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The wave towards a new steady state: effects of earthworm invasion on soil microbial functions

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Abstract Earthworms are ecosystem engineers that cause a long cascade of ecological effects when they invade previously earthworm-free forests. However, the consequences of earthworm invasion for soil microbial functions are poorly understood. Here, we used two well-studied invasion fronts of European earthworms in northern North American hardwood forests previously devoid of earthworms in order to investigate three stages of earthworm invasion: uninvaded, the front of the leading edge of earthworm invasion and locations invaded at least 10 years previously. Soil microbial biomass, respiration and metabolic quotient were measured. Earthworms had marked effects on soil microbial biomass (−42%) and respiration (−32%). At both sites, impacts were most pronounced at the leading edge of the invasion front, significantly decreasing soil microbial C use efficiency. This was most likely due to the disturbance of the soil microbial community caused by water stress. Based on these results, we hypothesize that effects of earthworm invasion on native soil

ecosystem functioning are most pronounced at the peak of the invasion wave. After experiencing this wave, ecosystems possibly enter a new steady state with altered biotic compositions and functions.

Keywords Ecosystem change · Exotic earthworms · Forest floor · Microbial biomass · Microbial respiration · Northern hardwood forests

Introduction

Anthropogenic biotic exchange threatens biodiversity and can compromise the functioning of ecosystems (e.g. Sala et al. 2000). Despite a considerable bias in favour of attention to aboveground invasions, the spread of exotic earthworms has received increasing notice in the last two decades (Bohlen et al. 2004; Hendrix et al. 2008). Earthworms are ecosystem engineers that cause a long cascade of ecological effects when they invade previously earthworm-free forests, including changes in soil nutrient status, soil bulk density, soil microbial processes, and reduction of native plant and soil microarthropod species richness (Frelich et al. 2006; Eisenhauer et al. 2007; Hendrix et al. 2008). The notable impacts of exotic earthworms on the nutrient cycling and functioning of native ecosystems (Burtelov et al. 1998; Bohlen et al. 2004; Groffman et al. 2004) have evoked concern about the consequences for carbon dynamics (Eisenhauer et al. 2007; Huang et al. 2010). Although understanding of

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effects of exotic earthworms on microbial community composition and activity is crucial for predicting future changes in carbon and nutrient dynamics (Huang et al. 2010), this topic has been insufficiently explored. Exotic earthworms have been shown to either decrease (e.g. Eisenhauer et al. 2007) or increase (e.g. Li et al. 2002) soil microbial biomass and activity. Reviewing the previous literature on exotic earthworm effects on soil microbes, McLean et al. (2006) concluded that earthworms change microbial community composition towards a smaller, but more active microbial community. They moreover hypothesized that impacts of exotic earthworms on soil microbes depend on the stage of invasion: initial decreases in soil microbial respiration and metabolic quotient may be followed by a decrease in microbial C use efficiency. Similarly, Straube et al. (2009) found nonlinear effects of exotic earthworms on soil microbial biomass and microarthropods, reinforcing the need for long-term studies. They posited a wavelike invasion pattern with most severe impacts in newly invaded habitats.

Here, we investigate two well-studied invasion fronts of European earthworms in northern hardwood forests in North America previously devoid of earthworms (Hale et al. 2005; Holdsworth et al. 2007). Transects perpendicular to the leading edges of earthworm invasion allowed us to discriminate between three invasion stages: uninvaded, the front of the leading edge, and invaded. Soil microbial biomass, respiration and metabolic quotient were determined as proxy measures for soil microbial functioning. In accordance with McLean et al. (2006) and Straube et al. (2009), we expected earthworm effects to be most pronounced at the leading edge of earthworm invasion.

Materials and methods

The two sites were located 250 km apart in northern Minnesota (Chippewa National Forest) and Wisconsin (Chequamegon section of Chequamegon-Nicolet National Forest), USA, a region devoid of native earthworms. Both study sites were covered with mesic forests approximately 80–100 years old after logging in the early 1900s, and were dominated by sugar maple (*Acer saccharum*) on deep, well drained, light colored sandy loams (Eutroboralfs on the Minnesota site and Fragiorthods and Haplorthods in

Wisconsin). The climate is humid continental, cold temperate: annual precipitation 650 and 870 mm, mean January and July temperatures -15 and $+20^{\circ}\text{C}$ and -12 and $+20^{\circ}\text{C}$ at the Minnesota and Wisconsin sites, respectively. The two study sites were used in previously published studies of European earthworm invasion impacts on native plant community structure, and were chosen to include the leading edge of earthworm invasion (Hale et al. 2005, 2006; Holdsworth et al. 2007). At the Minnesota site (on the Ottertail peninsula at Leach Lake, named “Section 19”) three parallel transects 10 m apart and 150 m in length, had been placed perpendicular to the leading edge of earthworm invasion in 1999 (Hale et al. 2005). For the present study, one of these transects was used. As the visible leading edge of earthworm invasion had further advanced, the transect was extended to a total length of 390 m (at 443–449 m a.s.l.; front of leading edge at $47^{\circ}16'0.00''\text{N}$, $94^{\circ}23'48.60''\text{W}$). At the Wisconsin site (at Tower Lake in the Rainbow Lake Wilderness Area) one transect of 400 m length had been placed in 2001 (Holdsworth et al. 2007). As the state of the litter layer indicated that at least the epigeic earthworm species *Dendrobaena octaedra* had penetrated substantially farther into the forest stand, the transect was extended to a total length of 700 m for the present study (at 380–405 m a.s.l.; front of leading edge at $46^{\circ}26'3.06''\text{N}$, $91^{\circ}19'36.00''\text{W}$). According to Hale et al. (2005) and Holdsworth et al. (2007), areas with thick organic horizons and a light brown A horizon were characterized by no earthworms or a small biomass (ca. 1 g m^{-2} ash-free dry mass, AFDM) of *D. octaedra*, whereas the middle areas of the transects, near the visible front of the leading edge of invasion, with thin organic horizons, had a moderate biomass (ca. $1\text{--}3\text{ g m}^{-2}$ AFDM) of *Lumbricus rubellus*, *Aporrectodea* spp., and *Octolasion tyrtaeum*, while heavily invaded areas (with organic horizons absent at mid-summer) and black A horizons were characterized by the presence of high earthworm biomass (ca. $3\text{--}5\text{ g m}^{-2}$ AFDM) of *Lumbricus terrestris*, *L. rubellus* and *Aporrectodea* spp. The latter have been invaded at least 10 years previously.

The sites were sampled on September 24 (Minnesota) and October 3, 2010 (Wisconsin). Along the transects, three plots ($10\text{ m} \times 4\text{ m}$ oriented perpendicular to the transect) were sampled, representing virtually uninvaded, front of the leading edge of

invasion, and heavily invaded areas. The structure of the upper soil horizons and earthworm presence in larger soil cores taken in parallel within these plots (to sample mesofauna) confirmed our expectations concerning the stage of earthworm invasion. For the present study, 12 randomly situated soil cores were taken per plot to a depth of ca. 9 cm using a steel corer, including organic layers (2 cm diameter, 3.14 cm² surface area). The thickness of different soil layers varied between the three stages of invasion with thin organic layers at invaded locations. Each soil core (72 in total; 2 sites × 3 stages of invasion × 12 replicates) was stored separately in a closed plastic bag, transported to the laboratory, sieved (<2 mm) and stored frozen at -20°C until further processing. Microbial biomass [$\mu\text{g C}_{\text{mic}} \text{g}^{-1}$ soil dry weight], basal respiration [$\mu\text{l O}_2 \text{h}^{-1} \text{g}^{-1}$ soil dw], and metabolic quotient [$\mu\text{l O}_2 \text{h}^{-1} \text{mg}^{-1} \text{C}_{\text{mic}}$] were measured using an O₂ microcompensation apparatus (Anderson and Domsch 1978; Scheu 1992). Microbial respiration was measured at hourly intervals for 20 h at 22°C. Basal respiration was determined without addition of substrate; C_{mic} was calculated from the respiratory response to D-glucose (substrate-induced respiration method; Anderson and Domsch 1978). The average of the lowest three readings within the first 10 h was taken as “maximum initial respiratory response” (MIRR) and C_{mic} was calculated as 38 × MIRR ($\mu\text{l O}_2 \text{h}^{-1} \text{g}^{-1}$ soil dw; Beck et al. 1997). Glucose was added in appropriate amounts to saturate the catabolic enzymes of the microorganisms (20 mg g⁻¹ soil dw). Despite some criticism (Wardle and Ghani 1995), the metabolic quotient is regarded as an indicator of change in microbial metabolism in response to disturbance, i.e. microbial C use efficiency (Anderson and Domsch 1985). Gravimetric soil water content was determined for each sample by comparing soil fresh and dry weight ([%]; dried for 3 days at 70°C).

Effects of site and stage of earthworm invasion (uninvaded, leading edge, invaded) on soil microbial biomass, basal respiration, metabolic quotient and gravimetric soil water content were analyzed by two-factorial Analysis of Variance (ANOVA). Data on gravimetric soil water content were arc-sin-transformed to meet the requirements of ANOVA (normality and homoscedasticity of errors). Means (\pm SD) presented in text and figures were calculated using non-transformed data. Means were compared using

Tukey's HSD test ($\alpha = 0.05$). Additionally, regressions were performed between soil microbial functions and soil water content. All analyses were performed using STATISTICA 7 (Statsoft).

Results

Microbial biomass ($759.6 \pm 348.6 \mu\text{g C}_{\text{mic}} \text{g}^{-1}$ soil dw) was significantly affected by earthworm invasion status: microbial biomass was higher at uninvaded locations than at invaded locations (+57%) and at the leading edge of earthworm invasion (+89%; $F_{2,62} = 23.11$, $P < 0.001$). This pattern was more pronounced at the Minnesota site, resulting in a significant interaction between site and invasion stage ($F_{2,62} = 3.18$, $P = 0.048$; Fig. 1a). Basal respiration ($2.97 \pm 1.28 \mu\text{l O}_2 \text{h}^{-1} \text{g}^{-1}$ soil dw) was significantly higher at uninvaded locations than at invaded locations (+48%) and at the leading edge of earthworm invasion (+54%; $F_{2,62} = 10.10$, $P < 0.001$). This pattern was consistent in both forests (invasion stage × forest: $F_{2,62} = 0.44$, $P = 0.65$), though only significant at the Minnesota site (Fig. 1b). The metabolic quotient ($4.12 \pm 1.12 \mu\text{l O}_2 \text{h}^{-1} \text{mg}^{-1} \text{C}_{\text{mic}}$) was significantly higher at the leading edge of earthworm invasion than in uninvaded (+20%) and invaded areas (+16%; $F_{2,62} = 6.22$, $P = 0.003$). Similar to basal respiration, this pattern was similar in both forests (invasion stage × forest: $F_{2,62} = 1.52$, $P = 0.22$), though only significant at the Minnesota site (Fig. 1c). Gravimetric soil water content was significantly higher at uninvaded (+33%) and invaded locations (+30%) than at the leading edge of earthworm invasion ($F_{2,62} = 17.63$, $P < 0.001$). This pattern was more pronounced at the Wisconsin site, resulting in a significant interaction between site and invasion stage ($F_{2,62} = 12.42$, $P < 0.001$). Gravimetric soil water content was positively correlated with soil microbial biomass ($R^2 = 0.41$, $P < 0.001$) and basal respiration ($R^2 = 0.12$, $P = 0.005$), while it was negatively correlated with the metabolic quotient ($R^2 = 0.29$, $P < 0.001$; Fig. 2).

Discussion

Our results confirm previous studies showing that exotic earthworms significantly change soil microbial

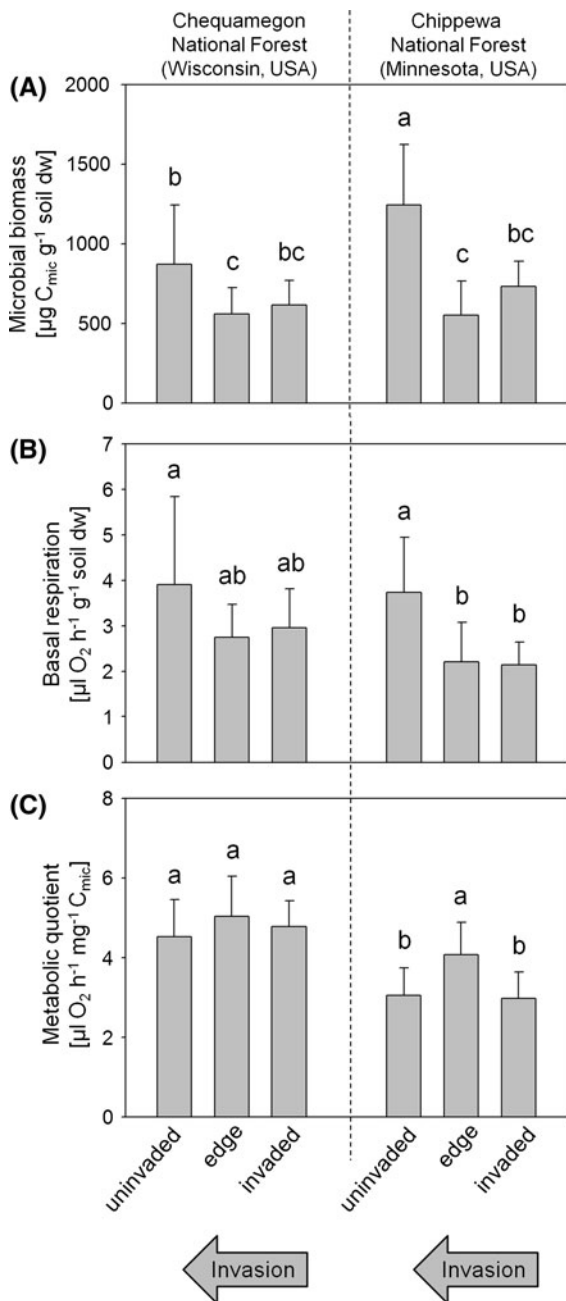


Fig. 1 Soil microbial biomass (a), basal respiration (b), and metabolic quotient (c) as affected by earthworm invasion (uninvaded, front of the leading edge of earthworm invasion, invaded) at the Wisconsin and Minnesota sites, respectively. Bars with varying letters differ significantly (Tukey's HSD test; $\alpha = 0.05$). Means with standard deviation

biomass and functions (Burtelow et al. 1998; Li et al. 2002; McLean et al. 2006; Eisenhauer et al. 2007). The present study moreover indicates that impacts are

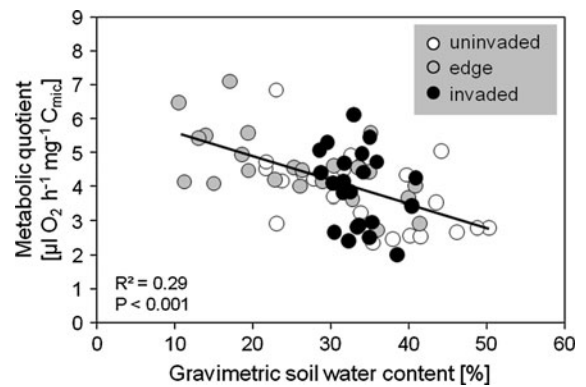


Fig. 2 Regression between gravimetric soil water content and metabolic quotient at the Minnesota and Wisconsin sites (pooled) differentiating locations without earthworms (uninvaded), the front of the leading edge (edge) and invaded locations (invaded)

most pronounced at the peak of the invasion wave, significantly decreasing soil microbial respiration and C use efficiency, and confirming our hypothesis. In line with the expectations of McLean et al. (2006), microbial biomass and respiration decreased significantly due to earthworm invasion. However, and in contrast to their expectations, the metabolic quotient was highest at the leading edge of earthworm invasion, suggesting disturbance of the soil microbial community, and declined at invaded locations to the level of uninvaded conditions. This pattern supports the findings of Eisenhauer et al. (2007) and Straube et al. (2009), showing the most pronounced impacts of invasive earthworms at the peak of the invasion wave. Although the metabolic quotient returned to the pre-invasion level at the invaded locations, the significant decrease in microbial biomass and respiration indicates a shift in microbial community composition. McLean et al. (2006) assumed a switch to a smaller, but more active microbial community, due to a decrease in the fungal:bacterial ratio. Earthworms may promote the dominance of more disturbance-resistant bacteria and reduce the biomass and diversity of fungi by disrupting hyphal networks of fungi (McLean et al. 2006). Bacteria have a lower C utilization efficiency than fungi, i.e. a higher metabolic quotient (Sakamoto and Oba 1994).

We found that the gravimetric soil water content was lowest at the leading edge of invasion, most likely due to soil compaction and burrow formation by earthworms (Hale et al. 2005). Earthworm burrows

are known to function as preferential flow pathways for soil surface water and to change soil water holding capacity (Edwards and Bohlen 1996; Shipitalo et al. 2004). Particularly *L. rubellus* may have played a crucial role in affecting soil water content since this species characterized the leading edge of the invasion front. Since soil microorganisms strongly depend on the availability of water (e.g. Coleman et al. 2004), the decrease in soil water content may have represented a major disturbance. This was supported by a significant negative correlation between soil water content and the metabolic quotient in the present study. The fact that patterns were similar at both sites suggests that our findings may be general.

Moreover, exotic earthworms mix dead organic matter with mineral soil, thereby mostly decreasing soil microbial biomass in the upper soil layers but increasing it in lower soil layers (McLean et al. 2006), and are stronger competitors for organic material than microbes (Tiunov and Scheu 1999). Earthworm effects observed in the present study may be partly related to changes in the structure of the soil profile with a decreased thickness of organic layers at invaded locations (down to virtually bare mineral soil at the heavily invaded sites). However, impacts of earthworm invasion on soil microbes may be more complex, depending on environmental conditions (Huang et al. 2010) and the earthworm ecological groups involved (Eisenhauer 2010).

Further, earthworms are known to change herbaceous plant community composition (Gundale 2002; Gilliam 2006), favoring graminoids at the investigated sites (Hale et al. 2006; Holdsworth et al. 2007), which may indirectly alter soil microbial communities. The investigation of shifts in soil microbial structure due to direct and indirect effects of the invasion of different earthworm species is a promising research direction that would lead to a better understanding of long-term effects of earthworm invasion on soil microbial functions.

The results of this study suggest that effects of earthworm invasion on native ecosystem functioning are most pronounced at the peak of the invasion wave. After experiencing this wave, ecosystems may enter a new steady state with altered biotic compositions and functions.

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