ORIGINAL PAPER

# Earthworm invasions of ecosystems devoid of earthworms: effects on soil microbes

M. A. McLean · S. Migge-Kleian · D. Parkinson

Published online: 4 July 2006 © Springer Science+Business Media B.V. 2006

Abstract Recent studies document North American earthworm invasions and their profound effects on the structure of the soil profile, which is the habitat for soil microorganisms (mainly fungi and bacteria). Dramatic alterations made to these layers during earthworm invasion significantly change microbial community structure and therefore microbial activities such as C transformations. Understanding the impacts of earthworm invasion on the microbes themselves will give insight into earthworm effects on microbial activities. Bacterial and actinomycete communities in earthworm guts and casts have not been studied in environments recently invaded by earthworms. Earthworm invasion tended to decrease fungal species density and fungal species diversity and richness. The presence of earthworms decreased zygomycete species abundance probably due to disruption of fungal

M. A. McLean (⊠) Department of Life Sciences, Indiana State University, Terre Haute, IN 47809, USA e-mail: lsmclean@isugw.indstate.edu

S. Migge-Kleian

Blumenbach-Institute of Zoology and Anthropology, University of Göttingen, Göttingen, Germany

D. Parkinson

Department of Biological Sciences, University of Calgary, Calgary, AB, Canada

hyphae. Physical disruption of hyphae may also explain decreased mycorrhizal colonization rates, decreased mycorrhizal abundance and altered mycorrhizal morphology in the presence of earthworms. Mixing of organic layers into mineral soil during earthworm invasion tended to decrease microbial biomass in forest floor materials while increasing it in mineral soil. In newly invaded forest soils, microbial respiration and the metabolic quotient tended to decline. In forests where either the microbial community has had time to adapt to earthworm activities, or where the destruction of the forest floor is complete, as in invasions by the Asian Amynthas hawayanus, the presence of earthworms tends to increase the metabolic quotient indicating a shift to a smaller, more active microbial community.

**Keywords** Bacteria · Disturbance · Earthworm invasion · Enzyme activity · Fungal community · Microbial biomass · Mycorrhizae

### Introduction

### Soil microbes

It is estimated that half of Earth's biomass is microbial (Whitman et al. 1998). Soils contain an incredible genetic and functional diversity of microbes (primarily bacteria and fungi) (Swift et al.

1979; Lavelle and Spain 2001; Buckley and Schmidt 2002). Microbes are enzymatically very versatile and are able to degrade a vast range of organic substrates including recalcitrant biopolymers such as lignin and cellulose (Atlas and Bartha 1998). Both bacterial and fungal nutrition are heterotrophic and absorptive in nature but differences in bacterial and fungal growth patterns account for their differing impacts in the soil habitat. Bacteria are unicellular, or, in the case of actinomycetes, finely filamentous, but both tend to be clustered in discrete microhabitats only a few micrometers in volume (Swift et al. 1979; Coleman and Crossley 1996). Bacterial cells are confined to the surface of soil particles and bacterial activity is related to the surface area of these particles (Gunnarsson et al. 1988). Their small size allows efficient dispersal through water films and permits colonization of all available crevices and pores where they rapidly exploit available resources (Swift et al. 1979). Actinomycetes can comprise 10 to 33% of the soil bacteria and are relatively drought tolerant (Swift et al.1979; Atlas and Bartha 1998; Brady and Weil 2002). In contrast, fungi are multicellular and their filaments (hyphae) penetrate many microhabitats where they secrete enzymes, degrade complex substrates and absorb the degradation products (Swift et al. 1979; Coleman and Crossley 1996; Carlile et al. 2001). These absorbed nutrients can be translocated along the hyphae to supply nutrients to parts of the hyphal network which are nutrient limited (Swift et al. 1979). Although many species of soil fungi occur as freeliving microbes, some occur as symbionts with plant roots, in which the fungus becomes integrated into the physical structure of the roots (Atlas and Bartha 1998). These mycorrhizae occur in most families of plants. Mycorrhizae extend from the root into the soil and increase plant uptake of nutrients, especially phosphorus and nitrogen and in return the fungal partner is supplied with carbon substrates (Carlile et al. 2001).

### Earthworm impacts on forest soil

In the absence of earthworms, forest soils typically contain organic layers of varying thickness, consisting of relatively unaltered leaf litter (Oi or L), fragmented, darkened litter (Oe or F) and amorphous, humified, unidentifiable litter (Oa or H). These organic layers are the site of the majority of microbial biomass and activity and contain a wide range of microhabitats and substrates for microbial exploitation (Atlas and Bartha 1998; Lavelle and Spain 2001). The invasion of earthworms results in reduction of the thickness, heterogeneity and organic matter content of these organic layers, an increase in the thickness of the upper mineral horizon, and increased or decreased organic matter content of the upper mineral horizon (Alban and Berry 1994; McLean and Parkinson 1997a; b; Burtelow et al. 1998; Hale 2004; Bohlen et al. 2004b). Earthworms also have profound effects on nutrient availability, through the addition of nutrients from urine, mucus and dead tissue as well as through chemical and physical alterations during gut passage (e.g. Scheu 1991; Brown et al. 2000). In general, earthworms increase N availability and may increase or decrease C accessibility depending on the type of earthworm, age of fecal material and soil layer (e.g. Brown et al. 2000; Lavelle and Spain 2001; also see Frelich et al., and Baker et al., this issue).

# Potential impacts of earthworms on soil microbes

The complex ways in which earthworm activities alter soil habitats for microbes may result in overall positive or negative effects on microbial activities and communities. Increased C and N availability in mucus and urine might be expected to increase microbial growth and activity, particularly in mineral soils where microbes are thought to be inactive most of the time. In contrast, gut passage may have positive or negative effects on microbes depending on the type of microbe and degree of disturbance. Degree of disturbance during gut passage relates to the length of time spent in the gut; typically, from 3–8 h for epigeics and 12–20 h for anecics (e.g. Parle 1963b; Edwards and Fletcher 1988).

Gut passage through epigeic earthworms results in litter fragmentation and some mixing with mineral material (Ponge 1991). Particle surface area is an important parameter for bacteria and there has been speculation that comminution, or the reduction to small fragments, would have potentially positive effects on bacterial activity (e.g. Gunnarsson et al. 1988). One might expect then that epigeic gut passage would result in increased bacterial activity and growth due to the increased particle surface area and the accompanying substrate accessibility. One might also expect that litter comminution during epigeic gut passage might either increase or decrease fungal growth and activity depending on the degree of hyphal disruption. The importance of the integrity of the hyphal links between parts of a fungal mycelium has led to much speculation about the potential effects of earthworm disturbance on fungi (e.g. Pattinson et al. 1997; McLean and Parkinson 2000; Lawrence et al. 2003).

In addition to causing physical disturbance, earthworms also chemically alter microbial substrates, thus altering nutrient availability (see Frelich et al.; Baker et al., in this volume for a discussion of earthworm effects on nitrogen; this review will be limited to earthworm effects on C transformations). Passage through guts of anecic or endogeic earthworms results in much more complete mixing of organic and mineral materials than through epigeic guts. The formation of stable organo-mineral complexes during anecic or endogeic gut passage may reduce C availability to microbes in casts depending on the time scale (e.g. Lavelle and Spain 2001). In the short term, (few days) microbial activity is often stimulated in casts, but over weeks, stabilization begins and over months to years, there is generally a protection of C in casts and a decrease in C mineralization (Brown et al. 2000). One might expect that anecic or endogeic gut passage would result in an initial stimulation and later a reduction in microbial activity due to the addition of soluble C in intestinal mucus followed by a long-term reduction in available C substrates. And anecic and endogeic gut passage is expected to have more negative impacts on fungal growth and activity due to more intense hyphal disruption, relative to that caused by epigeic gut passage.

A further potential impact of earthworms on microbes is that of transport of microbial propagules. Earthworm activities have been shown to increase microbial distributions in soils (Hutchinson and Kamel 1956; Doube et al. 1995; Harinkumar and Bagyaraj 1994; Daane et al. 1997) which may translate into overall effects on microbial growth and community structure.

In the following sections, we consider effects of earthworms on several aspects of soil microbial community structure and activity. In each case we first discuss the situation in which microbes and earthworms occur together in the same habitat, and then the situation where earthworms have recently invaded habitats devoid of earthworms.

### Earthworm impacts on microbial species and communities

Bacteria-co-occurring with earthworms

Whether the microbes observed in earthworm guts are permanent residents of the gut or are transient reflections of the microbial community on ingested materials is not yet clear. It seems likely that at least some bacteria may be permanent components of earthworm guts. That bacterial numbers varied in a uniform way from crop to hind gut in Lumbricus terrestris irrespective of litter substrate (Wolter and Scheu 1999) is consistent with this idea. Comparison of bacteria isolated from the guts of starved earthworms and earthworms fed farmyard manure led Toyota and Kimura (2000) to conclude that the Aeromonas species found in the guts of starved Eisenia foetida was indigenous to the earthworm. They also concluded that Bacillus species, which were abundant in earthworm guts reflected the bacterial community on the farmyard manure fed to the earthworms (Toyota and Kimura 2000).

Bacterial diversity in earthworm guts can be quite high; several hundred bacterial strains have been isolated from earthworm guts (Khambata and Bhat 1957; Márialigeti 1979). Gut bacteria are also functionally diverse, digesting a wide range of compounds including amino acids, sugars, cellulose, chitin, lignin, starch, polylactic acids (Khambata and Bhat 1957; Parle 1963a; b; Márialigeti 1979; Makulec 2002).

Bacterial abundances tend to be higher in earthworm guts, relative to the soil (Parle 1963b; Shaw and Pawluk 1986; Karsten and Drake 1997),

but see Makulec (2002). During passage through the gut bacterial numbers may increase or decrease depending on earthworm species, bacterial species and part of the gut (Parle 1963b; Kristufek et al. 1992; Pedersen and Hendriksen 1993; Karsten and Drake 1997). Increased bacterial abundance during gut passage was observed in the anecic L. terrestris as well as the epigeic L. rubellus, but not in the endogeic Aporrectodea caliginosa (Parle 1963b; Kristufek et al. 1994; Karsten and Drake 1997). These differences may be due to differences in earthworm digestion. For example, the release of labile substrates during comminution by epigeic and anecic earthworms may stimulate bacterial multiplication while ingestion of recalcitrant humified organic matter by endogeics may suppress bacterial multiplication in earthworm guts (Parle 1963b; Kristufek et al. 1992). Bacterial abundances increased logarithmically during L. terrestris gut passage suggesting bacterial growth and not selection of food materials rich in bacteria (Parle 1963b). Also differential susceptibility to digestive enzymes in L. rubellus and L. festivus foregut may explain the observed reduced bacterial abundance in the foregut, but increased abundance in the hind gut suggests growth (Pedersen and Hendriksen 1993). Differential susceptibility to digestive enzymes may in part relate to the ability of the bacterial species to insulate itself in bacterial microaggregates in the gut. In L. rubellus guts, Kristufek et al. (1994) observed metabolically active bacteria enveloped by layers of clay particles in an exopolysaccharide matrix. As biofilms protect bacteria from antibiotics and environmental stresses (Davey and O'Toole 2000), these microaggregates may protect the bacteria from digestive enzymes and abrasion.

Not only are bacteria found in earthworm guts, but bacteria added to earthworm substrates can survive passage through earthworm guts. Even gram-negative non-spore forming bacteria can survive gut passage through lumbricid earthworms (Pedersen and Hendriksen 1993; Thorpe et al. 1993; Doube et al. 1995; Daane et al. 1997). Bacterial communities in fresh earthworm casts reflect the particular bacterial community of the ingested substrate and the differential survival of bacterial cells and spores during gut passage. For example, lower abundance of *E. coli* and *Pseudomonas* spp. in casts than dung suggested that these species did not survive gut passage as well as *Enterobacter* spp. and *Aeromonas* spp. whose abundance was similar in dung and casts.

Earthworms may also alter the distribution of bacteria in the soil due to their burrowing activities. In laboratory microcosms, the influence of earthworm activities on plasmid transfer between spatially separated donor and recipient bacteria was studied (Daane et al. 1997). Limited vertical movement of donor and recipient bacteria occurred in the absence of earthworms while earthworm activities significantly increased vertical bacterial distributions (Daane et al. 1997).

### Bacteria-recent earthworm invasion

To our knowledge the bacterial communities in earthworm guts and casts in "pristine" environments have not yet been investigated. If at least some members of the earthworm gut bacterial community are permanent gut residents, then earthworms arriving in North America from Europe and Asia, for example, may still possess characteristic bacterial species. This needs to be determined. Since at least some members of the earthworm gut bacterial community appear to reflect the bacterial community on the substrates ingested, one might expect to find similar patterns in environments previously devoid of earthworms as those found in habitats with long-term earthworm occurrence.

# Actinomycetes—co-occurring with earthworms

Actinomycetes associated with earthworms have not been well studied. Not only is it unclear whether any of the actinomycetes isolated from earthworm guts are permanent residents or merely reflections of the actinomycete community on ingested substrates, studies are also lacking on interactions between earthworms and actinomycetes.

One estimate of temperate forest soil actinomycete diversity indicates that 90–130 species may be isolated from different soil layers (Lavelle and Spain 2001). Sixty species of actinomycetes

were isolated from the guts of Indian earthworms (Khambata and Bhat 1957). Kristufek et al. (1990) isolated 14 species of Streptomyces from meadow soil and 12 species from the guts of L. rubellus and Octolasion montanum. In both soil and earthworm guts, 5-36% of the bacteria isolated were actinomycetes, but this varied with earthworm species (Khambata and Bhat 1957; Kristufek et al. 1993; Atlas and Bartha 1998). Kristufek et al. (1993) attributed the low proportion in L. rubellus guts to the low proportion of actinomycetes on the organic matter consumed by L. rubellus. This suggests that the actinomycete community in earthworm guts reflects the actinomycete community on the ingested substrate rather than being permanent gut residents. Consistent with this idea are the observations that (i) different streptomycete species were dominant in L. rubellus and O. montanum guts, and (ii) the streptomycete communities in these earthworm guts were very similar to those in the soil in spring but very different from those in the soil in summer (Kristufek et al. 1990).

Actinomycete survival and abundance during earthworm gut passage depends on the earthworm species. Actinomycete abundance increased during *L. terrestris* gut passage but did not increase during *A. caliginosa* gut passage (Parle 1963b; Kristufek et al. 1992). These observations may indicate effects of differences in earthworm digestion and/or differential susceptibility to digestive enzymes in a manner similar to other bacteria (see above). Possibly longer gut transit time for *L. terrestris* would allow time for actinomycete multiplication while the short gut transit time for *A. caliginosa* would not.

Data on abundances of actinomycetes isolated from earthworm guts and casts are few and contradictory, and do not appear to relate to either the ecological strategy of the earthworm species or soil pH (Parle 1963b; Kristufek et al. 1993; Makulec 2002). These differences may reflect the different isolation media used in each study and point up the necessity for further investigation of this important microbial group. One microcosm study suggests that earthworm activities may also increase actinomycete abundance in the whole soil (as characterized by the phospholipid fatty acid (PLFA) 10Me18:0, which is an indicator of actinomycetes) (Saetre 1998).

Actinomycetes-recent earthworm invasion

To our knowledge there are no data on the effects of earthworm invasions on actinomycete communities in pristine soils. Since this group of microbes produce many antibiotics which may have significant negative impacts on the growth and activity of other soil microbes, this is a subject that needs to be explored.

Microfungi-co-occurring with earthworms

It seems likely that gut fungi are transient reflections of the substrate microbial community. This is supported by the observation that the fungal community in fresh *L. terrestris* casts appears to reflect the fungal community on the litter substrates consumed (Tiunov and Scheu 2000b). Fungal hyphal length was maximal in *L. terrestris* crop/gizzard but tended to decline during gut passage, and was generally greater than hyphal length in the soil; this indicated selective grazing on microbe-rich substrates (Wolter and Scheu 1999).

Data on fungal diversity in earthworm guts are very limited. Six fungal species were isolated on nitrogen-free media and 36 taxa were isolated on cellulose medium from the guts of Indian earthworms; 17 species were isolated from guts of *L. terrestris* and 16 from *Drawida calebi* (Hutchinson and Kamel 1956; Khambata and Bhat 1957; Dash et al. 1979). Presumably these are underestimates and more fungal species would have been isolated from these guts had the authors examined more samples or used more types of isolation media.

Transmission electron microscopy of *L. rubellus* earthworm gut contents revealed few fungal hyphae (Kristufek et al. 1994), although fungal colony-forming units (CFUs) increased during gut passage to a maximum in the hind gut (Kristufek et al. 1992). Dash et al. (1979) observed that size of hyphal fragments decreased during gut passage suggesting that increased CFUs is a result of comminution of hyphae into smaller, but still viable fragments during gut transit. Conversely, fungal CFUs decreased from the foregut to the

hindgut in A. caliginosa suggesting that endogeic gut passage results in more damage to fungal hyphae and fewer viable CFUs relative to epigeic gut passage (Kristufek et al. 1992). Further evidence that fungal survival during gut passage depends on the earthworm and the fungal species is given by Moody et al. (1996) and Shankar et al. (2002). Fungal spore viability differed between two anecic species, L. terrestris and A. longa. In general, gut passage through either earthworm species significantly reduced spore viability of all four fungal species tested except that Chaetomium globosum was slightly reduced by L. terrestris and significantly enhanced by A. longa (Moody et al. 1996). Germination of Mucor hiemalis spores was reduced by exposure to L. terrestris intestinal fluids and tended to be higher following mechanical abrasion (Moody et al. 1996).

Fungal diversity in earthworm casts is not well studied, ranging from 6 genera (not identified to species) to 61 species in the few observations that have been made (Parle 1963a; Tiwari and Mishra 1993; Tiunov and Scheu 2000b; Orazova et al. 2003). Even the highest estimates may underestimate actual species richness; plating more particles would probably yield more species. With these limitations in mind it appears that cast fungal community structure depends on the differential survival of fungal propagules, the substrates ingested by the earthworms and cast age. There are not enough data to conclude whether the species of earthworm is an important factor although it would be surprising if it were not, given differences seen in bacterial and actinomycete communities.

Fungal community structure in earthworm casts has recently been investigated in detail. Tiunov and Scheu (2000b) observed a tendency for two species of Mucorales and two species of *Alternaria* to be less abundant and for *Trichoderma* species to be more abundant in fresh casts than in the original litter, possibly due to differential survival of fungal propagules during gut passage. An investigation of the importance of improved microhabitat conditions in *L. terrestris* middens revealed that microhabitat conditions were less important than gut passage in structuring the cast fungal community (Orazova et al. 2003). Cast fungal communities are also affected by substrate consumed by earthworms. Fungal communities on fresh L. terrestris casts were more similar to communities on litter than to communities on soil (Tiunov and Scheu 2000b). Cast fungal communities of worms consuming beech (Fagus) differed from those consuming linden (Tilia) litter and these differences were maintained for at least 100 days during aging (Tiunov and Scheu 2000b). Cast age also affects cast fungal community structure. Fungal communities of aged casts were easily differentiated from those of fresh casts (Tiunov and Scheu 2000b). Aged casts containing either beech or lime litter retained their differences from each other and from aged casts without litter for 100 days (Tiunov and Scheu 2000b). As casts aged, the number of uncolonized particles decreased while the number of fungal taxa isolated, fungal and yeast CFUs, and abundance of two species of Mucorales increased (Parle 1963b; Tiunov and Scheu 2000b). Fungal CFUs in recently deposited casts were higher than in soil, possibly due to increased C availability in casts which contained 1.5 times more organic C and total N than the soil (Parle 1963a; Tiwari and Mishra 1993; Makulec 2002).

### Microfungi-recent earthworm invasion

Prior to earthworm invasion, the organic layers in soil provide many microhabitats and resources to support an abundant and diverse soil biota. The profound changes in soil organic layers resulting from earthworm invasion significantly alter microhabitats and resources for these microorganisms. However, data are very limited and refer only to effects of invasive earthworms on the fungal community in soil, not in earthworm guts or casts. Studies on gut and cast fungi of invasive earthworms are needed.

Effects of the invasion of *D. octaedra* into pine forest soil in Alberta were studied in laboratory mesocosms and in field plots, which are thought to represent the early and later stages in the invasion, respectively. Early in the invasion the number of fungal isolates per particle (a measure of competition between fungi) first increased, then decreased as worm activities intensified (McLean and Parkinson 1998). The increase in number of isolates per particle suggests that competition between fungi was reduced, possibly through the addition of nutrients or increasing spatial heterogeneity as the worms deposited casts in the organic layers. This was followed by a decrease in number of isolates per particle suggesting that further worm activities decreased resource availability or reduced spatial heterogeneity, as the organic layers became homogenized. Later in the invasion, fungal community diversity and richness decreased and dominance by one fungal species increased, apparently due to the disruption of fungal hyphae (McLean and Parkinson 2000). Evidence for this included (i) positive correlation of the occurrence of fast growing species such as Trichoderma polysporum with high worm biomass, (ii) negative correlation of the occurrence of slow growing species such as Oidiodendron echinulatum with high worm biomass, and (iii) decreased occurrence of Zygomycetes such as Mortierella ramanniana and species of Mucor in plots containing high worm numbers. Zygomycetes have few septa and are more susceptible to cell content leakage following hyphal damage than are fungi with more septa.

Given the differences between epigeic, endogeic and anecic earthworms in terms of their preferred food and effects on the soil profile, one might expect significant differences between the invasion of epigeic earthworms and either anecic or endogeic earthworms. In a laboratory mesocosm experiment, the anecic earthworm L. terrestris and/or the endogeic earthworm O. tyrtaeum were introduced into previously worm-free aspen forest soil. The presence of either or both earthworms did not affect fungal community diversity. The frequency of occurrence of Trichoderma species tended to decrease in the earthworm treatments. The frequency of occurrence of Oidiodendron species and Pseudogymnoascus roseus increased in the presence of L. terrestris, while that of Mortierella species decreased (Migge 2001).

With so few data, general conclusions about the effects of earthworm invasions on microfungal communities cannot be drawn, but some suggestions are possible. Frequency of occurrence of *Mortierella* species, which are Zygomycetes, was lower in the presence of epigeic, anecic or endogeic earthworms, reflecting their inability to tolerate hyphal disruption due to their lack of septa to prevent cell content leakage. That the occurrence of fast-growing species of *Trichoderma* was favoured by the presence of the epigeic *D. octaedra* but inhibited by the presence of anecic and/or epigeic earthworms may indicate that these microfungal species can only tolerate moderate amounts of disturbance induced by invasive earthworms.

#### Mycorrhizae-co-occurring with earthworms

Mycorrhizal spores may be better protected than microfungal spores from digestive enzymes and abrasion during earthworm gut passage. The few studies indicate that arbuscular mycorrhizal (AM) propagules and spores can survive gut passage and remain infective, and that casts may contain higher numbers and similar diversity of AM spores and propagules than surrounding bulk soil (Reddell and Spain 1991; Gange 1993).

Data on the interactions between earthworms and mycorrhizal colonization are few and somewhat contradictory. The presence of earthworms may increase (Patron et al. 1999; Brown et al. 2000), decrease (Pattinson et al. 1997; Brown et al. 2000) or not affect (Wurst et al. 2004) AM colonization. Positive effects of earthworms on mycorrhizal colonization may occur in poor soils where earthworm activities increase nutrient availability. For example, the addition of the tropical earthworm, Pontoscolex corethrurus, to unfertilized poor soil (low C, N, P) resulted in more root fragments infected by AM fungi (Patron et al. 1999; Brown et al. 2000). However, when NK fertilizers were added, earthworms decreased AM colonization (Brown et al. 2000). The observed decrease in AM colonization may reflect increased grazing on roots and AM fungal hyphae by the earthworms or the negative impacts of increased physical disturbance by earthworm activities.

#### Mycorrhizae—recent earthworm invasion

There are even fewer studies of the effects of invasive earthworms on mycorrhizae. Results from a study of the impacts of earthworm activities on mycorrhizal colonization of sugar maple are congruent with observations on microfungi. The presence of *L. rubellus*, *O. tyrtaeum* and *L. terrestris* all decreased colonization rates and total abundance of AM, likely due to physical disruption of fungal mycelium (Lawrence et al. 2003). Arbuscular mycorrhizal morphology was also altered in the presence of these earthworms, suggesting carbon stress due to loss of external hyphae (Lawrence et al. 2003).

In a greenhouse experiment investigating the effects of AM fungi and the endogeic earthworm A. caliginosa on plant growth and <sup>32</sup>P transfer between plants, there were very few significant effects (Tuffen et al. 2002). The authors speculated that the benefits of increased P availability due to earthworm activities was offset by the negative impacts of hyphal disruption on AM mycelium (Tuffen et al. 2002). To separate the effects of earthworm impacts on P availability from impacts via physical disruption of mycelium, the authors also included a mechanical disruption treatment. Mechanical disruption tended to produce shoot/root ratios more typical of nonmycorrhizal plants but few significant effects (Tuffen et al. 2002). Although these results suggest few significant effects of earthworms on AM and <sup>32</sup>P transfer between plants, it is likely that the experimental design hindered the observation of significant effects. Of necessity, earthworm activities and mechanical disruption occurred to only a quarter of the root/AM system; thus, although earthworms may have affected AM hyphal connections and activities, it was not possible to observe this.

Negative impacts on mycorrhizal colonization of seedlings have also been observed in earthworm-worked soils. Mycorrhizal colonization was reduced in soil from pure fir and mixed birch-fir stands that had been previously worked by earthworms, compared to controls not worked by earthworms (Welke and Parkinson 2003). Since earthworms were not present in the wormworked soils during the experiment, it is possible that differential spore survival during gut passage or preferential grazing on species observed only on Douglas fir reduced the inoculum in the wormworked Douglas fir soils. Another study showed that there is at least one mycorrhizal species that occurs only on these Douglas fir roots, which tends to support this idea (Jones et al. 1997).

Biol Invasions (2006) 8:1257-1273

These few studies, in combination with evidence that invasive earthworms can significantly decrease undisturbed forest floor thickness and alter nutrient cycling and fine root biomass, point up the need for further studies of the implications of earthworm invasion on mycorrhizal communities and functions.

#### Earthworm impacts on microbial biomass

# Microbial biomass—co-occurring with earthworms

As earthworm effects on microbial biomass have been recently reviewed by several authors (see Edwards and Bohlen 1996; Brown et al. 2000; Lavelle and Spain 2001), we will summarize the conclusions briefly. From the few data available, it appears that fungal biomass (hyphal length) is much greater in the earthworm crop/gizzard than in the soil, indicating selective grazing on microberich substrates by L. terrestris; the subsequent decline during gut passage may indicate digestion by the worms (Wolter and Scheu 1999). Microbial biomass tends to be higher in fresh casts, relative to uningested soil, and begins to decline within a few hours (Parle 1963a; Edwards and Bohlen 1996; Tiunov and Scheu 2000a). The relative increase in microbial biomass in casts depends on the available-C content of the uningested soil, with a higher relative increase in soils with low C content (Scheu 1987; Edwards and Bohlen 1996; Subler and Kirsch 1998; Haynes et al. 1999).

In an experiment to separate the effects of substrate and gut processes on microbial biomass and respiration, Tiunov and Scheu (2000a) used microcosms containing lime (*Tilia*) or beech (*Fagus*) soil in combination with lime or beech litter. In fresh cast material, type and quantity of ingested litter appeared to be more important than available C content. Microbial respiration, glucose-active biomass (GAMB) and biovolume were significantly higher in casts from treatments containing lime litter (Tiunov and Scheu 2000a). Fungal, bacterial and total microbial biovolume correlated significantly with litter content indicating the importance of the litter microbes (Tiunov and Scheu 2000a). The importance of the litter substrate and therefore litter microbes is also evident in an investigation of cast-lined *L. terrestris* burrow walls (Tiunov and Scheu 1999). GAMB was significantly higher in burrow walls than in bulk soils in both *Tilia* and *Quercus* forest but not in *Fagus* forest (Tiunov and Scheu 1999). In *Fagus* forest soil, *L. terrestris* burrow walls were not lined with casts, unlike those in *Tilia* forest soil; the authors suggested this was due to the inadequacy of *Fagus* litter as a food source, so overall soil GAMB did not increase (Tiunov et al. 2001).

The presence of earthworms may increase (Zhang et al. 2000; Scheu et al. 2002), decrease (Saetre 1998; Scheu et al. 2002; Caravaca and Roldán 2003) or not change (Zhang et al. 2000) microbial biomass in the bulk soil. The results depend on the earthworm species, the component of microbial biomass being assessed, the available C in the uningested soil and earthworm abundance. Endogeic species tend to decrease (Saetre 1998; Scheu et al. 2002) and anecic and epigeic species tend to increase (Zhang et al. 2000; Makulec 2002; Scheu et al. 2002) microbial biomass. A classic example of this is an experiment by Scheu et al. (2002) in which GAMB declined in the presence of endogeic earthworms but returned to control levels if both endogeic and epigeic earthworms were present; the authors suggested that epigeic incorporation of organic materials increased resource availability (Scheu et al. 2002).

The activities of earthworms tend to increase microbial biomass in low-C soils presumably by increasing C availability, while in high-C soils, earthworm activities tend to decrease microbial biomass, perhaps due to OM consumption. In low-C soil, intense short-term (24 h) activity by anecic earthworms increased total microbial biomass (chloroform fumigation), but decreased GAMB (Zhang et al. 2000). The results of Caravaca and Roldán (2003) appear to contradict the general trend of increased microbial biomass in low-C soils. The addition of *E. foetida* to sandy soil significantly decreased microbial biomass

while increasing water-soluble C and water-soluble carbohydrates (Caravaca and Roldán 2003). However, although the original sandy soil was low in C, the soils were amended with composted residues which significantly increased the C content (Caravaca and Roldán 2003). In effect, this was a high C situation, and as expected, microbial biomass decreased in the presence of earthworms. Earthworm activities also generally decrease the fungal:bacterial ratio in soil (Saetre 1998; Makulec 2002), presumably due to damage of fungal hyphae.

Microbial biomass—recent earthworm invasion

Studies of invasive earthworm impacts on microbial biomass are contradictory. Most occurred in different forest types invaded by different earthworm species, making it difficult to draw overall conclusions about the relative importance of earthworm species, available-C and soil type to microbial biomass.

To our knowledge there are no studies on microbial biomass in guts or casts of invasive earthworms. However, a study of L. terrestris burrow walls revealed significantly lower microbial biomass-C in burrow walls than in control maple-oak forest soil (Görres et al. 1997). This is an unexpected result, given that maple-oak leaf litter was presumably an adequate food resource for L. terrestris (see Tiunov et al. 2001). However, this may reflect the soil type or more complex interactions with other soil biota. The soil was very sandy and to ensure physical stability of the soil cores during the experiment, the A horizon was mixed with the C horizon resulting in a low initial microbial biomass-C content (Görres et al. 1997). The authors suggested that burrow walls attracted nematodes which grazed and reduced microbial biomass (Görres et al. 1997).

Studies of the invasion by several different earthworm species into maple forests in New York offer a chance to assess the importance of earthworm species on microbial biomass in the soil as a whole. In some maple stands invasion by a mixed community of lumbricids has been observed, in contrast to other stands where the Asian species *Amynthas hawayanus* has invaded. The mixed

community of lumbricids significantly reduced the forest floor in the previously uncultivated site, mixing the forest floor into the upper mineral horizon (Bohlen et al. 2004b) resulting in significantly lower forest floor microbial biomass C and N, increased readily mineralizable C and significantly higher microbial biomass C and N in the A horizon (Groffman et al. 2004). Although plots investigated by Burtelow et al. (1998) contained Aporrectodea species and no Amynthas, the forest floor had been completely transformed into a granular surface layer typical of Amynthas activities. The transformation of the forest floor into casts resulted in a significant decrease in forest floor OM but no increase in A horizon OM. indicating OM consumption by the earthworms (Burtelow et al. 1998). But the end result was increased microbial biomass C and N in the forest floor, increased readily mineralizable C and increased microbial biomass N in mineral soil of worm-worked patches (Burtelow et al. 1998). From these limited data, it appears that both types of earthworms release C, supporting a larger microbial biomass in the A horizon. Differences in the restructuring of the forest floor by these two earthworm types resulted in either increased or decreased forest floor microbial biomass.

A comparison of New York maple forests with differing land use histories illustrates the importance of previous cultivation on the effects of earthworm invasion. Previously cultivated (until 1920) versus logged (late 19th century) but never cultivated maple forests in NY contained a mixed community of lumbricids dominated by L. terrestris and L. rubellus L. terrestris or by L. terrestris (Bohlen et al. 2004a). In both forests, earthworm activities decreased forest floor depth and microbial biomass C and N, and increased A horizon microbial biomass C and N. But the magnitude of the effect related to the original forest floor available for incorporation into the mineral horizon (Groffman et al. 2004); in the previously cultivated site, there was little forest floor to be mixed into the mineral horizon and the increases in microbial biomass in the mineral horizon were less dramatic (Groffman et al. 2004).

Several studies were conducted in forests in Alberta, Canada, following the invasion of

epigeic earthworms into Populus and Pinus forests. Comparisons of these studies may indicate the importance of earthworm species and forest type to microbial biomass. In Populus microcosms, the activities of D. octaedra mixed organic layers into the upper mineral horizon, significantly decreasing GAMB in L/F layer and increasing GAMB in the H layer and A horizon (Scheu and Parkinson 1994a). Similar results were obtained in field microcosms although the effects were less pronounced (Scheu and Parkinson 1994b). These effects were attributed to the mixing of the organic layers into the mineral upper horizon since D. octaedra decreased GAMB in all layers and horizons when incubated separately (Scheu and Parkinson 1994a). Octolasion lacteum mixed organic and mineral materials in Populus microcosms more thoroughly than D. octaedra; effects on the GAMB were similar except that H layer GAMB was significantly decreased (Scheu and Parkinson 1994a) possibly reflecting more intense feeding activities in the H layer by this endogeic species, which is known to preferentially consume humified OM typical of H layers. In contrast, in Pinus mesocosms, D. octaedra significantly decreased GAMB in the F/H layer and mineral horizon (McLean and Parkinson 1997a) as was observed when the Pinus F/H layer was incubated separately (Scheu and Parkinson 1994a). Incorporation of OM from the upper layers deeper into the soil by D. octaedra cannot be the sole explanation here, since there was no corresponding increase in GAMB in the lower soil layers. The authors attributed this to consumption of organic matter by the worms (McLean and Parkinson 1997a).

In *Pseudotsuga*, *Betula* or mixed *Pseudotsuga* + *Betula* forest microcosms, the activities (but not the presence) of *A. caliginosa* did not affect forest floor GAMB (Welke and Parkinson 2003). However, in the mineral soil, the effect of *A. caliginosa* depended on the forest type (Welke and Parkinson 2003). GAMB was significantly lower in worm-worked *Betula* and mixed *Pseudotsuga* + *Betula* soils and significantly higher in pure *Pseudotsuga* soil (Welke and Parkinson 2003). Lower GAMB in the *Betula* and mixed *Pseudotsuga* + *Betula* soils may reflect consumption of OM and microbial biomass. Increased GAMB in *Pseudotsuga* mineral soil may reflect mixing of OM into the mineral horizon similar to that seen in aspen soil (Scheu and Parkinson 1994b). In general, the fungal:bacterial ratio was reduced by the activities of *D. octaedra* and *O. lacteum* (Scheu and Parkinson 1994a), presumably due to damage to fungal hyphae.

#### Earthworm impacts on microbial activity

Respiration and metabolic efficiency co-occurring with earthworms

Earthworm activities tend to increase microbial respiration in casts, burrow walls or in bulk soil (Shaw and Pawluk 1986; Scheu 1987; Haimi and Huhta 1990; Görres et al. 1997; Saetre 1998; Haynes et al. 1999; Zhang et al. 2000; Tiunov et al. 2001) due to the addition of readily accessible C substrates. However, the effects may depend on earthworm species, soil type, available C in uningested materials, and cast age. Also see reviews by Edwards and Bohlen (1996), Brown et al. (2000), Lavelle and Spain (2001).

Soil type affected the impact of *L. terrestris* on microbial respiration (Shaw and Pawluk 1986), which increased in clay loam and silty clay loam soil, but decreased in sandy loam soil. The decrease was accompanied by increased C associated with clay particles, indicating net storage of OM (Shaw and Pawluk 1986).

Several studies have shown that microbial respiration in casts depends on available C content (Scheu 1987; Haynes et al. 1999; Tiunov and Scheu 2000a) and cast age (Scheu 1987; Tiunov and Scheu 2000a). Scheu (1987) showed that although microbial respiration in A. caliginosa casts was higher than that of bulk soil, the cast microbial community was C-limited and respiration declined over time. In another study, Tiunov and Scheu (2000a) showed that in fresh L. terrestris casts, C content and litter type significantly correlated with cast respiration which declined over time. In L. terrestris burrow walls microbial respiration was significantly higher than in control soil (Tiunov and Scheu 1999); a small but significant proportion of the variation in respiration was explained by soil type (Fagus, Quercus and Tilia forest) and season (Tiunov and Scheu 1999).

Metabolic quotient  $(qCO_2)$  is the ratio of the amount of  $CO_2$  respired per unit microbial biomass and is an index of the relative activity of the microbial biomass. Earthworm activities tend to increase the metabolic quotient (Wolters and Joergensen 1992; Görres et al. 1997; Haynes et al. 1999; Tiunov and Scheu 1999; Zhang et al. 2000), suggesting a switch to a smaller, but more active microbial community. This may relate to the decrease in the fungal:bacterial ratio commonly seen in earthworm-worked soils and thought to result from disruption of fungal hyphal links. Bacteria are less efficient at assimilating C and therefore have a higher  $qCO_2$  than fungi (Adu and Oades 1978; Sakamoto and Oba 1994).

Litter quality affects  $qCO_2$  in *L. terrestris* burrow walls (Tiunov and Scheu 1999). The increase in  $qCO_2$  was relatively small in *Tilia* soil and larger in *Fagus* and *Quercus* soil (Tiunov and Scheu 1999). Other soil characteristics also affect the impact of earthworms on the  $qCO_2$  (Wolters and Joergensen 1992). The stimulation of  $qCO_2$  by *A. caliginosa* declined with increasing acidity, organic C, total N, exchangeable Ca and cation exchange capacity (Wolters and Joergensen 1992), suggesting that stimulation of microbes by earthworms is smaller in nutrient-rich soils.

Earthworm effects on microbial biomass and  $qCO_2$  in pasture soil are similar to results in forest soils given above, indicating a switch to a smaller but more active microbial community (Haynes et al. 1999). In arable soil, with much lower organic and microbial C, microbial biomass and  $qCO_2$  increased significantly (Haynes et al. 1999). Addition of either *A. caliginosa* or *E. foetida* to arable soils amended with organic residues significantly decreased microbial biomass and increased  $qCO_2$  in casts, also indicating a more active microbial community (Haynes et al. 1999; Caravaca and Roldán 2003).

# Respiration and metabolic efficiency—recent earthworm invasion

In general, earthworm activities increase soil respiration and thus earthworm invasions are expected to increase respiration in invaded sites

due to the increase in readily accessible C substrates through the addition of mucus. The underlying assumption is that microbes are C limited, but in newly invaded, C-rich organic layers this may not be true. That microbial respiration declined or was unaffected by invasive earthworms in several North American forests may reflect this. In aspen field microcosms microbial respiration in L/F material decreased and that in H material increased in the presence of D. octaedra (Scheu and Parkinson 1994b). In pine mesocosms representing the initial invasion by D. octaedra, earthworm density did not significantly affect microbial respiration although in field plots representing later stages of invasion, microbial respiration declined with increasing earthworm biomass particularly in the F/H layer (McLean and Parkinson 1997a, b). These data are consistent with microcosm studies indicating that aspen L, F and pine F/H layer microbial respiration was N-limited and therefore would not be enhanced by earthworm activities, whereas aspen H, A and pine L and B layer microbial respiration was C-limited and therefore more likely to be enhanced by earthworm activities (Scheu and Parkinson 1995).

Data from other forests suggest that this may be a general pattern in newly invaded forest soils. In fir, birch or mixed fir/birch forest floor worked by A. trapezoides microbial respiration was not significantly different from control forest floor (Welke and Parkinson 2003). However, respiration was significantly higher in worm-worked birch or fir/birch mineral soil and significantly lower in worm-worked fir mineral soil compared to the respective controls (Welke and Parkinson 2003). In New York maple forests with mixed communities of lumbricids the presence of earthworms did not significantly affect total soil respiration (Fisk et al. 2004). However, the forest floor and mineral soil were not separated in this study obscuring respiration differences between layers in the presence of earthworms.

In contrast, in maple forest plots worked by the invasive earthworm *Amynthas hawayanus*, microbial respiration from the organic horizon was significantly higher than in uninvaded plots (Burtelow et al. 1998). This earthworm species completely consumed the forest floor reducing it to granular cast material. This complete consumption of OM may have reduced microbial activity to the point where accessible C additions enhanced microbial respiration.

In soils invaded by earthworms, one might expect to see increased  $qCO_2$  indicating a switch to a smaller but more active microbial community similar to that seen in situations where the microbial community has evolved in the presence of earthworms. However, it may be that in recently invaded soils, there has not been enough time for the microbial communities to adapt to earthworm activities and therefore the switch to a more active microbial community is not observed. In maple-oak forest soil in Rhode Island, USA, there was evidence of a switch to a smaller, more active microbial community in L. terrestris burrow walls (Görres et al. 1997). In these burrow walls, microbial biomass C was significantly lower and qCO<sub>2</sub> was significantly higher than in control soil (Görres et al. 1997). The earthworm fauna of Rhode Island is completely exotic and these species have been present for at least 30 years (Reynolds 1973, 2002). We suggest that the addition of L. terrestris to a Rhode Island soil resulted in a switch to a smaller, more active microbial community because the microbial communities have had a chance to adapt to the presence of earthworms. A similar result was seen in aspen field microcosms in Alberta, Canada; low microbial biomass and high  $qCO_2$  in L/F material in the presence of D. octaedra suggest a switch to a smaller, more active microbial community in this layer (Scheu and Parkinson 1994b). Earthworms were first observed in this study site in 1984, 8 years before this experiment was conducted. We suggest that 8 years is long enough for the microbial community to adapt to the presence of earthworms.

In contrast, in more recently invaded forests, there is evidence that the microbial community has not yet adapted to the presence of earthworms. In mesocosms, representing the initial stages of the invasion of *D. octaedra* into *Pinus* forest, earthworm density did not significantly affect  $qCO_2$ , while in field plots representing later stages in the initial invasion by *D. octaedra*,  $qCO_2$ declined with increasing earthworm biomass (McLean and Parkinson 1997a, b). The activities

Biol Invasions (2006) 8:1257-1273

of *A. trapezoides* did not affect  $qCO_2$  in forest floor materials (Welke and Parkinson 2003). However,  $qCO_2$  was generally lower in wormworked *Pseudotsuga*, *Betula* or *Pseudotsuga* + *Betula* mineral soil compared to the respective controls (Welke and Parkinson 2003). The decreases in  $qCO_2$  observed in both studies suggest that the microbial community has not been able to switch to a smaller, but more active microbial community and/or that the fungal community remains dominant but is becoming less active in the presence of earthworms.

# C substrate use—co-occurring with earthworms

To our knowledge, there is only one investigation of the impact of earthworms on microbial C substrate use in sites with earthworms. In general, the presence of endogeic and/or epigeic earthworms decreased actual catabolic activity, attributed to decreased resource availability due to consumption by the earthworms (Scheu et al. 2002). The presence of endogeic and/or epigeic earthworms increased potential catabolic activity indicating a shift by the microbial community to more rapidly respond to resource pulses (Scheu et al. 2002). This is congruent with the observation that the presence of earthworms tends to produce a shift to a more active microbial community (see Wolters and Joergensen 1992; Görres et al. 1997; Haynes et al. 1999; Tiunov and Scheu 1999; Zhang et al. 2000). The earthworm species and number of species in an ecological group also affected actual and potential microbial catabolic potentials. Although two earthworm species may be epigeic, differences in gut transit, preference for organic or mineral material may significantly affect microbial C substrate use, emphasizing the importance of earthworm species diversity (Scheu et al. 2002).

### C substrate use-recent earthworm invasion

Data on  $qCO_2$  from recently invaded soils suggest that microbial communities in these soils have not yet developed into the smaller, more active communities typical in the presence of earthworms. If these communities are not yet adapted to the presence of earthworms, one might also reasonably expect that the presence of earthworms would not increase the diversity of C substrates used by these microbes. Contrary to this expectation, results from recently invaded forests in British Columbia, Canada, indicate that the activities (though not the presence) of A. trapezoides increased total potential bacterial activity, substrate richness and diversity (as assessed using BIOLOG plates) compared to control soils (Welke and Parkinson 2003). Similar results were obtained using BIOLOG plates in a field study where endogeic and anecic lumbricids had recently invaded aspen forests in Alberta (Migge 2001). These observations suggest that the microbial community is able to respond rapidly to resource pulses, including those caused by earthworm activities, due to high enzymatic versatility and not necessarily to changes in community structure (Lavelle and Spain 2001; Buckley and Schmidt 2002).

## Enzyme activities—co-occurring with earthworms

Activities of a broad range of enzymes have been reported from earthworm guts, including cellulase, chitinase, phosphatase, protease, xylanase, mannanase, urease, dehydrogenase, and various glucosidases (Khambata and Bhat 1957; Parle 1963b; Márialigeti 1979; Zhang et al; 1993, 2000; Mba 1997; Lattaud et al. 1998; Haynes et al. 1999; Makulec 2002; Prat et al. 2002; Caravaca and Roldán 2003).

There has been much discussion about whether these enzymes are of microbial or earthworm origin. Enzyme analysis of earthworm gut contents and gut wall tissue culture enabled Zhang et al. (1993) to differentiate enzymes produced by gut microbes from those produced by the tropical endogeic earthworm P. corethrurus. Gut (gut wall + contents) activities included N-acetylglucosaminidase, maltase, laminaribiase, cellulase, as well as a range of polysaccharides (including amylase, xylanase, glucomannanase, carboxymethylcellulase, and galactomannanase etc.) (Zhang et al. 1993). Tissue culture of the washed gut wall revealed a similar range of enzyme activities with the exception of cellulase and mannanase (Zhang et al. 1993). Of three tropical endogeic earthworms, gut wall tissue cultures showed that *Polypheretima elongata* was able to synthesize all its required enzymes while *Millsonia anomala* relied on microbes for cellulose and mannan digestion (Lattaud et al. 1998).

Different earthworm species have different ranges of enzymatic capabilities depending on their normal substrates: epigeic earthworms tend to possess higher cellulase activities than anecic or endogeic earthworms, reflecting relatively undecomposed organic matter as their primary food resource (e.g. Urbasek 1990; Zhang et al. 2000; Prat et al. 2002). High chitinase activity is thought to indicate the importance of fungi as a food resource (Zhang et al. 1993). Gut enzyme capabilities also differ between species within an ecological group, for example between three tropical endogeic earthworms (Lattaud et al. 1998). High amylase and maltase specific activities in P. elongata guts suggest that this species feeds mainly on roots, while glucosidic activities in M. anomala indicate that this species can degrade fungal cell walls and roots (Lattaud et al. 1998).

Gut enzyme activities differ both between species and in location of major activity within the gut (Zhang et al. 1993; Lattaud et al. 1998; Prat et al. 2002). In *P. corethrurus* and *M. kerguelensis* the majority of the activity was observed in the foregut and midgut, while in *D. rubidus tenuis*, the highest enzyme activities occurred in the anterior tract (Zhang et al. 1993; Prat et al. 2002).

# Enzyme activities—recent earthworm invasion

To our knowledge, there are no studies on gut enzymes in earthworms invading areas historically devoid of earthworms. Earthworms invading new areas are expected to carry with them those microbes that are a permanent component of their guts. These, presumably, would be the producers of essential enzymes for degradation of their normal substrates. Transient gut microbes (and their enzymatic capabilities) are expected to vary between habitats and with differences in ingested substrates.

### Conclusions

It is not yet clear whether or which earthworm gut microbes are permanent gut residents or merely transients reflecting the microbial community on consumed substrates. To resolve this, comparisons of the gut microbes of the same species ingesting the same or different litters are needed. Further, comparisons across habitats where earthworms and microbes have co-occurred for years with those where earthworms have recently invaded would clarify to what extent invading earthworms carry their original gut microbes with them (also see Hendrix et al., this issue).

Earthworm interactions with bacteria and microfungi have been studied more thoroughly than those with other microbes. However, although it is commonly assumed that the negative impacts of earthworm invasions on fungi are due to hyphal disruption, this has yet to be tested convincingly. Studies are particularly needed for Zygomycetes which appear to experience more severe negative impacts from hyphal disruption.

Despite the importance of actinomycete antibiotic production which may have negative impacts on other soil microbes, and the importance of mycorrhizae for plant growth, remarkably little is known about the interactions of either of these groups with earthworms. Both groups need more thorough study in habitats where they co-occur with earthworms and in recently invaded ones. Additionally, mycorrhizal studies should include a range of mycorrhizal types (AM, ectomycorrhizae, ericaceous, orchidaceous) to allow predictions of the probable impact of earthworm invasions on different plant species.

Earthworms tend to increase microbial biomass and respiration in low-C soils and decrease them in high-C soils. This becomes particularly important when considering the effects of earthworm invasions in forest soils, which typically have high organic matter contents in the forest floor. Studies of the effects of earthworm invasion in low-C soils would provide an interesting test of the importance of soil C status.

From the studies available, it appears that the microbial community adapts to the presence of earthworms by switching to a smaller, but more active microbial community. Long-term studies of the invasion of earthworms could assess whether or how quickly the various components of the microbial community adapt to the presence of earthworms.

There is evidence that earthworm gut enzymes reflect their primary food substrates. Further studies are needed to determine whether these are (i) of earthworm or microbial origin, (ii) independent of food substrates and (iii) carried with the earthworms as they invade a new environment.

Acknowledgments We thank Peter Groffman, Institute of Ecosystem Studies, and an anonymous reviewer whose comments improved the manuscript.

#### References

- Adu JK, Oades JM (1978) Utilization of organic materials in soil aggregates by bacteria and fungi. Soil Biol Biochem 10:117–122
- Alban DH, Berrry EC (1994) Effects of earthworm invasion on morphology, carbon and nitrogen of a forest soil. Appl Soil Ecol 1:243–249
- Altas RM, Bartha R (1998) Microbial ecology: fundamentals and applications. Addison Wesley Longman, Menlo Park, pp 694
- Bohlen PJ, Groffman, PM, Fahey TJ, Fisk MC, Suárez E, Pelletier DM, Fahey RT (2004a) Ecosystem consequences of exotic earthworm invasion of north temperate forests. Ecosystems 7:1–12
- Bohlen PJ, Pelletier DM, Groffman PM, Fahey TJ, Fisk MC (2004b) Influence of earthworm invasion on redistribution and retention of soil carbon and nitrogen in northern temperate forests. Ecosystems 7:13–27
- Brady NC, Weil RR (2002) The nature and properties of soils, 13th edn. Prentice-Hall, Upper Saddle River, NJ, pp 960
- Brown GG, Barois I, Lavelle P (2000) Regulation of soil organic matter dynamics and microbial activity in the drilosphere and the role of interactions with other edaphic functional domains. Euro J Soil Biol 36:177–198
- Buckley DH, Schmidt TM (2002) Exploring the biodiversity of soil—a microbial rain forest. In: Staley JT, Reysenback A-L (eds) Biodiversity of microbial life. Wiley-Liss, New York, pp 183–208
- Burtelow AE, Bohlen PJ, Groffman PM (1998) Influence of exotic earthworm invasion on soil organic matter, microbial biomass and denitrification potential in forest soils of the northeastern United States. Appl Soil Ecol 9:197–202
- Caravaca F, Roldán A (2003) Effect of Eisenia foetida earthworms on mineralization kinetics, microbial biomass, enzyme activities, respiration and labile C fractions of three soils treated with a composted organic residue. Biol Fertil Soils 38:45–51

- Carlile MJ, Watkinson SC, Gooday GW (2001) The fungi. Academic Press, San Diego, pp 588
- Coleman DC, Crossley DA (1996) Fundamentals of soil ecology. Academic Press, San Diego, pp 205
- Daane LL, Molina JAE, Sadowsky MJ (1997) Plasmid transfer between spatially separated donor and recipient bacteria in earthworm-containing soil microcosms. Appl Environ Microbiol 63:679–686
- Dash MC, Mishra PC, Behera N (1979) Fungal feeding by a tropical earthworm. Trop Ecol 20:9–12
- Davey ME, O'Toole GA (2000) Microbial biofilms: from ecology to molecular genetics. Microbiol Mol Biol Rev 64:847–867
- Doube BM, Ryder MH, Davoren CW, Meyer T (1995) Earthworms: a down-under delivery service for biocontrol agents of root disease. Acta Zool Fennica 196:219–223
- Edwards CA, Bohlen PJ (1996) Biology and Ecology of Earthworms. Chapman and Hall, London, pp 426
- Edwards CA, Fletcher KE (1988) Interactions between earthworms and microorganisms in organic matter breakdown. Agricul Ecosys Environ 24:235–247
- Fisk MC, Fahey TJ, Groffman PM, Bohlen PJ (2004) Earthworm invasion, fine-root distributions, and soil respiration in north temperate forests. Ecosystems 7:55–62
- Gange AC (1993) Translocation of mycorrhizal fungi by earthworms during early succession. Soil Biol Biochem 25:1021–1026
- Görres JH, Savin MC, Amador JA (1997) Dynamics of carbon and nitrogen mineralization, microbial biomass, and nematode abundance within and outside the burrow walls of anecic earthworms (*Lumbricus terrestris*). Soil Sci 162:666–671
- Groffman PM, Bohlen PJ, Fisk MC, Fahey TJ (2004) Exotic earthworm invasion and microbial biomass in temperate forest soils. Ecosystems 7:45–54
- Gunnarsson T, Sundin P, Tunlid A (1988) Importance of leaf litter fragmentation for bacterial growth. Oikos 52:303–308
- Haimi J, Huhta V (1990) Effects of earthworms on decomposition processes in raw humus forest soil: a microcosm study. Biol Fertil Soils 10:178–183
- Hale CM (2004) Ecological consequences of exotic invaders: interactions involving European earthworms and native plant communities in hardwood forests, PhD Dissertation, University of Minnesota. St. Paul, MN
- Harinikumar KM, Bagyaraj DJ (1994) Potential of earthworms, ants, millipedes and termites for dissemination of vesicular-arbuscular mycorrhizal fungi in soil. Biol Fertil Soils 18:115–118
- Haynes RJ, Fraser PM, Tregurtha RJ, Piercy JE (1999) Size and activity of the microbial biomass and N, S and P availability in earthworm casts derived from arable and pasture soil and arable soil amended with plant residues. Pedobiologia 43:568–573
- Hutchinson SA, Kamel M (1956) The effect of earthworms on the dispersal of soil fungi. J Soil Sci 7:213–218
- Jones MD, Durall DM, Harniman SMK, Classen DC, Simard SW (1997) Ectomycorrhizal diversity on Betula papyrifera and Pseudotsuga menziesii seedlings

grown in the greenhouse or outplanted in single-species and mixed plots in southern British Columbia. Can J Forest Res 27:1872–1889

- Karsten GR, Drake HL (1997) Denitrifying bacteria in the earthworm gastrointestinal tract and in vivo emission of nitrous oxide (N<sub>2</sub>O) by earthworms. Appl Environ Microbiol 63:1878–1882
- Khambata SR, Bhat JV (1957) A contribution to the study of the intestinal microflora of Indian earthworms. Archiv für Mikrobiologie 28:69–80
- Kristufek V, Pizl V, Szabó IM (1990) Composition of the intestinal Streptomycete community of earthworms (Lumbricidae). In: Lésel R (ed) Microbiology in poicilotherms. Elsevier Science Publishers, pp 137–140
- Kristufek V, Ravasz K, Pizl V (1992) Changes in densities of bacteria and microfungi during gut transit in *Lumbricus rubellus* and *Aporrectodea caliginosa* (Oligochaeta: Lumbricidae). Soil Biol Biochem 24:1499–1500
- Kristufek V, Ravasz K, Pizl V (1993) Actinomycete communities in earthworm guts and surrounding soil. Pedobiologia 37:379–384
- Kristufek V, Tajovský K, Pizl V (1994) Ultrastructural analysis of the intestinal content of earthworm *Lumbricus rubellus* Hoffm. (Annelida, Lumbricidae) Acta Microbiologica et Immunologica Hungarica 41:283– 290
- Lattaud C, Locati S, Mora P, Rouland C, Lavelle P (1998) The diversity of digestive systems in tropical geophagous earthworms. Appl Soil Ecol 9:189–195
- Lavelle P, Spain AV (2001) Soil Ecology. Kluwer Academic Publishers, Dordrecht, pp 654
- Lawrence B, Fisk MC, Fahey TJ, Suárez ER (2003) Influence of nonnative earthworms on mycorrhizal colonization of sugar maple (*Acer saccharum*). New Phytol 157:145–153
- Makulec G (2002) The role of *Lumbricus rubellus* Hoffm. in determining biotic and abiotic properties of peat soils. Polish J Ecol 50:301–339
- Márialigeti K (1979) On the community structure of the gut microbiota of *Eisenia lucens* (Annelida, Oligo-chaeta). Pedobiologia 19:213–220
- Mba C (1997) Rock phosphate solubilizing *Streptosporangium* isolates from casts of tropical earthworms. Soil Biol Biochem 29:381–385
- McLean MA, Parkinson D (1997a) Changes in structure, organic matter and microbial activity in pine forest soil following the introduction of *Dendrobaena octaedra* (Oligochaeta, Lumbricidae). Soil Biol Biochem 29:537–540
- McLean MA, Parkinson D (1997b) Soil impacts of the epigeic earthworm *Dendrobaena octaedra* on organic matter and microbial activity in lodgepole pine forest soil. Can J Forest Res 27:1907–1913
- McLean MA, Parkinson D (1998) Impacts of the epigeic earthworm *Dendrobaena octaedra* on microfungal community structure in pine forest floor: a mesocosm study. Appl Soil Ecol 8:61–75
- McLean MA, Parkinson D (2000) Field evidence of the effects of the epigeic earthworm *Dendrobaena octae-dra* on the microfungal community in pine forest floor. Soil Biol Biochem 32:351–360

- Migge S. (2001) The effect of earthworm invasion on nutrient turnover, microorganisms and microarthropods in Canadian aspen forest soil. Ph.D. Thesis, Technische Universität Darmstadt. Cuvillier Verlag, Göttingen
- Moody SA, Piearce TG, Dighton J (1996) Fate of some fungal spores associated with wheat straw decomposition on passage through the guts of *Lumbricus terrestris* and *Aporrectodea longa*. Soil Biol Biochem 28:533–537
- Orazova MK, Semenova TA, Tiunov AV (2003) The microfungal community of *Lumbricus terrestris* middens in a linden (*Tilia cordata*) forest. Pedobiologia 47:27–32
- Parle JN (1963a) A microbiological study of earthworm casts. Journal of general Microbiology 31:13–22
- Parle JN (1963b) Micro-organisms in the intestines of earthworms. J Gen Microbiol 31:1–11
- Patron JC, Sanchez P, Brown GG, Brossard M, Barois I, Gutiérrez C (1999) Phosphorus in soil and *Brachialis decumbens* plants as affected by the geophagous earthworm *Pontoscolex corethrurus* and P fertilization. Pedobiologia 43:547–556
- Pattinson GS, Smith SE, Doube BM (1997) Earthworm Aporrectodea trapezoides had no effect on the dispersal of a vesicular-arbuscular mycorrhizal fungi, Globus intraradices. Soil Biol Biochem 29:1079–1088
- Pedersen JC, Hendriksen NB (1993) Effect of passage through the intestinal tract of detritivore earthworms (*Lumbricus* spp.) on the number of selected Gramnegative and total bacteria. Biol Fertil Soil 16:227–232
- Ponge JC (1991) Food resources and diets of soil animals in a small area of Scots pine litter. Geoderma 49:33–62
- Prat P, Charrier M, Deleporte S, Frenot Y (2002) Digestive carbohydrases in two epigeic earthworm species of the Kerguelen Islands (Subantarctic). Pedobiologia 46:417–427
- Reddell P, Spain AV (1991) Earthworms as vectors of viable propagules of mycorrhizal fungi. Soil Biol Biochem 23:767–774
- Reynolds JW (1973) The earthworms of Rhode Island (Oligochaeta: Lumbricidae). Megadrilogica 1:1–4
- Reynolds JW (2002) Additional earthworm (Oligochaeta: Lumbricidae and Megascolescidae) records from Rhode Island, USA. Megadrilogica 9:21–27
- Saetre P (1998) Decomposition, microbial community structure, and earthworm effects along a birch-spruce soil gradient. Ecology 79:834–846
- Sakamoto K, Oba Y (1994) Effect of fungal to bacterial biomass ratio on the relationship between CO<sub>2</sub> evolution and total soil microbial biomass. Biol Fertil Soils 17:39–44
- Scheu S (1987) Microbial activity and nutrient dynamics in earthworm casts (Lumbricidae). Biol Fertil Soils 5:230–234
- Scheu S (1991) Mucus excretion and carbon turnover of endogeic earthworms. Biol Fertil Soil 12:217–220
- Scheu S, Parkinson D (1994a) Effects of earthworms on nutrient dynamics, carbon turnover and microorganisms in soils from cool temperate forests of the Canadian Rocky Mountains—laboratory studies. Appl Soil Ecol 1:113–125

- Scheu S, Parkinson D (1994b) Effects of invasion of an aspen forest (Canada) by *Dendrobaena octaedra* (Lumbricidae) on plant growth. Ecology 75:2348– 2361
- Scheu S, Parkinson D (1995) Successional changes in microbial biomass, respiration and nutrient status during litter decomposition in an aspen and pine forest. Biol Fertil Soils 19:327–332
- Scheu S, Schlitt N, Tiunov AV, Newington JE, Jones TH (2002) Effects of the presence and community composition of earthworms on microbial community functioning. Oecologia 133:254–260
- Shankar SG, Ranganathan S, Ranjith MS, Vijayalakshmi GS (2002) Did earthworms contribute to the parasitic evolution of dermatophytes? Mycoses 45:399–401
- Shaw C, Pawluk S (1986) Faecal microbiology of Octolasion tyrtaeum, Aporrectodea turgida and Lumbricus terrestris and its relation to the carbon budgets of three artificial soils. Pedobiologia 29:377–389
- Subler S, Kirsch AS (1998) Spring dynamics of soil carbon, nitrogen, and microbial activity in earthworm middens in a no-till corn field. Biol Fertil Soils 26:243–249
- Swift MJ, Heal OW, Anderson JM (1979) Decomposition in terrestrial ecosystems. University of California Press, Berkeley, pp 372
- Thorpe IS, Killham K, Prosser JI, Glover LA (1993) Novel method for the study of the population dynamics of a genetically modified microorganism in the gut of the earth worm *Lumbricus terrestris*. Biol Fert Soils 15:55–59
- Tiunov AV, Scheu S (1999) Microbial respiration, biomass, biovolume and nutrient status in burrow walls of *Lumbricus terrestris* L. (Lumbricidae). Soil Biol Biochem 31:2039–2048
- Tiunov AV, Scheu S (2000a) Microbial biomass, biovolume and respiration in *Lumbricus terrestris* L. cast material of different age. Soil Biol Biochem 32:265–275
- Tiunov AV, Scheu S (2000b) Microfungal communities in soil, litter and casts of *Lumbricus terrestris* L. (Lumbricidae): a laboratory experiment. Appl Soil Ecol 14:17–26
- Tiunov AV, Bonkowski M, Alphei J, Scheu S (2001) Microflora, Protozoa and Nematoda in *Lumbricus*

*terrestris* burrow walls: a laboratory experiment. Pedobiologia 45:46–60

- Tiwari SC, Mishra RR (1993) Fungal abundance and diversity in earthworm casts and in uningested soil. Biol Fertil Soil 16:131–134
- Toyota K, Kimura M (2000) Microbial community indigenous to the earthworm *Eisenia foetida*. Biol Fertil Soils 31:187–190
- Tuffen F, Eason WR, Scullion J (2002) The effect of earthworms and arbuscular mycorrhizal fungi on growth of and <sup>32</sup>P transfer between Allium porrum plants. Soil Biol Biochem 34:11027–1036
- Urbášek F (1990) Cellulase activity in the gut of some earthworms. Revue d'Éologie et du Biologie du Sol 27:21–28
- Welke SE, Parkinson D (2003) Effect of Aporrectodea trapezoides activity on seedling growth of Pseudotsuga menziesii, nutrient dynamics and microbial activity in different soils. Forest Ecol Manag 173:169–186
- Whitman WB, Coleman DC, Wiebe WJ (1998) Prokaryotes: the unseen majority. Proc Natl Acad Sci, USA 95:6578–6583
- Wolter C, Scheu S (1999) Changes in bacterial numbers and hyphal lengths during the gut passage through *Lumbricus terrestris* (Lumbricidae, Oligochaeta). Pedobiologia 43:891–900
- Wolters V, Joergensen RG (1992) Microbial carbon turnover in beech forest soils worked by *Aporrectodea caliginosa* (Savigny) (Oligochaeta: Lumbricidae). Soil Biol Biochem 24:171–177
- Wurst S, Dugassa-Gobena D, Langel R, Bonkowski M, Scheu S (2004) Combined effects of earthworms and vesicular-arbuscular mycorrhizas on plant and aphid performance. New Phytol 163:169–173
- Zhang BG, Rouland C, Lattaud C, Lavelle P (1993) Activity and origin of digestive enzymes in gut of the tropical earthworm *Pontoscolex corethrurus*. Euro J Soil Biol 29:7–11
- Zhang B-G, Li G-T, Shen T-S, Wang J-K, Sun Z (2000) Changes in microbial biomass C, N and P and enzyme activities in soil incubated with the earthworms *Metaphire guillelmi* and *Eisenia fetida*. Soil Biol Biochem 32:2055–2062