ORIGINAL PAPER

Influence of clay content and acidity of soil on development of the earthworm *Lumbricus rubellus* and its population level consequences

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Abstract In this paper we report on the influence of clay content and acidity of soil on growth and reproduction of the epigeic earthworm species *Lumbricus rubellus* (Hoffm.), which is common in most temperate soils and abundant in grasslands. Growth, cocoon production and survival of *L. rubellus* were tested in 12 Dutch soils which differed in soil properties. A matrix model was used to assess the population-level consequences of changes in growth and reproduction. Soil acidity had a strong negative effect on earthworm survival, and the maturation weight decreased with clay content. Individual weight gain in *L. rubellus* decreased with both acidity and clay content. The acidity of soils had a larger influence on population growth rate than the clay content. The acidity of the soil also changed the population composition towards younger age classes, whereas in soils rich in clay, the population composition did not change. The average individual weight of *L. rubellus* in clayey soils, however, was lower compared with that in soils low in clay, a result that agrees with literature data.

Keywords *Lumbricus rubellus* · Soil properties · Maturation weight · Population growth rate · Population model

Introduction

Earthworms form the largest part of the animal biomass in most temperate soils (Lavelle and Spain 2001; Paoletti 1999). They play an important role in soil fertility (Edwards

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and Lofty 1977) and are a major food source for many species such as other invertebrates, amphibians, reptiles, birds and mammals (Lee 1985). Soil properties have long been known to affect abundance and biomass of earthworms (Edwards and Lofty 1977; Guild 1948). Among these are pH (Edwards and Bohlen 1996) and organic matter (OM) content (Curry 1976; Edwards and Bohlen 1996; Edwards and Lofty 1977). Soil properties may affect earthworms directly or indirectly through vegetation and management [e.g. cropping system (Jordan et al. 2004), fertilization, tractor traffic (Hansen and Engelstad 1999)]. Some soil properties may even enhance other soil properties like clay content that increases water retention capacity, which improves soils for earthworms in arid environments (Baker et al. 1998). If soil properties have a direct influence on the abundance and biomass of earthworms, then they must have an impact on the vital rates: survival, growth and reproduction. Reported studies demonstrate how field abundance and biomass of earthworms relate to the above-mentioned soil properties (Auserwald et al. 1996; Didden 2001; Guild 1948; Hendrix et al. 1992); however, virtually no studies investigated how soil properties influence survival, growth pattern and cocoon production in earthworms.

In this paper our hypothesis is that soil properties have a direct influence on the energy acquisition of earthworms, and we report on the effect of clay content and acidity of soils on individual survival, growth and cocoon production in the epigeic species *Lumbricus rubellus* (Hoffm.). This species is common in most temperate soils and is one of the dominant earthworm species in grasslands (Didden 2001). We selected 11 Dutch soils, covering a range of soil properties relevant for the Netherlands (Edelman 1984), and our standard reference soil used in earlier studies on *L. rubellus* (e.g. Klok and de Roos 1996). Growth develop-

ment of hatchlings and reproduction of adult earthworms was assessed in bioassays using these 12 field soils. Survival, individual weight, development and reproduction of earthworms were monitored on a monthly basis. To integrate effects on growth and reproduction into population effects, we used a simple version of the Dynamic Energy Budget (DEB) model (Kooijman 2000) combined with a density-independent matrix model (Klok and de Roos 1996).

Methods

Soil characteristics

All analyses were preformed according to standard operational procedures (SOPs) from Alterra. Soil samples were dried at 40°C (Alterra SOP E-0001). Organic matter content of soil samples was determined by placing samples in porcelain crucibles which were weighed and incinerated in a furnace at 550°C for 4 h (Alterra SOP E-0100). The weight loss upon ignition (LOI) was considered to represent the total OM content. To determine acidity, 10 mg of soil was added to a 50-ml solution of KCl (74.5 g KCl in 1 l distilled water) and homogenized for 1 h, and pH values were measured after 24 h with a pH meter (Beckman pH·34) (Alterra SOP E-0103). Clay content of soils (<2 μm) was determined by sedimentation (Robinson pipette method; Robinson 1922) after first treating soil samples with H_2O_2 (30%) to remove OM.

Bioassays

At 12 locations, soil samples were collected from the top −10-cm layer after removal of aboveground vegetation (Table 1). Collected soil was homogenized, oven dried at 105°C for 42 h and passed through a 4-mm mesh to eliminate all cocoons. Five hundred grams of soil was treated with distilled water to reach field capacity, corresponding to a moisture content of approximately 50%, and added to 1-l jars (n=36); each treated soil was replicated three times. Adult L. rubellus from culture were used to produce F1 cocoons. Recently hatched juveniles [mean weight±standard deviation (SD), 16±2.5 mg] were allotted randomly to the 36 jars, six individuals to each jar. Worms were fed with alder leaves (Alnus glutinosa). This food type is much appreciated by the species and used in our standard tests (Klok et al. 2006; Ma 1984). Alder leaves were re-moisturized with distilled water before placement on top of the soil surface. The leaves were provided ad libitum, and jars were stored in climate rooms at 15°C, 61% humidity and continuous light to keep the earthworms in the soil. Earthworms were sorted from the

soils each month to assess survival, individual biomass (including gut contents) and developmental status. Clitellate worms were recorded as adult and those with a full tubercula pubertatis but no clitellum as subadults. Within a jar, individuals could not be identified. We therefore ranked the individuals within a jar according to their weight and assumed that individual growth rates did not change over time such that the rank order did not change during the test period. Before jars were emptied, leftover alder leaves were taken from the top of the soil surface to reduce mixture of leaves with soil. Earthworms were sorted from the soil by hand, which completely destroyed the burrowing structure created by the earthworms. After measurement, earthworms and soil were returned to the original jars, distilled water was added if necessary and food provided ad libitum on top of the soil. After 11 months, soils were wet sieved to assess the total number of cocoons produced.

Data analysis and modelling

Bioassay data were analysed with SPSS 10.1 for Windows. Data on life-history parameters, development, reproduction and survival were modelled with the DEB modelling framework (Kooijman 2000) to integrate soil effects on these parameters. The DEB theory is based on closed energy and mass balances and describes in a mechanistic way the acquisition and use of energy by individuals. We applied a simple version of DEB (Kooijman and Metz 1984) for which, under constant environmental conditions, individual growth can be described by the Von Bertalanffy growth curve:

$$l(a) = l_m - (l_m - l_b)e^{-\gamma \cdot a} \qquad (1)$$

and reproduction by:

$$m(a) = r_m[l_m - (l_m - l_b)e^{-\gamma \cdot a}]^2 \quad \text{for} \quad l(a) \geq l_{ad} \qquad (2)$$

where $l(a)$ equals the body weight to the power of one third (further denoted as $W^{1/3}$) of an individual of age a (day), l_m the maximum attainable weight ($mg^{1/3}$), l_b the weight ($mg^{1/3}$) at hatching, γ the rate constant in the Von Bertalanffy growth curve (day^{-1}), $m(a)$ the reproduction rate of an individual of age a, r_m the maximum reproduction rate and l_{ad} maturation weight ($mg^{1/3}$).

A stage-structured matrix model developed for L. rubellus (Klok and de Roos 1996) was used to translate soil-specific DEB parameters into effects on the population growth rate. This model divides the life cycle of the species into four stages: cocoon, juvenile, subadult, and adult. Starting with a certain number of individuals in each stage, the population development over time is given by:

$$n(t + 1) = Mn(t) \qquad (3)$$

Table 1 Origin, type and properties of 12 Dutch soils used for bioassays of growth and reproduction in *L. rubellus* and survival of hatchling earthworms

Soil	Site soil type[a,b]	Origin	pH KCl	OM (%)	Clay<2 μm (%)	Survival day (mean±SD)
1	Korenburgerveen Fibric Histosol Initial raw peat soil	52°00'N,6°40'E	2.6	5.7	2	0±0
2	Hackfort Gleyic Arenosol Sandy hydrovague soil	52°04'N,6°16'E	3.2	3.4	8	0±0
3	Kampina Carbic Podzol Ordinary hydropodzol soil	51°36'N,5°16'E	3.3	7.1	7.8	0±0
4	Drunense Heide Haplic Arenosol Sandy xerovague soil	51°40'N,5°09'E	3.4	0.28	<1.0	0±0
5	Fortmond Haplic Arenosol Sandy xerovague soil	52°22'N,6°05'E	3.7	1.2	5.8	328±28
6	Berkenwoude Terric Histosol Clayey earthy peat soil	52°03'N,4°42'E	4.1	17	59	333±23
7	Blauw grasland bij de Zijdebrug Terric Histosol Clayey earthy peat soil	51°53'N,4°45'E	4.5	45	55	330±26
8	Blauwe Hel Terric Histosol Initial raw peat soil	51°99'N,5°35'E	5	87.9	<2.0	248±68
9	Reference soil Fimic Anthrosol Sandy loam soil	53°02'N,6°40'E	5.1	5.7	2	315±5
10	Eendenkooi 't Broek Calcaric Fluvisol Clayey hydrovague soil	51°49'N,5°14'E	5.6	10.1	60	285±56
11	Oeverlanden Linge Aric Anthrosol Clayey hydroearth soil	51°58'N,5°07'E	6.9	16	49	157±20
12	Duin en Kruitberg Haplic Arenosol Sandy xerovague soil	52°24'N,4°34'E	7.4	<1.0	<2.0	317±6

Means and standard deviations were based on three replicates.

[a] Soil type according to FAO (1988)

[b] Soil type according to Dutch soil classification (De Bakker and Schelling 1989)

where $n(t)$ represents a vector denoting the number of individuals in the different developmental stages at time t, and M the population projection matrix

$$M = \begin{pmatrix} P_1 & 0 & 0 & F_4 \\ G_1 & P_2 & 0 & 0 \\ 0 & G_2 & P_3 & 0 \\ 0 & 0 & G_3 & P_4 \end{pmatrix} \quad (4)$$

where P_i, G_i and F_4 are the probability of remaining in the same stage, the probability of moving to the next stage and the number of cocoons produced per individual, respectively, and i indicates the life stage.

The DEB parameters are directly linked to these matrix entries (for details see Klok and de Roos 1996). When the environment remains stable, after some fluctuations (depending on the initial conditions), populations will grow at a constant rate (the maximum population growth rate), and their demographic composition will become fixed (the stable stage distribution). The maximum population growth rate r is directly related to the largest eigenvalue of M ($\lambda_m = \exp^r$), where λ_m equals the largest eigenvalue. The stable stage distribution is given by the right eigenvector of matrix M. The relative contribution of the matrix entries to λ_m can be calculated by assessing the

Fig. 1 Survival of *L. rubellus* in 12 Dutch soils. Each point represents the mean of three replicates, each comprising six worms at the start (i.e. 18 worms per soil). *Open circle*: soils 1, 2, 3 and 4; *solid diamond*: soils 5, 6, and 7; *solid block*: soil 8; *solid triangle*: soils 9 and 12; *open block*: soil 10; *open triangle*: soil 11
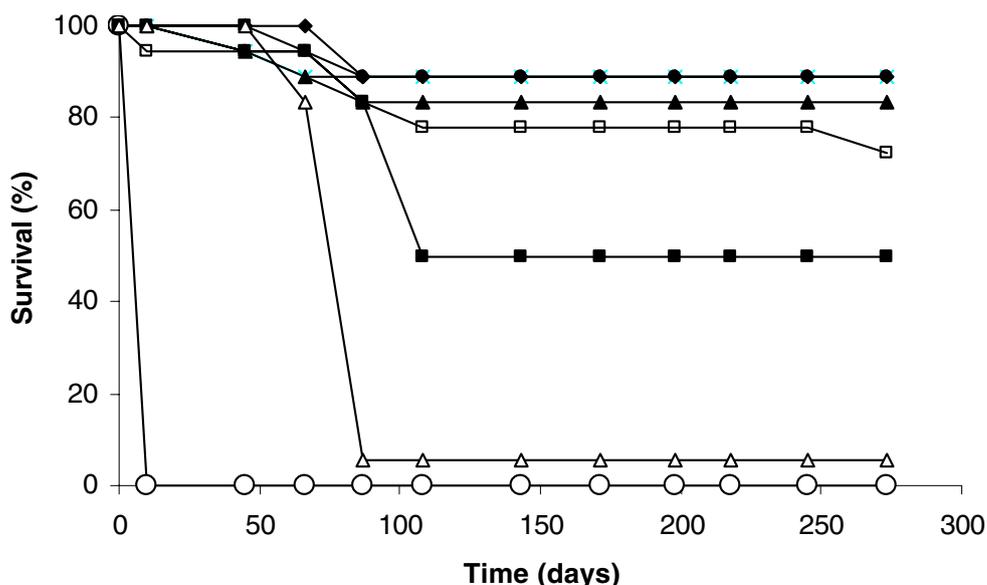
elasticity of the matrix entries (see Klok and de Roos 1996).

Model fit

For analysis of growth and reproduction, we used only those jars in which at least five worms survived the study period since individual weight development of worms in jars with less than five worms strongly differed from those with five and six individuals. Growth data were fitted to the Von Bertalanffy growth curve by least-squares estimation on the basis of average weights per jar. Some worms grew very slowly and failed to mature within the test period. These slow-growing worms were considered outliers when their weight was lower than the median minus the interquantile range calculated for all worms for a given tested soil and were excluded from the fitting procedure (cf. Jager et al. 2006). Data on cocoon production were used to calculate r_m by dividing the number of cocoons produced per worm per day by the mean squared adult weight to the power of one third during the period of adulthood until the end of the test.

Results

Origin and properties of tested soils

The origin of the 12 soils and the soil type and properties are shown in Table 1. As can be inferred from Table 1, the 12 soils represent a range of soil properties, with pH KCl values varying from 2.6 to 7.4, OM content from 0.28% to 88% and clay from <1.0% to 60%. Clay, OM and pH were not correlated (Pearson's two-tailed $p>0.05$).

Survival

All worms died in the first month when cultured in soils 1, 2, 3 and 4 (Fig. 1). In the eight remaining soils, survival decreased after 3 to 4 months. This decrease was drastic in soil 11, where only 5% survived the full test period, and in soil 8, where 50% survived. In the other soils, survival was more than 70% over the test period.

Development, reproduction and DEB model parameters

In the remaining six soils, where survival was higher than 70% (soils 5–7, 9, 10, 12), earthworms gained weight over time (Fig. 2). Individuals reached a significantly higher final weight in the reference soil (soil 9) compared with that
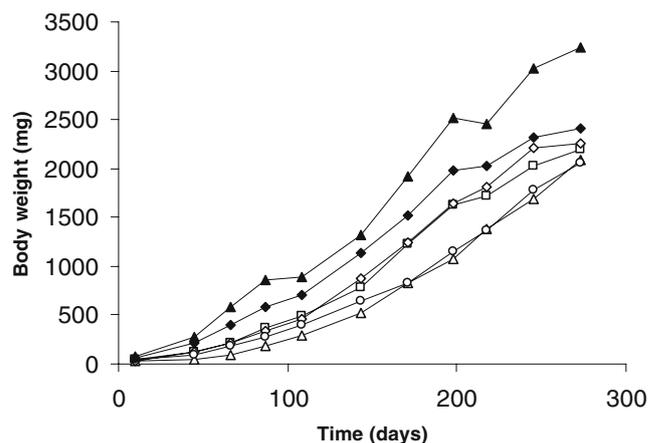

Fig. 2 Mean weights of *L. rubellus* in six soils in which five or more worms survived the test period at least in two jars (number of jars in brackets). *Solid triangle*: reference soil 9 (3); *open block*: soil 10 (2); *open triangle*: soil 6 (3); *open diamond*: soil 5 (2); *solid diamond*: soil 12 (3); *open circle*: soil 7 (2)

Table 2 Bioassay results of cultured *L. rubellus* in six Dutch soils

Soil	Adult weight (mg)	Adult age (day)	Final weight (mg)	Fertility[a] (cocoons worm^{-1} day^{-1})
5	1,396^{AB}±28	187^{AB}±3	2,191^A±229	0.31±0.02
6	1,150^{BC}±74	205^B±16	2,032^A±38	0.38±0.14
7	1,155^{BC}±52	198^{AB}±7	2,017^A±67	0.29±0.10
9	1,591^A±212	163^A±9	3,105^B±271	0.53±0.04
10	857^C±25	158^A±21	2,196^A±291	0.32±0.18
12	1,428^{AB}±159	167^A±7	2,311^A±140	0.36±0.04

Weight and age when reaching development stages, final weight at end of experiment and fertility. Mean values and standard deviation (*n*=3).

Mean values followed by same letter are not statistically different (ANOVA Tukey test *p*>0.05).

[a] Cocoons worm^{-1} day^{-1} is calculated from the total number of cocoons produced per jar divided by the number of adult days.

Table 3 Dynamic Energy Budget parameters of *L. rubellus* cultured in six Dutch field soils, mean values and standard deviation

Soil	γ (day^{-1}) mean±SD	l_m (mg$^{1/3}$) mean±SD	r_m ((mg$^{1/3}$)$^{-2}$ day^{-1}) mean±SD
5	0.0078^{A,B}±0.0025	14.54±2.01	0.0025±0.0002
6	0.0049^A±0.0008	15.98±2.28	0.0035±0.0015
7	0.0105^{A,B}±0.0007	11.38±0.28	0.0027±0.0010
9	0.0143^B±0.0003	13.29±0.23	0.0039±0.0003
10	0.0098^{A,B}±0.0045	13.48±3.60	0.0035±0.0019
12	0.0129^B±0.00019	12.76±0.61	0.0028±0.0005

Mean values followed by same letter are not statistically different (ANOVA Tukey test *p*>0.05).

in the other tested soils [analysis of variance (ANOVA) Tukey test, *F*=12.7, *p*<0.001] (Fig. 2). The weight when reaching the adult development stage differed significantly between soils (Table 2). Only maturation age of earthworms living in soil 6 was statistically higher than ages of those maturing in soils 9, 10 and 12 (Table 2). Cocoon production did not differ significantly between soils (Table 2). Of the DEB model parameters, only the Von Bertallanfy growth constant (γ) differed significantly between soils (ANOVA Tukey test, *F*=8.4, *p*<0.005) (Table 3).

Influence of clay content and acidity of soil on the parameters of the population model

Table 1 shows the survival of the cultured earthworms ranked by soil pH. The results indicate that earthworms did not survive in soils with pH values equal to or smaller than 3.4. Regression of the maturation weight as a function of

the clay content of the soil explains a large part of the variation in this weight, maturation weight (in milligrams) = 1,507−7.6 (clay %) (ANOVA, *F*=27.36, *p*=0.0002, R^2=0.68). The Von Bertallanfy growth constant (γ) relates with both pH and the clay content [γ (day^{-1})=0.005+0.001 (pH)−5.8*10^{-5} (clay %) (ANOVA, *F*=6.67, *p*=0.011, R^2=0.53)].

Population effects

Population growth rate was calculated from the significant relations between soil properties on the Von Bertalanffy growth constant and the weight of reaching the adult stage (l_{ad}), and with average values of the maximum attainable weight (l_m) and the maximum reproduction rate (r_m). Figure 3 shows the calculated population growth rate as a function of clay content for three soil pH values. As indicated by the lines in the graph, population growth declines slowly with increase in clay content of the soil; this decline is more apparent at low pH values than at high values. Figure 3 also shows that pH has a larger influence on the growth rate than does clay content. Whereas an increase in clay content from 10% to 60% reduces the

Fig. 3 Calculated population growth rate for *L. rubellus* as a function of clay contents of the soil for three pH KCl values of the soil. *Solid line*: pH=7; *dashed line*: pH=5.5; *dotted line*: pH=4
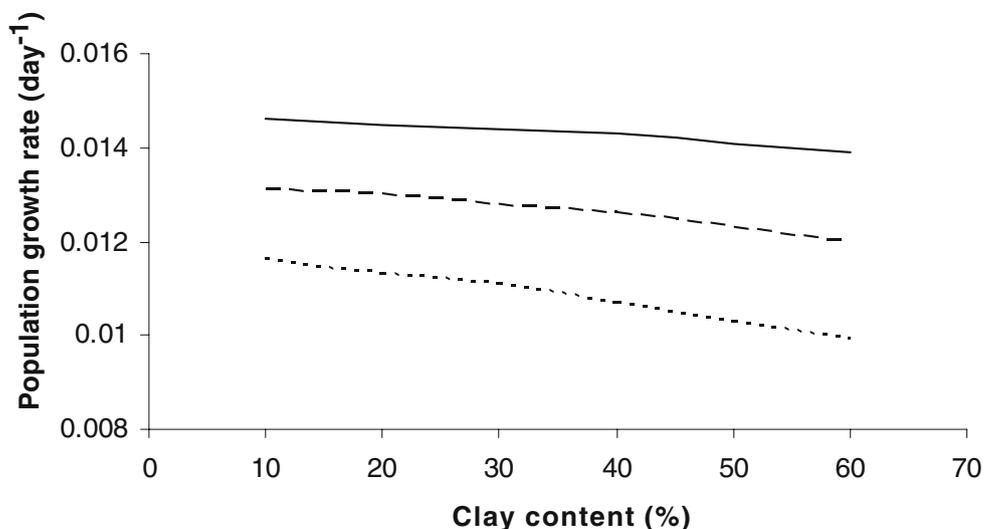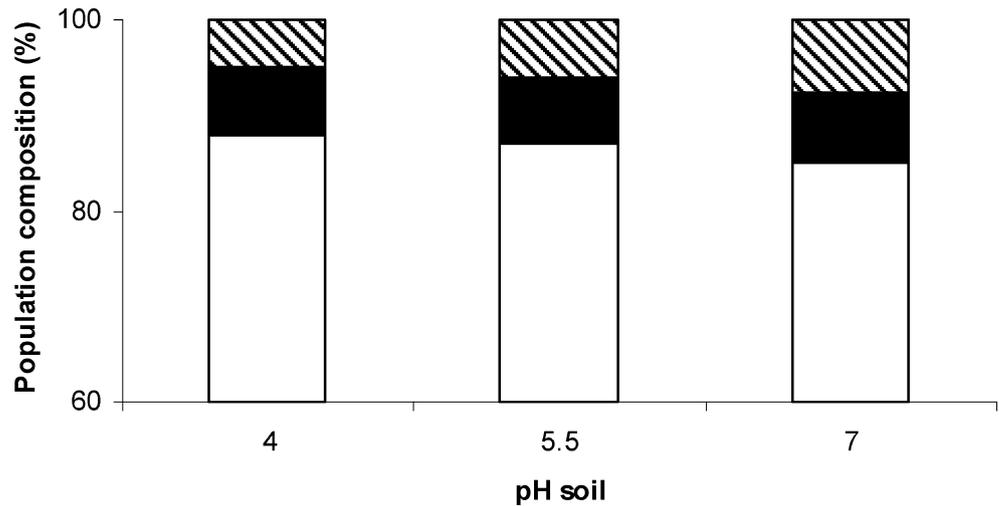
Fig. 4 Calculated population composition (stable stage distribution) of *L. rubellus* at three soil pH KCl values. Juveniles: *white*; subadults: *black*; adults: *hatched bar*. Note *y*-axis starts at 60%

growth rate by about 15%, a decrease in pH from 7 to 4 reduces the growth rate by 28%. The clay content of the soil had a negligible effect on the stable stage composition. An increase in pH, however, changed the population composition towards a larger proportion of adults (Fig. 4).

Discussion

Effect of clay content and acidity of soil on individual earthworms

In this study we detected a strong influence of soil acidity on survival in *L. rubellus*, earthworm survival being limited to soils with pH KCl values equal to or above 3.4. This result agrees with literature data that indicate that the

acidity of a soil has a negative influence on earthworm populations (Edwards and Lofty 1977), and earthworms are generally absent in soils with pH values below 3.5 (Curry 2004). Epigeic species such as *L. rubellus* are generally assumed to be more acid tolerant than species which live in deeper soil layers, and can survive in soils with pH values ranging from 3.5–7 (Satchell 1955).

Clay content of soil was negatively correlated with the maturation weight. The age at reaching this stage, however, did not change with clay content. The fact that clay content also has a negative influence on the Von Bertallanfy growth constant implies that weight gain per time unit of individuals is reduced. The DEB theory indicates that if environmental factors affect the Von Bertallanfy growth constant, whereas l_m (the maximum attainable weight) and r_m (the maximum reproduction rate) remain constant, the

Fig. 5 Mean individual weight of earthworms living in soils with different clay contents (after Auserwald et al. 1996). Individual weight (milligrams)= 308−3.4 (clay %) (ANOVA, $F=11.5$, $p<0.002$, $R^2=0.28$)
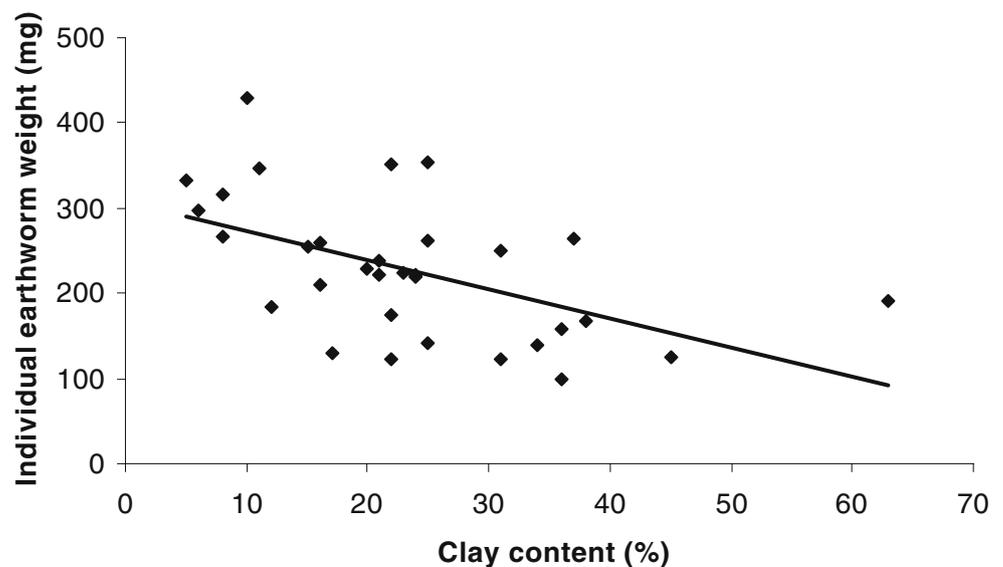
volume specific cost for growth changes (Kooijman 2000). This implies that earthworms in clayey soils need more energy to gain weight compared with those living in soils with lower clay content. A possible explanation for such higher energy costs is the higher penetration resistance of clayey soil compared with soil with low clay content. This higher resistance may cause earthworms to spend more energy in burrowing and moving through the soil in search of food in a clayey soil compared with a soil with low clay content.

Organic matter levels were relatively low in the tested soils; OM was above 20% only in two soils, which is considered optimal for earthworms (Curry et al. 2002). Organic matter content is often considered a good predictor of earthworm abundance (Edwards and Bohlen 1996) since OM provides at least part of the food base for earthworms. In our laboratory study, food was provided ad libitum, which may have confounded possible stronger effects of OM.

Effects of clay content and acidity of soil on earthworm populations, extrapolation to the field

Our model results imply that populations grow at a lower rate in soils with high clay levels. This, however, does not result in a change in the demographic composition of the population. Slower growth of individuals does not result in a later onset of reproduction since individuals mature at a lower weight in these clayey soils (Table 2). Because of this compensatory relationship, the flow of individuals through their life stages is almost independent of the clay content of the soil. However, since individuals grow slower, we expect a lower mean individual weight in soils with high clay content. A negative effect of clay on mean individual weight was observed by Vandecasteele et al. (2004). Such a relation was not noticed by Auserwald et al. (1996) in their extensive study of the influence of soil properties on earthworm populations. They used 32 different soils and reported that only soil moisture correlated with earthworm abundance and biomass. If, however, the mean individual weight is calculated from the data on total biomass and density given in Auserwald et al. (1996) (Table 1) and related to the clay content, a strong negative correlation is found (Fig. 5) (Pearson's two-tailed $p<0.01$). This result strengthens our finding that earthworms mature at a lower weight in clayey soils.

High earthworm densities in light and medium loam compared with clayey soils were reported by Guild (1948), who found that total earthworm density in light loam equaled twice the density in clay soil, and this difference was even five times higher for *L. rubellus*. Other authors, however, reported an increase in earthworm abundance with clay content of soil (Baker et al. 1998; Hendrix et al.

1992; Nordström and Rundgen 1974). Baker et al. (1998) noted that clay content affects many soil properties, such as water retention and cation exchange capacity, which may positively influence earthworms. Our study has shown that under optimal laboratory conditions, with comparable humidity, high clay content increases the volume specific cost for individuals to gain weight, which results in a lower population growth rate. Whether this lower growth rate also results in lower abundance of earthworms under field conditions is difficult to assess on the basis of this study only. Obviously, field abundance is the result of many abiotic (e.g. climate, management) and biotic (e.g. competition for food, predation) factors of which soil properties are just one.

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