RESEARCH ARTICLE

Soil Organism and Plant Introductions in Restoration of Species-Rich Grassland Communities

Paul Kardol,¹,² T. Martijn Bezemer,¹,³,⁴ and Wim H. Van Der Putten¹,³

Abstract

Soil organisms can strongly affect competitive interactions and successional replacements of grassland plant species. However, introduction of whole soil communities as management strategy in grassland restoration has received little experimental testing. In a 5-year field experiment at a topsoil-removed ex-arable site (receptor site), we tested effects of (1) spreading hay and soil, independently or combined, and (2) transplanting intact turfs on plant and soil nematode community development. Material for the treatments was obtained from later successional, species-rich grassland (donor site). Spreading hay affected plant community composition, whereas spreading soil did not have additional effects. Plant species composition of transplanted turfs became less similar to that in the donor site. Moreover, most plants did not expand into the receiving plots. Soil spreading and turf transplantation did not affect soil nematode community composition. Unfavorable soil conditions (e.g., low organic matter content and seasonal fluctuations in water level) at the receptor site may have limited plant and nematode survival in the turfs and may have precluded successful establishment outside the turfs. We conclude that introduction of later successional soil organisms into a topsoil-removed soil did not facilitate the establishment of later successional plants, probably because of the “mismatch” in abiotic soil conditions between the donor and the receptor site. Further research should focus on the required conditions for establishment of soil organisms at restoration sites in order to make use of their contribution to grassland restoration. We propose that introduction of organisms from “intermediate” stages will be more effective as management strategy than introduction of organisms from “target” stages.

Key words: aboveground–belowground linkages, nematodes, plant–soil organism interactions, principle response curves, soil spreading, turf transplantation.

Introduction

Conversion of agricultural land into species-rich grasslands is a common practice in order to restore plant species diversity. After agricultural land abandonment, the major abiotic constraint for development of species-rich grasslands is high soil fertility. As plant species diversity is highest at intermediate soil fertility (Grime 1973), the majority of restoration projects on ex-arable land are treated by addition of carbon-rich substrates (e.g., Blumenthal et al. 2003), topsoil removal (Marrs 1985; Van Diggelen et al. 1997), grazing, or hay removal (e.g., Bakker & Olff 1995; Pywell et al. 2002) in order to reduce soil fertility. Also, the absence of propagules of later successional “target” species can be an important constraint when restoring species-rich grasslands (Bakker & Berendse 1999). So far, nature restoration has made little use of recent studies showing that on smaller spatiotemporal scales, interactions with soil organisms can strongly affect competitive interactions and successional replacements of plant species (De Deyn et al. 2003, 2004; Kardol et al. 2007).

Soil organisms can affect plant community composition both directly and indirectly. Direct effects can be attributed to altered competitive ability of plants that accumulate parasites, pathogens, and root herbivores or mutualistic symbionts (Johnson et al. 1991; Van der Putten et al. 1993). Indirectly, decomposers affect plant community interactions by releasing nutrients from litter, root exudates, and soil organic matter (Wardle 2002). In secondary grassland succession, soil organisms can selectively suppress plant species from production grasslands, thereby enhancing the relative abundance of later successional plant species (De Deyn et al. 2003). On ex-arable land, soil organisms may play a similar role in enhancing initial succession and retarding succession when time after abandonment proceeds (Brown & Gange 1992; Kardol et al. 2006). However, this concept has not yet been tested as management strategy in restoration of species-rich grasslands.

As plant and soil communities are mutually dependent upon each other (Wardle et al. 2004), it is likely that synchronized introductions of plant propagules and soil organisms are required to successfully establish their mutual relationship. Different approaches can be used to introduce later successional plant propagules and soil organisms.

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First, hay and soil originating from a reference habitat can be spread at the restoration site. Seeds present in hay collected from species-rich grassland may ensure introduction of desired species (e.g., Kiehl & Wagner 2006). Spreading a thin layer of topsoil obtained from a target habitat may introduce the desired soil organisms. However, establishment of late successional soil communities could be inhibited by the resident soil community (De Deyn 2003). Introductions of invertebrates and soil microorganisms were successful in sterilized soil in greenhouse conditions (De Deyn et al. 2003; Kardol et al. 2007). Probably, elimination of the resident soil community by topsoil removal could enhance establishment of introduced soil organisms.

Alternatively, intact turfs obtained from a target habitat, containing both soil and vegetation, could be introduced to function as “stepping stones” for later successional plants and soil organisms (Bullock 1998). Established turfs may provide a local pool from which plant species can spread by seeds into the receptor site (Pywell et al. 1995). Alternatively, plant species may gradually colonize the receptor site by vegetative growth and enhance the area of target habitat. So far, studies involving turf transplantation, as well as studies involving addition of topsoil, evaluated the effects on ecosystem restoration by measuring changes in plant species composition and chemical or physical soil conditions (e.g., Pywell et al. 1995; Bullock 1998; Standen & Owen 1999; Kailová 2000; Vécir & Muller 2003). However, concomitant effects on soil organisms, essential for establishment of later successional plant–soil organism relationships, are usually ignored (Pywell et al. 2007).

We tested the hypothesis that simultaneous introduction of plant propagules and soil organisms originating from a later successional, species-rich *Cirsio-Molinietum* fen meadow (the donor site) enhances plant species diversity and community development of the restoration area (the receptor site) toward the target (donor) plant community and stimulates soil community development. We analyzed community composition of soil nematodes because they are abundant, trophically diverse, sensitive to disturbances, and are indicative of ecosystem functioning (Bongers & Ferris 1999) and successional changes in the soil subsystem (Kardol et al. 2005). In a 5-year field experiment at an ex-arable field from which the topsoil had been removed (the receptor site), we tested our hypothesis using two alternative approaches: (1) spreading hay and soil, independently or combined, and (2) transplanting turfs. We also compared plant and soil community development in the transplanted turfs with that in the donor site and assessed “turf survival.” Finally, we compared effects of topsoil removal with no topsoil removal.

**Methods**

**Site Description and Experimental Design**

The experiment was carried out on a former agricultural field in Lievelde, The Netherlands (lat 52°01’N, long 5°36’E). The field site was located on sandy deposits and had been cultivated most recently by maize. Between December 2000 and March 2001, 50 cm of the topsoil had been removed down to the mineral subsoil. In August 2001, we set up an experiment in which we examined effects of introducing later successional plant species and soil organisms by (1) spreading hay; (2) spreading soil; (3) spreading soil + hay; and (4) transplantation of intact turfs, containing both soil and vegetation. Material for the hay (i.e., fresh harvested shoots and seed capsules), soil, and turfs was obtained from a reference, or donor site located 250 m from the receptor site. We also included control plots where no further actions had been taken. The five treatments were carried out in a randomized block design, with five replicates of each treatment.

The donor site was a species-rich *Cirsio-Molinietum* fen meadow, dominated by Vernal grass (*Anthoxanthum odoratum*), Carnation sedge (*Carex panicea*), French hardhead (*Centaurea jacea*), Heath spotted orchid (*Dactylorhiza maculata*), Devil’s bit scabious (*Succisa pratensis*), and Yellow loosestrife (*Lysimachia vulgaris*) (Jongejans 2004). Early August, hay was collected after mowing and drying for 3 days, and soil and turfs of 25 × 25 × 10 cm³ were collected from the upper 10 cm of a 2 × 5-m² randomly chosen area. Hay and soil treatments were applied by spreading the material homogeneously over the plots, which were scarcely covered by vegetation at the time of application. Soil was spread as 2.5 L/m², hay was spread so that 50–75% of the soil surface was covered, and turfs were placed at a density of 1/m² (Appendix).

Plots were 5 × 5 m² and separated by 1-m border rows. Owing to slight differences in elevation, soil moisture differed across the field, and we placed two blocks 35 m from the other blocks in order to include some of the environmental heterogeneity in our design. Additionally, we installed five 5 × 5-m² plots within the field margin where the topsoil had not been removed (“no topsoil removal”). To compare plant and soil nematode community development at the transplanted turfs with plant community development at the donor site, we installed five plots of 0.75 × 0.75 m² (i.e., the aggregated size of nine transplanted turfs) at the donor site, adjacent to the area from which the turfs had been excavated. The receptor and the donor site were mown each year at end of August after which the hay was removed.

**Plant Community**

The percent cover of each vascular plant species was recorded in the inner 3 × 3 m² of each plot annually from August 2003 to August 2006. For turf transplantations, vegetation recordings were made separately for the inner nine turfs (“transplanted turfs”) and for the area in between the turfs (“turf-receiving” treatments) (Appendix). The vegetation was also recorded annually in the 0.75 × 0.75–m² plots at the donor site.
Soil Properties
In August 2003 and 2005, nine soil samples of Ø 3.5 cm and 10 cm depth were collected from each plot according to a grid pattern and then bulked, homogenized, and stored at 4°C until analysis. For turf transplantations, soil samples were collected from the center of the inner nine turfs and from nine positions in-between the turfs (i.e., turf-receiving treatment) corresponding to the pattern of the samples in the other plots (Appendix). Also from the plots at the donor site, nine soil samples were collected. Soil samples from 2003 were analyzed for ergosterol to determine fungal biomass as indicator of successional development of the soil (Van der Wal et al. 2006), chemical properties, and nematodes. Soil samples from 2005 were analyzed for moisture content and nematodes.

Soil samples for ergosterol and chemical properties were sieved (4-mm mesh) prior to analysis. Ergosterol was measured using a disruptive extraction (Van der Wal et al. 2006). For each sample, the net N mineralization or immobilization was determined after incubation of 50 g fresh soil at 20°C for 6 weeks, as the difference between the initial and the final amount of mineral N (NH₄+ + NO₃−). Mineral N, potential available P, total N and P, soil organic matter, pH, and moisture content were measured according to Van der Wal et al. (2006). Nematodes were extracted by Oostenbrink elutriators (Oostenbrink 1960) and analyzed according to Kardol et al. (2005). Nematodes were allocated to feeding groups according to Yeates et al. (1993).

Data Analysis
Soil properties, ergosterol content, and number of nematodes (total and within feeding groups; separately for 2003 and 2005) were analyzed using one-way analysis of variance (ANOVA), with treatment as fixed factor. Plant species richness was measured as the number of species per plot and analyzed by repeated measures ANOVA, with treatment (control, hay, soil, hay + soil, turf receiving, and no topsoil removal) as fixed factor and year as repeated measure. A contrast was specified to analyze differences in species richness between no topsoil removal and topsoil-removed treatments. When the assumptions of normality and homogeneity of variances were not met, data were log(x + 1) transformed or analyzed using a non-parametric Kruskal–Wallis test. Univariate analyses were performed using STATISTICA (release 7.1). Response of the taxonomic composition of soil nematodes to treatments (control, hay, hay + soil, turf receiving, transplanted turfs) was analyzed by principal component analysis (PCA) (samples from the non–topsoil-removed plots, which markedly differed from all other treatments, were excluded). Differences in taxonomic composition among control, hay, soil, hay + soil, and turf-receiving treatments, and transplanted turfs were tested using redundancy analysis (RDA) with Monte Carlo permutation tests (999 unrestricted permutations). The analyses were carried out separately for 2003 and 2005. Rare nematode taxa (found in ≤2 samples) were excluded from the analyses.

We analyzed effects of the experimental manipulations on plant community development using RDA and principal response curves (PRCs) according to Lepš and Šmilauer (2003). We tested the overall treatment effect on plant community development by including all treatment × year interactions (i.e., hay × year, soil × year, [hay + soil] × year, turf receiving × year) as explanatory variables in RDA. In detailed analyses, we separately tested the effect of each treatment (hay, soil, hay + soil, and turf receiving) on the temporal changes in plant species composition by testing the explanatory power of each particular treatment × year interaction, whereas defining the remaining treatment × year interactions, except the control × year interaction, as covariables. Significance of treatment × year interactions was tested with Monte Carlo permutation tests restricted to split-plot design to reflect the repeated measurements.

PRC is an extension of RDA and expresses treatments as deviations from a reference treatment (Van den Brink & ter Braak 1999). PRC first accounts for variation in species composition due to time and then attributes the remaining variation to the experimental treatments. We generated PRC diagrams by plotting the first principal component of the treatment effects against time. PRC diagrams were generated by comparing the treatments to (1) the control and (2) the donor site (both including and excluding no topsoil removal treatments). To compare the community composition of the transplanted turfs relative to area between the turfs (i.e., turf receiving), a separated PRC diagram was generated including data of the control treatment, the transplanted turfs, and the turf-receiving treatments, using the donor site as the reference treatment. We tested the significance of the first and of higher order PRCs by Monte Carlo permutations tests. We interpreted the directional changes in plant community composition by integrating the response of the individual plant species in the PRC diagrams, using a species weight diagram showing the affinity of the plant species with the treatment responses. Multivariate analyses were performed using CANOCO, version 4.5 (Ter Braak & Šmilauer 2002).

Because plant communities of the transplanted turfs originated from the donor site, we were explicitly interested in the similarity between these two plant communities over time. Therefore, we calculated Sørenson’s quantitative index (Magurran 1988), hereafter referred to as Sørenson’s similarity (Cs). For each year, we calculated the similarity between the transplanted turfs and the plots at the donor site as well as among the plots at the donor site. For each of the two datasets, we performed a linear regression analysis. To analyze if the similarity between transplanted turfs and the plots at the donor site differed from the similarity among plots at the donor site, we tested the null hypotheses that the intercepts and slopes of
the regression analyses did not differ (t tests). For nematode communities, we calculated Sørenson’s similarity between transplanted turfs and plots at the donor site and tested the difference in similarity between 2003 and 2005 by one-way ANOVA.

**Results**

**Soil Properties**

We did not observe differences in nematode abundance among control, hay, soil, hay + soil, and turf-receiving treatments for any of the feeding groups (Fig. 1). In 2003, the total number of nematodes was approximately 4-fold higher at the donor site than at the receptor site, whereas the total number of nematodes in the transplanted turfs was intermediate between numbers in the donor site and the turf-receiving treatments. In 2005, numbers of nematodes did not differ among treatments at the receptor site, the transplanted turfs, and the donor site (Fig. 1). Both in 2003 and in 2005, the number of bacterial feeders was significantly higher in no topsoil removal plots than in plots at the topsoil-removed site.

Nematodes were classified into 40 different taxa, mostly to family or genus level. The taxonomic composition of the nematode community did not differ among control, hay, soil, hay + soil, and turf-receiving treatments (RDA with permutation tests for all canonical axes for 2003 and 2005: $F = 1.01, p = 0.44$ and $F = 0.85, p = 0.64$, respectively). However, in 2003, PCA analysis revealed clear

![Figure 1. Densities of nematodes per feeding group in no topsoil removal (NTSR), control (C), hay (H), soil (S), hay + soil (HS), and turf-receiving (TR) treatments, and in the transplanted turfs (T) and the donor site (D). Data are $X \pm SE (n = 5)$. The statistical results indicate overall treatment effects for 2003 and 2005. Different letters denote significant differences between means based on one-way ANOVA, followed by Tukey post hoc tests or Kruskal–Wallis test with multiple comparisons of mean ranks ($p < 0.05$).](image-url)
separation of nematode communities in the donor site and in the treatments at the receptor site (Fig. 2). Root (hair-)feeding Tylenchidae and Dolichodoridae and omnivorous Dorylaimidae were particularly abundant at the donor site. The transplanted turfs clustered separately from the donor site and appeared to develop “toward” the surrounding communities at the topsoil-removed soil. Although the distinction was less clear, the ordination pattern for 2005 resembled the 2003 situation (Fig. 2). Similarity in nematode community composition of the transplanted turfs to the plots at the donor did not differ between 2003 and 2005 ($F_{1,8} = 0.25, p = 0.63$). Both in 2003 and in 2005, the nematode community composition in no topsoil removal treatments differed considerably from the treatments at the topsoil-removed site. Bacterial-feeding taxa, such as Rhabditidae and Metateratocephalus,

Figure 2. Ordination diagram (species–samples biplot) of PCA for the soil nematode community in 2003 and 2005. Each data point represents one treatment plot. Ovals are drawn to highlight clusters of samples from replicate treatments. Samples from the control, hay, soil, hay + soil, and turf-receiving treatments could not be clustered separately. Eigenvalues along the axes indicate the amount of explained variability in species composition. For clarity, only taxa with the best-fit range are shown.
were more abundant in no topsoil removal treatments than in treatments at the topsoil-removed site (data not shown).

Abiotic soil properties in hay, soil, hay + soil, and turf-receiving treatments did not differ from those in the control or from each other (Table 1). Compared to no topsoil removal, the treatments at the topsoil-removed receptor site were characterized by lower N mineralization, P availability, total N, total P, and organic matter content and moisture content. The donor site was characterized by low pH, high organic matter content, high moisture content, and relatively low P availability. Values of chemical soil properties of the transplanted turfs were generally in between the values of the donor site and the topsoil-removed site. Ergosterol content, as indicator of fungal biomass, was particularly high in the transplanted turfs.

Plant Community
The plant community development differed among the experimental treatments (RDA with permutation tests: \( F = 1.67, p = 0.03 \)). Detailed analyses showed that hay and hay + soil spreading significantly explained variation in plant community development (\( F = 3.31, p = 0.01 \) and \( F = 2.70, p = 0.03 \), respectively), whereas soil spreading alone and turf transplantation did not (\( F = 1.92, p = 0.09 \) and \( F = 1.30, p = 0.25 \), respectively). Plots with hay and hay + soil had higher abundance of grasses and other monocot species, such as Red fescue (\( Festuca rubra \)), Anthoxanthum odoratum, and Carex panicea, whereas control plots had higher abundance of Common bent (\( Agrostis capillaris \)) and Purple loosestrife (\( Lythrum salicaria \)) (Fig. 3A). However, for hay and hay + soil spreading, there was no clear directional development in plant community composition away from the control treatment. Instead, plant community composition converged to that of the control after 2005 (Fig. 3A). The differences in PRC scores between the treatments in the year 2003 should be attributed to divergence in plant community development during the first 2 years after the treatments had been installed.

Plant community composition of all treatments (including the control) at the receptor site tended to converge toward the donor site (Fig. 3B). Hay and hay + soil treatments were most similar to the donor site. Nevertheless, 5 years after initiating the treatments, the overall difference between the topsoil-removed receptor site, irrespective of treatments, and the donor site was substantial (indicated by the high scores on the y-axis). The donor site was characterized by higher abundance of An. odoratum and Lysimachia vulgaris, whereas the topsoil-removed receptor site was characterized by higher abundance of Lesser hawkbit (\( Leontodon saxatile \)), L. salicaria, and different rushes. Irrespective of treatments, some rare plant species, which could be defined as “target species” for restoration of species-rich grasslands, such as Red centaury (\( Centaurea erythraea \)), Dactylorhiza maculata, Heath grass (\( Dianthus decumbens \)), Moor matt grass (\( Nardus stricta \)), and Little green sedge (\( Ca. oederi ssp. oederi \)), established at the receptor site. The no topsoil removal treatment differed considerably from the other treatments and was far more different from the donor site than all the topsoil-removed treatments (Fig. 3C). The no topsoil removal treatment had few species in common with the topsoil-removed site and was dominated by ruderal, nitrophilous species, such as Stinging nettle (\( Urtica dioica \)).

Plant community composition of the transplanted turfs tended to diverge over time from that of the donor site (Fig. 3D), and most plant species of the transplanted turfs did not expand outside the turfs (P. Kardol 2006, Netherlands Institute of Ecology [NIOO-KNAW], personal observation). For Lys. vulgaris, which was among the dominant plant species at both the donor site and the transplanted turfs, we could detect spread from the turfs into the whole plot. Probably, this was also the case for

**Table 1.** Soil characteristics (0–10 cm) at the donor site and at the treatment plots in August 2003.

<table>
<thead>
<tr>
<th></th>
<th>N Mineralization (N, mg/kg)</th>
<th>P-Olsen (mg/kg)</th>
<th>Total N (g/kg)*</th>
<th>Total P (mg/kg)*</th>
<th>% SOM</th>
<th>pH</th>
<th>% Moisture</th>
<th>Ergosterol (mg/kg)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor site</td>
<td>14.84 ± 1.83a</td>
<td>2.92 ± 0.32b</td>
<td>2.77 ± 0.06a</td>
<td>239 ± 6b</td>
<td>6.28 ± 0.17a</td>
<td>4.87 ± 0.03b</td>
<td>37.2 ± 0.6a</td>
<td>37.3 ± 0.9a</td>
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<tr>
<td>Control</td>
<td>3.29 ± 0.86b</td>
<td>9.24 ± 4.15b</td>
<td>0.21 ± 0.04a</td>
<td>72 ± 15a</td>
<td>1.14 ± 0.15c</td>
<td>6.28 ± 0.09b</td>
<td>19.8 ± 1.7b</td>
<td>22.1 ± 1.1bc</td>
</tr>
<tr>
<td>Hay</td>
<td>1.10 ± 0.32b</td>
<td>4.36 ± 1.36b</td>
<td>0.21 ± 0.03a</td>
<td>47 ± 6a</td>
<td>1.04 ± 0.15c</td>
<td>6.33 ± 0.35a</td>
<td>20.8 ± 2.8b</td>
<td>21.6 ± 1.5bc</td>
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<tr>
<td>Soil</td>
<td>1.46 ± 0.51b</td>
<td>5.39 ± 1.86a</td>
<td>0.24 ± 0.02a</td>
<td>57 ± 9a</td>
<td>1.19 ± 0.13c</td>
<td>6.15 ± 0.04b</td>
<td>20.6 ± 1.3a</td>
<td>23.7 ± 1.2bc</td>
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<tr>
<td>Hay + soil</td>
<td>2.64 ± 1.27b</td>
<td>6.42 ± 1.69b</td>
<td>0.25 ± 0.03a</td>
<td>73 ± 13a</td>
<td>1.23 ± 0.10c</td>
<td>6.14 ± 0.04b</td>
<td>21.0 ± 2.5b</td>
<td>22.0 ± 2.1c</td>
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<td>Turf receiving</td>
<td>2.26 ± 0.74b</td>
<td>5.08 ± 1.66b</td>
<td>0.22 ± 0.01a</td>
<td>64 ± 10a</td>
<td>1.10 ± 0.08c</td>
<td>6.25 ± 0.05a</td>
<td>18.5 ± 1.0b</td>
<td>19.2 ± 1.3c</td>
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<tr>
<td>Transplanted</td>
<td>1.87 ± 0.66b</td>
<td>3.33 ± 0.63b</td>
<td>1.44 ± 0.06b</td>
<td>137 ± 5b</td>
<td>3.94 ± 0.16b</td>
<td>5.34 ± 0.02b</td>
<td>30.4 ± 2.3b</td>
<td>34.5 ± 1.5b</td>
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<tr>
<td>turf</td>
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<td></td>
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<tr>
<td>No TSR</td>
<td>41.49 ± 12.07a</td>
<td>93.16 ± 10.34a</td>
<td>2.86 ± 0.27a</td>
<td>933 ± 72a</td>
<td>6.77 ± 0.63a</td>
<td>5.78 ± 0.04b</td>
<td>26.6 ± 2.3a</td>
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<td>One-way ANOVA</td>
<td>F[7,32]</td>
<td>19.60</td>
<td>14.03</td>
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<td>77.03</td>
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</table>

Moisture content is also given for August 2005. For turf transplantation, data are presented for the receiving plots (turf receiving) and for the transplanted turfs. Data are \( X ± SE \) (\( n = 5 \)). Different letters denote significant differences between means based on one-way ANOVA, followed by Tukey post hoc tests or Kruskal–Wallis test (indicated by an asterisk) with multiple comparisons of mean ranks (\( p < 0.05 \)). No TSR, no topsoil removal; SOM, soil organic matter.
Figure 3. PRCs for the first RDA axes showing response over time of the plant community to the treatments relative to a chosen reference treatment. Reference treatments are presented as a horizontal line along the time axis. (A) Deviation in plant community development for hay, soil, hay + soil, and turf-receiving treatments relative to the control. First RDA axis: eigenvalue = 0.07, $F = 10.21$, $p = 0.001$. Second and subsequent axes of RDA were not significant. (B) Deviation in plant community development for control, hay, soil, hay + soil, and turf-receiving treatments relative to the donor site. First RDA axis: eigenvalue = 0.16, $F = 30.91$, $p = 0.001$. Second and subsequent axes of RDA were not significant. (C) Deviation in plant community development for control, hay, soil, hay + soil, turf-receiving, and no topsoil removal treatments relative to the donor site. First RDA axis: eigenvalue = 0.24, $F = 41.84$, $p = 0.001$. Second RDA axis: eigenvalue = 0.17, $F = 36.98$, $p = 0.002$ (not shown). Third and subsequent axes of RDA were not significant. (D) Deviation in plant community development for control, turf-receiving treatments, and the transplanted turfs relative to the donor site. First RDA axis: eigenvalue = 0.32, $F = 36.88$, $p = 0.001$. Second RDA axis: eigenvalue = 0.07, $F = 9.03$, $p = 0.008$ (not shown). Third and subsequent axes of RDA were not significant. The vertical one-dimensional plots at the right side of the diagrams are species weight diagrams showing the relative abundance of each species compared to the reference treatment. A positive score indicates an increase in abundance and a negative score indicates a decline. For clarity, only species with the best fit to the first ordination axes are shown.
An. odoratum. However, for An. odoratum, we could not exclude arrival through dispersal from adjacent plots because across years, the cover of An. odoratum in the turf-receiving treatments did not significantly differ from the control (repeated measures ANOVA, $F_{[1,8]} = 3.00$, $p = 0.12$). Other species that were present in the turfs at the time of transplantation, such as Dac. maculata and Tormentil (Potentilla erecta), had difficulties to persist in the turfs and were not able to spread into the surrounding area. In contrast, species such as Le. saxatile, Greater bird’s foot (Lotus pedunculatus), and Ranunculus repens invaded the turfs from the surrounding plot. In 2006, the turf-receiving treatments and the transplanted turfs converged to each other and the turf-receiving treatment converged to the donor site (Fig 3D). However, in 2006, also the control treatments converged to the donor site (Fig 3B & 3D), indicating that convergence of the turf-receiving treatments to the donor site was not necessarily attributable to the turf transplantation. Moreover, the similarity in plant community composition of the turfs to the plots at the donor site was significantly lower than the similarity among the plots at the donor site (comparison of intercepts: $t = 3.63$, $p < 0.001$) and the difference increased over time (comparison of slopes: $t = 2.79$, $p < 0.01$) (Fig. 4).

Across years, a total of 87 plant species were found. Species richness in hay, soil, hay + soil, and turf-receiving treatments did not differ from the control (Tukey post hoc tests, $p > 0.05$; data not shown). Species richness in no topsoil removal treatments was significantly lower than that in the topsoil-removed treatments, averaging 20 and 8 species/plot, respectively (contrast analysis after ANOVA: $F_{[4,29]} = 42.57$, $p < 0.001$). Overall, species richness significantly increased over time (year: $F_{[3,72]} = 56.61$, $p < 0.001$). However, the temporal development in species richness differed between treatments, as indicated by the significant treatment × year interaction ($F_{[15,72]} = 11.25$, $p < 0.001$): in the no topsoil removal treatment, species richness did not increase over time.

**Discussion**

Our hypothesis that introduction of later successional soil organisms into the topsoil-removed site would facilitate the establishment of later successional plant species was not confirmed in this 5-year field experiment. Spreading hay from the donor site affected plant community composition, whereas spreading soil from the donor site did not. Soil spreading did not affect the soil nematode community composition, and introduction of soil organisms by means of turf transplantation was not successful either. Soil community composition depends strongly on environmental conditions (Bongers & Ferris 1999). Differences in chemical, physical, and hydrological soil properties between the donor and the receptor site, therefore, may have been responsible for the lack of establishment of the introduced soil organisms (Pywell et al. 2007). Soil organic matter content at the donor site was more than 5-fold higher than that in the topsoil-removed receptor site. This will have limited resource input into the soil food web, as well as available habitat for primary decomposers and their predators. Indeed, in 2003, bacterial- and fungal-feeding nematodes, as well as semi-carnivores, were significantly lower in the receptor site than in the donor site and were not affected by spreading soil from the donor site.

The first years after transplantation, numbers of nematodes in the turfs decreased when compared to the donor site from which they originated, and the taxonomic composition of the nematode community in the turfs developed toward the receptor site. Although community similarity between the turfs and the donor site stabilized after 2003, this suggests limited survival of the nematodes from more mature ecosystems within the isolated turfs. This might be due to intrinsic loss through habitat fragmentation (Rantalainen et al. 2006). In those conditions, it is to be expected that the nematode community becomes dominated by bacterial- and fungal-feeding nematodes, which are at low trophic level positions within the soil food web (Wright & Coleman 1993). However, all nematode feeding types decreased in abundance just about the same extent. Moreover, soil nematodes act on small spatial scales relative to the turf size (Ettema & Yeates 2003) and are probably not particularly sensitive to reductions in habitat size (Rantalainen et al. 2004). Therefore, turf size per se is not expected to have affected soil nematodes. Instead, changes in environmental conditions that result from the edge-to-area ratio of the turfs could have caused

![Figure 4. Temporal change in plant community similarity (Sørensen’s similarity, $C_{S}$) of the transplanted turfs to plots at the donor site and among plots within the donor site. Data are $X \pm SE$ ($n = 5$).](image-url)
reduction in nematode abundance. Particularly, the turfs were placed in minerol soil, which is poorly buffered to seasonal soil water fluctuations (high in winter and low in summer). In summer, this resulted in dehydration of the turfs (P. Kardol, personal observation), which could have had direct or indirect detrimental effects on soil nematodes (Lindberg et al. 2002). Compared to the donor site, soil organic matter content was strongly reduced in the transplanted turfs. Probably, the rate of decomposition increased due to soil water level fluctuations at the receptor site (Ise & Moorcroft 2006). Alternatively, burrowing soil animals may have mixed soil from the turfs with mineral soil.

Our results also suggest that the turfs did not function as a pool from which nematodes could colonize the topsoil-removed receptor site. Other studies have suggested that many soil organisms can survive on bare mineral soil at least for 1 or 2 years (Siiri-Pietikäinen et al. 2003). There are different reasons as to why we did not record nematode dispersal outside the turfs. First, dispersal by nematodes from the transplanted turfs depends mainly on active locomotion, and this may result in slow colonization rates (Warwick 1984). Possibly, nematodes colonized the topsoil-removed receptor site but just not as far as the positions of the soil samples (Appendix). However, it is also possible that due to a mismatch in abiotic soil conditions between the turfs and the receptor site, nematodes did not disperse from the turfs. Moreover, in our study, unfavorable soil conditions and differences in substrate type could have led to limited survival of nematodes outside their “home habitat.” Finally, disturbance of turfs, by lifting, transport, and relaying, could have contributed to the decline of soil nematodes originally present in the turfs. In an analogous experiment, colonization of soil mites from transplanted turfs was not successful either (Gormsen et al. 2006).

As for nematodes, we showed limited survival of plant communities in the turfs. Plant species composition at the transplanted turfs became less similar to that in the donor site, and most plant species did not expand into the receiving plots. This indicates that also for the plant community, there was a mismatch between the donor site and the receptor site. Apparently, abiotic or biotic soil conditions of the mineral soil at the receptor site were unfavorable for the plant species at the turfs. Inherent time lags in the response of plant communities to environmental change may have tempered the rate of diversification between the transplanted turfs and the donor site. As discussed for soil organisms, fluctuating soil water levels at the receptor site may have disfavored some plant species at the turfs. Unfortunately, we do not have records of the plant species composition of the turfs at the time of transplantation. Moreover, the depths of the turfs (10 cm) could have damaged deep rooting species (Bullock 1998), such as the orchid *Dactylorhiza maculata*, which was present at the turfs the first year after transplantation (P. Kardol, personal observation) but has not been observed thereafter.

Hay spreading has been recommended as a successful method to introduce later successional plant species on a variety of soil types (e.g., Manchester et al. 1999; Patzelt et al. 2001; Kiehl & Wagner 2006). However, our results showed that particularly the more common plant species were introduced and that the amount of variation in plant species composition explained by hay treatments was low. After 5 years, plant species composition of hay-amended plots still differed substantially from that in the donor site. Probably, more attention should be given to the seed presence in the hay (Pywell et al. 1995) or the germination potential of the plant species at the restoration site. The majority of seeds present in hay comprises grass species (Smith et al. 1996). This can explain the high abundance in hay-amended plots of *Festuca rubra* and *Anthoxanthum odoratum*, which were dominant species at the donor site at the time the hay was collected. Topsoil from the donor site could have contained seeds or vegetative fragments from species other than those that were present in the hay (Manchester et al. 1999). However, our results suggest that seeds or other plant propagules in the topsoil were scarce or that germination and establishment were constrained, as we did not find plant species exclusively in plots where soil was spread.

After land abandonment, a major limitation for establishment of species-rich grassland vegetation is high nitrogen and phosphorus availability in soils (Marrs 1993). Obviously, in absence of topsoil removal, soil fertility was high, vegetation was dominated by nitrophilous species, and plant species composition did not appear to succeed toward the species-rich donor site. On the topsoil-removed receptor site, fertility was reduced and plant species composition tended to diverge toward the donor site; however, the vegetation remained rather dissimilar to the target *Cirsio-Molinietum* fen meadow. Nevertheless, the plant community at the topsoil-removed site was classified as low-productive, species-rich grassland. Plots of all treatments (including the control) contained several red list species and other species that could be defined as target species (Bal et al. 2001). These species must have been able to disperse from local or regional species pools (Soons et al. 2005) or seeds may have been present in the subsoil. This suggests that seed limitation was not a major constraint for grassland restoration in our case.

**Implications for Practice**

- Although laboratory studies suggest an important role of soil organisms in plant community dynamics as well as mutual interdependence of later successional plants and soil organisms, we could not demonstrate the use of soil organisms in grassland restoration on topsoil-removed ex-arable land.
- Spreading hay from target vegetation enhanced plant community development toward the target vegetation. Spreading soil had no additional effect.
Transplanting intact turfs from target habitat into the receptor site was successful neither for plants nor for soil organisms.

Adverse environmental conditions at the receptor site may have limited survival and establishment of the later successional soil organisms. These could have been (1) larger fluctuation of wet/dry conditions due to lowering the soil surface level by topsoil removal; (2) the top layer consisted of mineral substrate representing harsh conditions for soil organisms to colonize and survive; and (3) absence of vegetation cover at the time of soil organism introductions.

To prevent a mismatch in abiotic soil conditions between the donor and the restoration site, introduction of turfs or soil from intermediate stages may be more effective than soil or turfs from target sites. Or, the topsoil may be removed incompletely, leaving a thin layer of organic substrate.

To enhance the use of soil organisms in nature restoration and to improve establishment of their mutual relationship with target plant species, we recommend future restoration studies to unravel establishment and survival conditions of introduced soil organisms at restoration sites in relation to the conditions of the topsoil and initial vegetation cover.

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LITERATURE CITED


Appendix. Diagram showing the positions of vegetation recordings (2003–2006) and soil samples (2003) in turf transplantation plots and in other plots.