d, e)** forest soil without (Ctrl) and with maple, beech and woodgarlic litter after 282 days of incubation. Means of 4 replicates with 1 S.D.; bars sharing the same letters are not significantly different ($P < 0.05$, Tukey's HSD test).

Microbial activity and biomass

At the end of the experiment, basal respiration of the arable soil ranged between 0.37 and 1.24 µl O_2 g^{-1} dry wt h^{-1} (Fig. 4.6a). Addition of litter significantly increased basal respiration by 31.8, 36.6 and 52.1% in the Rye, Maize and Rape treatment, respectively (Table 4.2). Earthworms also significantly increased basal respiration, on average by 66.7%. However, effects of earthworms differed between litter types (significant earthworm × litter interaction; Table 4.2). The stimulating effect of earthworms was most pronounced in the Ctrl treatment (+179.6%), and decreased from the Rye (+98.0%) to the Maize (+45.6%) to the Rape (+20.6%) treatment. Overall, litter and earthworms accounted for 20.3% and 58.1% of the variation, and the earthworm × litter interaction accounted for an addition of 10.2% of the variation. Basal respiration of the forest soil ranged between 2.7 and 4.6 µl O_2 g^{-1} dry wt h^{-1} (Fig. 4.6b). Addition of litter significantly increased basal respiration by 19.4, 22.2 and 13.6% in the Maple, Beech and Woodgarlic treatments, respectively, and litter accounted for 19.0% of the variation. Earthworms decreased basal respiration by 29.9, 31.7, 16.6 and 14.8% in the Ctrl, Maple, Beech and Woodgarlic treatment and this accounted for 54.7% of the variation.

Microbial biomass (C_{mic}) in the arable soil ranged between 0.07 and 0.30 mg C_{mic} g^{-1} dry wt (Fig. 4.6c). Addition of litter significantly increased C_{mic} by 71.7, 85.9 and 206.8% in the Rye, Maize and Rape treatment, respectively and litter accounted for 91.7% of the variation (Table 4.2). Earthworms affected C_{mic} only in the Rye treatment where it was reduced by 4.7%; the earthworm × litter interaction explained 8.0% of the total variation. In the forest soil C_{mic} ranged between 1.33 and 2.09 mg C_{mic} g^{-1} dry wt (Fig 4.6d). C_{mic} was significantly increased by 15.3, 12.5 and 44.1% in the Maple, Beech and Woodgarlic treatments, respectively. Litter accounted for 78.0% of the variation. Earthworms generally decreased C_{mic} on average by 4.8% and this explained 2.3 % of the variation. However, in the Woodgarlic treatment C_{mic} was increased; the earthworm × litter interaction accounted for additional 9.9% of the variation.

Specific respiration (qO_2) was significantly decreased by litter, and litter accounted for 25.2% of the variation. However, the effect of litter was more pronounced in treatments with *O. tyrtaeum* and the earthworm × litter interaction accounted for an

additional 10.1% of the variation (Fig. 4.6e; Table 4.2). Earthworms increased qO_2 and this was most pronounced in the Ctrl treatment; in the litter treatments the effect of earthworms declined with increasing quality of the litter, and the earthworm × litter interaction accounted for 49.4% of the variation. Litter also affected qO_2; in the forest soil it was generally decreased in litter and earthworms treatments and this accounted for 38.5 and 28.9% of the variation, however, the effect of earthworms and litter depended on each other (significant earthworm × litter interaction; Fig. 4.6f; Table 4.2) and this accounted for an additional 8.1% of the variation. In contrast to the others qO_2 was not decreased by earthworms in the Beech treatment.

4.4 Discussion

Response of Octolasion tyrtaeum

Cast production and burrowing activity of earthworms was lower in the arable than in the forest soil experiment. Only 10-40% (treatment without litter) and about 80% (treatments with litter) of the soil in the microcosms was processed by *O. tyrtaeum* in the arable soil experiment, whereas in the forest soil experiment the soil was processed almost completely in each of the treatments after 282 days of incubation. In the arable soil without litter, earthworms died and this explains the low processing of soil. Obviously, the arable soil which is low in soil organic matter contains insufficient food resources for *O. tyrtaeum*. The survival of *O. tyrtaeum* in the forest soil without litter indicates that more food resources for endogeic earthworms are available in this soil. However, the food resources in this soil also were scarce and did not allow earthworms to grow.

Earthworms benefited from the addition of litter in both the forest and arable soil. In the forest soil the earthworm body mass increased irrespective of the quality of the added litter, whereas in the arable soil earthworms lost less weight in treatments with low quality litter. Endogeic earthworm species, such as *O. tyrtaeum*, prefer food materials rich in nitrogen and/or soluble carbohydrates (Lee 1987). Rye straw is poor in nitrogen and its roughness further suggests low palatability. Low loss in body weight in the rye straw treatment was therefore unlikely to be due to high food

quality of rye straw itself; rather it indicates that during the decomposition of rye straw, soluble carbohydrates are released which served as food substrate (Swift et al. 1979, Sörensen et al. 1996). It has been documented that endogeic earthworms are limited by soluble carbon rather than nitrogen compounds in soil (Tiunov & Scheu 2004). In comparison to maize and rape litter the decomposition of rye straw was slow, thus more rye residues and therefore more soluble carbohydrates were available later in the experiment, and this may have contributed to a more continuous availability of food resources for *O. tyrtaeum*. This indicates that in the long term, low quality litter may better serve the need for food resources of endogeic earthworms than high quality litter which is decomposed rapidly by microorganisms. Curry and Byrne (1992) also suggested that straw ploughed into the soil may substantially contribute to the nutrition of endogeic earthworms. Similarly, Boström (1987) concluded that decomposing barley straw prolongs the activity of endogeic earthworms.

In general, smaller specimens gained more (forest experiment) and lost less (arable experiment) body mass than larger individuals. This indicates a more pronounced limitation of the available food resources for larger specimens in the microcosms than for smaller ones. Larger earthworm specimens may exploit limited pools of food resources faster than smaller ones, because they are able to process a larger amount of soil at one time. The period of starvation due to limited food availability might therefore be longer for larger individuals.

Effects of earthworms on nutrient mineralisation and litter decomposition

Cumulative N_{min} leaching per unit soil in Ctrl treatments was higher in the forest soil than in the arable soil. Presumably, this was due to the higher amount of nitrogen in the forest soil. However, the fraction of total nitrogen in the soil leached as N_{min} from the arable soil (53.7 mg N_{min} g^{-1} N) was higher than that from the forest soil (35.5 mg N_{min} g^{-1} N). This suggests that nitrogen in the forest soil was more protected from leaching than that in the arable soil. The quality and amount of soil organic matter affects N mobilisation (Compton & Boone 2002) and it is known that the forest soil used in the experiment is rich in stabilised humus compounds (Jörgensen et al. 1983).

Most mineral nitrogen from high quality litter treatments leached during the first weeks. Watkins and Barraclough (1996) reported rapid N mobilisation from litter rich in nitrogen within the first weeks after incorporation into the soil. Cumulative N_{min} leaching was reduced strongly by the addition of litter low in nitrogen (Rye and Maple) suggesting that it was transferred into the litter by microorganisms (net N immobilisation; Jensen et al. 1997, Garnier et al. 2003). In contrast to previous studies (c.f. Jensen 1994), the classical concept that addition of litter with a C-to-N ratio higher than 20-30 results in net N immobilisation, whereas litter with a C-to-N ratio lower than 20 cause net N mineralisation fits the results of the present study surprisingly well (Black 1968, Allison 1973, Swift et al. 1979).

Earthworms increased (arable) or tended to increase (forest) nitrogen leaching from low quality litter treatments, suggesting that *O. tyrtaeum* counteracted microbial N immobilisation. A number of mechanisms might be responsible for this process, including feeding on microorganisms (Blair et al. 1997), modifying the microbial environment (Anderson et al. 1985) and competition with microorganisms for easy available C resources (Tiunov & Scheu 2004). Microbial biomass was not consistently reduced by earthworms and therefore feeding on microorganisms is not likely to have contributed to reduced N immobilisation. Also, the reduction in N_{min} leaching in presence of *O. tyrtaeum* in the treatment with woodgarlic litter where microbial biomass was increased suggests that the earthworms did not mobilise nitrogen bound in microbial biomass.

The cumulative CO_2 production per unit soil in the forest soil exceeded that in the arable soil by a factor of 16.8. However, the fraction of total carbon in soil evolved as CO_2 from the forest soil exceeded that from the arable soil by a factor of 1.8 only. Overall, therefore carbon compounds in the forest soil were less stable than those in the arable soil.

The addition of litter generally increased CO_2 production rates, particularly during the first weeks of the experiment but the time course of CO_2 production and therefore of litter decomposition differed between litter treatments with high quality litter generally decomposing faster than low quality litter (Swift et al. 1979). In general, each of the litter materials decomposed in a two phase pattern: the first phase with rapid mineralisation corresponds to the degradation of water soluble amino acids,

amino sugars and carbohydrates; during the second phase with slower mineralisation, cell walls and structural components are decomposed (Watkins & Barraclough 1996). The observed slower decomposition rates of low quality litter such as rye and beech leaves during the first phase and higher rates during the second phase reflect restricted access for microbial degradation during the first phase, but suggest that microorganisms effectively decomposed these litter materials later (Swift 1983, Scheu & Parkinson 1995). Entry and Backman (1995) concluded that the litter C-to-N ratio is a poor predictor of organic matter decomposition since it ignores differences in the quality of carbon compounds, and they suggest that the cellulose-to-lignin ratio more accurately predicts the decomposition of litter materials than the litter C-to-N ratio.

Cumulative carbon mineralisation was not significantly affected by earthworms in the arable soil during the 282 days of incubation. However, decreasing rates of CO_2 production in earthworm treatments (except the control) indicate that the litter became more protected against decomposition with time of incubation. Therefore, similarly to the forest soil, organic matter enclosed in casts of the arable soil is stabilised in the long-term. However, stabilisation of organic matter in the forest soil started earlier and was most pronounced during the last three months of incubation. Stabilisation of organic matter enclosed in earthworm casts has been reported before (Martin 1991, Scheu & Wolters 1991b), however, this study documents for the first time that the stabilisation processes of endogeic earthworms appear to be similar for high and low quality litter materials.

Microbial biomass

Basal respiration of the arable soil processed by earthworms was higher than in the respective treatments without earthworms. This is in contrast to the reduced CO_2 production rates at the end of incubation for soil in the microcosms with treatments with earthworms. Basal respiration (and also microbial biomass) was measured after sieving of the soil samples. The sieved samples were incubated for 10 days at room temperature for acclimation. Nevertheless, sieving resulted in a disruption of the cast aggregates formed by the earthworms. Presumably, due to the disruption nutrients and carbon resources physically protected in the cast aggregates became available to

microorganisms. Lower basal respiration in earthworm casts of the forest soil (compared to the respective treatments without earthworms) suggests that the organic matter therein is more intensively stabilised. This is likely to be related to the higher content of silt and clay in the forest (67% silt, 29% clay; S. Marhan & S. Scheu, unpubl. data) compared to the arable soil (20% silt, 10% clay; Garz et al. 1996). Soil organic matter is known to be stabilised by intimate associations with clay in fine textured soils; on the contrary, sand associated organic matter is known to be labile (Hassink 1994). During the gut passage, organic matter is intimately mixed with the inorganic soil matrix due to the addition of water and soil, and the physical action of the gut. On the contrary, due to reabsorption of water and mucus in the hindgut, casts may form stable soil aggregates with increased water holding capacity (Ziegler & Zech 1992), and microorganisms growing in casts contribute to this stabilisation by producing carbohydrates fostering the binding between the enclosed organic matter and the inorganic matrix (Haynes & Beare 1997).

Microbial biomass (C_{mic}) in the forest soil exceeded that in the arable soil by a factor of 18.8. Microbial biomass is known to be closely correlated to soil organic matter (Anderson & Domsch 1986, Wolters & Joergensen 1991, Wardle 1992); indeed, the carbon content of the forest soil exceeded that of the arable soil by a factor of about ten. The addition of plant litter generally increased the microbial biomass in both the arable and forest soil; obviously, litter resources consumed by microorganisms were used for biomass production (Haynes 2000). It is known that microbial biomass may rapidly increase after addition of rape straw and the increase may last for more than a year (Jensen et al. 1997). The increase in microbial biomass in the present study was positively correlated with the nitrogen content of the litter materials added, but this relationship was only significant in the arable soil. Presumably, microorganisms in the arable soil were more strongly limited by nitrogen than those in the forest soil. Earthworms generally reduced microbial biomass, which is consistent with the hypothesis that they compete with microorganisms for food resources (Tiunov & Scheu 2004). However, in the treatment with woodgarlic litter earthworms increased microbial biomass, possibly due to a shift in the structure of the microbial community towards more efficient species (cf. Wolters & Joergensen 1992).

Specific respiration (qO_2) has been used as indicator for the efficiency of the microbial community to use carbon resources (Anderson 1992, Scheu 1992a). It was generally higher in the arable than in the forest soil suggesting a more efficient use of carbon resources in the forest soil which likely is related to the different soil texture (cf. Kiem & Kandeler 1997). Earthworms increased specific respiration in the arable soil, whereas qO_2 was reduced in the forest soil compared to the respective control treatments. This might be explained by an arable soil microflora which is more stimulated by the earthworm gut passage (Haynes et al. 1999).

Conclusions

Results of the present study showed that effects of and on *O. tyrtaeum* varied with the quality of the added litter and between the two soils studied. Nitrogen mobilisation and immobilisation strongly depended on the quality of the litter materials added and on earthworms, and these were interdependent. Immobilisation of nitrogen by low quality litter was counteracted by earthworms; on the other hand, in the long-term, low quality litter appeared to support endogeic populations better than high quality litter. The interdependence of litter and earthworms for total carbon mineralisation was less pronounced. Generally, early during decomposition earthworms stimulated carbon mineralisation, however, in the long term litter resources enclosed in earthworm casts were stabilised, which was more pronounced in the forest soil with higher silt and clay content. In general, the effects of *O. tyrtaeum* on carbon mineralisation were almost independent from the quality of the litter introduced into the soil. More studies are needed to improve the understanding of long-term mobilising and stabilising processes in earthworm casts of differently textured soils.

5. Mixing of mineral soil layers of different organic matter content by endogeic earthworms (Lumbricidae, *Octolasion tyrtaeum*): effects on carbon and nitrogen mineralisation

Abstract

The effect of the endogeic earthworm species *Octolasion tyrtaeum* Savigny on stabilisation of uniformly ^{14}C labelled lignin (lignocellulose) in dependency of lower mineral soil was studied in two different upper mineral soils from forests on limestone. The two forest soils represented different stages of succession; a mature beech (*Fagus sylvatica* L.) and an ash (*Fraxinus excelsior* L.) forest in a state of humus accumulation. Four soil treatments were set up consisting of sieved upper mineral soil without (Ash and Beech treatment) and with a lower mineral soil (Ash + Min and Beech + Min treatment). Labelled lignin was homogenously mixed into the upper mineral soil and to half of the microcosms one *O. tyrtaeum* was added. Leaching of mineral nitrogen, CO_2 and $^{14}CO_2$ production were monitored continuously throughout 100 days of incubation.

It is hypothesised that endogeic earthworms may stabilise lignin and the organic matter derived from the upper mineral soil by mixing intimately with the lower unsaturated mineral soil. Earthworm body mass increased during the experiment but the increase was more pronounced in Ash (+ 89.2%) and Ash + Min (+ 81.0%) than in Beech (+ 22.3%) and Beech + Min (+ 9.8%) treatments. Cumulative mineral nitrogen (N_{min}) leaching was higher in the upper mineral soil only treatment from the ash forest than in that from the beech forest. Cumulative carbon mineralisation was increased by earthworms (+ 9.1%) and by addition of lower mineral soil (+ 6.2%). Effects of lower mineral soil were more pronounced in Beech (+ 10.8%) than in Ash treatments (+ 1.8%). In total 8.4 and 6.6% of the lignin added was mineralised in Ash and in Beech treatments without earthworms, respectively. Lignin mineralisation

was strongly increased by earthworms in the Beech treatments (+ 24.6%) whereas in Ash treatments the effect of earthworms was negligible. Results showed that earthworms predominantly colonised the upper mineral soil during the experiment. The effect of mixing of upper and lower mineral soil was therefore less than expected. Soil organic matter and lignin decomposition were not reduced by lower mineral soil but instead the strengthening effect on mineralisation by *O. tyrtaeum* was less pronounced in combination with lower mineral soil (+ 8.6%) than without (+ 14.1%). In conclusion, this indicates that thorough mixing of organic matter with C-unsaturated lower mineral soil by endogeic earthworms protects organic matter from microbial decomposition. By this soil organic matter becomes stabilised in the long-term which is supposed to be of crucial importance for the formation of mull soil rich in soil organic matter.

5.1 Introduction

Endogeic earthworms are of prominent importance for bioturbation of soils. Ultimately, the activity of endogeic earthworms leads to the formation of mull-type soils (Müller 1950, Bal 1982, Scheu 1987, Bernier 1998). Mull soils are characterised by a complete mixing of fragmented litter into the mineral soil layers by the soil macrofauna resulting in an accumulation of soil organic matter in mineral soil horizons (Kubiena 1948). Mull type soils are common in mature deciduous forests with a relative high pH. The base rich soil facilitates endogeic and anecic earthworm species which reach high biomasses as for example in the Göttinger Wald, a 130 year old beech forest on a limestone plateau near Göttingen (Germany) (Schaefer 1991). This forest represents a typical climax ecosystem on limestone parent rock in Central Europe. On the same limestone plateau close to this beech forest there is an ash dominated forest representing an earlier stage of forest succession. The ash forest developed from a fallow field which has been left uncultivated for about 60 years. The upper mineral soil of this forest is also rich in humus and soil organic matter. However, in contrast to the beech forest, the humus rich mineral soil is only 3-5 cm deep and overlaying a mineral soil layer poor in

organic matter consisting mainly of loess material. The soil has been assumed to be in a state of humus accumulation (Thöle & Meyer 1979, Scheu 1990). Humus accumulations during secondary succession have been widely studied (Kögel-Knabner 1993, Swift 2001, Six et al. 2002). Accumulation of soil organic matter starts in the upper soil layers followed by enrichment of the lower mineral soil in later successional stages. Lower mineral soil layers become enriched in organic matter until the inorganic matrix of the mineral soil is saturated (Hassink et al 1997). To this day the role of earthworms in the stabilisation processes of soil organic matter by mixing material rich in organic matter with an unsaturated inorganic matrix is little understood.

The present study investigates effects of earthworms on the mineralisation of organic matter in absence and presence of lower mineral soil with low organic matter content, i.e. with an unsaturated inorganic matrix. By using upper mineral soils from two forest ecosystems with different stages of humus accumulation, effects of the degree of saturation with organic matter on mineralisation processes were investigated. For studying the effect of earthworms the endogeic species *Octolasion tyrtaeum* Savigny was chosen because *O. tyrtaeum* is one of the dominant endogeic earthworm species in the studied forests. The use of ^{14}C labelled lignin allowed detailed analysis of carbon mineralisation of one of the major structural carbon components of litter. Lignin has been assumed to be an important precursor of humus materials in soils (Haider 1988, Kögel-Knabner 1993).

5.2 Material and Methods

Sampling

Soil samples were taken from a beech and ash forest representing two different stages of secondary succession in April 2002. The forests are located on a limestone plateau east of Göttingen at 420 m above sea level. The beech forest (*Fagus sylvatica* L.) is ca. 130 year old (c.f. Schaefer 1990) and forms the climax stage on limestone in the submontane belt of Central Europe (Scheu 1992). The ash forest (*Fraxinus excelsior* L.) represents an earlier successional stage established on fallow land about

60 years after cessation of cultivation (Scheu 1990). The soils of the two forests are of the *Rendzina*-type, shallow and to some extent containing loess. The mineral soil of the beech forest is rich in organic matter to the depth were it reaches the limestone bedrock typically at 10-30 cm. In contrast, in the ash forest only the upper mineral soil of 5-10 cm is rich in humus overlaying a mineral soil layer of low organic matter content. Samples of the upper mineral soils of both forests were taken from the top 0-10 cm layer after removal of the litter material. Soil from the lower mineral layer was taken only from the ash forest from about 20 cm below the soil surface. The soils were passed through a 4 mm sieve, homogenised and plant roots and smaller stones were removed by hand. To kill any earthworms, the soil was frozen at - 28°C for two weeks. Data on soil carbon and nitrogen contents and C/N ratios of the soil materials used in the experiment are given in Table 5.1. Earthworms were sampled by hand in the beech forest at the time when the soil samples were taken. The earthworms were stored in plastic buckets filled with soil from the beech forest at 5°C until the experiment was set up.

Table 5.1
Content of carbon and nitrogen, and C/N ratio of the soil materials used; means and SD of three replicates.

	C (%)		**N** (%)		**C/N** ratio	
	Mean	SD	Mean	SD	Mean	SD
Beech forest						
upper mineral soil	9.01	0.35	0.72	0.01	12.54	0.34
Ash forest						
upper mineral soil	6.60	0.09	0.58	0.01	11.39	0.13
lower mineral soil	1.97	0.03	0.20	0.01	9.73	0.02

The experiment

Before the experiment was set up soil samples were placed at 5°C for one week and for acclimation one further week at 20°C. Incubation was conducted in microcosms consisting of perspex tubes (height 150 mm, Ø 60 mm) fixed air tight on ceramic plates. Leaching water was sampled in vessels placed underneath the microcosms in the box. The microcosms were closed at the top by a lid which had a small vessel

attached to the underside. The vessel could be filled with alkali to absorb CO_2 evolved from the soil.

The microcosms were filled with soil equivalent to 50 g dry wt per layer. The same amount of upper and lower mineral soil was used. The following treatments were established: (1) ash forest upper mineral soil only (Ash), (2) ash forest upper mineral soil on top of lower mineral soil from the ash forest (Ash + Min), (3) beech forest upper mineral soil only (Beech) and (4) beech forest upper mineral soil on top of lower mineral soil from the ash forest (Beech + Min). Ten replicates were established per treatment.

After soil placement the microcosms were watered weekly for four weeks. Leachate of this pre-incubation period was not included in the calculations. To each microcosm 100 mg ^{14}C labelled lignin was added and homogeneously mixed into the upper mineral soil. ^{14}C labelled lignin (lignocellulose) material was obtained by incubating beech twigs with homogeneously ^{14}C labelled vanillic acid as described in more detail in Scheu (1993). Specific activity of the labelled material was 155.6 kBq g^{-1}. One specimen of *O. tyrtaeum* was placed into half of the microcosms of each treatment one day after the ^{14}C material had been added. Before the earthworms were added they were placed on wet filter paper to void their guts for three days. Initial earthworm body mass was 189 (±10) mg fresh wt.

The microcosms were placed in a climatic chamber at 20°C and incubated in darkness for 100 days. Microcosms were irrigated with 10 ml distilled H_2O at six day intervals. Leaching water was collected and pooled samples of 4 weeks were analysed for the amount of ammonium (NH_4^+-N) and nitrate (NO_3^--N) in the leachate. Total nitrogen leached (N_{min}) during the incubation was calculated by summing the amounts of NH_4^+-N and NO_3^--N. Concentrations of ammonium (NH_4^+) and nitrate (NO_3^-) in the leachate were measured by ion-selective ISE-electrodes (Winlab, Windaus, Germany).

For measurement of CO_2 production the microcosms were closed by rubber stoppers and incubated for 6 days. CO_2 evolved during this period was trapped in 3 ml alkali (1 N KOH) and measured titrimetrically after precipitation of carbonate with saturated $BaCl_2$ solution using 0.1 N HCl (Macfadyen 1970). An aliquot of the alkali (0.5 ml) was taken from each microcosm and mixed with 4.5 ml scintillation fluid

(Luma-Safe Plus, Lumac-LSC, Netherlands) to measure the amount of ^{14}C evolved from the labelled lignocellulose. Radioactivity was measured immediately after sampling using a liquid scintillation analyser (Model 1600 TR; Packard, Meriden, USA).

At the end of the experiment earthworms were sampled and individual body mass was measured. Soil organic carbon (C_{org}) and nitrogen (N_{org}) were determined by an elemental analyser (Model 1400; Carlo Erba, Milan, Italy).

Statistical analyses

Data on cumulative CO_2 production, $^{14}CO_2$-C evolution and mineral N (N_{min}) leaching after 100 days of incubation were analysed by three factor analysis of variance (ANOVA). Factors were earthworm (without and with *O. tyrtaeum*, EW), type of upper mineral soil (beech and ash) and lower mineral soil (with and without lower mineral soil of the ash forest, Min). Data on water content was calculated separately for treatments with and without lower mineral soil by two-factor ANOVA. Changes in earthworm body mass were analysed by two-factor ANCOVA using the initial earthworm body mass as covariable. ANOVA's data was inspected for homogeneity of variance (Levene test) and log-transformed if required in advance. Results of the ANOVA calculations are presented as F and *P* values and as the proportion of the total variation (sum of squares, SS) accounted for by a particular factor. Tukey's HSD-test was used for comparison of means. Statistical analysis was done using the STATISTICA 6.0 software package (Statsoft, Germany).

5.3 Results

Earthworm burrowing

By the end of the experiment the upper soil layers had been almost entirely transformed into casts by *O. tyrtaeum*. In contrast, only 20-40% of the lower soil layer was processed and only few earthworm burrows crossed this layer. The water content of the upper soil was generally higher in the Beech treatments without *O. tyrtaeum* (43.6% dry wt) than in the respective Ash treatments (34.4%; $F_{1,16}$ =

2764.17, P < 0.001). Earthworms increased soil water content in both upper soils to 45.2 and 36.6% for Beech and Ash treatments, respectively ($F_{1,16}$ = 127,87, P < 0.001). In treatments with lower mineral soil water content was also affected by the upper mineral soil type ($F_{1,14}$ = 1692.60, P < 0.001) and increased by earthworms ($F_{1,14}$ = 158.16, P < 0.001). Water contents of Beech + Min (34.9 and 36.4%) were higher than those of the Ash + Min treatments (28.9 and 30.9%) without and with *O. tyrtaeum*, respectively.

Earthworm body mass

All specimens of *O. tyrtaeum* placed into the microcosms survived until the end of the experiment and increased in body mass. However, the increase in Ash (89.2 ±20.9%) and Ash + Min treatments (81.0 ± 33.4%) was significantly higher than in Beech (22.3 ± 16.3%) and Beech + Min treatments (9.8 ± 10.7%; $F_{1,15}$ = 56.59, P < 0.001 for the effect of type of upper mineral soil).

Nitrogen leaching

Leaching of mineral nitrogen (N_{min}) decreased during the experiment. In particular the amount of nitrogen leached as nitrate (NO_3^--N) strongly decreased during the first 65 days, whereas the amount of ammonium nitrogen (NH_4^+-N) in the leachate remained more constant (data not shown). Overall, N_{min} leaching occurred mainly as nitrate with the nitrate-to-ammonium nitrogen ratio differing significantly between the Ash (25.9) and the Ash + Min treatment (157.1) and between the Beech (67.0) and the Beech + Min treatment (157.8; significant effect of type of upper mineral soil and lower mineral soil, Table 5.2).

Differences between treatments without and with lower mineral soil were more pronounced in the treatments with ash soil (significant upper mineral soil × lower mineral soil interaction. Leaching of N_{min} as nitrate was higher in treatments with upper soil from the ash forest than in beech forest treatments (Fig. 5.1a, Table 5.2).

Upper soil type accounted for 31.8% of the total variation. Presence of lower mineral soil decreased cumulative NH_4^+-N leaching significantly, on average by 71.0% (44.9% of the total variation) compared to treatments without lower mineral soil. The decrease was more pronounced in treatments with upper mineral soil of the

ash (-77.8%) than in the beech treatments (- 47.9%). The upper mineral soil type × lower mineral soil interaction was highly significant and further accounted for 21.5% of the total variation. Leaching of N_{min} as nitrate was also higher in ash than in beech treatments; type of upper soil accounted for 54.5% of total variation (Fig 5.1b, Table 5.2). In contrast to ammonium leaching, presence of mineral soil significantly increased leaching of NO_3^--N on average by 21.9% (23.6% of the total variation; Table 5.2). Increase was more pronounced in ash (+ 25.5%) than in beech upper soil treatments (+ 17.2%). The significant upper mineral soil type × lower mineral soil interaction further accounted for 2.4% of the total variation. *O. tyrtaeum* also significantly increased cumulative leaching of ammonium and nitrate nitrogen on average by 9.8% and 14.8%, respectively, but the effect of earthworms accounted for only 0.7 and 5.3% of the total variation.

Effects of the factors studied on cumulative nitrogen leaching (N_{min}) resembled those on NO_3^--N leaching since most nitrogen leached as NO_3^--N (Table 5.2). However, the amount of N_{min} leached relative to the total amount of soil nitrogen in the microcosms (mg N_{min} g^{-1} N) was not affected by 'lower mineral soil' in treatments without earthworms. This indicated that nitrogen bound in the lower mineral soil of the ash forest was mobilised to a similar extent than that bound in upper mineral soils (Fig. 5.1c).

Table 5.2
ANOVA table of *F*-values and the effects of earthworms (with and without, EW), presence of a lower mineral soil layer (with and without, Min) and the type of upper mineral soil (ash and beech forest, Soil) on cumulative nitrogen leaching as ammonium (NH_4^+-N) and nitrate (NO_3^--N) by the system, nitrate to ammonium nitrogen ratio and on cumulative mineral nitrogen leaching (N_{min}) leaching relative to initial nitrogen content and cumulative carbon mineralisation by the system incubated for 100 days.

	df	NH_4^+-N	NO_3^--N	nitrate to ammonium N ratio	N_{min} (mg N_{min} g^{-1} N)	CO_2-C (mg CO_2-C)
EW	1	34.2***	13.1**	0.4ns	14.9***	121.4***
Min	1	2183.7***	58.1***	753.8***	15.9***	57.9***
Soil	1	1542.9***	134.3***	110.5***	374.9***	0.5ns
EW × Min	1	11.5**	3.0ns	0.8ns	5.4*	4.3*
EW × Soil	1	5.2*	1.7ns	0.3ns	0.7ns	3.5ns
Min × Soil	1	1045.4*	5.9*	99.4***	1.2ns	28.4***
EW × Min × Soil	1	5.4*	0.2ns	0.0ns	0.2ns	0.7ns

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns = not significant

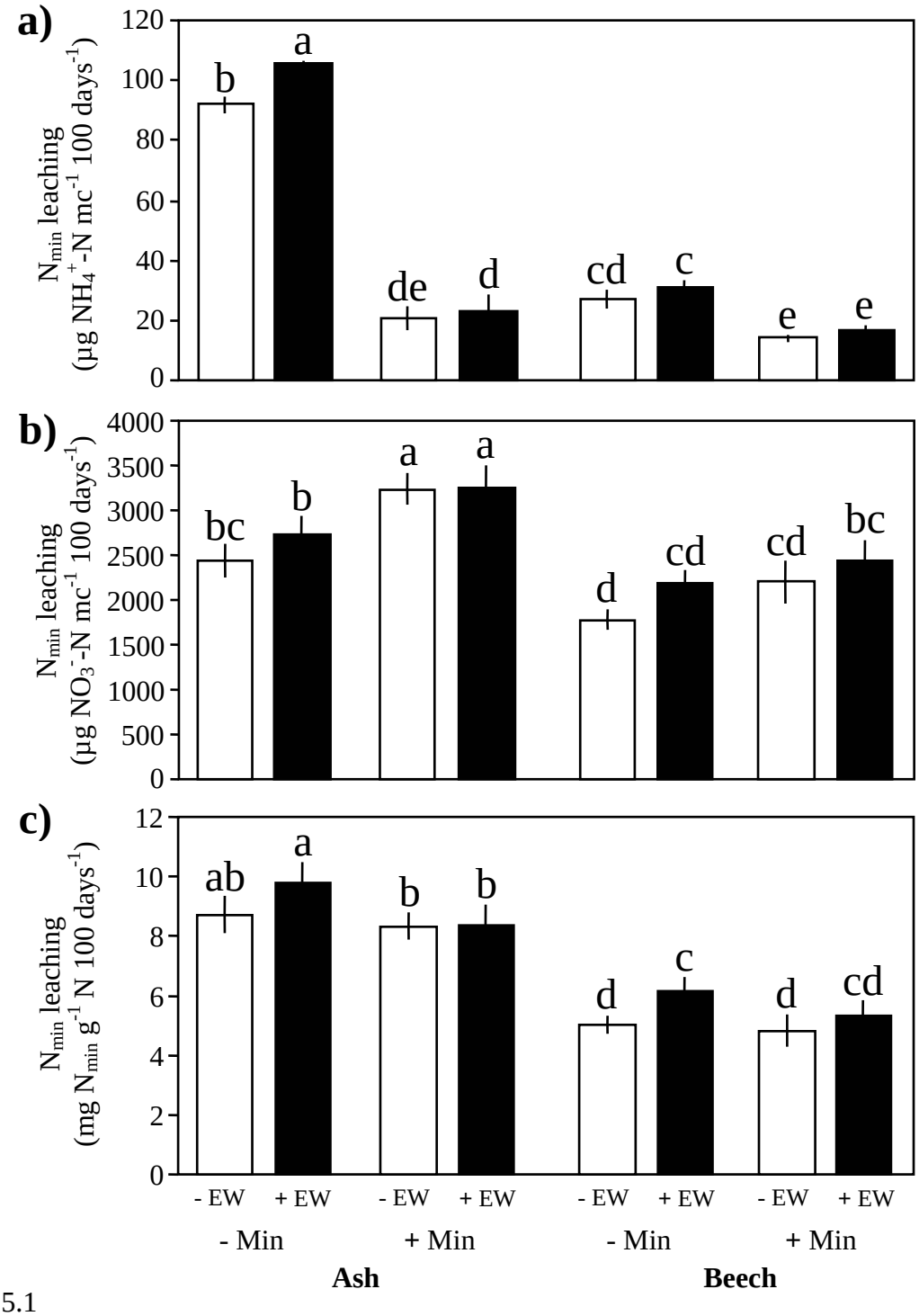

Fig. 5.1
Effects of type of upper mineral soil (ash and beech forest), lower mineral soil (without, - Min and with + Min) and earthworms (without, - EW and with + EW) on cumulative leaching by the systems of **(a)** ammonium (NH_4^+-N), **(b)** nitrate (NO_3^--N), and **(c)** leaching of mineral nitrogen (NH_4^+-N + NO_3^--N) relative to the total amount of soil nitrogen during 100 days of incubation. Bars sharing the same letter are not significantly different (Tukey's HSD test, $P < 0.05$).

CO_2 production

Rates of CO_2 production in treatments without earthworms declined throughout the experiment (Fig. 5.2a) except in ash forest soil treatments where they increased initially. CO_2 production in earthworm treatments generally exceeded that of the control (Fig. 5.2b). The effect of *O. tyrtaeum* on CO_2 production increased early in the experiment reaching a maximum at day 20. Then rates of CO_2 production decreased but the decrease was faster in Ash than in Beech treatments.

Cumulative CO_2 production during the experiment ranged between 160 and 196 mg CO_2-C per microcosm (Fig. 5.3a). Each of the factors studied significantly affected total CO_2 production (Table 5.2). Overall, earthworms increased total CO_2 production by 9.1% which accounted for 48.8% of the variation. Increases by earthworms differed between treatments with and without lower mineral soil (earthworm × lower mineral soil interaction), i.e. earthworms increased CO_2 production in the Ash (10.0%), Ash + Min (5.0%), Beech (12.5%) and Beech + Min (9.1%) treatment. The presence of lower mineral soil also increased carbon mineralisation but only in the Beech treatments (+ 10.8%); the increase in Ash treatments was negligible (+ 1.8%). The interaction between type of upper mineral soil and lower mineral soil was highly significant and accounted for an additional 11.4% of the variation.

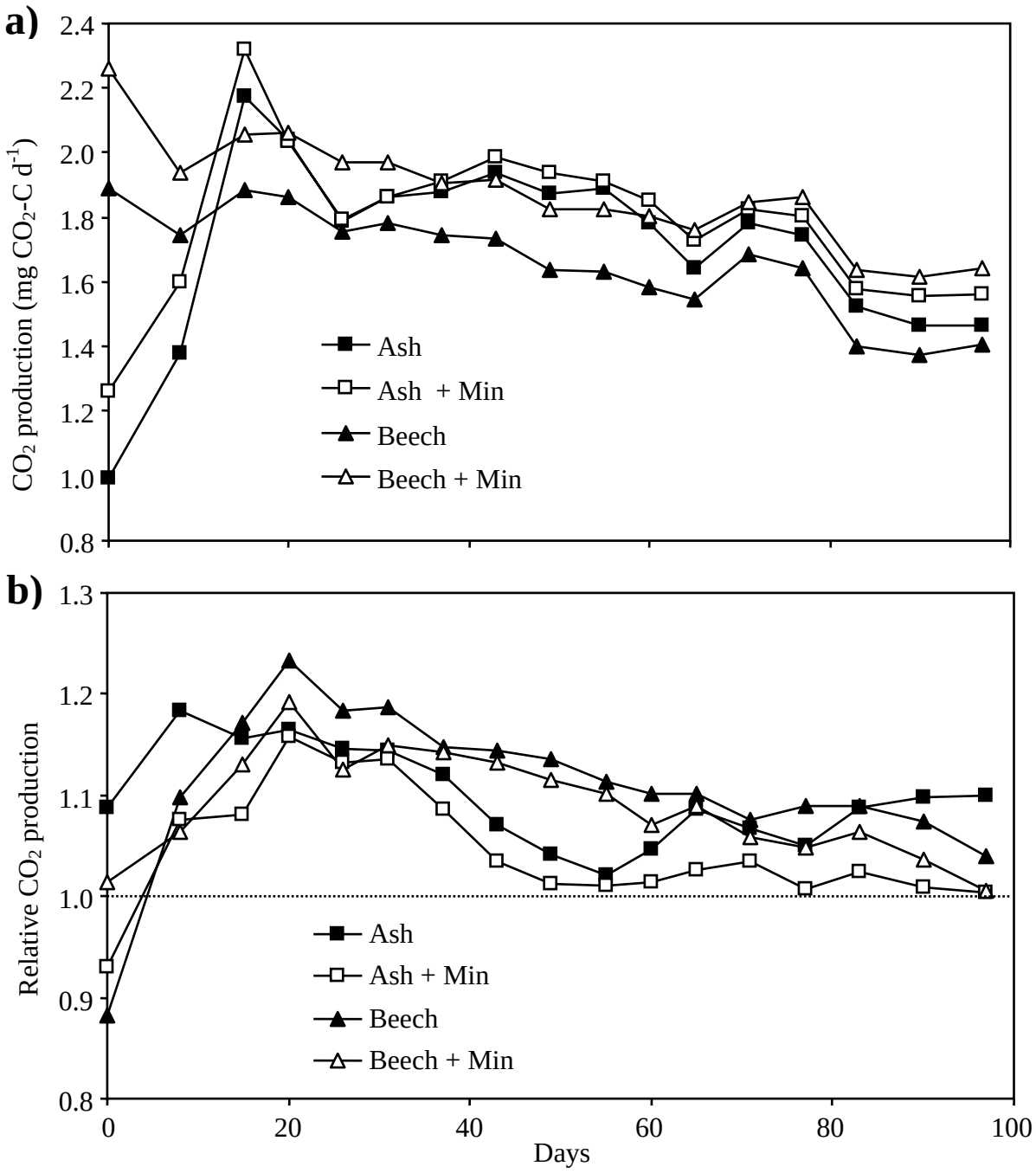

Fig. 5.2
Effects of type of upper mineral soil (ash and beech forest) and lower mineral soil (without, - Min and with + Min) on **(a)** rates of carbon mineralisation (mg CO_2-C per day and microcosm) in treatments without earthworms, and **(b)** the effect of earthworms on carbon mineralisation plotted relative to respective treatments without earthworms.

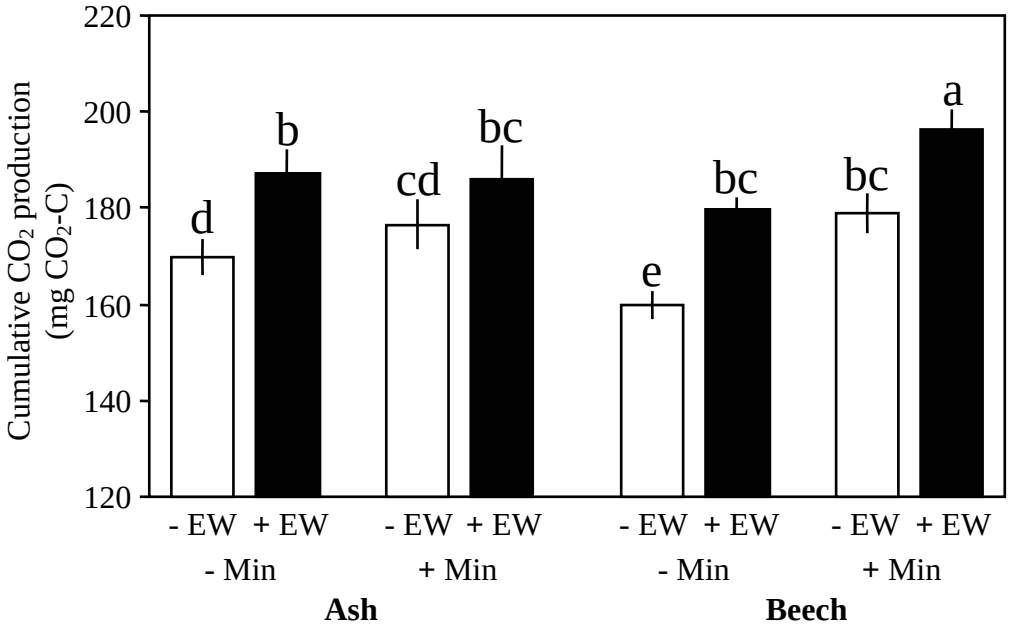

Fig. 5.3
Effects of type of upper mineral soil (ash and beech forest), lower mineral soil (without, - Min and with + Min) and earthworms (without, - EW and with + EW) on cumulative carbon mineralisation (mg CO_2-C per microcosm) during 100 days of incubation. Bars sharing the same letter are not significantly different (Tukey's HSD test, $P < 0.05$).

Mineralisation of ^{14}C labelled lignin

No ^{14}C could be found in the leaching water suggesting that lost of lignin derived carbon as dissolved organic carbon (DOC) was negligible in this experiment. Loss of lignin derived carbon from the system happened only via mineralisation as $^{14}CO_2$-C. Changes in mineralisation rates of lignin differed between Beech and Ash treatments but were hardly affected by the presence of lower mineral soil. Mineralisation rates strongly increased during the first 15-20 days of incubation reaching 0.25 and 0.17% of initial ^{14}C d^{-1} in the Ash and Beech treatments without earthworms respectively. (Fig. 5.4a). After the maximum mineralisation rates declined slowly but continuously in Beech treatments to a level of about 0.13% of initial ^{14}C d^{-1}. In contrast, in Ash treatments $^{14}CO_2$-C production sharply declined for 12 days and then increased again reaching a second maximum of 0.24% of initial ^{14}C d^{-1} on day 49. After this mineralisation rates declined slowly to a level similar to that in Beech treatments.

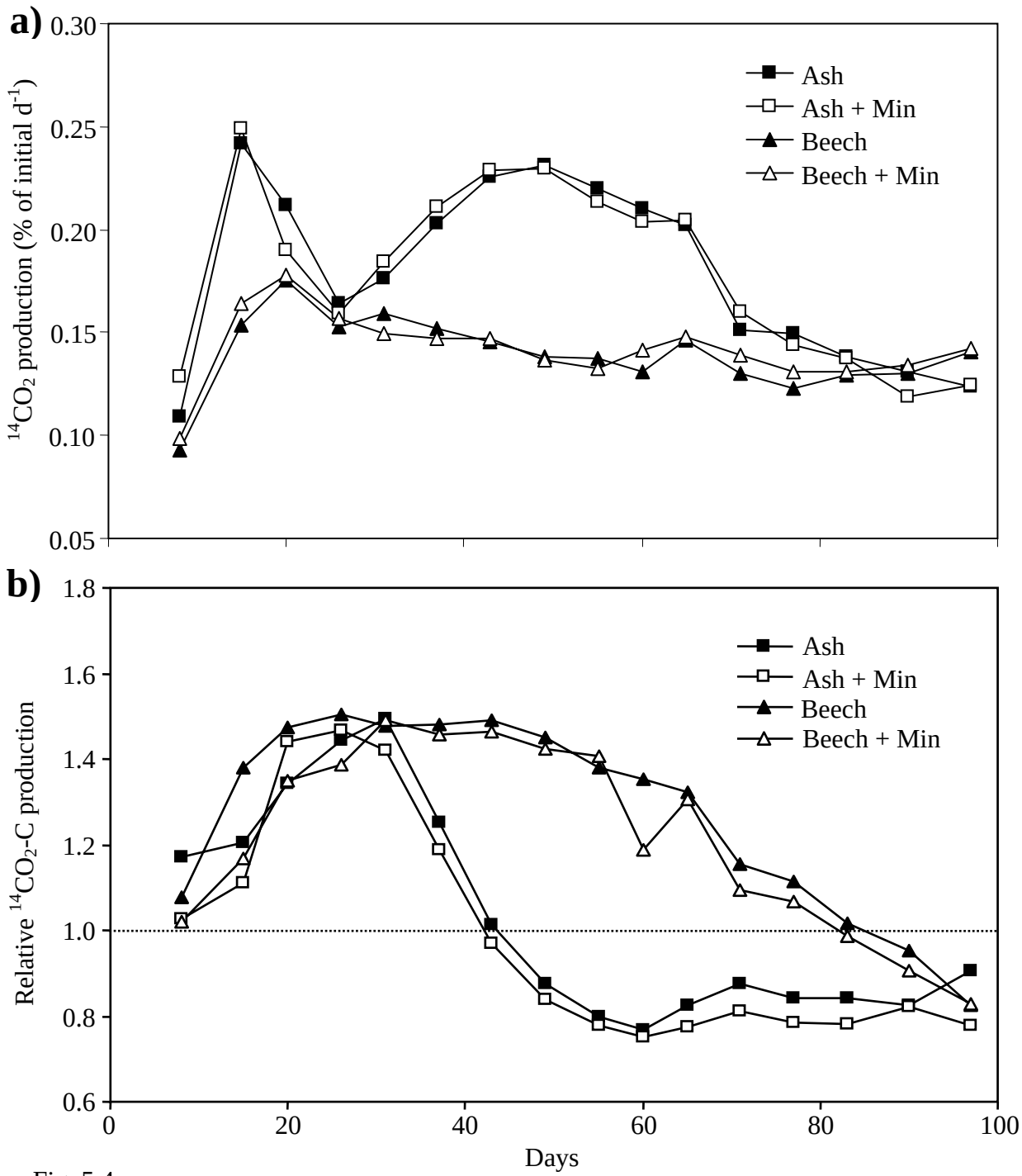

Fig. 5.4
Effects of type of upper mineral soil (ash and beech forest) and lower mineral soil (without, - Min and with + Min) on **(a)** mineralisation rates of ^{14}C labelled lignin (% of initial per day) in treatments without earthworms, and **(b)** mineralisation of ^{14}C labelled lignin in treatments with earthworms plotted relative to respective treatments without earthworms.

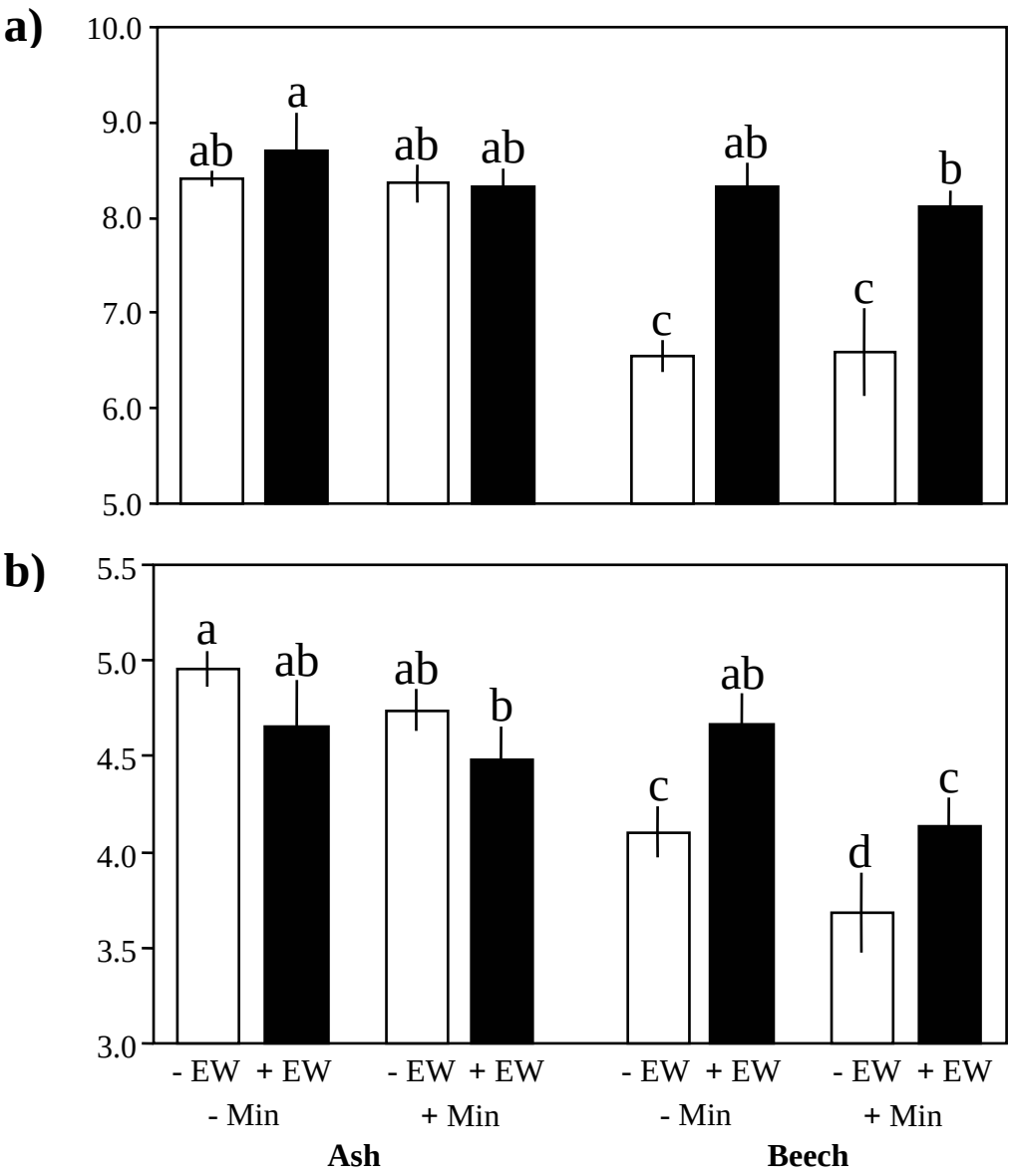

Fig. 5.5
Effects of type of upper mineral soil (ash and beech forest), lower mineral soil (without, - Min and with + Min) and earthworms (without, - EW and with + EW) on **(a)** cumulative mineralisation of ^{14}C labelled lignin and **(b)** cumulative mineralisation of ^{14}C labelled lignin relative to total carbon mineralisation during 100 days of incubation. Values within rows sharing the same letter are not significantly different (Tukey's HSD test, $P < 0.05$).

O. tyrtaeum strongly increased the mineralisation of lignin for 42 and 82 days in Ash and Beech treatments respectively (Fig. 5.4b). Later, mineralisation rates in earthworm treatments were lower than in the respective treatments without earthworms. At the end of the experiment mineralisation rates in Ash and Beech treatments were about 20% lower than in treatments without earthworms.
In total 8.4% of the initial lignin carbon was mineralised during the experiment more or less similarly in each ash treatment. In contrast, cumulative lignin mineralisation was lower in the Beech treatments without (6.6% of initial) than in those with *O. tyrtaeum* (8.3% of initial). Earthworms significantly increased lignin mineralisation on average by 11.3% by accounting for 27.5% of the total variation. Upper soil type alone accounted for 39.8% of total variation. However, effects of earthworms were much more pronounced in Beech treatments (by 24.6%). The earthworm × type of upper mineral soil interaction was highly significant and additionally accounted for 22.3% of the variation (Table 5.2).

In total, presence of lower mineral soil alone did not affect cumulative mineralisation of lignin whereas earthworm × lower mineral soil interaction was significant (additional 1.5% of total variation). The strengthening effect on mineralisation by *O. tyrtaeum* was less pronounced in combination with lower mineral soil (+ 8.6%) than without (+ 14.1%).

Mineralisation of lignin relative to total CO_2 production by the systems was on average by 11.9% lower in upper mineral soil from the beech than from the ash forest, thus accounting for 39.8% of the variation. The presence of lower mineral soil decreased the relative ^{14}C mineralisation by 3.7% in the Ash and 10.3% in the Beech treatments (significant type of upper mineral soil × lower mineral soil interaction). The effect of earthworms on the relative mineralisation of lignin differed between Ash and Beech treatments (significant earthworm × type of upper mineral soil interaction, 22.3% of total variation). In Ash treatments, earthworms reduced relative mineralisation rates (- 6.1%), whereas in Beech treatments they were increased (+ 12.5%).

5.4 Discussion

Forest soils often contain higher amounts of soil organic matter, which is predominantly formed by the accumulation of stable humus substances, such as lignin (Schaefer 1991, Joergensen 1991). The potential of the soil matrix to bind organic material such as lignin is important for the accumulation and stabilisation of soil organic matter (Six et al. 2002). In the present experiment two forest soils were compared; representing different stages of humus accumulation. Total mineralisation of lignin derived carbon was 1.3 times higher in treatments containing ash forest soil, which is supposed to be in the state of humus accumulation, than in that containing humus rich soil from a matured beech forest. Cumulative CO_2 production from ash soil therefore slightly exceeded that of beech soil. Microorganisms are predominantly responsible for the degradation and mineralisation of soil organic matter (Swift et al. 1979). It is known that few soil microorganisms are able to attack the lignin molecule (Crawford 1981) and lignin degradation was shown not to be correlated with the microbial biomass (Entry et al. 1987). The extent of lignin mineralisation therefore strongly depends on the composition of the soil microflora. By this fact, it may be assumed that the microbial community of the upper mineral soil from the ash forest proportionally contained more microorganisms which are able to mineralise lignin than the beech forest soil. However, lignin mineralising microorganisms are also affected by resource availability and it is shown that lignin mineralisation is increased if easily degradable C resources, such as carbohydrates are available (Reid 1979, Kirk & Farell 1987). Presumably, higher contents of easily available and degradable carbon resources in the upper mineral soil from the ash than from the beech forest may also explain the differences in lignin decomposition. The content of easily available and degradable carbon sources has not been measured directly in the present experiment. Nevertheless, the increase in body mass of the earthworms which was more pronounced in the ash than in the beech treatments indicates that contents of easily available carbon resources should be higher in ash than in beech upper mineral soil. It can be assumed that the diet of endogeic earthworms relies substantially on easily available carbon resources and the growth of juvenile specimens of *O. tyrtaeum* has been shown to be enhanced by the addition of glucose

solution which functioned as a source of easily available carbon (Tiunov & Scheu 2004). The addition of glucose was also shown to increase numbers of endogeic earthworms in the beech forest (Scheu & Schaefer 1998). One may therefore assume that lignin degrading microorganisms are limited by carbon in the beech forest.

Lignin mineralisation rates strongly differed between the treatments during incubation period. *O. tyrtaeum* strongly increased lignin mineralisation during the first couple of weeks of incubation in the ash forest soil whereas in the beech forest soil the increasing effect of earthworms lasted for almost twice the time. The enzyme system of earthworms was shown to be not important in degrading the lignin molecule (Neuhauser et al. 1978). The different mineralisation patterns might therefore be partly explained by changes in the microbial community structure which is able to degrade lignin (Scheu 1992). Changes in the microbial community structure might be caused by favourable conditions during the earthworm gut passage and in the earthworm casts. Microbial growth is often limited by the availability of nutrients, mainly by nitrogen and/or phosphorous in addition to carbon (Anderson & Domsch 1978, Joergensen & Scheu 1999). For the ash forest, microbial growth was shown to be limited by both, carbon and phosphorous, whereas in the beech soil growth is primarily limited by phosphorous (Scheu 1987, 1990). Lignin mineralisation was shown to be limited by the availability of phosphorous (Reid 1979). Endogeic earthworms are able to mobilise phosphorous and increased amounts of phosphate have been frequently reported in earthworm casts (Graff 1971, Aldag & Graff 1975, Haynes et al. 1999). By this it is assumed that increased mineralisation rates of soil organic matter and lignin in the beech forest soil are caused by an increased availability of phosphorous. The pronounced mineralisation in ash forest soil treatments with earthworms during the first weeks of incubation may be caused by increased availability of nutrients in the earthworm casts. However, to a lesser extent also carbon may become more easily available for microbial use in the earthworm casts. Substantial amounts of carbon are deposited by the earthworms in the form of mucus which is concentrated in the lining of their burrows and in the casts. This additionally provides easily available carbon resources for microbial degradation (Lavelle 1983, Scheu 1991). Increased nutrient and/or

carbon availability is likely to be an important factor in trying to explain the parallels of increased mineralisation rates in casts of both forests during the first weeks of incubation.

Reduced lignin mineralisation rates in later stages of incubation in earthworm treatments may also have been caused by different factors: The steepest decline of lignin mineralisation rates occurred in the earthworm treatment with ash forest soil. Essential nutrients for lignin decomposition probably became limited for microbial use due to increased leaching of nitrogen and possibly also phosphorous from the earthworm treatments. This is supported by the fact that for both soils leaching of mineral nitrogen was most pronounced in the first weeks of the incubation and this was higher in earthworm treatments. Decomposition of lignin by microorganisms is supposed to be favoured by carbon which is mobilised by *O. tyrtaeum* (see above). However, earthworms and microorganisms may also compete for easily available carbon resources (Tiunov & Scheu 2004). The better competitor will exploit the available carbon pool more rapidly, thereby limiting the carbon availability for the opponent. In the present experiment, *O. tyrtaeum* probably exploited the carbon pool more efficiently and shortened this pool for the microorganisms; this would then be followed by a reduction of lignin mineralisation. This supports that an external source of C is necessary for microorganisms to degrade lignin and that lignin degradation stops when the carbohydrate supply depletes (Crawford & Crawford 1976, Kirk et al. 1976).

In general, the incorporation of soil organic matter into cast aggregates is assumed to reduce carbon mineralisation (c.f. Lee 1985, Edwards & Bohlen 1996). The incorporated organic particles were shown to become encrusted and served as nuclei for new stable aggregates during the earthworm gut passage (Shipitalo & Protz 1989). Lignin particles have also been found to be protected against microbial attack in the inner compartments of the cast aggregates due to a physical separation (Guggenberger et al. 1995). By this fact, access of microorganisms to nutrients located in the inner compartments of stabilised cast aggregates is reduced and this is assumed to limit decomposition of soil organic matter (c.f. Wolters 2000). This may

explain the reduced lignin mineralisation rates in treatments with upper mineral soil towards the end of the experiment.

However, lower mineral soil also affected lignin mineralisation; the strengthening effect on cumulative lignin mineralisation by *O. tyrtaeum* was less pronounced when lower mineral soil was additionally present (+8.6%) compared to treatments with only upper mineral soil (+14.1%). The close association of organic particles with a C-unsaturated inorganic matrix was shown to increase the stabilisation of organic matter (Six et al. 2002). For the mechanisms by which C accumulates in the mineral-associated fraction it is suggested that inputs of organic matter were rendered relatively rapidly into particles or colloids that are associated with mineral matter and thus are physically protected thereby slowing decomposition (Jastrow 1996). Lignin molecules in the present study may have been stabilised and protected against microbial degradation by this close association with the lower mineral soil matrix due to intimately mixing during earthworm gut passage.

The fact that lignin mineralisation from earthworm casts was less pronounced when lower mineral soil was present supports my hypothesis and let me come to the conclusion that intimately mixing of organic material with an C-unsaturated inorganic matrix during the earthworm gut passage increases the stabilisation of carbon in the earthworm cast. At the long-term, the pool of stabilised carbon will be increase until the C-unsaturated matrix becomes more and more saturated due to constantly mixing by endogeic earthworms. The formation of mull soils, rich in organic matter therefore will be fostered by the earthworm mediated incorporation of upper soil into the lower mineral soil horizons and in reversion. However, at the end of the incubation the burrowing activity of *O. tyrtaeum* in the lower mineral soil was low and it indicates that lower mineral soil is unfavourable for *O. tyrtaeum*, probably because of the low organic matter content. The formation of mull soils by the activity of endogeic earthworms is therefore supposed to be a process which will take decades of years.

6. Use of $^{13}CO_2$ signatures for studying the mobilisation of stable soil organic carbon by the endogeic earthworm *Octolasion tyrtaeum* (Lumbricidae)

Abstract

Endogeic earthworms ingest large amounts of organic matter enclosed in mineral soil. Part of the soil organic matter is mobilised during the gut passage through earthworms but the overall effect of earthworms on the dynamics of soil organic carbon (SOC) is little understood since the origin and age of the SOC pool which is mobilised is unknown. To investigate if endogeic earthworms are able to mobilise old soil organic carbon pools we studied the effect of endogeic earthworms (*Octolasion tyrtaeum* Savigny) on ^{13}C signatures of CO_2 evolved from soil of a maize field. Cultivation on this field had changed from wheat (C_3 plant) to maize (C_4 plant) 23 years ago. The study was conducted in microcosms, half of them were filled with soil from the maize the other half with soil from an adjacent wheat field which served as control for $^{13}CO_2$-C measurements.

Earthworms decreased in body mass during the experiment. After 150 days the remaining body mass was lower in the maize than in the wheat soil. Rates of mineral nitrogen leaching and carbon mineralisation were higher in the wheat than in the maize soil. Earthworms increased nitrogen leaching and CO_2 production to a similar extent in both soils. During the incubation $\delta^{13}C$ signatures in CO_2 became more enriched in the wheat (from -25.8‰ to -25.4‰) and more depleted in the maize treatments (from -17.1‰ to -18.6‰). In general, CO_2 was more enriched in ^{13}C than the carbon of the bulk wheat (-26.6‰) and maize soil (-21.4‰). Earthworms did not affect $\delta^{13}C$ signatures in CO_2 in the wheat soil but they decreased $\delta^{13}C$ signatures in the maize soil at the last two sampling dates. Compared to the initial signature (-22.8 ±0.1‰) $\delta^{13}C$ signatures of *O. tyrtaeum* were more depleted in the wheat (-23.6 ±0.3‰) and more enriched in the maize soil (-21.0 ±0.4‰) at the end of the

experiment. Results of the study indicate that endogeic earthworms contribute to the mobilisation of old carbon pools, however, carbon from earthworm body tissue may also have contributed to the shift in ^{13}C signatures in CO_2 in the maize soil.

6.1 Introduction

For understanding soil organic matter dynamics it is necessary to know the parameters affecting the stability of soil organic carbon (SOC). SOC consists of different constituents that vary in mass, age and rate of turnover, the active, slow and resistant fraction. Plant residues and root exudates contribute to the labile fraction, while resistant humic substances withstand decomposition for more than 1000 years (Collins et al. 2000). Degrading and mineralising soil organic matter is mainly due to the activity of microorganisms. Soil animals, such as earthworms, alter the environmental conditions for microorganisms and therefore the decomposition of organic material (Edwards & Fletcher 1988, Martin 1991, Wolters 2000). In casts of endogeic earthworms microbial biomass is reduced, while microbial activity (specific respiration) is enhanced (Scheu 1993c, Tiunov & Scheu 2000b). The mobilisation of nutrients, such as nitrogen and phosphorous, but also of easily available carbon sources was shown to be responsible for the enhanced activity of soil microorganisms in earthworm casts (Scheu 1987c).

Carbon assimilation efficiency of endogeic earthworms is low (1-2%) and this is compensated for by the consumption of large quantities of soil (Bolton & Phillipson 1976, Scheu 1987b, 1992b). Little is known on which carbon pool endogeic earthworms actually mobilise and assimilate. The analysis of natural carbon stable isotope signatures ($\delta^{13}C$) might be a powerful tool for investigating the age of SOC pools mobilised by soil organisms (Flessa et al. 2000, Roscoe et al. 2001). Generally, the isotopic signatures of SOC reflect that of the local plant community (Deines 1980, Cadisch & Giller 1996). Using soil from sites on which C_3 plants had been replaced by C_4 plants, the incorporation of plant derived carbon into the SOC pool can be determined (Balesdent et al. 1987, Balesdent & Balabane 1996, John et al. 2003). The $\delta^{13}C$ signatures of microorganisms (C_{mic}) and of the respired CO_2 reflect

those of SOC. Analysing $\delta^{13}C$ signatures of CO_2 evolved from soils allow to trace the carbon pool which actually is mineralised (Santruckova et al. 2000).

The objective of the present study was to determine the age of the SOC fraction which is mobilised by endogeic earthworms (*Octolasion tyrtaeum* Savigny). For this purpose two soils of different cropping history were analysed. One originated from a field where cultivation shifted 23 years ago from wheat to maize; the other was taken from a field which had been planted with wheat since 1969. By analysing the $\delta^{13}C$ signature in CO_2 from treatments with and without earthworms, we intended to estimate the age of the SOC fraction mobilised by earthworms.

6.2 Material and Methods

Soil sampling

Soil samples were taken from the long-term field experiment of the Landbauschule Rotthalmünster (Bavaria, Germany). The site is located 360 m above sea level, the mean annual precipitation is 886 mm and the mean annual temperature is 8.7°C. The soil is a *Gleyic Luvisol* composed of 11% sand, 72% silt and 17% clay (Kleber 2003). Soil from two fields was taken: a field continuously planted with wheat since 1969 and fertilised with NPK (wheat treatment) and a field continuously planted with maize since 1979; before, this field had also been cultivated with wheat. Both fields were fertilized with standard amounts of NPK.

Soil samples were taken from the upper 30 cm (plough horizon) in September 2002, immediately after the harvest of wheat and before the harvest of maize. Soil samples were taken from at least four locations per field and pooled. Plant residues and stones were excluded by sieving (4 mm). By the end of the experiment earthworms were collected and killed by freezing at -28°C. Soil samples were stored frozen (-20°C) in plastic bags.

The soil carbon and nitrogen content of the wheat field was 12.8 mg C_{org} g^{-1} dry wt and 138 µg N_{tot} g^{-1} dry wt, respectively. Respective values of the maize field were 12.3 mg C_{org} g^{-1} dry wt and 134 µg N_{tot} g^{-1} dry wt. The $\delta^{13}C$ signature of the soil of the wheat field was -26.6 ±0.1‰ and that of wheat –26.8 ±0.1‰. The $\delta^{13}C$ signature of the soil of the maize field was -21.4 ±0.2‰ reflecting long-term maize cropping.

The $\delta^{13}C$ signature of maize was -12.7 ±0.2‰. At both fields straw materials were left on the field and ploughed into the soil to a depth of 30 cm. As indicated from a more intense sampling about 35.1% of the total C_{org} in the Ap horizon of the maize field originated from maize carbon (John 2003). The pH measured in 0.01 M $CaCl_2$ solution of the soil used was 6.34 and 6.54 for the wheat and maize field, respectively.

For the experiment subadult specimens of the endogeic earthworm species *O. tyrtaeum* were extracted by hand from a 130 year old beech forest near Göttingen ("Göttinger Wald") in October 2002.

The experiment

Soil samples were placed at 5°C for one week, and for acclimation one further week at 20°C before the start of the experiment. The experiment was set up in microcosms consisting of perspex tubes (height 150 mm, Ø 60 mm) which were fixed air tight on ceramic plates. The microcosms allow drainage of soil materials at semi-natural conditions by lowering the atmospheric pressure in a box below the ceramic plate. Leaching water from each of the microcosms was sampled in vessels placed underneath the microcosms in the box. The microcosms were closed at the top by a lid which had a small vessel attached to the underside. This vessel could be filled with alkali to absorb CO_2 evolved from the soil.

The microcosms were filled with fresh soil equivalent to 200 g dry wt. The following treatments were established: (1) wheat soil and (2) maize soil. For each treatment 20 microcosms were set up; to ten of each one *O. tyrtaeum* individual was added; the other half served as control without earthworms. Before the earthworms were added they were placed on wet filter paper for three days to void their guts; the filter paper was changed twice. Mean initial body mass of the added *O. tyrtaeum* individuals was 255 (±50) mg fresh wt. Smaller and larger specimens were added to each of the treatments. The microcosms were incubated in a climate chamber in darkness at 20°C for 150 days.

Microcosms were watered weekly with 10 ml distilled H_2O. Leaching water was collected at regular intervals and pooled samples of four weeks were analysed for

mineral nitrogen. Concentrations of ammonium (NH_4^+) and nitrate (NO_3^-) in the leachate were measured by using ion-selective ISE-electrodes (Winlab, Windaus, Germany). From these data the total amount of mineral nitrogen leached (N_{min}) during the incubation period was calculated. CO_2 evolved in the microcosms was trapped in 3 ml 1 N KOH. Prior to addition of alkali used for determination of CO_2, the chambers were pre-incubated with alkali for 24 h. This was done to prevent sampling of CO_2 from dissolved carbonates during the subsequent incubation. Openings in the lid ensured free gas exchange between CO_2 determinations. For CO_2 determination the microcosms were closed by rubber stoppers and incubated for 120 h at 2 week intervals. From 10 microcosms of each soil treatment (five with and without *O. tyrtaeum* in each case) trapped CO_2 was measured by titration with 0.1 N HCl after precipitation of carbonate with $BaCl_2$ (Macfadyen 1970).

For determination of the content of ^{13}C in CO_2 alkali from the remaining 20 microcosms was taken at the same dates. The alkali was quickly removed with a pipette and filled in 50 ml centrifuge vessels containing 3 ml saturated $BaCl_2$ solution. Vessels were immediately filled up to 50 ml with carbonate free distilled water and tightly closed to minimise the trapping of CO_2 from the air. Vessels were shaken by hand and centrifuged for 2 min at 2500 rpm. After centrifugation the supernatant was discarded, the trapped CO_2 remained as a solid pellet of $BaCO_3$ at the bottom the vessel. The vessels were immediately filled again with H_2O and were closed, shaken and centrifuged. This washing procedure was replicated five times until the supernatant was of neutral pH (pH 6.8-7.0) thereby excluding further trapping CO_2 from the air. Then the samples were dried in an oven at 80°C for 48 h. Earthworms were sampled by hand and individual body mass was measured.

The initial soil organic carbon (C_{org}) and nitrogen content (N_{tot}) was measured by an elemental analyser (Model 1400, Carlo Erba, Milan, Italy).

Microbial biomass and respiration

Soil samples taken at the end of the experiment were analysed for microbial biomass (C_{mic}), basal respiration and specific respiration (qO_2) by the substrate-induced respiration method (SIR) (Anderson & Domsch 1978). Measurements were done

using an automated respirometer system based on electrolytic O_2 microcompensation (Scheu 1992a). Control and earthworm worked soil were supplemented with 4 mg glucose g^{-1}. Glucose was added as an aqueous solution adjusting the water content to 80% of the water holding capacity. Oxygen consumption rates at 22°C were measured every 0.5 h. The mean of the eight lowest measurements during the first 11 h after glucose addition was taken as the maximum initial respiratory response (MIRR). Microbial biomass C (C_{mic}; µg g^{-1} dry wt) was calculated as 38 × MIRR (µl O_2 g^{-1} h^{-1}) (Beck et al. 1997). For basal respiration, the average O_2 consumption rate (µg O_2 g^{-1} h^{-1}) in samples not amended with glucose during hours 15-30 after attachment to the respirometer system was used. From data of microbial biomass and basal respiration the specific respiration (qO_2; µl O_2 mg^{-1} C_{mic} h^{-1}) was calculated.

Stable isotope analysis

Isotope ratios ($^{13}C/^{12}C$) were expressed using the δ notation; $\delta^{13}C$ signatures were calculated as $\delta^{13}C$ (‰) = $[(\delta_{sam}/\delta_{std}) -1] \times 10^3$, with δ_{sam} representing the $^{13}C/^{12}C$ ratio of the sample, and δ_{std} that of the standard (Pee Dee Belemnite; PDB). Acetanilide ($\delta^{13}C$ = -29.5‰; Merck, Darmstadt) and $BaCO_3$ ($\delta^{13}C$ = -38.1‰; Merck, Darmstadt) were used as internal standards. The accuracy of the measurements was 0.1‰.

Stable isotope signatures were determined by a coupled system of an elemental analyser (NA 1500, Carlo Erba, Milan) and an isotope ratio mass spectrometer (MAT 251, Finnigan) (Reineking et al. 1993). For measurement of $\delta^{13}C$ in CO_2 0.3 mg $BaCO_3$ was weighed into tin capsules (4 × 6 mm). The $\delta^{13}C$ signature of the initial soil organic carbon was determined from sieved soil samples after drying and grinding in a ball mill. Soil samples containing ca. 250 µg C were measured. $\delta^{13}C$ signatures of the earthworms were determined by analysing tissue material from the anterior part of the specimens. The earthworms were killed by freezing; the anterior part was cut and dried at 60°C. About 1.6 mg dry wt from each individual earthworm was analysed.

Statistical analysis

Data on cumulative mineral nitrogen leaching, CO_2 production, basal respiration, microbial biomass and specific respiration were analysed by two-factor analysis of variance (ANOVA). Factors were earthworm (without and with *O. tyrtaeum*) and type of soil (wheat and maize soil). Changes in earthworm body mass were analysed by ANOVA with the factor type of soil; the initial individual earthworm body mass was used as covariance. Signatures of $\delta^{13}C$ in CO_2 were analysed by single ANOVAs separately for the wheat and maize treatments and for the sampling dates. Prior to ANOVAs data were inspected for homogeneity of variance (Levene test) and log-transformed if required. Results of the ANOVAs are presented as F and *P* values and as the proportion of the total variation (sum of squares, SS) accounted for by a particular factor. Tukey's HSD-test was used for comparison of means. A statistical probability $P < 0.05$ was considered significant. STATISTICA 6.0 software package was used for statistical analyses (Statsoft, Hamburg, Germany).

6.3 Results

Earthworm burrowing and body mass

All introduced *O. tyrtaeum* individuals survived until the end of the experiment in the wheat soil, whereas in the maize soil one earthworm died. This replicate was excluded from the following calculations. Body mass of the surviving earthworms was significantly higher in wheat than in maize soil with 79.7 and 58.9% of the initial biomass, respectively ($F_{1,9} = 13.52$, $P < 0.01$). The soil in the wheat treatments was almost entirely processed and transformed into casts by *O. tyrtaeum* whereas in the maize treatment only ca. 80% of the soil was transformed into casts.

Soil water content of the wheat (25.6% dry wt^{-1}) and maize (26.4% dry wt^{-1}) control treatments did not differ significantly. However, the water content of the earthworm treatments of the wheat soil (29.1% dry wt^{-1}) slightly but significantly exceeded that of the maize soil (28.4% dry wt^{-1}) ($F_{1,35} = 67.54$, $P < 0.001$).

Nitrogen leaching

Leaching of mineral nitrogen (N_{min}) decreased almost parallel in each treatment during the experiment; in wheat treatments it decreased from an initial of 28 µg N_{min} g^{-1} dry wt day^{-1} to 6 µg N_{min} g^{-1} dry wt day^{-1} at the end of the incubation period. In maize treatments it decreased from 21 to 3 µg N_{min} g^{-1} dry wt day^{-1}. Nitrogen leached mainly as nitrate, but the nitrate-to-ammonium nitrogen ratio differed significantly between the soil treatments (log-transformed data; $F_{1,34} = 790.49$, $P < 0.001$). The nitrate-to-ammonium nitrogen ratio was about 3.4 fold higher in maize than in wheat treatments, which was due to higher leaching of ammonium from the wheat soil. Type of soil accounted for 97.1% of the total variation. The nitrate-to-ammonium nitrogen ratio was also significantly increased by the presence of *O. tyrtaeum* ($F_{1,34} = 8.61$, $P < 0.01$) but earthworms accounted for only 0.7% of the total variation.

Cumulative N_{min} leaching was also affected by both, soil type ($F_{1,34} = 151.40$, $P < 0.001$) and earthworms ($F_{1,34} = 232.69$, $P < 0.001$), accounting for 36.0 and 55.3% of the variation. Overall, the amount of nitrogen leached from wheat soil exceeded that from the maize soil by 25.9% (Fig. 6.1). Earthworms increased N_{min} leaching on average by 33.1%.

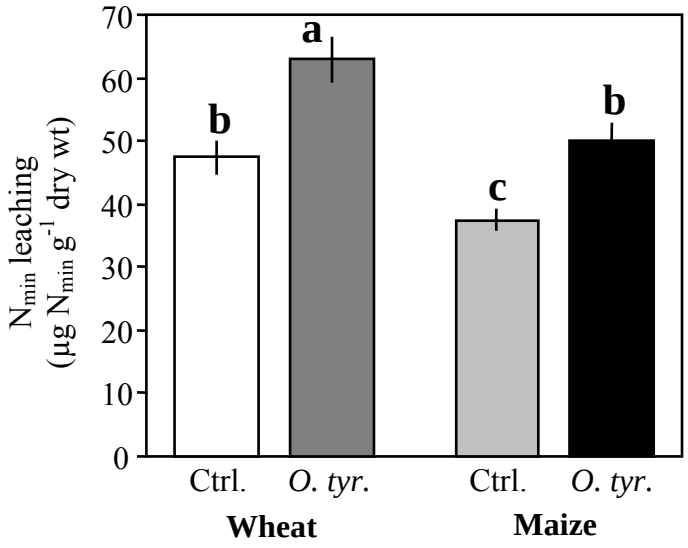

Fig. 6.1

Effects of *Octolasion tyrtaeum* (without, Ctrl, and with *O. tyr.*) on cumulative mineral nitrogen leaching from soil of the wheat and maize field during 150 days of incubation. Means of 10 replicates with 1 S.D.; bars sharing the same letters are not significantly different ($P < 0.05$, Tukey's HSD test).

CO_2 production

Rates of CO_2 production increased within the first 40 days of incubation and then declined almost parallel (Fig. 6.2). CO_2 production in earthworm treatments exceeded that in treatments without earthworms during the whole incubation period. Cumulative CO_2 production was significantly higher in wheat than in maize treatments ($F_{1,15} = 95.12$, $P < 0.001$) (Fig 6.3). Soil type accounted for 56.4% of the total variation. Earthworms significantly increased cumulative CO_2 production on average by 16.6% ($F_{1,15} = 55.71$, $P < 0.001$), accounting for 33.0% of the variation.

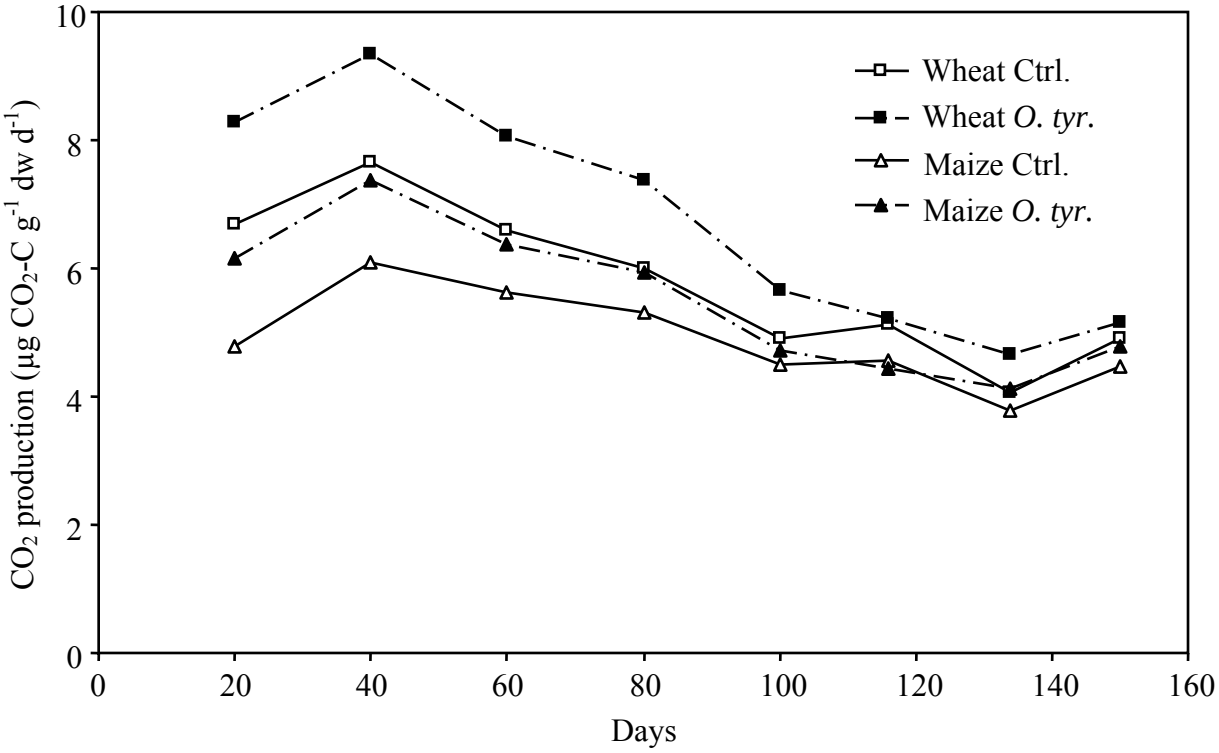

Fig. 6.2
Effects of *Octolasion tyrtaeum* (without, Ctrl., and with, *O. tyr.*) on rates of CO_2 production from soil of the wheat and maize field during 150 days of incubation.

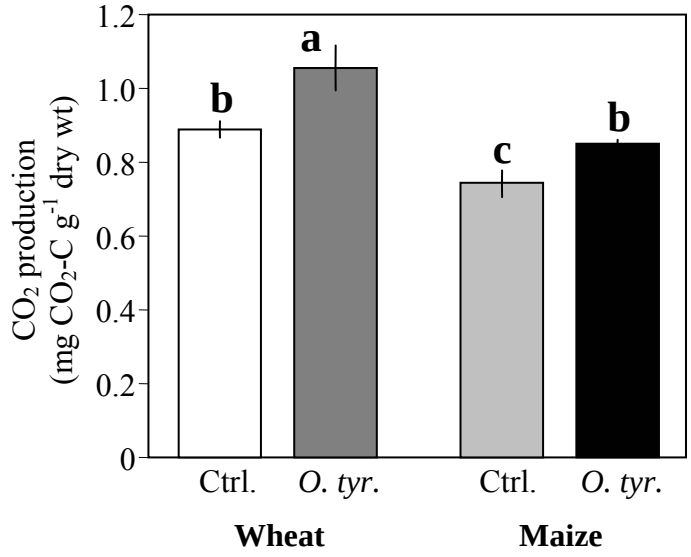

Fig. 6.3
Effects of *Octolasion tyrtaeum* (without, Ctrl, and with, *O. tyr.*) on cumulative production of CO_2 from soil of the wheat and maize field during 150 days of incubation. Means of 5 replicates with 1 S.D.; bars sharing the same letters are not significantly different ($P < 0.05$, Tukey's HSD test).

Microbial biomass

At the end of the experiment basal respiration and specific respiration were not significantly different between treatments and neither affected by the type of soil nor by earthworms. In contrast, microbial biomass in the wheat treatments exceeded that in the maize treatments by 22.1%. Earthworms tended to reduce the microbial biomass in both soils but this was not significant ($F_{1,12} = 4.27$, $P = 0.06$).

^{13}C signatures

As expected, $\delta^{13}C$ signatures of CO_2 evolved from the soil were more depleted in wheat than in maize treatments (Fig. 6.4). $\delta^{13}C$ signatures differed among the sampling dates in each treatment. In the wheat treatments CO_2 evolved from the soil tended to become more enriched in ^{13}C during incubation. In contrast, CO_2 evolved from the maize soil became more depleted in ^{13}C towards the end of the experiment. Earthworms did not affect $\delta^{13}C$ signatures of the CO_2 evolved from the wheat soil, whereas in maize treatments with earthworms the CO_2 evolved from the soil was significantly more depleted in ^{13}C at the third ($F_{1,8} = 15.16$, $P = 0.005$) and fourth sampling date ($F_{1,8} = 17.02$, $P = 0.003$).

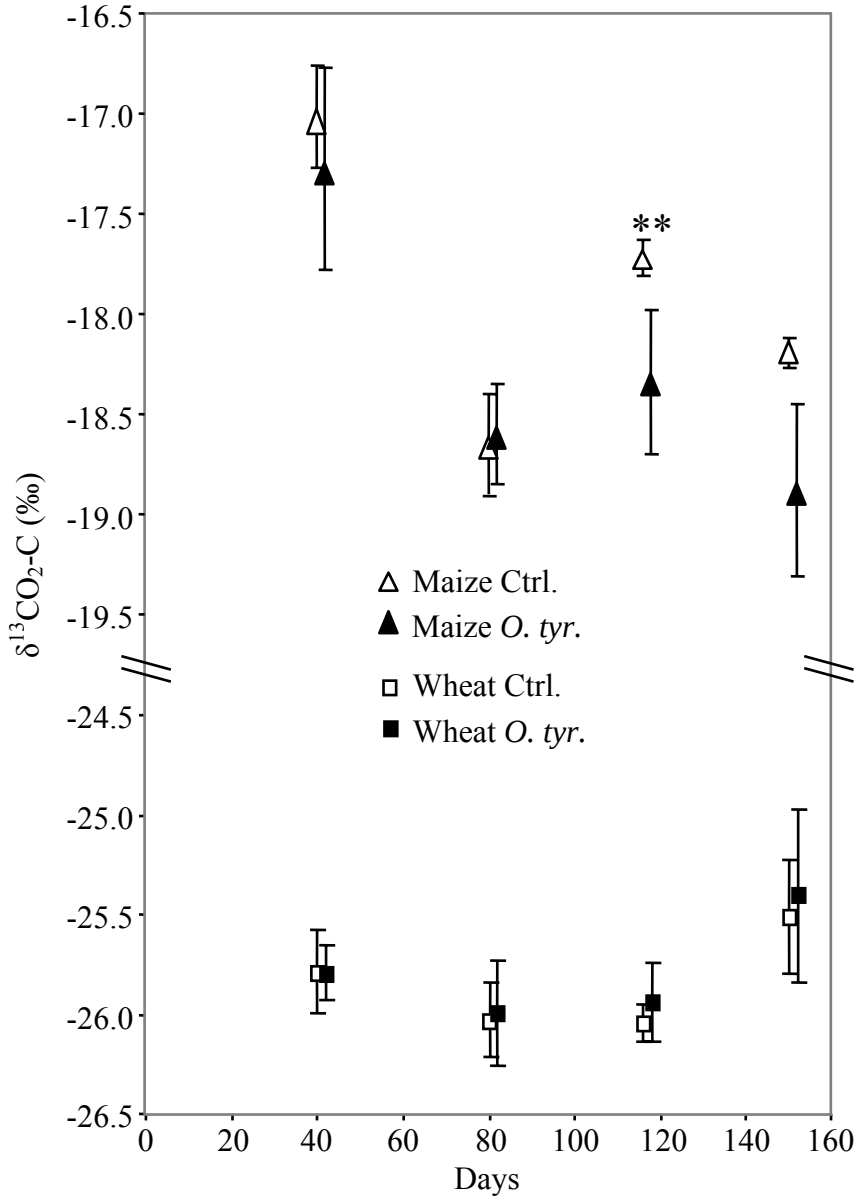

Fig. 6.4
Effects of *Octolasion tyrtaeum* (without, Ctrl., and with, *O. tyr.*) on the $\delta^{13}C$ signatures of CO_2 evolved from soil of the wheat and maize field. Means of 5 replicates with 1 S.D. Significant differences ($P < 0.01$) between treatments with and without *O. tyrtaeum* are indicated by asterisks (**).

The initial $\delta^{13}C$ signature of *O. tyrtaeum* specimens was -22.8 ±0.1‰. At the end of the experiment earthworms were more depleted in ^{13}C (-23.6 ±0.3‰) in treatments with soil from the wheat field. In contrast, in soil from the maize field they were more enriched (-21.0 ±0.4‰).

6.4 Discussion

Earthworm body mass decreased in both soils during the 150 days of incubation, indicating a lack of food resources. Decrease of earthworm body mass during microcosm incubation experiments has been reported frequently (c.f. Scheu 1993a, 1994b) and the incubation conditions of the present experiment were unfavourable for earthworm growth, the used arable soils were poor in carbon and nitrogen (on average 1.25% C_{org} and 0.0136% N_{tot}) and the incubation temperature (20°C) was high relative to the natural living conditions of *O. tyrtaeum*. High temperatures resulted in high metabolic activity and carbon loss by respiration. Earthworms were unable to compensate for these losses by increasing consumption rates as observed previously (Phillipson & Bolton 1976). The decrease in body mass of *O. tyrtaeum* was less pronounced in the wheat soil treatments than in the maize soil treatments. The organic carbon and nitrogen content was only slightly higher in the wheat soil than in the maize soil and it is unlikely that these small differences were responsible for the differences in earthworm body mass. Soil samples were taken in September when the wheat plants had been harvested whereas maize plants were still growing. Both soils were sieved and visible plant residues were excluded before placement into the microcosms. During this procedure more plant residues were picked out from the wheat soil. Likely the amount of labile organic matter originating from fresh plant material incorporated into the soil was higher in the wheat soil. Also, higher rates of mineral nitrogen leaching indicated that the size of the labile organic matter pool was greater in the wheat soil. High amounts of ammonium leaching are indicators for early plant residue decomposition (De Neve & Hofman 1998). Early in the experiment CO_2 production rates were much higher in wheat than in maize treatments whereas rates were similar at the end of the incubation. The microbial biomass was significantly higher in the wheat treatments at the end of the experiment, whereas basal respiration and specific respiration did not differ between the wheat and maize treatments. These findings support the hypothesis the labile organic matter pool in the wheat soil exceeded that of the maize soil but they also suggest that the pool of labile organic matter in the wheat soil depleted quickly

during the incubation period and reached the size of the maize soil by the end of the experiment.

Rates of mineral nitrogen leaching and CO_2 production were increased in the presence of *O. tyrtaeum*. Enhanced mobilisation of nitrogen in arable soils by earthworms has been reported frequently (Christensen 1987, Bohlen & Edwards 1995, Marinissen & De Ruiter 1993). A number of studies also showed an increased mineralisation of carbon in soil processed by earthworms (see introduction of this Chapter).

During the experiment earthworm tissue became more depleted in ^{13}C in the wheat soil, (-0.8 δ units) whereas in the maize soil it became more enriched (+1.8 δ units). The $\delta^{13}C$ signature of the earthworms used in the experiment was initially -22.8‰ and therefore, earthworms were considerably more enriched compared to the wheat soil. The depletion in $\delta^{13}C$ signatures in earthworm body tissue in wheat soil therefore likely resulted from an incorporation of the more depleted carbon from that soil. Compared to the initial signature earthworms became more enriched in ^{13}C in the maize soil reflecting that they incorporated maize derived carbon. At the end of the experiment the $\delta^{13}C$ value of *O. tyrtaeum* was even more enriched than that of carbon of the maize bulk soil suggesting that the earthworms preferentially used the younger maize derived carbon pools. Earthworms mainly feed on recent soil organic matter pools and assimilate soil organic matter with the same age distribution as the overall decomposers in the soil (Martin et al. 1992). $\delta^{13}C$ signatures of a tropical endogeic earthworm species, *Millsonia anomala*, indicated that the animals preferentially assimilated carbon originating from fresh plant debris (Martin & Lavelle 1992). For *Pontoscolex corethrurus*, another tropical endogeic earthworm species, it was shown that tissue $\delta^{13}C$ signatures resemble those of their diet (Spain & Le Feuvre 1997).

Generally, $\delta^{13}C$ signatures of the CO_2 evolved resembled the $\delta^{13}C$ signatures of the soil. $\delta^{13}C$ signatures of the CO_2 evolved from the wheat soil was slightly enriched compared to the bulk soil (on average by 0.8 δ units), whereas ^{13}C signatures of CO_2 from the maize soil were much stronger enriched (on average by 3.3 δ units). In addition, $\delta^{13}C$ signatures in CO_2 varied with the time of incubation. Differences of $\delta^{13}C$ signatures between the bulk soil and the evolved CO_2 may result from microbial

fractionation. CO_2 from microbial respiration has been reported to be depleted in ^{13}C by 2.2‰ compared to microbial biomass carbon, whereas the microbial biomass carbon has been reported to be enriched by 2.0‰ compared to soil C_{org} (Santruckova et al. 2000, Potthoff et al. 2003). These fractionations resulted in similar $\delta^{13}C$ signatures of CO_2 and C_{org} and this was also the case in the present study. In addition, the isotopic discrimination depends on the growth stage of microbial populations; over a 40 day incubation period CO_2 became enriched in ^{13}C, whereas C_{mic} became depleted in ^{13}C (Santruckova et al. 2000). In the present study rates of CO_2 production decreased during incubation suggesting that part of the microbial biomass became less active or died. Decomposition of microbial tissue enriched in ^{13}C may have been responsible for the slightly increased $\delta^{13}C$ signatures in the CO_2 evolved from the wheat soil. In contrast, the large changes in $\delta^{13}C$ signatures in the CO_2 evolved from the maize soil cannot be explained in this way. Microorganisms in the maize soil predominantly must have used younger less stable carbon pools derived from maize residues ($\delta^{13}C$ value of about -12.70‰). Presumably, however, these pools became depleted during the 150 days of incubation and therefore older carbon pools less enriched in ^{13}C had to be mobilised by microorganisms.

Using the ^{13}C methodology and soil containing carbon pools of different ^{13}C signatures the present study aimed at investigating which carbon pool is mobilised by endogeic earthworms. In the wheat soil $\delta^{13}C$ signatures in CO_2 did not differ between control and earthworm treatments. In contrast, in the maize soil $\delta^{13}C$ signatures in CO_2 from earthworm treatments were more depleted than those of control treatments at the last two sampling dates suggesting that the use of older carbon pools was increased.

However, carbon from earthworm tissue may also have contributed to the more depleted $\delta^{13}C$ signatures in CO_2. The decline in earthworm body mass was equivalent to an average amount of carbon of 4.37 mg. By assuming that the loss of earthworm body mass will be most pronounced during the second half of the incubation period when labile carbon sources became limited (see discussion above) and that this amount of carbon was converted entirely into CO_2 about 6.6% of the total evolved CO_2 derived from the earthworm tissues. We calculated the possible contribution of earthworm derived carbon to the $\delta^{13}C$ signature of the CO_2 by using a

two-source mixing model (Phillipps & Gregg 2001). Mean calculated $\delta^{13}C$ signature for CO_2 evolved from earthworm treatments was -18.30‰. This was rather close to the measured $\delta^{13}C$ signature of the CO_2, which has a $\delta^{13}C$ signature on average of -18.61‰. In addition, $\delta^{13}C$ signature of the living earthworm body mass shifted during the incubation time and therefore the resulting $\delta^{13}C$ signature of the CO_2 will be further depleted by earthworm derived carbon than calculated above, reaching levels similar to the CO_2 evolved from the control treatments.

Overall, results of the present experiment first suggest that endogeic earthworms contribute to the mobilisation of old carbon pools in soil but unfortunately, due to the loss of carbon from animal tissue during the experiment this conclusion remains tentatively.

7. Growth and functioning of the endogeic earthworm *Octolasion tyrtaeum* (Lumbricidae) as affected by mineral and organic fertilisers

Abstract

Endogeic earthworms play an important role in mobilisation and stabilisation of carbon and nitrogen in forest and arable soils. Soil organic matter is the major food resource for endogeic earthworms. However, little is known about the size and origin of the organic matter pool on which the earthworms actually live. We measured changes in body mass of juvenile endogeic earthworms (*Octolasion tyrtaeum* Savigny) in soils with different C and N contents resulting from different fertiliser treatments. The soil was taken from a long-term experiment (Statischer Düngungsversuch, Bad Lauchstädt, Germany). The treatments included (1) non-fertilised soil, (2) NPK fertilised soil, (3) farmyard manure and (4) NPK + farmyard manure fertilised soil. The soil was incubated in microcosms with and without one juvenile *O. tyrtaeum* for 80 days.

Earthworm biomass decreased in non-fertilised soil by 48.6%, in NPK soil by 9.4%, but increased in farmyard manure soil by 19.7 and 42.8% (soil with additional NPK application). In farmyard manure treatments the biomass of bigger individuals (> 155 mg fresh wt) decreased, but in smaller individuals (< 150 mg fresh wt) it increased. In NPK fertilised soil without farmyard manure only small *O. tyrtaeum* (< 70-75 mg fresh wt) increased in body mass, whereas in the non-fertilised soil all individuals decreased in body mass. Generally, soil respiration correlated positively with soil carbon content. Earthworms significantly increased soil respiration and nitrogen leaching and this was most pronounced in farmyard manure treatments. Microbial activity was generally higher in farmyard manure soil indicating that farmyard manure increases labile organic matter pools in soil. Also, biomass of earthworms

and microorganisms was increased in farmyard manure soil, but the presence of earthworms reduced microbial biomass, suggesting that soil microorganisms and earthworms competed for labile organic matter pools in soil. The results document that NPK fertilisation on its own was insufficient to sustain *O. tyrtaeum,* whereas long-term fertilisations with farmyard manure enabled survival of endogeic species due to an increased pool of utilisable soil organic matter in arable soil.

7.1 Introduction

Bouché (1977) classified earthworm species into three different ecological groups; epigeic, anecic and endogeic. The major food resources of epigeic and anecic earthworms are known, both mainly live on fresh or little decomposed plant residues which they collect in the litter layer above the mineral soil (epigeic species) or which is collected and concentrated in middens at the entrance of their vertical burrows (anecic species). A number of studies investigated food preferences, growth rates and fecundity of epigeic and anecic species (Satchell & Lowe 1967, Daniel 1991, Butt 1993). Less is known about the food resources of endogeic earthworms, although endogeic species are the most abundant earthworms in many agricultural and forest soils (Phillipson et al. 1978, Lee 1985, Edwards et al. 1995). Food resources and food preferences of some tropical (Lavelle et al. 1980, 1983b, Martin & Lavelle 1992, Martin et al. 1992) and temperate endogeic species have been investigated (Bouché & Kretzschmar 1974, Boström & Lofs-Holmin 1986, Boström 1987, Hendriksen 1990, Scheu 1987b, 1991a, b). These studies documented that endogeic earthworms predominantly ingest soil, but prefer to feed on soil rich in organic matter and decomposing plant debris (Bolton & Philipson 1976). In general, they appear to be less selective than epigeic and anecic species. However, little is known on the origin and size of the organic matter pool endogeic earthworms really live on, i.e. the resources which are assimilated. Potential food resources include (1) plant residues or organic waste, such as farmyard manure, (2) soil bacteria and fungi and (3) well decomposed organic materials, such as humus substances. Knowledge on which organic matter pool is mobilised by endogeic earthworms is essential so as to

understand their effects on mobilisation and stabilisation of carbon and nitrogen in soil. Further, earthworms, including endogeic species, were proven to increase aeration and drainage of compacted arable soils and to enhance plant growth and crop yield by mobilisation of nutrients (Edwards et al. 1995, Scheu 2003). To foster these beneficial effects of earthworms, knowledge about how to establish a stable endogeic earthworm population in arable fields is necessary.

This study investigates the effects of long-term fertiliser applications to an arable cropping system on the growth of juvenile endogeic earthworms. Further, the effects of fertiliser applications and earthworm activity on microbial biomass, basal and specific respiration are studied. Using soils from long-term arable cropping systems, the effects of soil organic matter pools of different origin and size on earthworm growth and nutrient cycling are investigated.

7.2 Materials and Methods

Study site

Soil samples were taken from an arable field which forms part of a long-term fertilisation experiment in Bad Lauchstädt ("Statischer Düngungsversuch"; central Germany, Saxony-Anhalt). The “Statischer Düngungsversuch” is a long-term field experiment with continues treatments for about 100 years. The soil is a *Haplic Chernozem* loam (FAO-classification) with a clay content of 22%. The mean long-term annual temperature at the study site is 8.8°C and the annual precipitation is 480 mm (Körschens 1994). Soils from four different fertilisation treatments were chosen for the present experiment: (1) non-fertilised soil used as control (Ctrl) (1.61% C, 0.11% N), (2) NPK fertilised soil (NPK) (1.76% C, 0.15% N), (3) farmyard manure fertilised soil (FYM) (2.35% C, 0.12% N) and (4) NPK + farmyard manure fertilised soil (NPK + FYM) (2.43% C, 0.17% N). The mean application rates of nutrient in NPK fertilised plots were 95 kg N, 30 kg P, 115 kg K per hectare and year; to FYM treatments 30 t FYM ha^{-1} were added every second year. The last applications of NPK and FYM fertilisers took place in spring 2000 and November 1998, respectively.

A typical crop rotation of sugar beet, spring barley, potatoes and winter wheat had been established in this area. Soil samples were taken from the upper 10 cm of the soil in October 2000. The soil was passed through a 4 mm sieve to remove stones and plant residues and then stored in plastic bags at -28°C.

The experiment

Prior to placement in microcosms the soil samples were placed at 5°C for ten days. Juvenile specimens of *Octolasion tyrtaeum* (Savigny) were extracted by hand digging from a 130 year old beech forest near Göttingen ("Göttinger Wald") in October 2000. The earthworms were kept in containers with soil of the studied arable field at 5°C. For acclimation the earthworms were placed at 20°C one week before they were placed into the microcosms. The microcosms were filled with soil equivalent to 100 g dry wt. In total 52 microcosms were established; 13 from each fertilisation treatment. To ten of them one juvenile *O. tyrtaeum* was added, and three microcosms without earthworms served as control. Before the earthworms were placed into the microcosms they were kept on wet filter paper to void their gut for two days; the filter paper was changed after two days (Dalby et al. 1996). The mean body mass of *O. tyrtaeum* specimens was 122 mg (fresh wt) ranging from 30 to 270 mg. Smaller and larger specimens were homogenously distributed over the four treatments.

The microcosms consisted of Perspex tubes (height 150 mm, Ø 60 mm) fixed air tight on ceramic plates. They allowed drainage of soil materials at semi-natural conditions by lowering the atmospheric pressure in a box below the ceramic plate. Leaching water from each of the microcosms was sampled in a vessel placed underneath the microcosms in the box. The microcosms were closed at the top by a lid which had a small vessel attached to the underside. This vessel could be filled with alkali to absorb CO_2 evolved from the soil. The microcosms were watered weekly with 10 ml distilled H_2O. Leaching water was collected at regular intervals and pooled samples of four weeks were analysed for mineral nitrogen content (N_{min}; NH_4^+ and NO_3^-). From these data the total amount of nitrogen leached during the experiment was calculated. CO_2 evolved in the microcosms was trapped in 1 N KOH. Prior to the addition of alkali used for determination of CO_2 the chambers

were pre-incubated with alkali for 24 h. This was done to prevent sampling of CO_2 from dissolved carbonates during the subsequent incubation. For CO_2 determination the microcosms were closed with rubber stoppers and incubated for 96 h at two week intervals. Trapped CO_2 was measured titrimetrically with 0.1 N HCl after precipitation of carbonate with saturated $BaCl_2$ solution (Macfadyen 1970). Openings in the lid ensured free gas exchange between CO_2 determinations. The microcosms were incubated in a climate chamber at 20°C in darkness for 80 days. At the end of the experiment earthworms were sampled and individual body mass was measured.

Microbial biomass

Soil samples taken at the end of the experiment were analysed for basal respiration and microbial biomass (C_{mic}) by the substrate-induced respiration method (SIR) (Anderson & Domsch 1978). Measurements were taken using an automated respirometer system based on electrolytic O_2 microcompensation (Scheu 1992a). Control soil (n = 3) and earthworm worked soil (n = 4) were supplemented with 4 mg glucose g^{-1} dry wt. Glucose was added as an aqueous solution adjusting the water content to 80% of the water holding capacity. Oxygen consumption rates at 22°C were measured every 0.5 h. The mean of the eight lowest measurements during the first 11 h after glucose addition was taken as the maximum initial respiratory response (MIRR). Microbial biomass C (C_{mic}; µg g^{-1} dry wt) was calculated as 38 × MIRR (µl O_2 g^{-1} h^{-1}) (Beck et al. 1997). For basal respiration, the average O_2 consumption rate (µl O_2 g^{-1} dry wt h^{-1}) in samples not amended with glucose during hours 15-30 after attachment to the respirometer system was used. From data on microbial biomass and basal respiration the specific respiration (qO_2; µl O_2 mg^{-1} C_{mic} h^{-1}) was calculated.

Carbon and nitrogen content

The total carbon and nitrogen content of the initial soil and the soil after the incubation in microcosms were determined using an elemental analyser (Model 1400, Carlo Erba Company, Milan, Italy). Concentrations of ammonium (NH_4^+) and nitrate (NO_3-) in the leachate were measured by ion-selective ISE-electrodes

(Winlab, Windaus, Germany).

Statistical analysis

Data on cumulative CO_2 production, the amount of nitrogen leached and microbial biomass and respiration were analysed by three-factor analysis of variance (ANOVA). The factors were: earthworm (without and with *O. tyrtaeum*), NPK (without and with NPK fertilisation) and FYM (without and with FYM fertilisation). Changes in earthworm body mass were analysed by two-factor ANCOVA with the factors NPK and FYM; the initial individual earthworm body mass was used as covariate. Prior to ANOVAs data were inspected for homogeneity of variance (Levene test) and log-transformed if required. Results of the ANOVAs are presented as F- and *P*-values and as the proportion of the total variation (sum of squares, SS) accounted for by a particular factor. Tukey's HSD test modified for unequal N was used for comparison of means. A statistical probability $P < 0.05$ was considered significant. The STATISTICA 6.0 software package was used for statistical analyses (Statsoft, Tulsa, USA).

7.3 Results

Earthworms

During the experiment two *O. tyrtaeum* specimens died by accident and these two replicates were excluded from the calculations. Mean earthworm body mass decreased during the experiment in the Ctrl (-48.6%) and the NPK treatment (-9.4%), but increased significantly in the FYM (+19.7%) and NPK + FYM (+42.8%) treatment. Body mass was significantly increased by both NPK ($F_{1,33} = 20.44$, $P < 0.001$) and FYM ($F_{1,33} = 118.79$, $P < 0.001$), and they accounted for 7.6 and 44.1% of the total variation, respectively. The increase in earthworm body mass in NPK treatments was more pronounced without FYM than in those with FYM (significant NPK × FYM interaction; $F_{1,33} = 4.35$, $P < 0.05$). Changes in earthworm body mass significantly correlated with the total soil carbon content ($r = 0.95$; $F_{1,2} = 17.99$, $P = 0.051$) but not with the total soil nitrogen content ($r = 0.69$; $F_{1,2} = 1.87$, $P = 0.30$). Generally, changes in earthworm body mass strongly

depended on the initial fresh mass of *O. tyrtaeum* (Fig. 7.1). Smaller individuals increased in body mass, whereas the body mass of bigger specimens remained constant or declined. In FYM treatments the body mass of individuals smaller than 150-155 mg fresh wt increased, whereas in the NPK treatment only very small *O. tyrtaeum* individuals (< 70-75 mg fresh wt) increased in body mass. In the Ctrl treatment none of the earthworms increased in body mass.

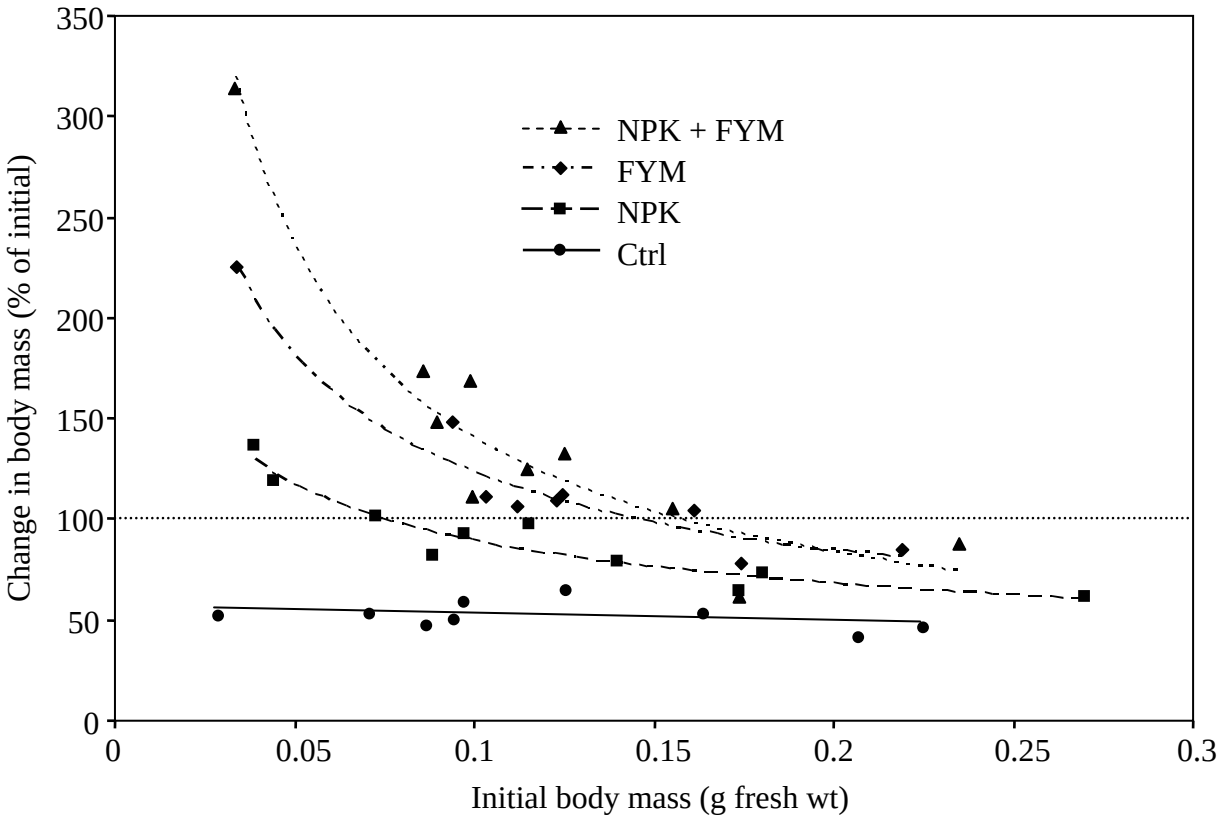

Fig. 7.1
Effects of fertilisers (without fertilisation, Ctrl; with mineral fertiliser, NPK; with farmyard manure, FYM and with mineral fertiliser and farmyard manure, NPK + FYM) on body mass of juvenile *Octolasion tyrtaeum* at the end of the experiment. Curves represent negative linear and exponential functions for changes in body mass (% of initial body mass); Ctrl, $y = -41.75 + 56.52$, $r^2 = 0.15$; NPK, $y = 36.01x^{-0.39}$, $r^2 = 0.91$; FYM, $y = 34.06x^{-0.56}$, $r^2 = 0.91$; NPK + FYM, $y = 24.26x^{-0.76}$, $r^2 = 0.82$.

Table 7.1
Effects of earthworms (*Octolasion tyrtaeum*) and fertilisation (Ctrl, NPK, FYM and NPK + FYM) on cumulative nitrogen leaching and CO_2 production, microbial biomass, basal and specific respiration. Values within rows sharing the same letter are not significantly different (Tukey's HSD test modified for unequal N, $P < 0.05$).

	Without earthworms				**With earthworms**			
	Ctrl	NPK	FYM	NPK + FYM	Ctrl	NPK	FYM	NPK + FYM
Nitrogen leaching (mg N_{min} g^{-1} N)	22.7d	22.8d	44.2c	39.7c	35.7cd	37.2c	72.0a	61.7b
CO_2 production (mg CO_2-C g^{-1} C)	41.7b	29.3c	26.7c	31.6c	52.4a	43.6b	44.6b	49.8a
C_{mic} (SIR) (µg C_{mic} g^{-1} dry wt)	230.2c	287.9abc	363.5ab	386.2a	217.9c	262.4bc	260.0bc	302.6abc
Basal respiration (µl O_2 g^{-1} dry wt h^{-1})	0.79a	1.06a	1.14a	1.28a	0.96a	0.92a	1.27a	1.25a
Specific respiration, qO_2 (µl O_2 mg^{-1} C_{mic} h^{-1})	3.57a	3.67a	3.12a	3.29a	4.38a	3.63a	4.86a	4.12a

Microbial biomass

Soil microbial biomass was in the range of 217.9 to 386.2 µg C g^{-1} dry wt and was at a maximum in the NPK + FYM treatment without *O. tyrtaeum* (Table 7.1). Each of the factors studied significantly affected the microbial biomass; NPK and FYM increased microbial biomass by 15.6 and 31.4%, respectively, accounting for 9.5 and 33.2% of the total variation (Table 7.2). Presence of *O. tyrtaeum* generally reduced microbial biomass by 17.7% accounting for 17.1% of the total variation. The increase in microbial biomass in FYM treatments was more pronounced in treatments without (+44.7%) than in those with *O. tyrtaeum* (+17.1%) (significant earthworm × FYM interaction; Table 7.2). Microbial biomass significantly increased with soil carbon ($r = 0.64$; $F_{1,26} = 18.39$, $P < 0.001$) and soil nitrogen content ($r = 0.67$; $F_{1,26} = 21.71$, $P < 0.001$).

Table 7.2
Three-factorial ANOVA table of F-values on the effects of earthworms (without and with *Octolasion tyrtaeum*, EW), NPK (without and with mineral fertiliser) and FYM (without and with farmyard manure application) on nitrate-to-ammonium nitrogen ratio, mineral nitrogen leached, cumulative CO_2 production, microbial biomass, basal respiration and specific respiration.

	df	Nitrate-to-ammonium ratio #	N_{min} (µg N g^{-1} dry wt)	CO_2 production	C_{mic}	Basal respiration	Specific respiration
EW	1	61.7***	135.8***	296.6***	10.7**	0.1ns	8.9**
NPK	1	17.6***	48.3***	0.8ns	5.9*	1.0ns	1.2ns
FYM	1	1752.4***	315.4***	131.4***	20.9***	11.1**	0.0ns
EW × NPK	1	2.0ns	1.9ns	1.7ns	0.0ns	1.6ns	2.5ns
EW × FYM	1	6.4*	15.5***	34.7***	4.7*	0.1ns	2.6ns
NPK × FYM	1	50.1***	0.8ns	51.7***	0.3ns	0.1ns	0.0ns
EW × NPK × FYM	1	1.3*	0.1ns	0.7ns	0.2ns	0.1ns	0.0ns

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns = not significant; #, log-transformed data

Basal respiration was only significantly affected by FYM; O_2 consumption was increased by 32.0% in FYM treatments (Table 7.2). Due to high standard deviations there was no significant difference between the treatments (Table 7.1). Specific respiration was only significantly affected by earthworms; it was increased by 36.8% and this accounted for 57.7% of the total variation.

Water content

At the end of the experiment the water content of incubated soil samples was significantly affected by each of the three factors (Table 7.2). Earthworms increased the soil water content by 12.5%, NPK fertilisation by 3.7% and FYM by 11.3%, accounting for 59.3, 4.9 and 42.2% of the variation, respectively. None of the interactions were significant. Water content of treatments without earthworms was positively correlated to soil carbon ($r = 0.94$; $F_{1,10} = 74.31$, $P < 0.001$) and soil nitrogen content ($r = 0.91$; $F_{1,10} = 49.38$, $P < 0.001$). In earthworm worked soils correlations with carbon ($r = 0.83$; $F_{1,36} = 81.20$, $P < 0.001$) and nitrogen content ($r = 0.85$, $F_{1,36} = 94.43$, $P < 0.001$) were less pronounced but still highly significant.

Nitrogen leaching

Nitrogen (N_{min}) was leached mainly as nitrate (NO_3^-) and ammonium (NH_4^+), concentrations of ammonium were generally low. Leaching of nitrate declined in a linear fashion during the experiment in each of the treatments. Leaching of ammonium remained almost constant during the first two sampling dates in the treatments without farmyard manure and decreased slightly in the FYM treatments, whereas, during the third sampling period it decreased strongly in each of the treatments (data not shown).

In microcosms without earthworms the nitrate-to-ammonium nitrogen ratio was highest in the control (281), reduced in the NPK (121) and lowest in FYM treatments (30 and 34 in FYM and NPK + FYM treatments, respectively). Generally, the lower nitrate-to-ammonium nitrogen ratios in FYM treatments originated from proportionately higher ammonium concentrations. The nitrate-to-ammonium nitrogen ratio was significantly affected by each of the factors studied (Table 7.2). Earthworms increased the nitrate-to-ammonium nitrogen ratio by 67.2 (Ctrl), 35.3

(NPK), 24.1 (FYM) and 22.4% (NPK + FYM). In NPK and FYM treatments the nitrate-to-ammonium nitrogen ratio was reduced by 30.1 and 80.8%, respectively. NPK decreased the nitrate-to-ammonium nitrogen ratio by 30.6%. Overall, *O. tyrtaeum* and NPK accounted for only 3.2 and 0.9% of the total variation of log-transformed data, whereas, FYM alone accounted for 90.7% (Table 7.2). In FYM treatments (+23.0%) the mobilising effect of earthworms was significantly less pronounced than in treatments without FYM (+53.7%); (significant earthworm × FYM interaction; Table 7.2). Furthermore, the nitrate-to-ammonium nitrogen ratio in FYM treatments was decreased by NPK fertilisation, whereas it was increased in treatments without FYM. The NPK × FYM interaction accounted for an addition of 2.6% of the variation.

The total amount of N_{min} leached was lowest in the control without earthworms (24.1 ±0.9 µg N g^{-1} dry wt) increased in the NPK and FYM treatments, and was highest in the NPK + FYM treatment with 34.2 (±0.5), 53.0 (±0.7) and 68.5 (±1.9) µg N g^{-1} dry wt, respectively (Fig. 7.2a). Overall, *O. tyrtaeum* increased leaching of N_{min} by 59.3% and this accounted for 24.3% of the variation. Also, in NPK and FYM treatments, total nitrogen leaching was increased significantly by 32 and 107%, respectively. NPK accounted for 8.6% and FYM for 58.5% of the variation. The increase in leaching of nitrogen by earthworms was more pronounced in treatments without FYM (60.6%) than in treatments with FYM (58.7%) but the interaction accounted only for 2.8% of the variation.

When total N_{min} leaching was calculated relative to soil nitrogen, leaching was higher in FYM than in NPK + FYM treatments and similar in the NPK and the Ctrl treatments (Table 7.1). Calculated on a dry weight basis NPK significantly increased total N_{min} leaching ($F_{1,41}$ = 48.30, P < 0.001), whereas nitrogen leaching was not affected by NPK when calculated relative to soil nitrogen ($F_{1,41}$ = 3.80, P = 0.06).

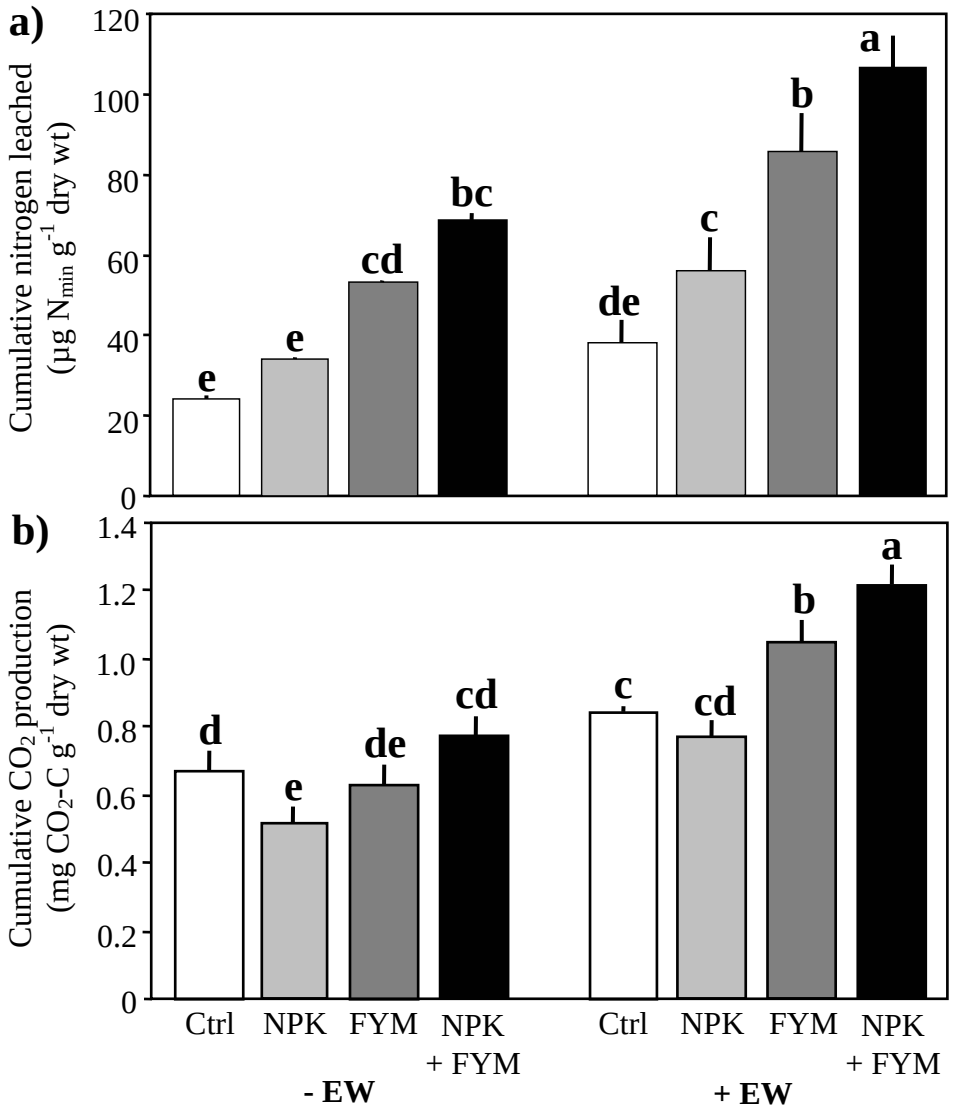

Fig. 7.2

Effects of earthworms (without *Octolasion tyrtaeum*, - EW; with *O. tyrtaeum* = + EW) and fertilisers (without fertilisation, Ctrl; with mineral fertiliser, NPK; with farmyard manure, FYM; with mineral fertiliser and farmyard manure, NPK + FYM) on **(a)** cumulative nitrogen leaching, and **(b)** cumulative CO_2 production during 80 days of incubation. Means of 3 (- EW) and 10 (+ EW) replicates with 1 S.D.; bars sharing the same letter are not significantly different ($P < 0.05$, Tukey's HSD test for unequal N).

CO_2 production

Daily production of CO_2 in chambers without *O. tyrtaeum* remained at almost constant levels of 2.7, 3.3 and 4.1 µg CO_2-C g^{-1} dry wt d^{-1} throughout the incubation for the NPK, FYM and NPK + FYM treatment, respectively (Fig. 7.3a). In contrast, daily production of CO_2 in the Ctrl treatment was high (4.9 µg CO_2-C g^{-1} dry wt d^{-1}) at the beginning, declined to 2.8 µg CO_2-C g^{-1} dry wt d^{-1} after 53 days and increased again to 4.0 µg CO_2-C g^{-1} dry wt d^{-1} during the last week of incubation. CO_2 production was generally increased by earthworms during the whole period of incubation, on average by factors of 1.2, 1.5 and 1.6 for Ctrl, NPK and FYM treatments, respectively (Fig. 7.3b). The effect increased until days 39-53 and then declined until the end of the experiment.

Cumulative CO_2 production was increased in FYM treatments, on average by 30.6% and at a maximum in the NPK + FYM treatment (Fig. 7.2b). Generally, cumulative CO_2 production was significantly increased in the presence of *O. tyrtaeum*, on average by 49.7% (Table 7.2). FYM and earthworms accounted for 23.5 and 53.0% of the total variation, respectively. The increase in CO_2 production by earthworms was most pronounced in FYM treatments (significant earthworm × FYM interaction; Table 7.2). In addition, the effect of FYM was more pronounced in treatments with NPK fertilisers (significant FYM × NPK interaction).

The effect of *O. tyrtaeum* on cumulative CO_2 production was even stronger when relating to unit soil carbon, accounting for 65.4% of total variation ($F_{1,42}$ = 304.51, $P < 0.001$). In microcosms without earthworms CO_2, production per unit soil carbon was similar in each of the treatments except the Ctrl where it exceeded that in NPK, FYM and NPK + FYM treatments by factors of 1.4, 1.6 and 1.3, respectively (Table 7.1).

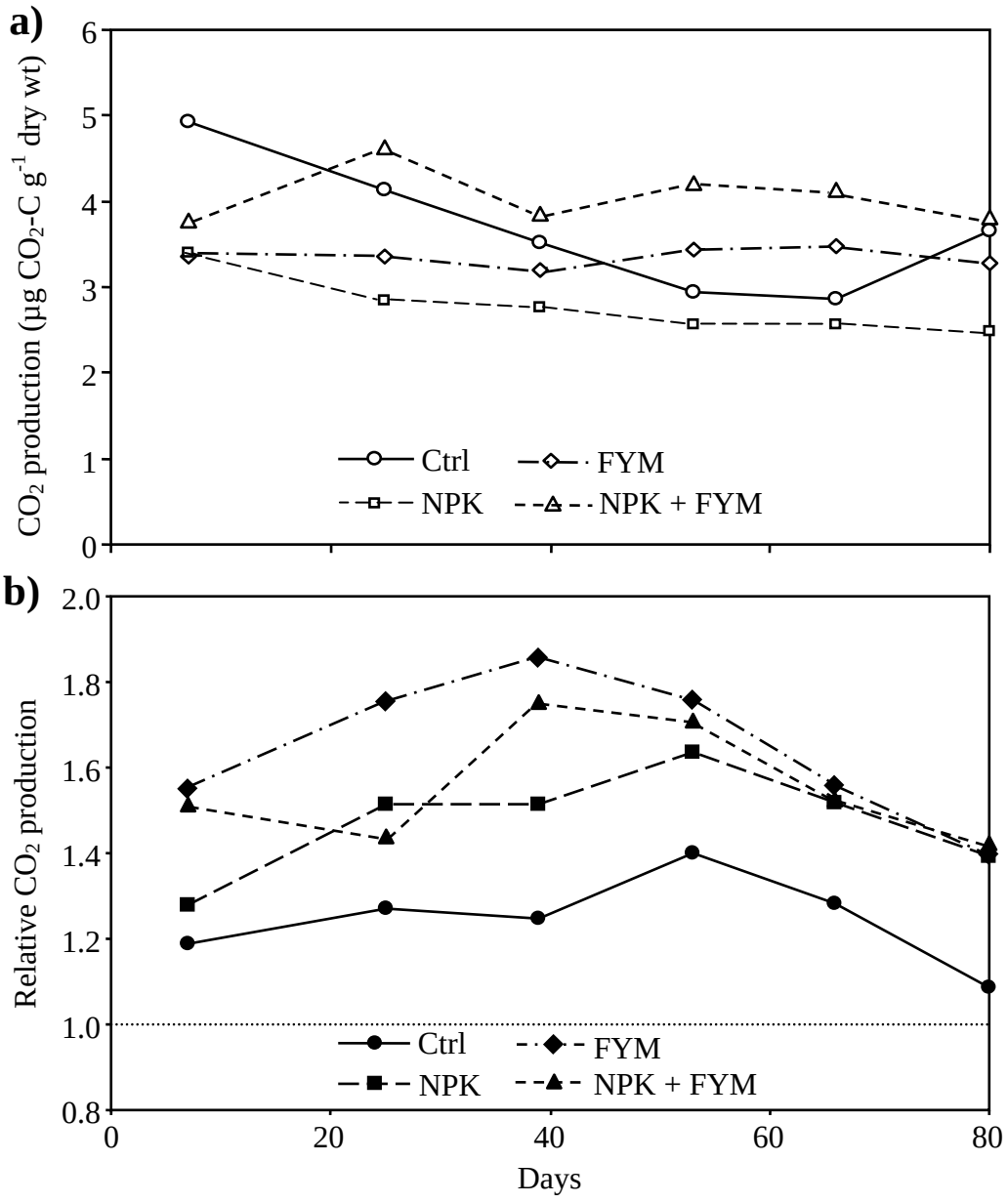

Fig. 7.3
Effects of fertilisers (without fertilisation, Ctrl; with mineral fertiliser, NPK; with farmyard manure, FYM and with mineral fertiliser and farmyard manure, NPK + FYM) on CO_2 production of **(a)** treatments without *O. tyrtaeum*, and **(b)** with *O. tyrtaeum*. Data are plotted relative to carbon mineralisation rates of the respective treatments without earthworms.

7.4 Discussion

Effects of fertiliser treatments on earthworm body mass

Changes in earthworm body mass strongly depended on the initial live body mass of the earthworms. In general, smaller individuals grew more vigorously than bigger individuals. Individuals larger than about 150 and 75 mg fresh wt did not grow in the FYM and NPK only treatments, respectively. The body mass specific growth of earthworms did not follow a straight line, rather smaller individuals gained proportionately more body mass than larger ones (Boström 1987). For *Eisenia fetida* it was shown that juvenile individuals are able to adapt more readily than adults to changes in food resources (Bouwman & Reinecke 1991). They suggested this to be due to the inability of the symbiotic microflora to adapt to changes in food resources. A minor impact of the gut microflora is supposed for *O. tyrtaeum* because of a short gut transit time of the ingested soil (Scheu 1987a). The quality of the food resources may also decide if growth of earthworms is possible. The trade-off between the body mass which becomes cubed during growth and the surface of assimilatory gut epithelium which becomes only squared may restrict the size of earthworms living in an environment where only low quality food is available.

Soil management affects the pool and turnover of soil organic matter through changes in the quantity and quality of plant residues entering the soil, their spatial and seasonal distribution, and through changes in fertiliser input (Christensen 1996). The soil organic matter content of the arable soil used in the present experiment was affected by the long-term application of different fertilisers (Körschens 1997). The differences in soil organic matter content between the four treatments significantly affected the body mass of juvenile *O. tyrtaeum*. In the Ctrl soil without fertiliser application, and with low soil carbon and nitrogen contents, the body mass of *O. tyrtaeum* decreased strongly. Obviously in this soil the organic matter content was insufficient to sustain growth of *O. tyrtaeum*. Long-term application of mineral fertilisers increased the soil organic matter content in the NPK only treatment, and the loss of earthworm body mass was lower. Inorganic fertilisers may contribute indirectly to the increase in earthworm populations due to increased amounts of crop residues entering the soil (Edwards & Lofty 1979, Lofs-Holmin 1983a, Hendrix et al.

1992). In the present experiment, however, earthworms only gained body mass in farmyard manure fertilised soil. The organic carbon content in the FYM and NPK + FYM soil was considerably higher than that in soil without organic fertilisation. The last manure application to the FYM sites of the present study dated back almost two years. Therefore, effects on earthworm body mass, nutrient leaching and CO_2 production are unlikely to have resulted from fresh manure application; rather the effects were due to long-term changes in soil organic matter due to continuous FYM fertilisation. However, the total nitrogen content in the NPK soil exceeded that in the FYM soil. The combined fertilisation with NPK and FYM resulted in the maximum soil N content, whereas, in comparison to the FYM only treatment, it affected earthworm body mass only a little. Therefore, soil nitrogen content and earthworm body mass correlated poorly. Similarly Boström (1987) reported that the growth of *Aporrectodea caliginosa* was not related to food nitrogen concentration.

In contrast to nitrogen, changes in earthworm body mass were well correlated with the soil organic carbon content. Presumably, FYM as a source of labile organic matter facilitated earthworm growth. This is consistent with the conclusion of Marshall (1977) that organic fertilisers increase earthworm populations. Curry (1976) and Andersen (1983) reported that farmyard manures and animal slurries beneficially effect earthworm populations. Animal wastes such as farmyard manure, are rapidly decomposed. Before the establishment of the microcosms, particulate plant residues were removed from the soil by sieving. Another endogeic species, *Aporrectodea caliginosa*, grew slowly in soil where visible organic particles were removed (Boström & Lofs-Holmin 1986). Boström (1987) found earthworm growth to be generally increased in soil with high concentration of plant residues. This suggests that decomposing plant residues form an essential food resource for endogeic earthworms. Other food resources of endogeic earthworms include dissolved organic carbon. The growth of juvenile *O. tyrtaeum* strongly increased when glucose as easily available carbon resource was added to a forest rich in organic matter and fallow soil low in organic matter (Tiunov & Scheu 2004). Replacement of soil was also shown to increase the body mass of *O. tyrtaeum* in this study. In treatments without soil replacement earthworm body mass remained constant in the forest soil and decreased in the fallow soil if no glucose was added,

indicating that the pool of easily available carbon resources is smaller in soils poor in organic matter than in soils with high C and N content. This supports the results of the present study that *O. tyrtaeum* selectively exploited easy available food resources such as labile organic matter derived from organic fertilisers or plant residues. This also may explain why bigger specimens lost body mass whereas smaller ones increased in body mass. Bigger specimens presumably exploited the limited resources in the microcosms more quickly than smaller ones, therefore the time of starvation during the incubation period was longer and thus the bigger lost more biomass.

Effects of Octolasion tyrtaeum *on soil organic matter and microbial biomass*

Cumulative mineral nitrogen leaching (N_{min}) was weakly correlated with the organic matter content of the soil and was not significantly correlated with the total soil nitrogen content. Higher N_{min} leaching in the FYM treatments in combination with higher rates of ammonium nitrogen leaching indicates enhanced mobilisation from residues of the farmyard manure. Nitrogen mobilisation by earthworms was slightly more pronounced in the treatments without FYM but overall, the percentage of the total nitrogen leached as N_{min} varied little between the treatments. Increased mobilisation of nitrogen by earthworms is well known (Marinissen & De Ruiter 1993, Scheu 1994b, Curry et al. 1995). Scheu (1994a) showed that there is an earthworm mobilisable nitrogen pool in soil. The similar amount of earthworm mobilised nitrogen in each of the treatments indicates that the earthworm mobilisable nitrogen pool is independent of the soil organic matter content and long-term fertiliser amendments.

Daily rates of CO_2 production of FYM and NPK treatments remained at almost constant levels throughout the incubation. This indicates that the pool of fast decomposing residues from plant residues and farmyard manure was small. In general, earthworms increased carbon mineralisation during the whole time of incubation with a maximum between days 39 and 53. Later in the experiment, however, the effects of the earthworms declined strongly. This indicates, that ultimately, the soil organic matter enclosed in earthworm casts is stabilised as suggested earlier (Haynes & Fraser 1998, McInerney & Bolger 2000a).

Microbial biomass and enzyme activities in soil are known to be increased by organic fertilisers, and microbial transformations are stimulated by organic amendments (Gunapala & Scow 1998, Kandeler et al. 1999a). In the present study, microbial biomass was reduced by *O. tyrtaeum* in each of the treatments with the decrease being more pronounced in FYM treatments where microbial biomass was high. Also, earthworm body mass increased in FYM treatments, suggesting that soil microorganisms and earthworms competed for food resources derived from labile and easily available soil organic matter pools. This is supported by the specific respiration of soil microorganisms, which was increased by earthworms. Increased specific respiration is an indicator for stress, suggesting that competition for food results in a higher metabolic activity of the soil microorganisms (Anderson 1992, Scheu 1987a, Tiunov & Scheu 2000b).

Overall, the results document that labile organic matter pools in arable soil are essential for soil microorganisms as well as for endogeic earthworms. *O. tyrtaeum* decreased the microbial biomass, which suggests that earthworms and soil microorganisms compete for food resources derived from labile organic matter pools. The amount and origin of soil organic matter in arable soils is crucial for the establishment of endogeic earthworm populations, and their activity beneficially feeds back to nutrient mineralisation and therefore plant productivity.

8. General Discussion

In the present work, effects of two earthworm species of two different ecological groups were investigated. Anecic earthworms, such as *L. terrestris*, are known to be predominantly responsible for the removal of surface plant litter and for the incorporation of plant residues into the mineral soil in both arable and forest ecosystems. Endogeic earthworms, such as *O. tyrtaeum*, are most important for the bioturbation of the organically rich upper mineral soil with the organically poor lower mineral soil. The activities of both groups of earthworms strongly affect the incorporation and the existing pool of organic matter in the soil. Factors known to significantly affect the stabilisation and mobilisation effect of earthworms on soil organic matter have been studied in six laboratory experiments (Chapter Two to Seven). Laboratory experiments have the advantage of controlled environmental conditions such as constant temperature and humidity. Each of the experiments has been conducted at the same temperature (20°C) and the humidity of the soils was kept at a constant level throughout the incubation periods. The studies therefore allow evaluation of the different factors which influence the effects of earthworms on the soil organic matter pools. The ageing of earthworm casts was exclusively monitored in casts of *L. terrestris* (Chapter Two and Three). In the experiments with *O. tyrtaeum*, earthworms were present in the experimental chamber during the whole period of incubation. However, the proportion of earthworm casts increased with time and the differences between treatments with and without *O. tyrtaeum* became more pronounced later during the incubation period. Nevertheless, processes in ageing casts were masked in part by those in fresh deposited casts.

Mineralisation of carbon

The effects of earthworms on carbon mineralisation changed with time of incubation; four phases can be distinguished (Figure 8.1).

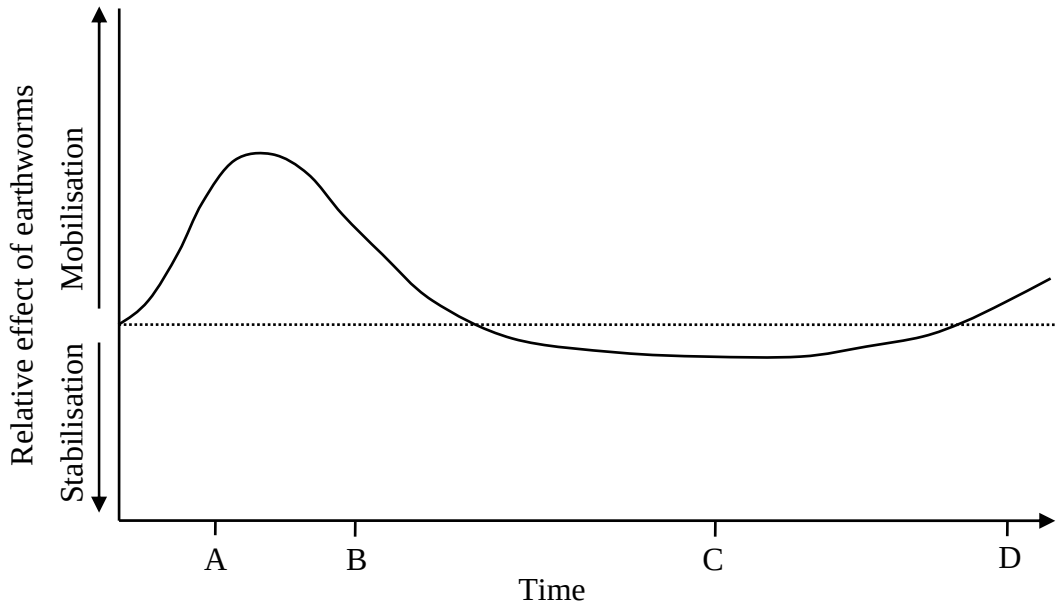

Fig. 8.1
Changes in the effect of earthworms on the stabilisation and mobilisation of organic matter. The graph represents carbon mineralisation rates in casts or treatments with earthworms relative to those in respective treatments without earthworms. Four different phases are distinguished (A-D; see text).

(A) Increased carbon mineralisation in fresh casts.

Carbon mineralisation in earthworm treatments strongly increased during the first five to seven weeks of incubation. This occurred in almost all experiments and every treatment with the exception of incubated cast material of *L. terrestris.* The latter consisted of pooled cast material that had been produced over a period of ten weeks.

The activity of soil microorganisms typically is increased in fresh casts which leads to high carbon mineralisation rates (Scheu 1987, Lavelle et al. 1992). Soil bacteria were shown to colonise fresh casts more intensively than fungi and the higher turnover rates of bacteria are assumed to be responsible for the high C mineralisation (Paul & Clark 1989). Bacteria utilising simple sugars and polysaccharides seem to profit from the increased accessibility of soluble organic compounds and nutrients which are derived from the destruction of soil aggregates and the comminution of organic matter during the gut passage through earthworm (Shaw & Pawluk 1986,

Barois et al. 1993, Devliegher & Verstraete 1997). Microbial activity is also favoured by the relatively high moisture content in fresh earthworm casts. Not only activity is shown to be increased, the numbers of microorganisms are also often increased in freshly deposited casts of earthworms (Lee 1985, Doube & Brown 1998). This might be caused by the fact that dormant microorganisms are activated during the gut passage (Lavelle 1997) and by the proliferation of early r-selected colonisers (Visser 1985).

In presence of litter the stimulation of carbon mineralisation by earthworms was less pronounced in both the arable and the forest soil (Chapter Four). This indicates that due to the availability of additional carbon and nutrient resources derived from the decaying litter saturated the microbial demand on carbon and nutrients mobilised during the gut passage therefore little further stimulated microbial activity. In contrast, the mineralisation of lignin was strongly increased by earthworms in the ash and the beech forest soil during phase (A) (Chapter Five). It can be assumed that the higher availability of nutrients and carbon in the earthworm casts facilitated the microbial attack of lignin. It is known that degradation of lignin strongly depends on the availability of more easily available carbon resources and nutrients, particularly phosphorus (Reid 1979, Kirk & Farell 1987).

The increase in carbon mineralisation was generally more pronounced in arable than in forest soil and was at a maximum in the arable soil fertilised with farmyard manure (Chapter Seven). The microbial biomass was high in the FYM fertilised soil. It is possible that due to strong competition for resources the microorganisms responded strongly to the increased availability of nutrients and carbon in the earthworm casts. However, the more pronounced response may also have resulted from the fact that a higher number of dormant cells were activated. During the gut passage labile carbon pools are mobilised and an incomplete resorption by the earthworm contributes to the increased availability of carbon in earthworm casts (Chapter Seven). However, results of Chapter Six indicate that if labile organic matter is scare also stabilised carbon pools may be mobilised during the gut passage through earthworms.

(B) Decline of microbial stimulation

During phase (B), carbon mineralisation was still higher in earthworm casts, but the degree of increase continuously declined. In arable soil the effect of earthworms declined rapidly, whereas in soil from the beech forest the decline was slower. The addition of beech litter to the forest soil further prolonged the period of increased carbon mineralisation.

The steeper decline of C mineralisation rates in the arable soils indicates that the pool of resources made available is faster exploited by microorganisms than in the forest soils. Resources rapidly exploited by microorganisms constitute of easily available carbon resources, such as sugars, and these possibly become limited due to the high metabolism of microorganisms in fresh casts. The pool of carbon and also that of easily available carbon was small in the arable soils studied, and this limited microorganisms and endogeic earthworms (Chapter Seven). The carbon content of the beech forest soil was high, although a great part of it is locked in stable organic matter pools. Nevertheless, substantial amounts were available for microbial decay as indicated by higher CO_2 production rates per unit carbon in the beech forest than in the arable soil (Chapter Four). Addition of litter increased the availability of carbon but carbon resources of high quality litter materials were also rapidly mineralised. In the long term, microorganisms and earthworms are likely to benefit more from low quality litter materials which provide a more continuous supply of easily available carbon resources (Chapter Four).

Increasing physical protection by the formation of cast aggregates may have also decreased the stimulating effect of earthworms on the mineralisation of soil organic matter (Barois et al. 1993). The stimulation of carbon mineralisation in earthworm casts is clearly modified by a number of factors and these are likely to also have affected the following phase during which carbon mineralisation was reduced.

(C) Carbon stabilisation in earthworm casts

Phase C only occurred in some of the experiments, partly due to the fact that the incubation period in some of the experiments was too short to reach this phase (Chapter Five, Six and Seven). Stabilisation of carbon in earthworm casts differed between arable and forest soil; it only occurred in the forest soil (Chapter Three).

Litter which was incorporated into the soil by *L. terrestris* was shown to be stabilised in the forest but not in the arable soil. In part, this might have been due to the different litter types used (beech and rye) but presumably soil texture also contributed to the more pronounced carbon retention in the forest soil (Hassink et al. 1997). Organic matter is known to be stabilised by the association with silt and clay (Sorensen 1972, Ladd et al. 1985, Feller & Beare 1997). Jenkinson (1977) stated that the more clay can be found in a soil, the greater is the retention of C in the long term. Earthworms were shown to increase the C content in the clay fraction (Chapter Two, Scullion & Malik 2000). The different extent of C stabilisation in the arable and forest soil (Chapter Three and Four) may therefore have been due to the higher clay content in the forest soil. Stabilisation of rye litter incorporated into casts of *L. terrestis* in the arable soil was probably limited by the low clay content (Chapter Three). The reduction of C mineralisation from the incorporated litter in casts with sand presumably resulted from increased comminution of litter particles during the gut passage through *L. terrestris*. C mineralisation was shown to be higher for larger particle sizes of litter than for smaller sizes (Gunnarsson et al. 1988).

(D) Destabilisation of earthworm casts

Destabilisation of earthworm casts refers to the break up of the cast aggregates and the associated exposure of the protected organic matter therein for microbial decay. In casts of *L. terrestris* from the forest soil rates of C mineralisation increased with time. The stability of cast aggregates was shown to strongly depend on the moisture level and on dying-rewetting cycles; wet aggregates are more fragile and drying increases the stability of earthworm casts (Marinissen & Dexter 1990, McInerney & Bolger 2000). In each of the microcosm experiments, the soil and cast materials were incubated at almost high moisture levels with weekly irrigations. The favourable moisture conditions may have facilitated the destruction of the cast aggregates with time. During destruction of the cast aggregates organic matter stabilised inside the aggregates presumably became available for decomposing microorganisms, thereby increasing rates of C mineralisation. Physical destruction of aggregates during particle size fractionation documented that the break up of aggregates in fact results in a strong increase in microbial activity (Chapter Two and Four).

Mobilisation of nitrogen

Mineral nitrogen leaching was uniformly increased by earthworms in the experiments. Nitrogen leaching was generally more pronounced in treatments with arable soil. Increased nitrogen mobilisation due to earthworm activity has been shown in a number of studies with arable and forest soil (Barley & Jennings 1959, Scheu 1994, Willems et al. 1996). The mobilisation of nitrogen by earthworms was shown to improve the performance of plants (Tomati & Galli 1995, Brown et al 1999, Scheu 2003). The effects of earthworms on soil nitrogen dynamics are strongly affected by the addition of litter and vary with the quality of litter (Chapter Four); *O. tyrtaeum* reduced N immobilisation by low quality litter but also the leaching of nitrogen from arable and forest soil with high quality litter. The change in the timing of immobilisation and mobilisation of nitrogen from decomposing litter is of prime importance for the synchronisation of nutrient availability and plant growth. Studies on interactions between litter materials of different quality and soil decomposers may help in developing more sustainable agricultural practices in the future.

Conclusions

In conclusion, stabilisation and mobilisation processes of organic matter by earthworms are strongly affected by the texture of the soil. High clay contents appear to be a prerequisite for the stabilisation of organic matter in earthworm casts and the presence of a C-unsaturated mineral matrix further increases C stabilisation. Litter materials of different qualities only slightly affected the effects of earthworms on carbon mineralisation. Earthworms strongly affected the mobilisation of nitrogen from litter of different quality. Endogeic earthworm populations of arable ecosystems are strongly affected by the kind of fertilisation and the quality of the incorporated plant residues. Fostering of earthworm populations by favourable management practices positively feeds back to soil organic matter, soil structure and fertility, and to the mobilisation of plant nutrients.

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