2 Soil fauna distribution in heterogeneous soils

D.A. Heemsbergen[ab], W. Dimmers[a], J.H. Faber [a], M.P. Berg[b] and H.A. Verhoef[b]

[a] Alterra: Wageningen University and Research Centre, P.O. Box 47 NL-6700 AA Wageningen, The Netherlands
[b] VU University, Amsterdam, De Boelelaan 1085, 1081 HV Amsterdam, The Netherlands

Abstract
Spatial heterogeneity in abiotic and biotic factors is an important habitat feature of ecosystems as it facilitates potential refuges for less favourable conditions. Heterogeneously contaminated soils can affect specific groups of organisms and their functioning, both directly by toxic effects and by avoidance of contaminated micro-sites. We tested the hypothesis that horizontally heterogeneous soil characteristics affect macrofauna distribution in a river floodplain. The horizontal pattern of zinc content showed a gradual increase in concentration in eastern direction and a high heterogeneity in the southern part of the grassland. Correspondence analyses showed that vegetation and soil moisture content explained most of the variation found between different sites. Abundances of species and vegetation were not affected by contamination which was most probably due to the low bioavailability of the contaminants. The temporal dynamics showed that to have a proper assessment of species' presence and abundances, one has to sample for consecutive weeks to ensure optimal capturing of the species present due to climatic fluctuations.

Keywords: soil characteristics, spatial heterogeneity, river floodplain, soil fauna, soil contamination

Introduction
Spatial heterogeneity in abiotic and biotic factors is an important habitat feature of ecosystems. It is a facilitating condition for the coexistence of species as it creates a variety of microhabitats. In this mosaic of microsites, different temporal and spatial competition occurs per microsite and competitors can be spatially and temporally separated (Ettema and Wardle, 2002; Hartly and Shorrocks, 2002; Hampton, 2004). Furthermore, spatial

heterogeneity creates potential refuges from unfavourable conditions from which species can recolonise sites from which they were driven to extinction. Therefore, spatial heterogeneity affects the coexistence of species. Different scales of heterogeneity, from microsites to larger landscape level, give different environmental factors affecting species distribution (Nichols et al., 1998; Chust et al., 2003). On a local scale, abundances of organisms depend mostly on local climate and weather, structure and composition of the sward and its nutritional quality and the quantity and quality of litter returned to the soil, and soil physical and chemical characteristics (Curry, 1994).

Heterogeneity in contamination can affect specific groups of organisms and their functioning, both directly by toxic effects and by avoidance of contaminated microsites. Avoidance of contamination has been observed in earthworms (Slimak, 1997; van Zwieten et al., 2004; Eijsackers et al., 2005; Natal da Luz et al., 2008) and springtails (Natal da Luz et al., 2008) and avoidance of contaminated litter by isopods (van Cappelleveen, 1986; Weiβenburg and Zimmer, 2003). Furthermore, variation in soil characteristics leads to variation in bioavailability of the already heterogeneous contamination (Bourg and Loch, 1995; Ritchie and Sposito, 1995).

The Dutch river floodplains show a high heterogeneity in soil characteristics, including contamination. As the contamination load of the river Rhine varied through the years, the floodplains are diffusely contaminated with different contaminants, mainly metals, PAHs, mineral oils and PCBs. The highest concentrations of contaminants have been deposited in the years 1950–1970 and are mostly found at a depth of 10–35 cm (Middelkoop, 1997). Spatial differences in sedimentation rates and anthropogenic disturbances have caused the contamination to be variably positioned within the soil profile. Therefore, the floodplains show heterogeneity in contamination on a vertical and horizontal scale.

We tested the hypothesis that horizontally heterogeneous soil characteristics affect macrofauna distribution in a river floodplain. Furthermore, we wanted to assess the scale at which heterogeneity in soil characteristics occurs in the floodplain. Soil characteristics measured included clay, water, soil organic matter and zinc content. Macrofauna were captured for 4 weeks using pitfall traps and determined on order level except for the millipedes and isopods which were determined on species level.

Materials and Methods

The study site is a grassland in the river floodplain Afferdense and Deestse Waarden from the river Waal (longitude 51˚54'N, latitude 5˚39'E), a contributory of the river Rhine (Figure 1). Lying at an altitude of 7.8 meters above sea level, it is inundated almost every year for 1-3 months in winter and early spring. The grassland is one of the few areas undisturbed in the floodplain for the last 100 years. In order to determine the spatial variation of soil characteristics in the field, 30 points were set out approximately 17-30 meters apart, forming a grid covering the field. As horses were grazing in the field, the pitfalls were not placed in low grass areas to avoid trampling and therefore the grid is irregular (Figure 1). A plastic plate attached to 2 stainless steel pins was placed 10 cm above the pitfalls to prevent rain fall into the pitfalls. Location of the points was determined using a GPS and then the point was marked. Soil samples were taken with a corer, diameter 10 cm, 20 cm deep and then cut into 0-10 cm and 10-20 cm depth. Pitfall traps (diameter 10 cm) were placed at each point for fauna trapping for a period of 4 weeks (calendar week 40-43, 2000). Formalin (4%) was used to conserve fauna trapped. Pitfalls were checked and emptied once a week. Fauna was determined mostly to taxa level, for earthworms, centipedes, millipedes and isopods it was determined on species level.

Figure 1. Location of grassland in the Afferdense and Deestse Waarden floodplains. The squares in the grassland refer to enclosures.

Soil analyses

Soil samples were dried at 40 °C for 48 hours after being homogenised and milled (using a mortar). Soil samples were analysed for water content, heavy metal contents and texture. Moisture content (w/w) was determined by drying moist soil for 24 hours at 55 ºC. Total metal concentrations were determined by digesting 1 g dry soil in a mixture of H_2O, concentrated HNO_3 (65%) and HCl (37%) at a volume ratio of 1:1:4 using a MARS5 microwave (Bongers, 2007). Quality control was maintained by digesting reference samples (SETOC), of which the measured Zn concentrations did not deviate more than 10% from the certified reference value. All zinc extracts were analysed using flame Atomic Absorption Spectrometry (Perkin Elmer 1100B AAS). Soil samples for soil particle size analyses were pre-treated to remove organic matter (using H_2O_2) and carbonates (using HCl) to obtain only mineral soil particles (Konert and van den Berghe, 1997). Clay content of the soil was determined using laser diffraction size analysis (Konert and van den Berghe, 1997).

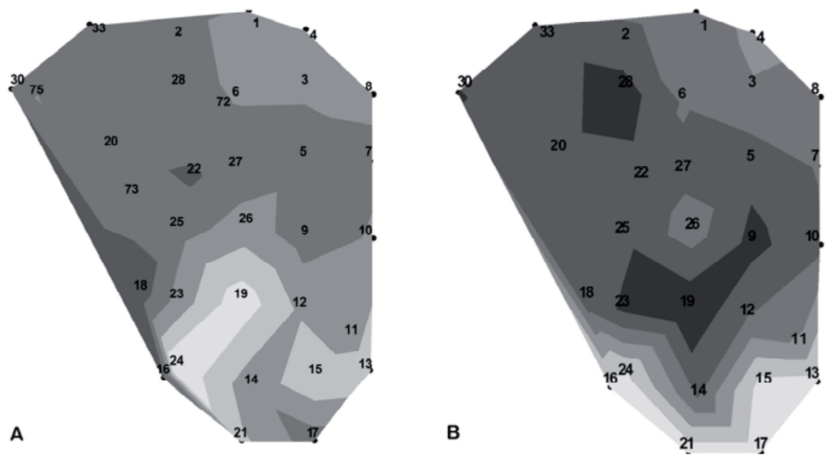
Spatial mapping and statistics

The spatial data on soil characteristics was interpolated using the GIS program ArcView 3.2a (ESRI) using a triangular irregular network. The scale of spatial heterogeneity of the soil characteristics was determined by making a semi-variogram of the 30 points (Genstat 7.0). For analysis, sample points were classified for vegetation in 10 classes, based on the percentage of high/medium/low vegetation surrounding the sample points with a radius of 5 meter. Fauna trapped in pitfalls were summed over four weeks and analysed per taxa. A canonical correspondence analyses was done to analyse relations between environmental and fauna variables using CANOCO (version 4.5 for Windows). Pearson correlation was used for correlation analyses between variables.

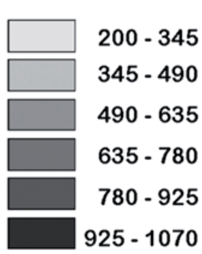
Results

Vegetation in the grassland consisted mainly of grass species and herbs. High vegetation was dominated by the species *Potentilla reptans* L., *Potentilla anserina* L., *Urtica dioica* L., *Rumex crispus* L., *Lolium perenne* L., *Agrostis stolonifera* L.and *Cirsium arvense* L.and low vegetation was dominated by *Bellis perennis* L., *Trifolium repens* L., *Lolium perenne* L. and *Agrostis stolonifera* L. The edges of the grassland showed short vegetation, while the centre of the grassland showed high vegetation cover. In the centre of the grassland, horse grazing created short vegetation corridors through the high vegetation cover.

The distribution of zinc concentrations in the top 10 cm of the soil showed a different pattern than at 10–20 cm (Figure 2a and b). Total zinc concentrations varied between 170 and 1050 mg/kg dry soil. Zinc content showed a rather homogeneous pattern in the northern part of the grassland. In the southern part of the grassland both high and low zinc concentrations were located close together, which was most profoundly in the zinc concentration at 10-20 cm depth (Figure 2b). The metal concentrations (copper, lead, cadmium and zinc) showed strong positive correlations. Furthermore, calcium, copper, lead and zinc concentrations were positively correlated with clay content.



Legend. Zinc concentration (mg/kg DW):



Semi-variogram of zinc concentrations at 0-10 cm in soil profile.

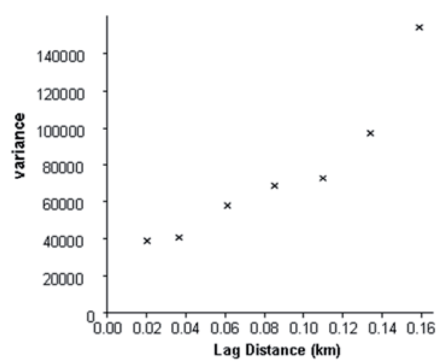
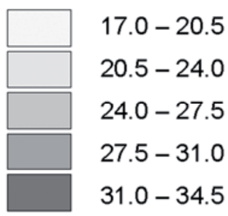
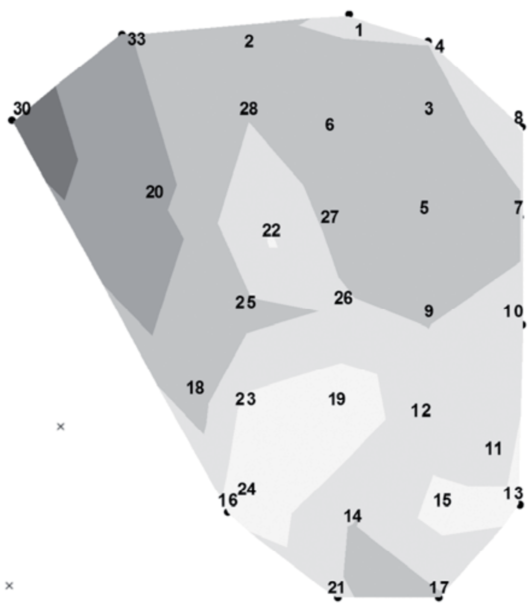


*Figure 2. Total zinc concentration (mg/kg DW) at **a**, 0-10 cm and **b**, 10-20 cm in the soil profile of the Afferdense and Deestse Waarden.*

Soil water content varied between 17-34.5% FW (Figure 3). The western part of the grassland showed an increase in water content which was due to an adjacent ditch containing water during the sampling period. Semi-variogram of moisture content showed a linear increase of variation with increasing distance (Figure 3). Figure 4 shows the clay content of the soil in the top 10 cm of the profile. Highest clay content was found in the eastern part of the grassland, while lowest clay content was found in the southern part of the grassland. Semi-variogram of clay content showed an inconclusive relation.

Legend. Soil moisture content (% FW).





Semi-variogram of soil moisture content at 0-10 cm in the soil profile.

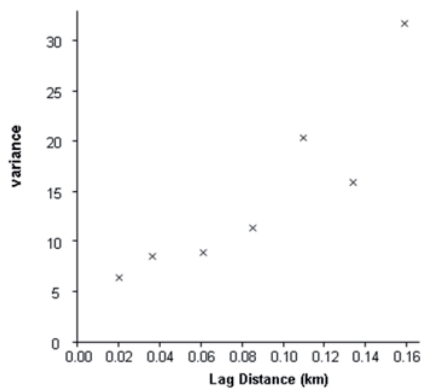
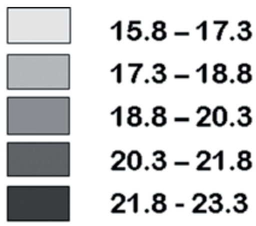
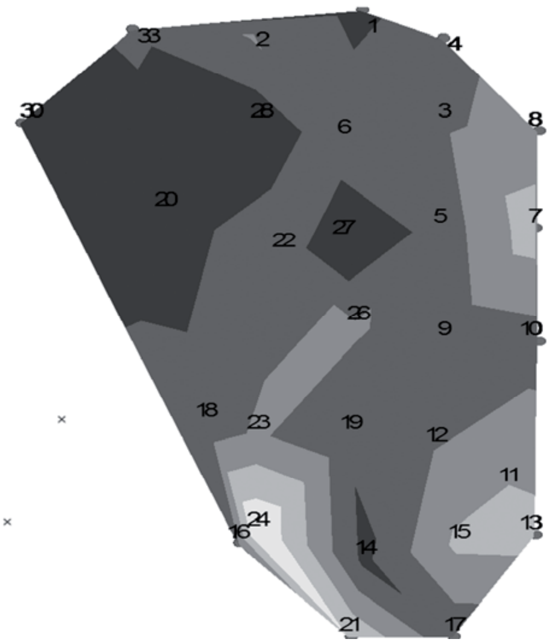


Figure 3. Soil moisture content at 0-10 cm in the soil profile (% FW) of the Afferdense and Deestse Waarden.

Legend. Clay content (% DW).





Semi-variogram of clay content at 0-10 cm (% DW).

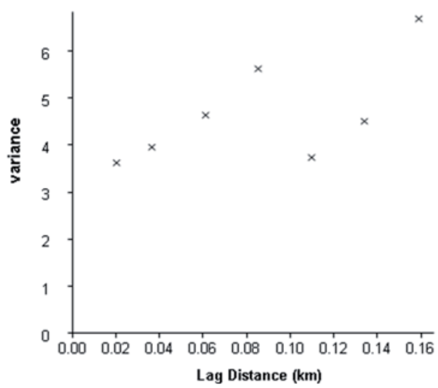
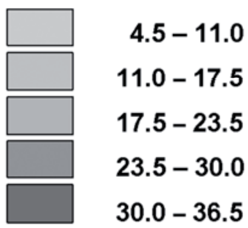


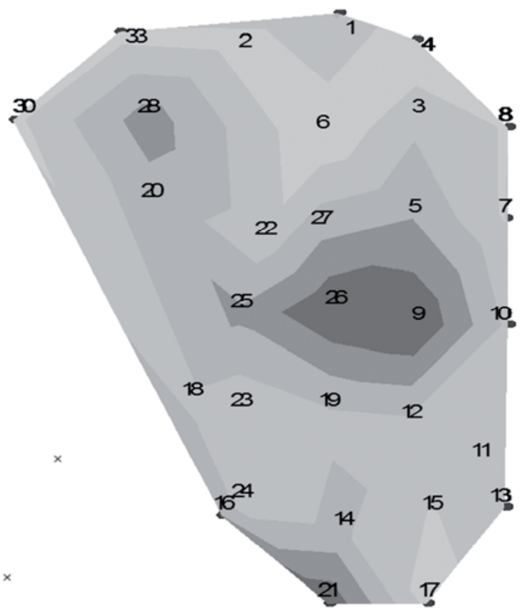
Figure 4. Clay content at 0-10 cm (% DW) in the soil profile of the Afferdense and Deestse Waarden.

Coleoptera numbers varied between 3 and 93 individuals per pitfall in a week time. Highest numbers of Coleoptera were found in the centre and south of the grassland (Figure 5). Coleoptera numbers caught were similar in the 4-week sampling period. Variance in Coleoptera numbers increased with increasing distance (Figure 5 semi-variogram).

Legend.

Number of Coleoptera:





Semi-variogram of total number of Coleoptera.

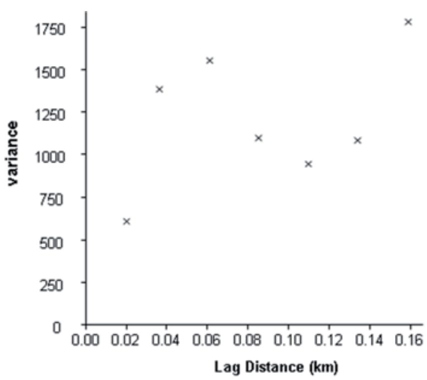


Figure 5. Average number of Coleoptera caught in pitfall traps per weeks in the Afferdense and Deestse Waarden.

Variance in number of Araneida was lower than Coleoptera (Figure 6) being 0-41 numbers per pitfall. Highest numbers were found in the centre and north-eastern part of the grassland. During the sampling period, the number of Araneida dropped significantly after the first week from an average of 9 to 4 individuals per pitfall. Semi-variogram of Araneida showed no relation with distance but a sharp drop in variance occurred at the largest lag distance.

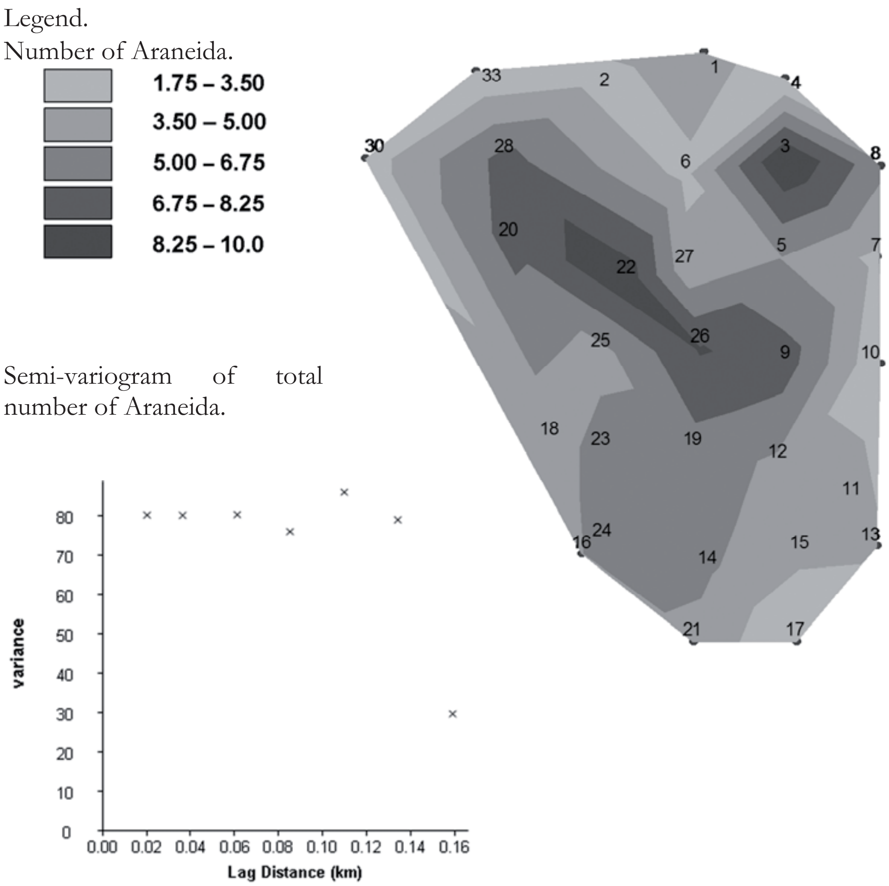


Figure 6. Average number of Araneida caught in pitfall traps per week in the Afferdense and Deestse Waarden.

Total numbers caught of Hymenoptera, Heteroptera, Diptera, Annelida and larvae of Coleoptera and Diptera are shown in Table 1. Individuals caught from the order Hymenoptera were mostly species of the family Formicidae, averagely 70%. Homoptera consisted mostly of species of the families Cicadellidae and Aphidoidea.

Table 1. Median numbers caught of Hymenoptera, Heteroptera, Diptera, Annelida and larvae of Coleoptera and Diptera in pitfall traps over four weeks.

Order	Median	Lowest	Highest
Hymenoptera	25	6	56
Homoptera	14	5	76
Diptera	14	5	23
Annelida	7	1	32
Coleoptera larvae	5	1	13
Diptera larvae	10	1	38

Only a few millipede species were caught in the floodplain, being *Polydesmus denticulatus* (20 ind.) and *Brachyiulus pusillus* (1 ind.). All but one of these specimens were found in the last 2 weeks of sampling. The caught individuals were located next to the enclosures in the grassland and in the north-eastern part of the grassland. Isopods were more abundant, but still in low numbers, *Trachelipus rathkii* (7 ind.), *Philoscia muscorum* (1 ind.), *Hyloniscus riparius* (2 ind.). Two centipedes species were present, *Lamyctes fulvicornis* (60 ind.) and *Lithobius curtipes* (4 ind.). Also amphibian species were caught in the pitfalls, including *Rana esculenta* (11 ind.), *Triturus vulgaris* (5 ind.), *Triturus cristatus* (4 ind.) and *Bufo bufo* (1 ind.). The amphibian species were caught throughout the grassland and did not show a distinct distribution pattern.

The biplots of the canonical correlation analyses (Figure 7) showed that vegetation and soil moisture content explained most of the variation found and were therefore the two main factors of the two first axes explaining 28 to 56% of the variation found. Only in week 43, the first axes was significant in explaining the macrofauna distribution (being 56%). Individual correlations showed that Hymenoptera was positively correlated in week 41 with soil water content (r = 0.407, P = 0.029) and clay (r = 0.405, P = 0.029). Vegetation showed positive correlations with Coleoptera in week 40 (r = 0.377, P = 0.044), and negatively with Annelida (wk 42, r = -0.398, P = 0.033) and Diptera larvae (wk 43, r = -0.402, P = 0.028). In week 43, both Coleoptera and Araneida showed a correlation with the Y coordinate (r = -0.377, P = 0.040; r = -0.566, P = 0.010 respectively) implying that more specimens were caught in the south than in the north of the grassland.

Correlations between species were frequently found of which Araneida and Coleoptera, Coleoptera and Hymenoptera, Araneida and Hymenoptera were observed twice in the 4 weeks of observations (all P <

0.05). Annelida and Coleoptera larvae both showed negative correlations with Hymenoptera and Homoptera, although being it in different weeks (42 and 43 respectively).

Discussion

The river floodplain grassland contains locations where zinc concentrations exceed the Dutch risk assessment level 4, which stands for high ecological risk (VROM, 2000). Zinc concentrations were positively correlated with other heavy metal concentrations. This is in accordance with results of Middelkoop (1997) who found strong correlations between metal concentrations, and clay content and metal concentrations. Furthermore, this author found a strong negative relationship between field height and metal concentrations. This was not observed in our field, as the low southern part of the field showed low metal concentrations. These low concentrations are due to the geomorphological differences of the soil, as the south part of the grassland used to be a bank of the river side channel (Schoor, 1994). The soil characteristics of the old side channel bank showed a higher content in sand and contain therefore lower metal concentrations. This resulted in relatively high heterogeneity in the south part of the grassland and therefore the geomorphological history of floodplain grassland should be taken into account with sampling strategy on soil characteristics to prevent a potential underestimation of heterogeneity in soil characteristics.

In general, the numbers of invertebrates caught were similar to other temperate grasslands that were subject to grazing (Curry, 1994). Correlation analyses showed that Diptera larvae and Annelida were negatively correlated with vegetation. As horses grazed in the grassland there were no pitfall traps placed in completely low vegetation to prevent trampling. Therefore, most Diptera larvae and Annelida were caught at locations with low-medium height in vegetation where horses grazed more intensely compared to the high vegetation. In general, Diptera larvae and Annelida are found to be positively related to soil moisture content and input of dead organic matter (Frouz, 1999; De Bruyn et al., 2001). It could well be that extra input of dead organic matter was caused due to horses dropping grass while grazing and input of horse manure. We must note that Diptera larvae and Annelids are soil dwellers and the pitfall capture method is not optimal for capturing soil dwelling fauna (Krebs, 1989).

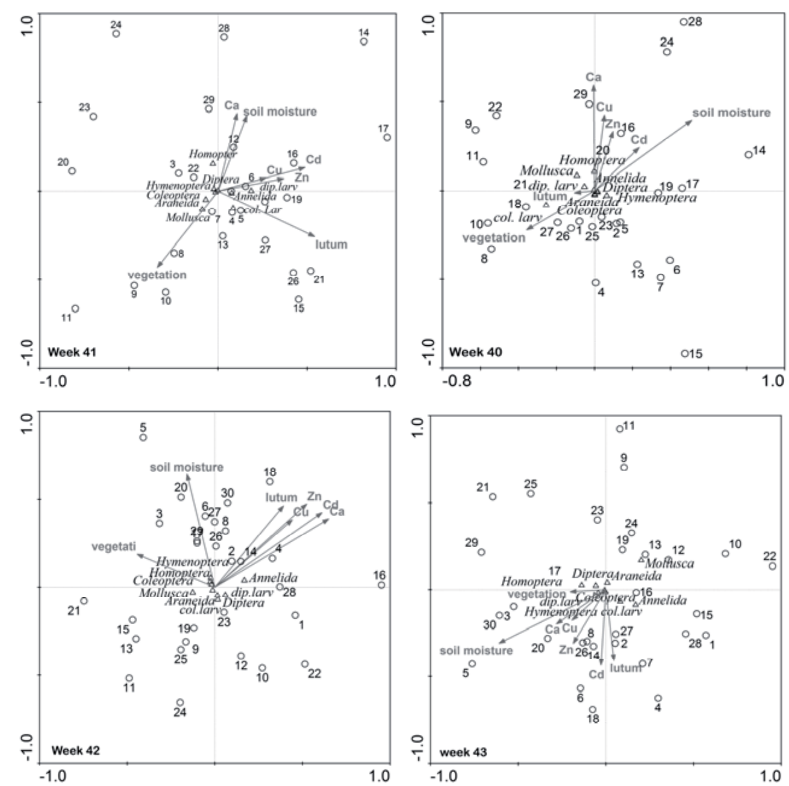


Figure 7. Canonical correspondence analysis of the 30 location in the field on environmental data and taxa variables for the 4 weeks sampled. Abbreviations used: Ca, calcium; col.larv Coleoptera larvae; Cu, copper; dip.larv, Diptera larvae. Metal concentrations are total concentrations (mg/kg DW).

Numbers of Homoptera, mostly species of the Cicadellidae and Aphidoidea families, showed a positive trend with soil moisture content. Arthropoda are known to prefer moist habitats due to water loss through their epicuticle if relative humidity is below 99% (Verhoef and Daan, 1995; Villani et al., 1999). Regarding Homoptera, egg hatching, survival and diapause termination is moisture dependent (Hodek, 2003; Moriyama and Numata, 2006). Therefore effect of soil moisture on Homoptera appears to be season dependent. Powell et al. (2007) found a positive correlation between population density of grasshoppers and soil moisture content between September to April and a negative correlation between grasshopper numbers and soil moisture content. Furthermore, diapause termination appears to be positively related to soil moisture content, besides temperature and photophase, after summer drought (Hodek, 2003).

Therefore the higher numbers of Homoptera found in this study are likely due to the high soil moisture content in October.

Numbers of Araneida did not show any correlations with soil characteristics and vegetation. Highest numbers were found in high vegetation areas in the centre of the grassland. Lower numbers were caught at the edge of the field, which was reflected in the remarked drop in variation at large distance in the semi-variogram. Other studies show that the Araneida numbers caught in the centre of the field were within normal range of temperate grasslands (Curry, 1994). Coleoptera had a similar distribution to Araneida but a second hotspot was in the southern part of the field.

Abundances of species and vegetation did not seem to be affected by contamination. Although the total concentrations of the contaminants were high, results from other studies in the river floodplains of the river Waal indicated low bioavailability of the contamination. Hobbelen et al. (2004) found total metal concentration of 1140 mg/kg zinc, but the $CaCl_2$ extractable fraction of zinc was only 0.81 mg/kg. Measurements of Zorn (2004) in the same grassland as this study showed $CaCl_2$ extractable fraction of 10-66 μg/kg at locations with a total Zn concentration of 500 mg/kg. This low availability of the contamination is probably due to the ageing of the contamination in the soil and the high pH, clay and organic matter content of the soil (Ritchie and Sposito, 1995; Middelkoop, 1997).

Overall, soil water content and vegetation explained most of the variation in soil fauna between sites. Although vegetation showed a distinct pattern in the field it was not correlated to abiotic characteristics, as the grazing of the horses was mostly affecting the vegetation pattern. Grazing alters floral composition and vegetation height leading to structurally heterogeneous sward and a change in micro-climate and all these factors are known to affect invertebrate distribution in the field (Curry, 1994; Dennis et al., 1998). Therefore, any potential effect of contamination on soil fauna distribution was most likely out-scaled by the natural effects of grazing on soil fauna and vegetation cover and their interactions.

Temporal dynamics

Over the four week, Coleoptera numbers caught remained similar. However, for Diptera, Homoptera, Hymenoptera and Araneida, the number of specimens caught were decreasing with time. This was most probably due to the decreasing temperatures in October 2000 (KNMI, 2000), and hence the decrease in activity of these species. In contrast, the millipede *Polydesmus denticulatus* was mostly caught in the last 2 weeks of the sampling period. This could be due to the relative wet conditions in the grassland after considerable amount of rain had fallen in the first 2 weeks (44 mm, KNMI, 2000).

In the last week of sampling, Coleoptera and Araneida moved southwards in the field, probably due to the wet conditions in the north of the field. October had 61 mm rain during the 4 weeks of monitoring (KNMI, 2000) and the average evaporation of grasslands in October is 17 mm per month in the Netherlands (Massop et al., 2005). Therefore the soil in the northern part was highly saturated with water, while in the southern part there seemed less water saturation probably due to the lower clay content in the soil, and therefore a higher infiltration rate.

Conclusions

Vegetation and soil water content were the two main factors explaining part of the variation found in macrofauna. Abundances of species and vegetation were not affected by contamination which was most probably due to the low bioavailability of the contamination. Any potential effect of contamination on soil fauna was most likely out-scaled by the natural effects of grazing, vegetation cover and soil water content. The temporal dynamics showed that to have a proper assessment of species' presence and abundances, one has to sample for consecutive weeks to ensure optimal capturing of the species present due to climatic fluctuations.

Acknowledgements

We thank Martin Konert for his support on the soil particles size analyses. The investigations were supported by the Research Council for Earth and Life Sciences (ALW) with financial aid from the Netherlands Organisation for Scientific Research (NWO) and the Dutch Ministry of Agriculture, Nature and Food Quality (BO-01-002-204). Matty P. Berg was financially supported by the Royal Dutch Academy of Arts and Sciences (KNAW).