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Secondary succession of terrestrial isopod, centipede, and millipede communities in grasslands under restoration

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Abstract The aim of this study was to investigate the impact of restoration management on the composition of a macro-invertebrate community in a formerly, nutrient-poor grassland. Four grassland plots were selected that were last fertilised 7, 11, 24 or 29 years before sampling in 1996. In the same plots it was observed that nutrient impoverishment as a restoration tool resulted in a decrease in primary production and a directional shift in vegetation composition after cessation of fertiliser application. Terrestrial isopods, millipedes, and centipedes were sampled with pitfall traps in the four plots. The directional shift observed in vegetation composition before this study was not accompanied by a directional change in macro-invertebrate composition. Both the field poorest in nutrients and the one richest in nutrients showed the lowest density and species richness, while the species composition was similar across intermediate succession stages. By far the most specimens and species were caught in the field that had not received fertilisers for 24 years. Succession theory could only partly explain the observed results. Canonical correspondence analysis of the data revealed that only a small part of the pattern could be explained by the nutrient status of the grasslands. The C accumulation due to secondary succession of plants was hypothesised to influence the densities and diversity of macro-invertebrate communities in these grasslands.

Keywords Chilopoda · Diplopoda · Biodiversity · Isopoda · Secondary succession

Introduction

During recent decades, the vegetation composition of nutrient-poor grasslands in north-western Europe has been affected by nutrient supply by fertilisers and soil acidification (Roem and Berendse 2000). These formerly species-rich plant communities are now dominated by a small number of common and highly productive plant species (Fuller 1987; Bobbink 1991). Formerly nutrient-poor grasslands can be restored, to a certain extent, if the effects of nutrient enrichment on the vegetation are counteracted. Cessation of fertiliser application in combination with grassland management practises, like mowing followed by removal of the hay, can result in nutrient impoverishment. The effect of such nutrient impoverishment on the vegetation has been intensively studied in grasslands under restoration succession (Olf and Bakker 1991; Olf et al. 1990, 1994). These studies show that, due to restoration management, production of these grasslands has decreased, nutrient-limiting conditions for plant growth have been restored, and plant species richness increased.

Aboveground and belowground compartments of terrestrial ecosystems interact. Indirect effects between these two components are increasingly becoming appreciated as important in determining vegetation succession and soil community composition (Wardle 2002). Secondary succession of plant communities under restoration management might indirectly affect soil communities through an influence on aboveground plant production and vegetation structure, for example by changing the quality and diversity of plant litter (Scheu and Schulz 1996; Bardgett and Shine 1999) or structure and micro-climate of the vegetation (Blomqvist et al. 2000). Little is known about the consequences of changes in plant communities on the dynamics of soil animal populations (but see Brussaard et al. 2004). Further, soil animal community composition at

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different stages of secondary succession has scarcely been studied (Pizl 1992; Wasilewska 1994; Scheu and Schulz 1996; Tajovski 2000; Verschoor et al. 2001, 2002; Verschoor 2002; Hemerik and Brussaard 2002).

This study aimed to analyse whether the community composition of soil macro-invertebrates tracked observed changes in vegetation production and shifts in vegetation composition associated with nutrient impoverishment in grasslands. Our hypotheses were that: (1) the composition of the macro-invertebrate community is specific for a certain stage of vegetation succession, i.e. nutrient impoverishment; and that (2) litter-feeding soil animals react more strongly than do carnivores to above-mentioned changes in the vegetation.

To achieve the aim of the study a detailed sampling program was carried out in 1996 in four acid, species-poor grasslands under restoration succession. The time since last fertilisation differed for these four grasslands, i.e. 7, 11, 24 and 29 years, respectively. To test the hypotheses, we analysed the effects of management practices on the number of individuals, species richness, and assemblage composition of three groups of macro-invertebrates, i.e. terrestrial isopods and millipedes (both detritivores), and centipedes (polyphagous predators). Changes in the diversity and species composition of macro-invertebrates under restoration management are discussed in the light of general succession theory.

Materials and methods

Research area

Sampling was carried out in the stream valley of the Drentsche Aa, situated in the north-eastern part of the Netherlands (Fig. 1). In this stream valley, sites near the Anlooër Diepje brooklet (53°05'N, 6°40'E) were chosen to analyse the effect of restoration management on the composition of the soil macro-invertebrate community. The brooklet area mainly consists of grasslands. The soil type in these grasslands is loamy sand with a total clay plus silt content of 20–23%. Annual hay making for all plots is in August and hay is removed to reduce the nutrient availability in the soil.

Four grassland plots have been selected in the brooklet area and subjected to restoration succession. Prior to restoration these grasslands received on average 100–250 kg N ha⁻¹ year⁻¹ with sufficient P and K for plant growth (Verschoor 2001). Fertilisation of the plots stopped in 1989, 1985, 1972 and 1967, i.e. a final application of fertiliser to the plots 7, 11, 24 and 29 years ago, respectively (see Fig. 1 for the location in the brooklet area).

The experimental area acquired by the State Forestry Service in 1985 is situated at the border of a parcel with deciduous forest. Some general characteristics of the plots are given in Table 1.

Sampling programme

The four grassland plots were sampled from March to December 1996. Within each of the grasslands, a plot of 30×15 m² was randomly selected. Each plot was divided into three subplots of 10×15 m². A small tent or soil photoeklektor (see Hemerik and Brussaard 2002) was placed randomly within each of the subplots. To avoid severe damage to the vegetation, these photoeklektors were moved every fortnight to another randomly selected spot within the same subplot. Photoeklektors were never returned to a previously used spot. Each photoeklektor contained two pitfall traps (diameter 12 cm) filled with a 2% formaldehyde solution in which surface-dwelling invertebrates were captured. The traps were emptied weekly to collect the macro-invertebrates. When the traps were

Fig. 1 Location of the field site in the Netherlands. The *grey arrow* indicates the location of the sampling sites at the bank of the Anlooër Diepje brooklet (*upper right inset*). The *circled numbers* indicate the time (years) since last fertilisation and the *black arrows* the positions of the four plots in the area

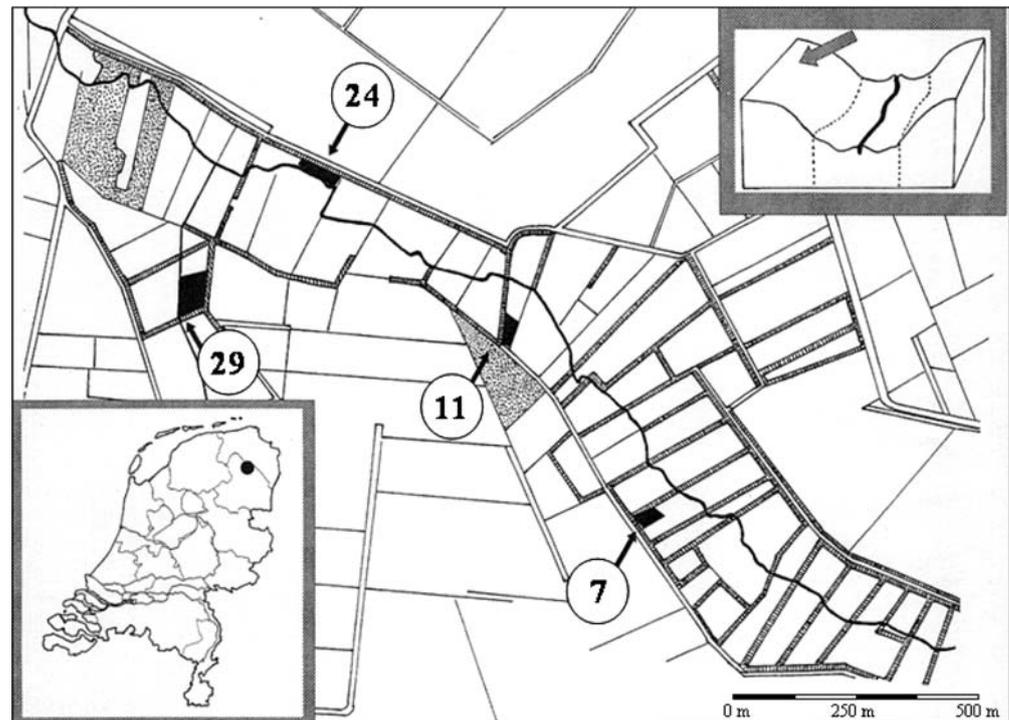


Table 1 Characteristics of the plots given in order of time since last fertiliser application (see Verschoor 2001)

Time of restoration (years)	Distance to brooklet (m)	pH _(H₂O) ^{a,b} mean (SD)	C/N ^b ratio	Moisture ^b (mean) (range in parentheses) % dry weight	Primary production ^b (g m ⁻²)	Dominant plant species (in order of dominance)
7	90	4.8 (0.24) a	19.3	27.8 (11.2–50.4)	422±107	<i>Agrostis stolonifera</i> , <i>Lolium perenne</i> , <i>Ranunculus repens</i> , <i>Holcus lanatus</i>
11	90	4.0 (0.28) b	24.4	22.0 (9.1–38.6)	257±96	<i>H. lanatus</i> , <i>A. stolonifera</i> , <i>Anthriscus sylvestris</i>
24	10	3.8 (0.08) c	23.5	29.1 (7.2–57.5)	236±101	<i>Agrostis capillaris</i> , <i>Festuca rubra</i> , <i>Anthoxanthum odoratum</i>
29	50	3.5 (0.08) d	35.0	31.8 (12.8–72.7)	252±38	Mosses, <i>F. rubra</i> , <i>A. capillaris</i> , <i>Galium saxatile</i>

^a*n*=5, non-bulked samples, results of a Kruskal-Wallis test and the pair-wise Mann-Whitney *U*-test are given (*different letters indicate significant differences at P*<0.05).

^bDepth 0–10 cm; *n*=3, non-bulked samples.

placed, soil was dug out, brought to the laboratory and hand-sorted to collect soil macro-invertebrates.

Additional data

We used the mean air temperature and the total weekly precipitation at Eelde Airport (see Hemerik and Brussaard 2002) in the week

before emptying the traps or collecting the soil samples in the analyses. Air temperature was highly correlated with soil temperature (RD-Data logger; Omega Technologies; 25-min intervals at a depth of 5–10 cm in all four plots). The wet weight of the soil was determined after hand sorting for invertebrates. Subsequently, soil was dried for 20 h at 70°C and weighed in order to determine the moisture content (as % dry weight). Data on soil pH_(H₂O), C/N ratio,

Table 2 Species-specific characteristics of terrestrial isopods, millipedes, and centipedes (after Berg 1995a,b, 1996)

Species	Body size (mm)	Soil humidity	Soil type	Trophic group	Biotope specificity	Occurrence (no. 10×10-km ² grid squares)	Rank (no10×10-km ² grid squares)
Terrestrial Isopoda							
<i>Ligidium hypnorum</i>	9	Hygrophilic	Clay/peat	Detritivores	Stenotope	83	7
<i>Oniscus asellus</i>	16	Indifferent	Indifferent	Detritivores	Eurytope	171	3
<i>Philoscia muscorum</i>	11	Indifferent	Indifferent	Detritivores	Eurytope	195	2
<i>Porcellio scaber</i>	17	Indifferent	Indifferent	Detritivores	Eurytope	199	1
<i>Trichoniscus pusillus</i>	5	Hygrophilic	Indifferent	Detritivores	Eurytope	160	4
Diplopoda							
<i>Brachydesmus superus</i>	9.5	Hygrophilic	Clay	Detritivores	Eurytope	56	7
<i>Craspedosoma raulinsii</i>	14.7	Hygrophilic	Sand/peat	Detritivores	Stenotope	91	6
<i>Cylindroiulus caeruleocinctus</i>	30.7	Indifferent	Clay	Detritivores	Eurytope	35	11
<i>Cylindroiulus latestriatus</i>	16.5	Indifferent	Sand/peat	Detritivores	Eurytope	61	9
<i>Cylindroiulus punctatus</i>	24.0	Indifferent	Sand	Detritivores	Stenotope	184	2
<i>Julus scandinavicus</i>	33.8	Indifferent	Indifferent	Detritivores	Eurytope	142	1
<i>Nemasoma varicorne</i>	13.7	Indifferent	Sand	Detritivores	Stenotope	21	21
<i>Ommatoiulus sabulosus</i>	38.5	Xerophilic	Sand	Detritivores	Stenotope	36	14
<i>Polydesmus denticulatus</i>	17.0	Hygrophilic	Sand/peat	Detritivores	Eurytope	101	3
<i>Proteroiulus fuscus</i>	13.0	Indifferent	Sand/peat	Detritivores	Eurytope	133	5
Chilopoda							
<i>Brachygeophilus truncorum</i>	13	Hygrophilic	Sand	Carnivores	Eurytope	99	6
<i>Lamyctes emarginatus</i>	9.3	Hygrophilic	Sand/peat	Carnivores	Stenotope	4	28
<i>Lithobius forficatus</i>	30	Indifferent	Indifferent	Carnivores	Eurytope	241	1
<i>Lithobius microps</i>	7.5	Hygrophilic	Clay	Carnivores	Eurytope	81	3
<i>Lithobius crassipes</i>	11.5	Indifferent	Sand	Carnivores	Eurytope	118	4
<i>Necrophlaeophagus flavus</i>	35	Indifferent	Clay	Carnivores	Eurytope	59	7
<i>Schendyla nemorensis</i>	17	Hygrophilic	Sand	Carnivores	Eurytope	99	5

and above-ground primary production were taken from Verschoor (2001).

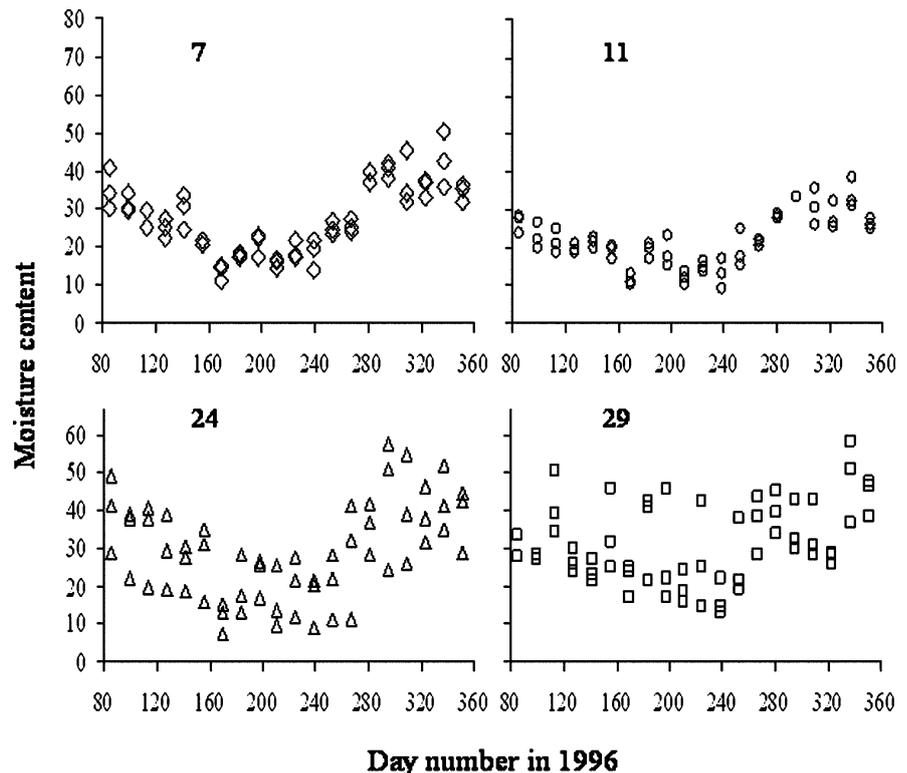
Identification and ecological profiles of macro-invertebrates

Terrestrial isopods, millipedes and centipedes were identified to the species level using the keys of Hopkin (1991), Schubart (1934), and Berg and Evenhuis (2001), respectively.

For each species an ecological profile was made, based on information on body size, trophic position in the food web, soil type and soil moisture preferences, biotope specificity, and distribution status in the Netherlands (Table 2). Body sizes were calculated from body length records obtained from the literature. For isopods the body sizes published by Hopkin (1991) were taken. Adult body length of millipedes was based on the average of the maximum length of males and females given in Schubart (1934). Adult body length of centipedes was calculated as the average of the body size range given in Berg and Evenhuis (2001).

Ecological information for the sampled isopods, millipedes, and centipedes was taken from Berg (1995a,b, 1996, respectively). Soil humidity preference classification was based on the occurrence of species in certain soil types. Five main types of soils occur in the Netherlands: sand, peat, loess, clay and brook valley soils. If a species has been recorded from clay and/or peat only it was assumed to be hygrophilic, whereas species only recorded in sandy soils were assumed to be xerophilic. Species indifferent to soil humidity are found in all soil types. Distribution status was based on the occurrence of species in hectads and on the number of records of species. Occurrence was expressed as the number of $10 \times 10\text{-km}^2$ grid squares in which a species has been recorded in the Netherlands since 1970, and ranking was based on the total sum of records per species over the period 1970–1995. The higher the number of occupied $10 \times 10\text{-km}^2$ grid squares and rank the more common a species is. Classification of species as either stenotopic or eurytopic depended on the number of biotopes in which a species has been recorded. Moreover, eurytopic species often have a high occurrence, and commonly are indifferent to soil type and/or soil humidity. Trophic group classification was based on Wallwork (1970).

Fig. 2 Moisture contents per fortnight for each of the treatments. For each sample date the moisture contents (% on dry weight basis) of the three subplots are given. Treatments are indicated by the time (7, 11, 24, 29 years) since last fertilisation



Statistical analysis

For the statistical analysis we first determined the percentage of the specimens found in soil samples and traps. Only 4% of the specimens were found in the soil samples. We analysed all data together, because we assume that all specimens are from the surface area covered by one tent. Each data point shows the content of one soil sample and trap at a specific date in the 9-month sampling period.

CANOCO for Windows version 4.0 (Ter Braak and Smilauer 1998) was used to summarise the data on species composition in the four plots during the 9 months in an ordination diagram. The resulting diagrams were interpreted with knowledge of plot variables, i.e. sampling day number, air temperature, precipitation and moisture, time since last fertiliser application and species-specific characteristics. The data were analysed by means of canonical correspondence analysis (Jongman et al. 1995) using Hill's scaling. Detrending was not selected. The significance of the first three axes was tested with a Monte-Carlo permutation test (Ter Braak and Smilauer 1998).

Results

Environmental variables in relation to field management

Soil $\text{pH}_{(\text{H}_2\text{O})}$ differed significantly between the four plots (Table 1). Between 7 and 11 years after ceasing fertilisation of the plots the pH dropped almost one unit. Thereafter, the decrease in pH was slower.

The C/N ratio of the soil was significantly correlated with time since fertilisation last applied ($r=0.81$, $n=4$, $P_{\text{one-sided}}=0.098$), with similar values for the plots 11 and 24

years after fertilization (Table 1). The increase in the C/N ratio was due to an increase in total C content, from 0.53 g cm⁻³ in plot 0 to 0.79 g cm⁻³ in the plot 29 years after fertilisation, while total N remained fairly constant (Verschoor 2001).

Aboveground primary production was highest, 7 years after ceasing fertilisation ($r=-0.444$, $n=12$, $P_{\text{one sided}}=0.074$) and was almost halved from then on in all subsequent plots (Table 1). No differences could be observed in aboveground primary production between the last three treatments.

Soil moisture contents did not differ among the treatments, although in the plots 24 and 29 years after cessation of fertilisation the variation in moisture content among the subplots was larger (Fig. 2).

Species observed

Five species of terrestrial isopods were caught during the sampling period (Tables 2, 3). Of these, *Ligidium hypnorum*, is moderately common in the Netherlands, while the four others, with *Philoscia muscorum* as the dominant species, belong to a group of very common and eurytopic species, with no preferences for a specific soil type (Table 2). Average density of isopods was not different among the treatments.

Ten millipede species were captured during the sampling period (Tables 2, 3). *Polydesmus denticulatus* was the dominant species. Average density for this species was highest in treatment C, i.e. 24 years since last fertilisation, and lowest in treatment K, i.e. 29 years after cessation of fertilisation. Within the group of millipedes, a clear range in body size, soil type and moisture preference was present (Table 2). Half the species were classified as eurytopic, the other half as stenotopic. Most of the stenotopic species prefer nutrient-poor, sandy or peaty soils.

The assemblage of centipedes comprised seven species of which only one was found in the plot with the longest period since the last application of fertiliser (Tables 2, 3). One stenotopic species was found in large densities, *Lamyctes emarginatus*, a rather hygrophilic and rare species with a restricted distribution (Table 2). Significant differences in average densities were found (Table 3). The other species observed are common to very common, and eurytopic. The most abundant species in the Netherlands, i.e. *Lithobius forficatus* had generally a very low abundance in our plots (Table 2). With the exception of *Necrophloeophagus flavus*, and to a lesser extent *Brachygeophilus truncorum* and *Schendyla nemorensis*, the observed species prefer to live at the soil surface.

Species that were found at intermediate vegetation succession stages, 11 and 24 years after fertiliser application, could generally be characterised as eurytopic species without preference for a certain soil moisture level,

Table 3 Average number of specimen captured per metre square in the plots during the total sampling period ($n=3$; SD in parentheses). For each species the average densities were first tested with a Kruskal-Wallis test, and when this test indicated a difference ($\alpha=0.10$) between the four plots under restoration succession pair-wise comparison was performed with the Mann-Whitney *U*-test (Siegel 1956). Different letters within a row indicate significant differences between the treatments. Treatments are indicated by the number of years since last fertiliser application

Species	Treatment			
	7 Years	11 Years	24 Years	29 Years
Terrestrial Isopoda				
<i>Ligidium hypnorum</i>	– a	– a	2.00 (2.00) b	– a
<i>Oniscus asellus</i>	0.33 (0.58) a	– a	0.33 (0.58) a	– a
<i>Philoscia muscorum</i>	21.67 (12.01) a	24.33 (6.66) a	25.00 (5.57) a	9.00 (3.00) a
<i>Porcellio scaber</i>	– a	– a	0.33 (0.58) a	– a
<i>Trichoniscus pusillus</i>	0.33 (0.58) a	0.67 (1.15) a	10.67 (9.71) a	0.67 (1.15) a
Diplopoda				
<i>Brachydesmus superus</i>	– a	1.33 (1.15) a	1.00 (1.73) a	– a
<i>Craspedosoma raulinsii</i>	2.00 (2.65) a	2.00 (1.00) a	1.67 (2.08) a	0.33 (0.58) a
<i>Cylindroiulus caeruleocinctus</i>	– a	1.33 (1.53) b	– a	– a
<i>Cylindroiulus latestriatus</i>	– a	– a	0.67 (0.58) b	– a
<i>Cylindroiulus punctatus</i>	0.33 (0.58) a	1.00 (1.00) a	– a	– a
<i>Julus scandinavicus</i>	– a	0.67 (0.58) a	1.00 (1.00) a	– a
<i>Nemasoma varicorne</i>	– a	– a	0.33 (0.58) a	– a
<i>Ommatoiulus sabulosus</i>	– a	0.67 (0.58) a	– a	– a
<i>Polydesmus denticulatus</i>	5.00 (3.61) a	4.67 (3.21) a	19.00 (12.00) b	1.00 (1.00) c
<i>Proteroiulus fuscus</i>	0.33 (0.58) a	– a	1.33 (0.58) b	0.33 (0.58) a
Chilopoda				
<i>Brachygeophilus truncorum</i>	4.67 (1.15) a	2.67 (3.79) a	1.33 (2.31) a	– a
<i>Lamyctes emarginatus</i>	– a	11.00 (2.00) b	4.00 (1.73) c	1.67 (1.15) d
<i>Lithobius crassipes</i>	– a	1.33 (1.53) a	1.67 (2.08) a	– a
<i>Lithobius forficatus</i>	– a	0.33 (0.58) a	0.67 (0.58) a	– a
<i>Lithobius microps</i>	0.33 (0.58) a	– a	1.33 (2.31) a	– a
<i>Necrophlaeophagus flavus</i>	– a	– a	0.67 (1.15) a	– a
<i>Schendyla nemorensis</i>	– a	0.67 (1.15) a	0.67 (0.58) a	– a

and with a common to very common occurrence nationally (Tables 2, 3). No general pattern in preference of a certain soil type was found.

The canonical correspondence analysis showed that a small but significant part (2%, $P=0.005$) of the variation in the isopod, millipede and centipede community between samples was explained by the factors temperature, moisture, soil pH, C/N ratio, and primary production. We found previously that the abiotic factors temperature and precipitation were strongly correlated to each other ($r=0.97$). Therefore, we did not include precipitation as a factor in the final analysis, only the time since last fertiliser application, temperature, moisture and day number. The first canonical axis in the analysis with 2% variation explained is correlated with temperature and moisture.

Total density and diversity

All three groups of soil macro-invertebrates showed a similar pattern of changes in density and diversity since cessation of fertilisation. With increasing time since last fertiliser application, the total density of the community increased from 105 to 221 specimens, and then dropped to only 39 specimens in the plot 29 years since last fertilisation (Fig. 3a). Similarly, total diversity increased with time since last fertiliser application up until 24 years, i.e. from nine to 19 species, and declined to six species in

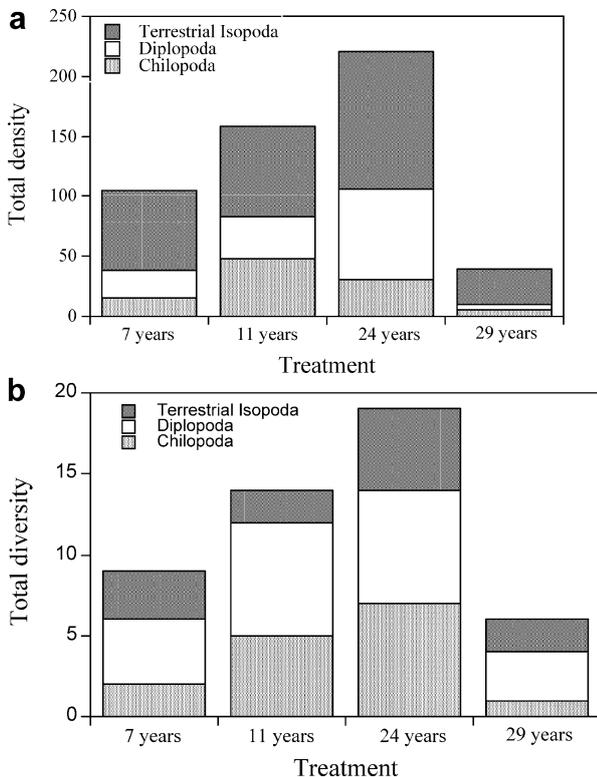


Fig. 3 **a** Total density in numbers recovered per plot and **b** total diversity in number of identified species for the four treatments. The totals are sums over the total sampling period of 9 months. Treatments are indicated by the time since last fertilisation

the plot which had not been fertilised for 29 years (Fig. 3b).

The increase in total density was accompanied by an increase in the total diversity ($r^2=0.93$; Fig. 4). No correlation between soil pH and aboveground primary production and diversity or density of macro-invertebrates was found.

Discussion

Restoration management stimulated the density of macro-invertebrates during the first 24 years following the final application of fertiliser. The observed increase in total C content (Verschoor 2001) and C/N ratio, and the decline in soil pH and nutrient availability with time lapsed since cessation of fertilisation indicate an accumulation of organic matter in the topsoil. There is evidence from field studies that bottom-up control, i.e. an increase in basal resources, can be important in regulating soil fauna groups. The formation of a C-rich surface layer (Scheu and Schulz 1996; Frouz 1997) and addition of organic substrates to field plots (Chen and Wise 1999) strongly stimulate the density of soil macro-invertebrates. Two processes play a role in this relationship. Firstly, accumulation of soil C results in the formation of a litter layer, which increases the habitable space for soil organisms. When organic matter accumulates soil water retention is enhanced, which might protect macro-invertebrates that are susceptible to water-loss from desiccation. Most species recorded in our study predominantly colonise the litter layer, and a large part of the species prefers moist soil conditions. Secondly, the large variety in micro-habitats and food resources present in accumulating litter favours the establishment of a highly diverse community (Anderson 1977; Lavelle and Spain 2001), which includes a large proportion of litter-feeding animals, such as isopods and millipedes. Scheu and Poser (1996) and Chen and Wise (1999) found an increase in the density of centipedes caused by accumulation of litter and

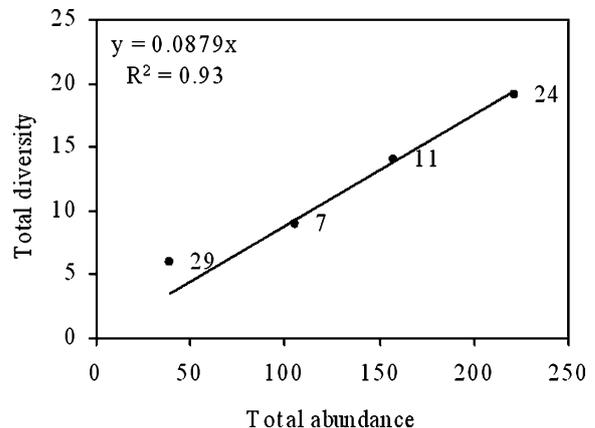


Fig. 4 Correlation between the total density per plot and the total number of species per plot during the total observation time. Treatments are indicated by the time since last fertilisation

argued that this can be explained by an increase in prey density.

The lowest numbers of invertebrate individuals were caught in the plot impoverished for 29 years, a trend that has also been observed for other taxonomic groups of soil fauna at the study site (Hemerik and Brussaard 2002). Although this plot showed the highest amount of organic matter, positive effects of soil C accumulation on macro-invertebrate density might here be counteracted by the combined detrimental effects of a low soil pH and nutrient status, the build-up of recalcitrant organic matter and the striking shift in vegetation composition. Most macro-invertebrate species are known to be acid-sensitive (Kopeszki 1991; Scheu and Poser 1996). At low soil pH the availability of Ca and Mg will strongly diminish, even across small differences in pH (Berg et al. 1997). The need of isopods and millipedes to accumulate Ca and Mg for their exoskeleton makes them vulnerable to low availability of these minerals (Hopkin and Read 1992; Warburg 1993). Millipedes appear to be selective feeders, with preference for litter with a high Ca content (Lyford 1943; Neuhauser and Hartenstein 1978), and isopods assimilate high percentages of Ca from their food (Radu et al. 1971; Coughtrey et al. 1980). Moreover, during decomposition of soil organic matter recalcitrant organic compounds, such as phenolics and humic substances, are formed (Killham 1994; Lavelle and Spain 2001). Recalcitrant organic matter is low in food quality for detritivores. Isopods preferentially ingest decomposing material with a low phenolic content (Neuhauser and Hartenstein 1978). The amount of palatable food items might be further reduced by the substantial moss cover, which equalled the total cover by grasses (Verschoor et al. 2002), that was observed 29 years after cessation of fertiliser application. Mosses also represent a low quality food source for soil fauna (Lawrey 1987).

According to succession theory a progressive and directional change in species composition during secondary succession is to be expected (Horn 1974; Connell and Slatyer 1977), because at each stage species modify the environment to make it less suitable for themselves (inhibition hypothesis), species prepare the environment for later successional species (facilitation hypothesis), or species utilise specific resources irrespective of the species that are present in the community (tolerance hypothesis). The plant species-rich vegetation specific for nutrient-poor grasslands has been restored after approximately 30 years of restoration management (Olf and Bakker 1991). Assuming that the four plots investigated represent sequential stages of secondary succession, a continuous change in plant composition was observed during this period. Seven years after the last fertiliser application four plant species were dominant, including one herb. During secondary succession the vegetation became dominated by fewer species, the dominance of grasses increased, and in each stage a different grass species prevailed. A drastic change occurred in species composition after 24 years since the cessation of fertilisation, when a substantial moss cover had developed. Secondary vegetation succession at this site is primarily driven by the reduced availability of nutrients in the soil (Olf and Bakker 1991; Olf et al. 1994).

Although a progressive and directional change was observed in the vegetation composition, as predicted by succession theory, this was not accompanied by a directional change in the composition of the macro-invertebrates. Although the diversity of all groups increased with time after cessation of fertiliser application no replacement of species occurred. No species were found that occurred exclusively in the plots 7 and 29 years after cessation of fertilisation. The community in each of these plots comprised a subset of the community found at intermediate time intervals. Almost all species present in the community after 29 years of restoration management were also present 7 years after the last fertiliser application. Moreover, in general the species that occurred at intermediate succession stages only were eurytopic, rather common regionally, and even known to prefer different soil types. Scheu and Schulz (1996) and Tajovski (2000), in one of the few studies on secondary succession of macro-invertebrates, found similar results. No replacement of macro-invertebrate species during secondary succession was observed after cessation of cultivation of an arable field, which developed into a beech forest (Scheu and Schulz 1996). Based on their results these authors concluded that changes in soil macrofauna communities could not be straightforwardly explained by succession theory. One reason might be that interspecific competition between soil animals is of less importance to the structuring of soil fauna communities than environmental changes induced by vegetation development during secondary succession.

Although we did not observe directional change in macro-invertebrate composition during secondary succession, vegetation development influenced the density and diversity of the macro-invertebrate community. Plant species are known to affect the soil food web by the quality and quantity of plant litter (Wardle 2002). Conversely, recent findings show that soil invertebrate fauna enhance grassland succession and diversity (Bradford et al. 2002; De Deyn et al. 2003). An understanding of the interactions between the aboveground and belowground parts of ecosystems, especially of the fauna that directly depends on the plants, and how this drives succession in ecosystems is fundamental for the conservation of macro-invertebrate and plant species-rich natural grasslands.

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