European Journal of Soil Biology 68 (2015) 56–60

Contents lists available at ScienceDirect

European Journal of Soil Biology

journal homepage: http://www.elsevier.com/locate/ejsobi

Original article

Collembolan reproduction in soils from a long-term fertilisation experiment opposes the Growth Rate Hypothesis

Birthe Schröder [a, *], Natalie Steiner [a], Ines Merbach [b], Martin Schädler [b], Juliane Filser [a]

[a] University of Bremen, UFT, General and Theoretical Ecology, Leobener Str., D-28359 Bremen, Germany
[b] Centre for Environmental Research Leipzig-Halle, Theodor-Lieser-Str. 4, 06120 Halle, Germany

ARTICLE INFO

Article history:
Received 21 November 2014
Received in revised form
13 March 2015
Accepted 23 March 2015
Available online 24 March 2015

Keywords:
Growth Rate Hypothesis
Phosphorus
Fertilization
Soil invertebrates
Folsomia candida

ABSTRACT

The Growth Rate Hypothesis (GRH) predicts better performance of fast-reproducing species in environments with increased P content. Thus far, most studies were performed in aquatic ecosystems; only few studies focused on soil ecosystems. In this study, soils from a long-term experiment (Static Fertilization Experiment, Bad Lauchstädt, Germany) that differ in P content by the factor 3 were used. We tested the influence of NPK fertilised, PK fertilised and unfertilised soils on the reproduction of *Folsomia candida*. In order to evaluate the effect of unit size, we compared the collembolan reproduction test as recommended by the OECD (large unit) with a recently introduced miniaturized version (small unit). Furthermore, the tests were combined with a predator–prey relationship using the gamasid mite *Hypoaspis aculeifer*. Even though significant differences between soils were found, reproduction was lowest at highest P content, which contradicts the GRH. In addition, predation and unit size had a strong influence on the reproduction. Both unit sizes proved feasible in predator–prey experiments, with stronger effects of mites on reproduction in the small unit due to higher relative predator density.

1. Introduction

The Growth Rate Hypothesis (GRH) predicts that fast-growing organisms profit from relatively high available P concentrations, because C:N:P stoichiometry of organisms is more constrained than the environmental stoichiometry due to the ability of organisms to actively take up or excrete elements [6]. In freshwater ecosystems, web structures are apparently determined by N:P availability, with low N:P ratios favouring organisms with a high growth rate [24,17]. Phosphorus is required for production of ATP, RNA, DNA and phospholipid molecules [27,6]. In the ocean, planktonic organisms and sea water C:N:P ratios closely match [23]. Redfield [23] accounted this to organic processes regulating the proportions of these major elements in the water.

In terrestrial systems, more attention should be paid to P than to N limitation [7]. However, the GRH has been mainly studied in aquatic systems and plants [24]. Few studies thus far have addressed soil organisms, which play a central role in nutrient cycling. Mulder & Elser [19] related biomass size spectra of soil microflora, nematodes and microarthropods to soil C:N:P stoichiometry. With higher P availability in the soil, more large-bodied invertebrates were found [19]. Schneider et al. [25] compared stoichiometric data of slow-growing cave arthropods (Collembola, Coleoptera, Orthoptera, millipedes, arachnids) to their surface-dwelling counterparts and found lower P contents in their body mass. Hambäck et al. [11] compared stoichiometry of Diptera diets and bodies and found that Diptera body N did not directly increase with diet quality. Relative P content largely decreased with body size. For detrivorous arthropods, Martinson et al. [18] compared allometry and nutritional stoichiometry of N and P. Body N content of detritivores was similar to herbivores and the P content was independent of trophic level. Adult cricket females (*Acheta domesticus*) fed diets with high amounts of P showed increased oviposition compared to those fed low amounts of P [30]. After application of superphosphate fertiliser over twelve years, a four-fold increase in Collembola and Acarina numbers related to the P content in the litter was found by King & Hutchinson [14]. However, in laboratory tests, chemicals are usually introduced into the soil immediately before test start. Because this leaves no time for degradation, organisms will be exposed to unnatural chemical

* Corresponding author.
E-mail address: bi_sc@uni-bremen.de (B. Schröder).

http://dx.doi.org/10.1016/j.ejsobi.2015.03.007
1164-5563/

speciations [29]. Results from such studies may therefore differ from patterns in natural systems with chronic differences on P availability and concomitant alterations in the microflora [1]: higher P contents should, e.g., favour bacteria rather than fungi [31]. The aim of this work was to study if there is an (indirectly) positive effect of phosphorus from long-term fertilisation on fast-reproducing soil fauna, represented by the standard species *Folsomia candida*.

GRH was tested using a reproduction test. Reproduction is not only affected by nutrient availability via food, but further strongly depends on intra- and interspecific interactions, particularly on population size and predation pressure. Therefore we included these aspects in our study. We used soils from a long-term fertilisation experiment that have developed different and stable levels of P after more than 100 years and an altered microflora [3]. Tests were performed with *F. candida* and varied with respect to soil volume and presence or absence of a predator, the gamasid mite *Hypoaspis aculeifer*. As Lang & Gsödl [15] showed with carabid beetles preying on aphids, predation impact depends not only on presence and value of alternative prey, but also on the escape efficiencies of the different prey species. Due to less room to escape and the smaller amount of soil we expected a higher predation rate in smaller test units.

The following hypotheses were tested:

1. *Soil*: For the three differently fertilised soils we expected the reproduction of *F. candida* to vary with soil P content. Highest reproduction was expected in soil fertilised with NPK, followed by PK, lowest in unfertilised soil.
2. *Predation*: We expected to find less reproduction in presence of the predator *H. aculeifer*.
3. *Unit size*: In comparison of two different sizes of the test unit, no difference in reproduction of *F. candida* was expected.
4. *Interactions*: Interactions of the factors soil, predation and unit size were expected. In the small unit a stronger effect of predation and a weaker effect of soil was expected due to the presence of less alternative prey in the reduced amount of soil.

2. Material and methods

2.1. Experimental design

We performed a fully factorial randomized design with 5 replicates, testing the impact of three factors: (i) soil, (ii) predation, (iii) unit size. The density of predators per unit of volume was 2.5* higher in the smaller units.

2.2. Test soil

The test soil originated from the Static Fertilization Experiment (SFE) in Bad Lauchstädt, Germany, where the Centre of Environmental Research (UFZ) is testing long-term effects of fertilisation. Since 1902, the impact of farmyard manure (0, 20 and 30 t ha^{-1} $year^{-1}$) linked with P, K and N fertiliser application on crop quality and quantity of four different field crops (sugar beet, spring barley, potato, winter wheat) is analysed. Further subjects to evaluation are C, N and P values of the soil as well as pH [2]. The soil samples used in this study (see Table 1) were taken on June 17th, 2013 from field section 6 planted with spring barley (*Hordeum vulgare* "Marthe") from plots 13 (NPK), 17 (PK) and 18 (no fertilisation). For maximum water holding capacity (WHC_{max}), pH, C_{org}, P contents and C:N ratio of the soil see Table 1.

2.3. Test animals

Collembola have proved valuable as bioindicators of soil quality due to their sensitivity. Due to their highly permeable cuticle they are vulnerable to contaminated soil [10]. The used Collembola species *F. candida* is found worldwide in humus-rich soils, leaf litter layers and caves. *F. candida* is a parthenogenetic, eyeless, unpigmented species with a furca. It is a favoured prey for a wide variety of endogeic and epigeic invertebrates such as mites. In nature, *F. candida* is omnivorous, under laboratory conditions it is reared with baker's yeast (*Saccharomyces cerevisiae*) [21]. The elemental composition of *F. candida* in percent of dry mass is 52.5 ± 1.2% C, 9.9 ± 0.7% N, 0.62 ± 0.10% P; thus the C:N:P ratio is 84.7:16:1 [16].

We used the mite *H. aculeifer* as a predator, which feeds on hexapoda like Collembola, their eggs and larvae, but also on fungivorous and herbivorous mites, enchytraeid worms and nematodes [12]. *H. aculeifer* is able to reproduce sexually as well as parthenogenetically. *H. aculeifer* shows a sexual dimorphism, with males growing to 0.55–0.65 mm and 10–15 μg. Females reach a size of 0.8–0.9 mm and a weight of 50–60 μg and are more pigmented [20].

2.4. Reproduction tests

The reproduction test with the species *F. candida* is a standard method for testing differences in soil quality on Collembola [21].

The main results of the reproduction test are the total number of juveniles produced by parent animals and the survival of parent animals. The main difference between the standard for testing chemicals on collembolan reproduction in soil (large unit [21]) and the miniaturized collembolan reproduction test (small unit [8]) is the number of Collembola and amount of soil added to the test. For the large unit, 10 individuals of *F. candida* were introduced to 30 g soil in 100 ml screw neck vials. In contrast in the small unit 4 individuals of *F. candida* were placed into 30 ml crimp neck vials with 10 g soil. The moisture content of the test soil was adjusted to 35% of the WHC_{max}. As test animals, 10 day-old synchronized *F. candida* were used. About 10 mg of dry baker's yeast was added on top of soils before placing the animals on the test soils and replenished on demand after two weeks. Seven days after the start of the test, two month old *H. aculeifer* females were added to the samples with predation, one mite per test vessel. For the test period of 28 days the test vessels were placed in a climate chamber at 20 °C ± 1 and a light–dark cycle (400–800 lux) of 16:8 h. Once a week, the samples were aerated; also, soil humidity was checked by weighing and adjusted when necessary. The positions of the test vessels were randomized weekly. At the end of the test, 100 ml of tap water and the content of the test vessel were mixed in a 250 ml cup and the

Table 1
Soil properties of the unfertilised (nihil) and the two fertilised soils (PK & NPK). Composition of used fertilisers for PK and NPK fertilised soils: N: Calcium ammonium nitrate 27%, P: Triple-superphosphate 40%, K: Potassium chloride 60%.

Fertilised soil	C_{org} [mg/100 g]	N [mg/100 g]	P [mg/100 g]	C:N ratio	N:P ratio	C:P ratio	pH	WK_{max} [% dry soil]
nihil	1580	130	3.0	11.9	44.0	523.2	5.91	47.6
PK	1570	130	7.1	11.8	18.8	222.2	5.78	48.8
NPK	1770	150	11.1	11.5	13.9	159.6	5.67	52.8

mixture was stirred for three minutes. Subsequently the Collembola floating on the surface of the mixture were photographed (Canon EOS 1100) and counted using the program ImageJ 1.44p (Wayne Rasband, National Institutes of Health, USA). The Collembola were separated by size into adults and juveniles.

2.5. Data analysis

Statistical analyses of the experiments were performed using R, version 3.0.1 [22]. First a Shapiro–Wilk normality test was performed ($p = 0.1446$). In addition a Levene test for homogeneity of variance was done ($p = 0.3985$). The influences of the factors soil, number of mites and unit size on the reproduction as well as the interaction of these factors were analysed using a full factorial three-way ANOVA. For pairwise comparisons Tukey tests were conducted. The reproduction was expressed in juvenile Collembola per added adult over 28 days in order to consider the difference in individual numbers between the two unit sizes.

3. Results

3.1. Total model of collembolan reproduction tests

The three-way ANOVA rendered significant differences for the reproduction of *F. candida* according to the factors soil and predation. In addition, significant interactions between the factors soil and unit size and between predation and unit size were found (Table 2).

In the large unit without mites (Fig. 1A) no significant differences between the soils were found ($p = 0.4$). Mean reproduction in unfertilised and PK-fertilised soil was 73 juveniles per adult; in NPK-fertilised soil it was 68 juveniles per adult. In the large unit with predation (Fig. 1B) highly significant differences were found ($p < 0.001$). The lowest mean reproduction was found in NPK-fertilised soil (35 juveniles per adult). Between PK-fertilised soil (57 juveniles per adult) and unfertilised soil (60 juveniles per adult) the difference in reproduction was negligible. In the small unit without predation (Fig. 1C) a highly significant difference between the soils was found ($p = 0.007$). Reproduction was lowest with 74 juveniles per adult in PK-fertilised soil, followed by NPK-fertilised soil (81 juveniles per adult). The highest reproduction was found in unfertilised soil with 94 juveniles per adult. In the small unit with predation (Fig. 1D) no difference was found between soil types ($p = 0.915$). In unfertilised and NPK-fertilised soil the mean reproduction was 36 juveniles per adult; in PK-fertilised soil it was 33 juveniles per adult.

In the small unit with predation, per vessel in average 190 juvenile Collembola less than without predation were found. In the large unit, 209 juvenile Collembola less than without predation were found. The mean overall loss in reproduction due to predation per test vessel was 200 ± 9 juveniles.

Table 2
Results of analysis of variance (ANOVA) for juveniles per adult of *F. candida* in three different fertilisation treatments with and without the predatory mite *H. aculeifer* in two different unit sizes ($n = 5$). Boldface indicates statistical significance.

Source	df	Sum sq	F value	Pr(>F)
Soil	2	1158.3	6.0692	**0.0045**
Predation	1	17587.4	184.3154	**<0.0001**
Unit size	1	65.4	0.6856	0.4118
Soil:predation	2	266.9	1.3983	0.2569
Soil:unit size	2	889.9	4.6629	**0.0141**
Predation:unit size	1	2683.4	28.1216	**<0.0001**
Soil:predation:unit size	2	682.7	3.5772	**0.0356**
Residuals	48	4580.2		

4. Discussion

F. candida reproduction significantly differed between the differently fertilised soils, as expected in the first hypothesis. The effect of P on the reproduction of Collembola, however, was exactly contrary to the expectations (Table 1). In spite of the relatively lowest P content, the highest reproduction occurred in the unfertilised soil. This result does not support the GRH, based on which the reproduction of Collembola was expected to benefit from soil with high P availability [6]. In general, the P concentrations are relatively high [28]. Taking a look at the soil parameters measured by the UFZ (Table 1), we found higher reproduction at low N:P and C:P ratios. Contaminations might explain the negative effect on *F. candida* reproduction found in the fertilised soils [9,29]. According to the Chemicals Unit of DG Enterprise [4], fertilisers with 20 mg Cd/kg P_2O_5 or more lead to long-time accumulation of Cd in soil. Soil salinity, especially chloride effects, can mask effects of heavy metals and other toxins. Long-term application of mineral fertilisers increases soil salinity and osmotic pressure. As a side effect, collembolan egg development might have been impaired [26]. Kaneda and Kaneko [13] found positive correlations between C:N ratios, pH values and collembolan growth [body length]. Reproduction is strongly dependent on body size of the adult; a strong correlation between adult size and reproduction was found, with reproduction being more sensitive than growth [5].

In the second hypothesis it was expected that the predation rate of *H. aculeifer* would have an effect on reproduction of *F. candida*. A highly significant negative effect of predation on *F. candida* reproduction was found (Table 2).

The comparison of the two different unit sizes of collembolan reproduction tests in the third hypothesis showed no differences, as expected. The reproduction in total differed due to the different numbers of adults introduced to the unit sizes, but not in number of juveniles per adult.

In the fourth hypothesis, an interaction in the predation effect with regard to soil type and predator presence was expected. For the mean reproduction per adult, this effect was found for unit sizes. In the tests with predation the large unit showed the higher mean values; the lowest reproduction values were found in the small unit with predation. These results conform to our expectations that unit size would be negatively correlated with the reproduction of *F. candida* in presence of *H. aculeifer*. This depends on the different predator:prey ratios in the large unit and the small unit collembolan reproduction test. The predator–prey ratio is 2.5 times higher in the small unit than in the large unit. However, the total number of juveniles lost to predation was similar in both unit sizes. No difference in effect of predation was found between soils. Indeed, in the large unit no difference between soils was found, in contrast to the small unit (Table 2, Fig. 1A+C). On one hand, predation had an effect in the large unit, but not in the small unit (Fig. 1A+C). In the small unit with predation, variation was considerably larger (Fig. 1B+D), most likely due to the lower number of introduced *F. candida*. On the other hand, as *H. aculeifer* is a polyphagous predator feeding on fungivorous and herbivorous mites, insect eggs and larvae, enchytraeid worms and nematodes [12], the availability of alternative prey should be higher in larger test units. Alternative prey in the NPK soil may have suffered worse than *F. candida*, which led to a higher consumption of *F. candida* in the large unit (cf. Fig. 1B), whereas in the small unit, due to the overall lower resource availability, alternative prey may generally have been insufficient for *H. aculeifer*.

Originally, the reproduction test was developed to analyse the possible effects of contaminations. When comparing different soils, weaker effects had to be expected. The effect of predation differed between unit sizes. This shows the importance of taking biotic

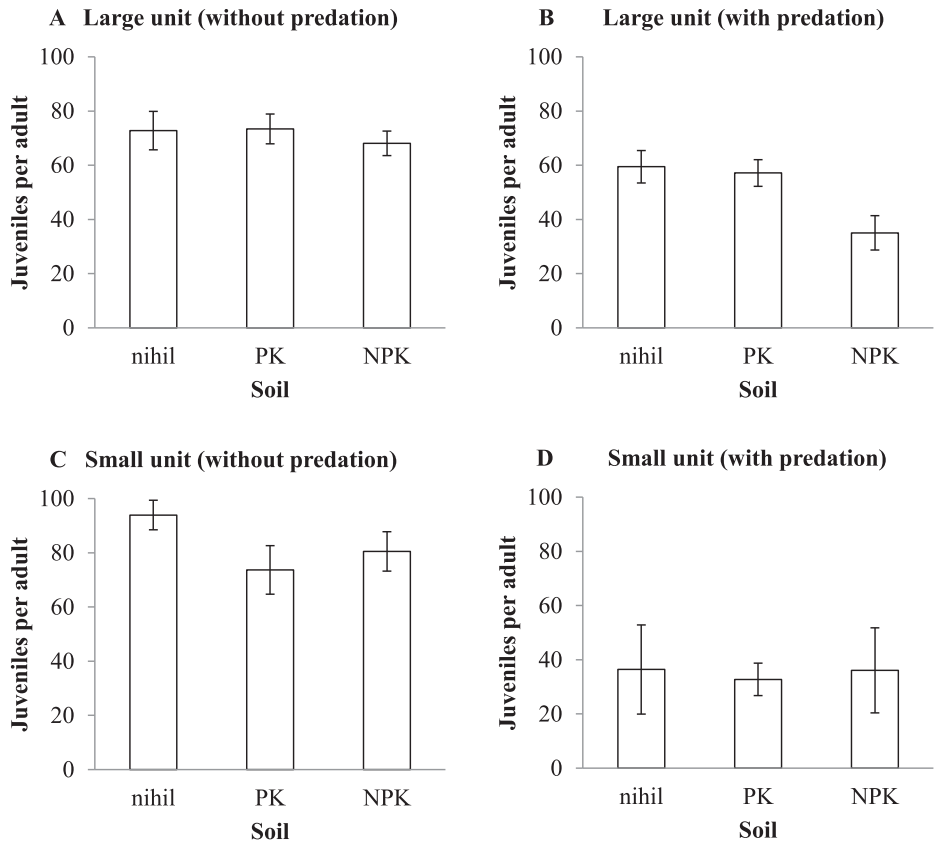

Fig. 1. Reproduction of *F. candida* (juveniles per adult) in the large unit without (A) and with predation (B) and the small unit without (C) and with predation (D). The small unit was performed with 4 individuals of *F. candida* and the large unit with 10 individuals. In samples with predation, one mite (*H. aculeifer*) was added to each test vessel. Means ± SE, n = 5.

interactions into account. In conclusion, effects of biotic interactions and abiotic factors can overrule GRH.

Acknowledgements

We thank Moira McKee for proofreading.

References

[1] R.D. Bardgett, E. McAlister, The measurement of soil fungal:bacterial biomass ratios as an indicator of ecosystem self-regulation in temperate meadow grasslands, Biol. Fertil. Soils 29 (1999) 282–290.
[2] N. Blair, R.D. Faulkner, A.R. Till, M. Körschens, E. Schulz, Long-term management impacts on soil C, N and physical fertility: part II: Bad Lauchstadt static and extreme FYM experiments, Soil Tillage Res. 91 (2006) 39–47.
[3] L. Böhme, U. Langer, F. Böhme, Microbial biomass, enzyme activities and microbial community structure in two European long-term field experiments, Agric. Ecosyst. Environ. 109 (2005) 141–152.
[4] Chemicals Unit of DG Enterprise, Proposal for a Regulation of the European Parliament and of the Council Relating to Cadmium in Fertilizers, 2003. Draft N°22.
[5] Y. Crouau, C. Moïa, The relative sensitivity of growth and reproduction in the springtail, *Folsomia candida*, exposed to xenobiotics in the laboratory: an indicator of soil toxicity, Ecotoxicol. Environ. Saf. 64 (2006) 115–121.
[6] J.J. Elser, D.R. Dobberfuhl, N.A. MacKay, J.H. Schampel, Organism size, life history, and N: P stoichiometry, BioScience 46 (1996) 674–684.
[7] J.J. Elser, W.F. Fagan, R.F. Denno, D.R. Dobberfuhl, A. Folarin, A.F. Huberty, et al., Nutritional constraints in terrestrial and freshwater food webs, Nature 408 (2000) 578–580.
[8] J. Filser, S. Wiegmann, B. Schröder, Collembola in ecotoxicology – any news or just boring routine? Appl. Soil Ecol. 83 (2014) 193–199.
[9] J. Filser, R. Wittmann, A. Lang, Response types in Collembola towards copper in the microenvironment, Environ. Pollut. 107 (2000) 71–78.
[10] M.T. Fountain, S.P. Hopkin, *Folsomia candida* (Collembola): a "Standard" soil arthropod, Annu. Rev. Entomol. 50 (2005) 201–222.
[11] P.A. Hambäck, J. Gilbert, K. Schneider, H.M. Martinson, G. Kolb, W.F. Fagan, Effects of body size, trophic mode and larval habitat on Diptera stoichiometry: a regional comparison, Oikos 118 (2009) 615–623.
[12] S. Jänsch, M.J. Amorim, J. Römbke, Identification of the ecological requirements of important terrestrial ecotoxicological test species, Environ. Rev. 13 (2005) 51–83.
[13] S. Kaneda, N. Kaneko, Influence of soil quality on the growth of *Folsomia candida* (Willem) (Collembola), Pedobiologia 46 (2002) 428–439.
[14] K.L. King, K.J. Hutchinson, Effects of superphosphate and stocking intensity on grassland microarthropods, J. Appl. Ecol. 17 (1980) 581–591.
[15] A. Lang, S. Gsödl, Prey vulnerability and active predator choice as determinants of prey selection: a carabid beetle and its aphid prey, J. Appl. Entomol. 125 (2001) 53–61.
[16] T. Larsen, M. Ventura, D.M. O'Brien, J. Magid, B.Aa. Lomstein, J. Larsen, Contrasting effects of nitrogen limitation and amino acid imbalance on carbon and nitrogen turnover in three species of Collembola, Soil Biol. Biochem. 43 (2011) 749–759.
[17] T.M. Main, D.R. Dobberfuhl, J.J. Elser, N:P stoichiometry and ontogeny of crustacean zooplankton: a test of the Growth Rate Hypothesis, Limnol. Oceanogr. 42 (1997) 1474–1478.
[18] H.M. Martinson, K. Schneider, J. Gilbert, J.E. Hines, P.A. Hambäck, W.F. Fagan, Detritivory: stoichiometry of a neglected trophic level, Ecol. Res. 23 (2008) 487–491.
[19] C. Mulder, J.J. Elser, Soil acidity, ecological stochiometry and allometric scaling in grassland food webs, Glob. Change Biol. 15 (2009) 2730–2738.
[20] OECD, Guidelines for the Testing of Chemicals, No 226, Predatory Mite (*Hypoaspis aculeifer*) Reproduction Test in Soil, Organization for Economic Cooperation and Development, Paris, 2008.
[21] OECD, Guidelines for the Testing of Chemicals, No 232, Collembolan Reproduction Test in Soil, Organization for Economic Cooperation and Development, Paris, 2009.
[22] R-Development-Core-Team, R: a Language and Environment for Statistical Computing, The R Foundation for Statistical Computing, Vienna, Austria, 2013.
[23] A.C. Redfield, The biological control of chemical factors in the environment, Am. Sci. 45 (1958) 205–221.
[24] J. Sardans, A. Rivas-Ubach, J. Peñuelas, The elemental stoichiometry of aquatic and terrestrial ecosystems and its relationships with organismic lifestyle and ecosystem structure and function: a review and perspectives, Biogeochemistry 111 (2012) 1–39.
[25] K. Schneider, A.D. Kay, W.F. Fagan, Adaptation to a limiting environment: the phosphorus content of terrestrial cave arthropods, Ecol. Res. 25 (2010) 565–577.

[26] G. Schrader, K. Metge, M. Bahadir, Importance of salt ions in ecotoxicological tests with soil arthropods, Appl. Soil Ecol. 7 (1998) 189–193.
[27] R.W. Sterner, J.J. Elser, Ecological Stoichiometry. The Biology of Elements from Molecules to the Biosphere, Princeton Univ. Press, Princeton, NJ, 2002.
[28] H. Stumpe, J. Garz, H. Scharf, Wirkung der Phosphatdüngung in einem 40jährigen Dauerversuch auf einer Sandlöß-Braunschwarzerde in Halle, in: Z. Pflanzenernähr. Bodenk, vol. 157, 1994, pp. 105–110.
[29] J. van Wensem, J.J. Vegter, N.M. van Straalen, Soil quality criteria derived from critical body concentrations of metals in soil invertebrates, Appl. Soil Ecol. 1 (1994) 185–191.
[30] L. Visanuvimol, S.M. Bertram, Dietary phosphorus availability influences female cricket lifetime reproductive effort, Ecol. Entomol. 35 (2010) 386–395.
[31] W. Zhong, Z. Cai, Long-term effects of inorganic fertilizers on microbial biomass and community functional diversity in a paddy soil derived from quaternary red clay, Appl. Soil Ecol. 36 (2007) 84–91.