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Restoration of species-rich grasslands on ex-arable land: Seed addition outweighs soil fertility reduction

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ABSTRACT

A common practice in biodiversity conservation is restoration of former species-rich grassland on ex-arable land. Major constraints for grassland restoration are high soil fertility and limited dispersal ability of plant species to target sites. Usually, studies focus on soil fertility or on methods to introduce plant seeds. However, the question is whether soil fertility reduction is always necessary for getting plant species established on target sites. In a three-year field experiment with ex-arable soil with intensive farming history, we tested single and combined effects of soil fertility reduction and sowing mid-successional plant species on plant community development and soil biological properties. A controlled microcosm study was performed to test short-term effects of soil fertility reduction measures on biomass production of mid-successional species. Soil fertility was manipulated by adding carbon (wood or straw) to incorporate plant-available nutrients into organic matter, or by removing nutrients through top soil removal (TSR). The sown species established successfully and their establishment was independent of carbon amendments. TSR reduced plant biomass, and effectively suppressed arable weeds, however, created a desert-like environment, inhibiting the effectiveness of sowing mid-successional plant species. Adding straw or wood resulted in short-term reduction of plant biomass, suggesting a temporal decrease in plant-available nutrients by microbial immobilisation. Straw and wood addition had little effects on soil biological properties, whereas TSR profoundly reduced numbers of bacteria, fungal biomass and nematode abundance. In conclusion, in ex-arable soils, on a short-term sowing is more effective for grassland restoration than strategies aiming at soil fertility reduction.

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1. Introduction

In industrialised countries, a common practice to counteract the loss of natural habitats is converting high-input arable land into low-input, species-rich grassland (Walker et al., 2004). So far, restoration of species-rich grasslands on ex-arable land has shown variable success. High nutrient availability after cessation of agricultural practices favors the competitiveness of early-successional plant species over later-successional ones, which typically results in initial dominance of fast-growing annual weeds and tall forbs (Marrs, 1993) and a slowdown of plant community succession (McLendon and Redente, 1992). Therefore, soil fertility reduction is a widely applied practice when restoring species-rich grasslands. The traditional way of removing or concentrating nutrients is by hay making or grazing without fertilizer application (Bakker and Berendse, 1999). Alternatively, soil fertility can be reduced by removing the entire top soil (Marrs, 1985; Van Diggelen et al., 1997; Walker et al., 2007), which is a short-term intervention with a long-term effect (Verhagen et al., 2001). However, top soil removal is drastic and expensive (Aerts et al., 1995) and has many side effects, such as removal of the seed bank (e.g., Pywell et al., 2002), adverse effects on soil biota, and changes in soil structure and water holding capacity.

An alternative to top soil removal is soil fertility reduction via microbial nutrient immobilisation (e.g., Blumenthal et al., 2003). As carbon availability often restricts microbial biomass production, excess of nitrogen could be immobilised by adding organic carbon (Zink and Allen, 1998; Averett et al., 2004; Eschen et al., 2007). Contrary to long-term effects of top soil removal, effects of soil micro-organisms on nutrient availability may be short-lived because of high microbial turnover rates (Reever Morghan and Seastedt, 1999). Effectiveness and duration of microbial nutrient immobilisation can depend on the substrate quality, e.g., as indicated by C:N ratio (Török et al., 2000), on the community composition of micro-organisms (Van der Wal et al., 2006a), or on top-down control by predatory soil organisms (Wardle et al., 2005). From a previous study by Van der Wal et al. (2006a), we know that addition of carbon substrates to ex-arable soils particularly results in a fast, but temporal, increase of opportunistic, fast-growing micro-organisms. A short-term increase of fast-growing micro-organisms by carbon addition may result in changes in soil organisms higher up in the soil food-web, which, in turn, can have long-term effects on the structure and functioning of the soil food-web (Wardle et al., 1995).

Apart from high soil fertility, grassland restoration can be constrained by the absence of seeds of later-successional plant species (Hutchings and Booth, 1996; Pywell et al., 2002); seed banks of ex-arable soils contain predominantly 'non-target' species (Bekker et al., 1997; Standish et al., 2007). Therefore, plant community and ecosystem development strongly depend on local and regional species pools as a source of plant propagules (Zobel et al., 1998). However, seed sources may be limited in agricultural landscapes (Piessens et al., 2005), dispersal possibilities are generally poor and colonisation and establishment of later-successional plant species is low (Verhagen et al., 2001). Introduction of later-successional plant propagules may help overcome dispersal limitation (e.g., Martin and Wilsey, 2006; Edwards et al., 2007). However, successful establishment of later-successional plant species may rely upon reduction of soil fertility. Although both soil fertility and dispersal limitation are key factors in limiting grassland restoration, relatively few studies have compared effects of fertility reduction and seed addition alone and in combination (Foster and Dickson, 2004).

In a 3-year field experiment we examined effects of carbon addition (to immobilise nutrients) and top soil removal (to discard nutrients) with and without sowing a mixture of eight later-successional plant species on plant and soil community development. To study short-term effects of carbon addition on biomass production of later-successional plant species under controlled conditions, an additional 1-year microcosm experiment in the greenhouse was performed. We tested the following hypotheses: (1) adding carbon increases microbial biomass, immobilises plant-available nutrients and reduces plant biomass for a relatively short-term; (2) top soil removal reduces plant-available nutrients, as well as biomass of plants and of soil biota for a longer-term; (3) soil fertility reduction suppresses early-successional, weedy species and promotes the effectiveness of sowing mid-successional plant species; and (4) changes in microbial biomass in response to carbon addition trickle-up to higher levels of the soil food-web. We used soil nematodes as indicators of changes in soil food-web structure because they display high taxonomic richness and occupy multiple trophic levels (Ferris et al., 2001). To test effects of differences in substrate decomposability, we used both straw and wood for carbon addition.

2. Methods

2.1. Site description and design of the field experiment

We conducted a 3-year field experiment on an ex-arable site in Assel, the Netherlands (52°21′N, 5°82′E), located on sandy glacial deposits and previously cultivated with maize (*Zea mays*). After abandonment in 2002, the site was exposed to low intensive grazing by red deer and rabbits, the main wild vertebrate herbivores in that area. In May 2004, we set up an experiment with four replicate blocks (Appendix 1, Suppl. data). Within each block, eight treatment plots (4 m × 4 m) were separated by 3 m wide border rows. Blocks were located at 5 m distance from each other. We established the following treatments: control (C), addition of straw (S), addition of wood fragments (ranging in size from <0.5 to ±2 cm^3) (W), and top soil removal (TSR). Wheat straw (*Triticum* spp.) and birch wood (*Betula pendula*) were obtained from local suppliers and evenly distributed over the appropriate treatments to obtain final concentrations of 2 mg C g^{-1} dry soil. A previous study by Van der Wal et al. (2006a) suggests that these concentrations are likely to stimulate microbial biomass production and, hence, immobilise plant-available nutrients. Straw and wood was mechanically disked into the top 10 cm of the soil; plots without wood or straw addition were also disked. TSR treatments were established adjacent to the other treatments in an area where the organic top layer of 40–50 cm was removed down to the mineral subsoil. TSR plots were not disked. All

treatments were applied in combination with and without sowing a mixture of four perennial mid-successional grasses (*Agrostis capillaris*, *Anthoxanthum odoratum*, *Briza media*, *Festuca ovina*) and four perennial forbs (*Achillea millefolium*, *Hypochaeris radicata*, *Plantago lanceolata*, *Rumex acetosella*). All species are characteristic of mid-successional stages of secondary succession after land abandonment on sandy soils in this region (Kardol et al., 2005). The seeds, provided by a specialised supplier (Cruydt-hoeck, Groningen, the Netherlands), were sown at densities of 500 grass seeds or 150 forb seeds per m^2.

2.2. Plant community

In July/August 2004, 2005 and 2006, percentage cover of each vascular plant species was recorded in the inner 2 m × 2 m of each plot. We used the cover of *Conyza canadensis*, which was the dominant weedy species at the start of the experiment, as indicator for weed suppression. Every year, peak standing shoot biomass was determined by clipping four (two in 2004) 25 cm × 25 cm subplots within each plot (Appendix 1, Suppl. data). In 2005 and 2006, in the centre of each 25 cm × 25 cm subplot a soil sample (5 cm diameter and 10 cm depth) was taken to determine root biomass. Roots were washed from the soil over a 2 mm sieve. Standing biomass of each subplot was determined after drying shoot and root material.

2.3. Soil parameters

In May 2004 (immediately after establishment of the treatments), and in May 2005 and 2006, from each plot 15 random soil samples (3.4 cm diameter and 10 cm depth) were collected, bulked, mixed and sieved (4 mm mesh). Subsamples were taken for physical, chemical and microbial soil properties and for isolating nematodes. Soil moisture content, pH, available P, NH_4, NO_3, K, total N, total P, soil organic matter and ergosterol were measured as described in Van der Wal et al. (2006b). Ergosterol, a sterol found in fungal cell membranes, was used as indicator for fungal biomass and extracted and quantified as described in Bååth (2001). Bacterial numbers were determined by microscopical counting. Nematodes were extracted by Oostenbrink elutriators, heat-killed and fixed using 35% formaldehyde diluted to 4%. Of each sample, 150–200 randomly selected nematodes were identified to family or genus level and to feeding group according to Yeates et al. (1993). A heterogenous group of omnivorous Dorylaimid nematodes were identified to the level of the super-family *Dorylaimoidea* (sensu Jarajpuri and Ahmad, 1992).

2.4. Microcosm experiment

After we established the field experiment, random soil samples (5 cm diameter, 10 cm depth) were collected within the border rows. Samples were bulked for each of the two areas (TSR and non-TSR). Soil was homogenised and sieved (4 mm diameter). Part of the non-TSR soil was enriched with 1 cm fragments of straw (S) or with wood fragments <0.5 cm^3 per particle (W). Non-enriched soil served as control (C). In a greenhouse, we established microcosms (18 cm × 18 cm × 18 cm) filled with the field soils. Microcosms were randomly placed on trolleys. Light regime was minimally 16 h/d of light, and natural day-light was supplemented with metal halide lamps (225 $\mu mols^{-1}\ m^{-2}$ PAR) to ensure minimum light supply and a L:D temperature regime of 21:16 °C. Each treatment (C, S, W, and TSR) was replicated five times. Initial soil moisture level was set at 10% (w:w) and was re-set twice a week by weighing. Each microcosm was planted with a mixed community composed of the same eight mid-successional species that had been sown in the experimental field. Seeds were germinated and grown according to Kardol et al. (2006). Each microcosm was planted with one seedling of each of the eight species in fixed positions and each replicate had a different plant configuration to minimise positioning effects.

After 56, 128, and 198 days, shoots were clipped at 2 cm above the soil surface and sorted into species. After 253 days, shoots were clipped at the soil surface and roots were washed from the soil over a 2 mm sieve. For each harvest, shoots (and roots) were weighed after drying. Roots could not be sorted to species. At the first, second and final harvests, three soil cores (1 cm diameter, 15 cm depth) were collected in each microcosm. Soil cores were bulked per microcosm and NH_4 and NO_3 contents were determined. Soil samples from the first and second harvest were also analysed for available P. At the final harvest, five soil cores were collected in each microcosm, bulked and used for nematode extraction.

2.5. Data analysis

Normality and homogeneity of variance in anova were checked with Kolmogorov-Smirnov and Levene's tests, respectively. If the assumptions were not met, data were log- or square-root-transformed, or a non-parametrical test was used. Univariate analyses were run in STATISTICA 7.1 (Statsoft Inc., Tulsa, OK, USA). Multivariate analyses were run in CANOCO 4.5 (Ter Braak and Šmilauer, 2002).

2.5.1. Field experiment

Data were analysed using two-way repeated measures anova with soil treatment (C, S, W, TSR) and sowing as between-subjects factors, and year as repeated measure. Blocks were used as random factor. Contrasts were specified for testing across-year differences among soil treatments. For shoot and root biomass in sown and unsown plots, contrasts were also specified for within-year treatment comparisons. Proportional cover of sown plant species was analysed for sown plots only using one-way repeated measures anova. Due to low values, proportional cover of *C. canadensis* could not be analysed for TSR treatments and for the year 2006. NH_4 content was analysed for 2006 only, because of too low values in previous years. Linear regressions were used to test within-year relationships between microbial parameters and soil moisture content, between bacterial numbers and numbers of bacterial-feeding nematodes, and between ergosterol and fungal-feeding nematodes.

Treatment effects on plant species and nematode taxon composition were analyzed using principal component analysis (PCA). To determine whether the soil treatments significantly explained variation in plant and nematode community composition, we used Monte Carlo permutation tests (999 permutations) in redundancy analysis (RDA). For each

treatment, the respective treatment × year interaction was included as explanatory variable and the remaining treatment × year interactions as covariables. To reflect our repeated measurements, permutation tests were restricted for split-plot design (Lepš and Šmilauer, 2003). Additionally, we performed variance partitioning to test which part of the total variation in plant and soil nematode community composition could be explained by sowing and which part by the groups of soil treatments. Treatment × year interaction terms were tested in partial redundancy analysis (pRDA). Each (group of) variables was partialled out as covariable at a time and the resulting percentage of variance explained by the pRDA was compared by the one obtained with the full RDA model. PCA and RDA analyses were run using log-transformed percentage cover and abundance data for plants and nematodes, respectively. In all RDA and PRC analyses block was used as covariable.

2.5.2. *Microcosm experiment*

Data were analyzed using repeated measures anova, similar as for the field experiment. We specified contrasts for shoot biomass to compare treatments within harvests. Cumulative total and species-specific shoot biomass (i.e., the sum of harvest 1–4), total root biomass, nematode densities (total and per feeding group) and NO_3 content at harvest 1 were analysed using one-way anova with Tukey hsd tests or Kruskall-Wallis tests with multiple comparison of mean ranks for individual comparisons. NO_3 content at harvests 2 and 4 and numbers of endo-parasitic plant feeders were close to zero and were not analysed.

3. Results

3.1. *Field experiment*

3.1.1. *Plant community*

Sowing drastically affected plant community development and had a much stronger effect than the soil treatments as indicated by the distances among treatment centroids (Fig. 1a). From the total 49.6% of explained variability, 35.6% could be explained solely by sowing (RDA: $F = 64.37$, $P < 0.01$) and 12.1% could be explained solely by the soil treatments (RDA: $F = 7.31$, $P = 0.01$). For the soil treatments, TSR significantly explained variation in plant community composition (RDA: $F = 10.10$, $P < 0.01$), while wood and straw addition did not (RDA: $F = 6.14$, $P = 0.22$ and $F = 7.87$, $P = 0.10$, respectively). From the second year, sowing resulted in strong dominance of mid-successional species (proportional cover 75–99%), not only in wood and straw treatments, but also in control treatments (Fig. 1b). Across years, the proportional cover of sown plant species was not affected by addition of straw or wood ($F_{1,12} = 1.09$, $P = 0.31$, and $F_{1,12} = 0.01$, $P = 0.92$, respectively). The significant treatment × year interaction ($F_{1,6} = 481.38$, $P < 0.01$) could be attributed to high proportional cover of sown species in TSR treatments in the first year of the experiment. However, the total cover of TSR treatments remained low throughout the experimental period (<10% for unsown plots and <20% for sown plots).

The proportional cover of *C. canadensis* was suppressed by straw addition ($F_{1,18} = 6.72$, $P = 0.02$), but not by wood ($F_{1,18} = 3.21$, $P = 0.09$). In the first year, cover was <2% in straw treatments and ranged from 8% to 27% in control and wood treatments. However, in the second year, the differences diminished and in the third year, the cover of *C. canadensis* was close to zero in all soil treatments. In the second year, the cover of *C. canadensis* was significantly lower in sown than in unsown plots (<1% and up to 25%, respectively; $F_{1,22} = 47.46$, $P < 0.01$). There was no interaction between carbon amendments and sowing ($F_{1,18} = 1.44$, $P = 0.26$). In TSR treatments cover of *C. canadensis* was always <1%.

3.1.2. *Shoot and root biomass*

Shoot and root biomass differed significantly among years (Fig. 2). There were significant effects of sowing and of soil treatments on biomass, and the interaction between sowing and soil treatments was also significant (Table 1). In sown plots, straw addition reduced shoot biomass in the first year when the community was still dominated by early-successional weeds, but increased shoot biomass in the second and third year when the community was already dominated by the sown species. In unsown plots, straw addition did not significantly affect shoot biomass, although shoot biomass tended to be lower in straw than in control treatments in the first year (Fig. 2). Wood addition did not affect shoot biomass. In sown plots, straw addition increased root biomass in the second and third year, while wood addition increased root biomass only in the third year. In unsown plots, root biomass was reduced by wood and straw in the second year, but not in the third. Across years, root and shoot biomass in TSR treatments were substantially lower than in other treatments (Fig. 2).

3.1.3. *Bacteria, fungi and nematodes*

Bacterial numbers and fungal biomass were strongly reduced by TSR ($F_{1,24} = 23.92$, $P < 0.001$ and $F_{1,24} = 82.94$, $P < 0.001$, respectively). On average, in plots with TSR bacterial numbers were two-fold and fungal biomass was 14-fold lower than in non-TSR plots. In contrast, bacterial numbers were not affected by straw and wood addition ($F_{1,24} = 1.84$, $P = 0.19$ and $F_{1,24} = 0.07$, $P = 0.79$, respectively), while fungal biomass tended to be stimulated by straw and wood addition ($F_{1,24} = 2.77$, $P = 0.11$ and $F_{1,24} = 4.23$, $P = 0.051$, respectively). Bacterial numbers were highest in the second year, whereas fungal biomass increased after the first year (Appendix 2, Suppl. data). Across years, bacterial counts were positively related to soil moisture content (linear regression: $R = 0.44$, $P < 0.001$), which was not the case for fungal biomass ($R = 0.14$, $P = 0.24$). Sowing did not affect bacterial numbers and fungal biomass ($F_{1,24} = 1.35$, $P = 0.26$ and $F_{1,24} = 0.44$, $P = 0.51$, respectively), and there were no soil treatment × sowing interactions ($F_{1,24} = 0.06$, $P = 0.98$ and $F_{1,24} = 0.20$, $P = 0.90$, respectively).

Total nematode abundance was unaffected by sowing ($F_{1,24} = 0.01$, $P = 0.94$), but there was a significant effect of soil treatments ($F_{3,24} = 25.47$, $P < 0.001$). This effect was caused solely by TSR where nematode abundance was strongly reduced (Fig. 1d; Appendix 3, Suppl. data). Except for TSR treatments, nematode abundance within feeding groups generally followed the pattern of the total number of nematodes, with higher numbers in the first year than in later years (Appendix

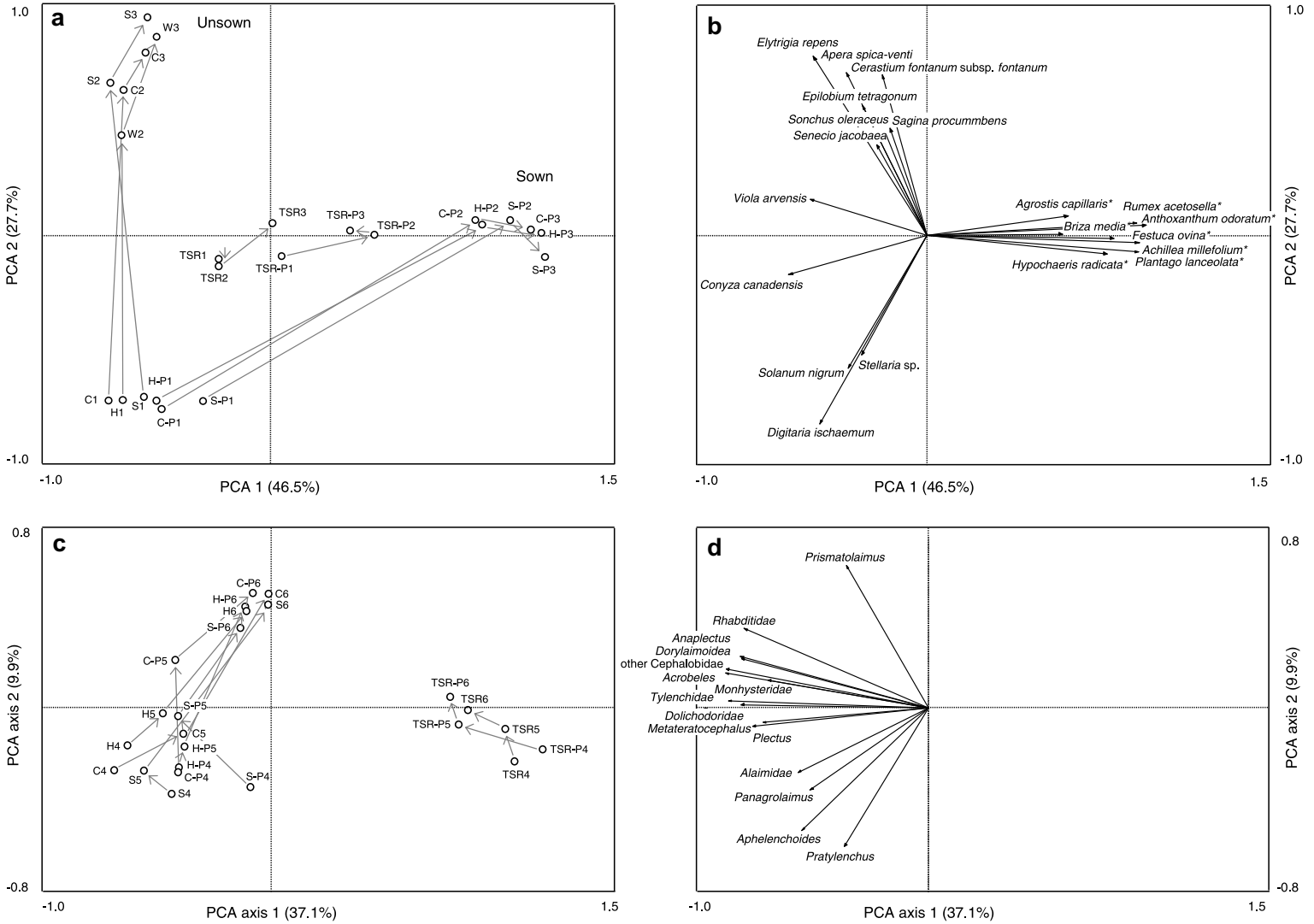

Fig. 1 – PCA of (a, b) plant and (c, d) soil nematode community composition. Shown are plots for samples (a, c) and for species/taxa (b, d). Percentages along the axes correspond to the amount of explained variation in community composition. Sample plots show centroid scores (i.e., weighted means of sample scores) for control (C), straw (S), wood (W), and top soil removal (TSR) for each year of monitoring. Numbers in treatment codes indicate the consecutive years: 2004, 2005, and 2006. 'P' in treatment codes indicates treatments applied in combination with sowing mid-successional plant species. Arrows in the sample plots indicate the temporal course of plant and soil nematode community development. For clarity, only the best fitting species/taxa are plotted in the species plots. * – Plant species included in the sowing treatment.

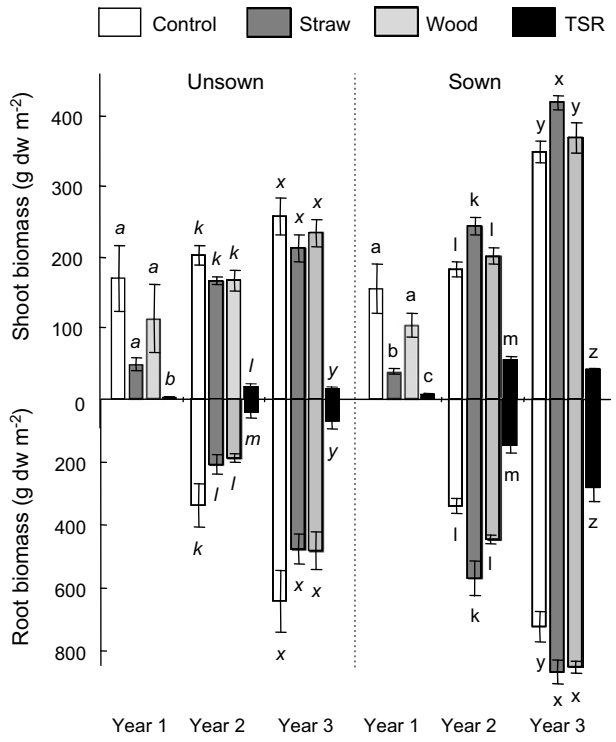

Fig. 2 – Shoot and root biomass (means ± SE) in control, straw, wood and top soil removal (TRS) treatments for unsown treatments (left) and sown treatments (right) in the field experiment. Within the two sowing treatments, different letters denote significant within-year differences among treatments according to within-year contrast analyses after repeated measures anova ($P < 0.05$).

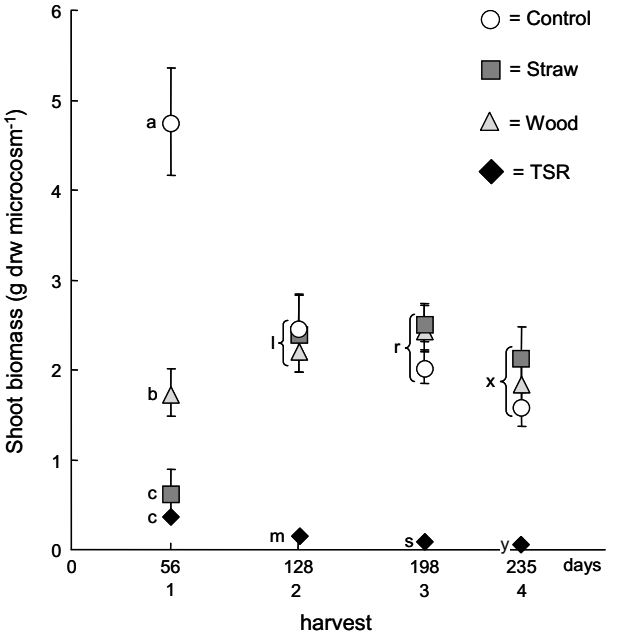

Fig. 3 – Shoot biomass (mean ± SE) in control, straw, wood and top soil removal treatments at four consecutive harvests in the microcosm experiment. Different letters denote significant differences between treatments according to contrast analyses after repeated measures anova ($P < 0.05$), separately for harvest 1 (a, b, c), harvest 2 (l, m), harvest 3 (r, s) and harvest 4 (x, y).

3, Suppl. data). Particularly, opportunistic bacterial-feeding *Acrobeles*, fungal-feedling *Aphelenchoides*, and plant-feeding *Pratylenchus* and *Dolichodoridae* decreased in abundance after the first year. Although total numbers of nematodes remained low in the TSR treatment, over time, more taxa colonised the TSR plots which resulted in convergence of nematode community composition towards the non-TSR treatments (Fig. 1c). From the total 19.3% of explained variability, 16.4% could be explained solely by the soil treatments (RDA: $F = 6.17$, $P < 0.01$) and 1.8% could be explained solely by sowing (RDA: $F = 2.01$, $P = 0.02$). For the soil treatments, TSR significantly explained variation in nematode community composition (RDA: $F = 12.44$, $P < 0.01$), while wood and straw addition did not (RDA: $F = 1.81$, $P = 0.13$ and $F = 1.90$, $P = 0.12$, respectively).

Across treatments, numbers of bacteria were significantly positively related to numbers of bacterial-feeding nematodes and fungal biomass was significantly positively related to numbers of fungal-feeding nematodes (linear regression, $P < 0.05$). However, these relationships were not found when TSR plots were excluded from the analyses.

Table 1 – Results of two-way repeated measures anova for shoot biomass and root biomass in the field experiment with soil treatment (control, straw, wood and top soil removal (TSR)) and sowing as between-subject factors

Source	Shoot biomass			Root biomass		
	df	*F*	*P*	*df*	*F*	*P*
Between subjects						
Soil treatment (*T*)	3	69.7	<0.001	3	49.6	<0.001
Sowing (*S*)	1	10.3	0.003	1	68.6	<0.001
$T \times S$	3	4.0	0.019	3	7.9	<0.001
Within subjects						
Year	2	153.8	<0.001	1	156.1	<0.001
Year × *T*	6	15.3	<0.001	3	8.8	<0.001
Year × *S*	2	0.8	0.45	1	3.9	0.06
Year × *T* × *S*	48	1.5	0.18	24	0.2	0.88

Table 2 – Number of nematodes per feeding group (100 g dry soil^{-1}) in control, straw and wood treatments in the microcosm experiment

Treatment	Ecto-parasites	Root hair-feeders	Bacterial- feeders	Fungal- feeders	Omni-carnivores
Control	22 ± 11^{ab}	226 ± 105^{bc}	399 ± 32^{b}	34 ± 4^{ab}	314 ± 34^{a}
Straw	70 ± 27^{a}	1442 ± 288^{a}	769 ± 106^{a}	26 ± 13^{b}	306 ± 37^{a}
Wood	11 ± 4^{b}	670 ± 157^{b}	659 ± 60^{a}	72 ± 19^{a}	363 ± 91^{a}
TSR	32 ± 7^{ab}	5 ± 2^{c}	113 ± 28^{c}	15 ± 1^{b}	69 ± 10^{b}
ANOVA					
$F_{3,16}$	3.65	16.96	25.95	5.65	7.47
P	0.035	<0.001	<0.001	0.008	0.002

Data are mean ± SE. Results from anova indicate overall treatment effects. Within columns, different letters denote significant differences between means based on Tukey hsd tests ($P > 0.05$).

3.1.4. *Abiotic soil parameters*

As expected, across years, NO_3 content, NH_4 content, available P, K, total N, total P, and SOM were strongly reduced by top soil removal (contrasts in anova, $P < 0.05$; Appendix 2, Suppl. data). Plant-available N and P in plots with wood or straw addition did not differ from control plots (contrasts in anova, $P > 0.05$), however, NO_3 content tended to be lower in plots with straw or wood addition (Appendix 2, Suppl. data). Available P decreased over time in all plots ($F_{2,48} = 82.27$, $P < 0.001$). K increased in plots with straw addition ($F_{1,24} = 48.54$, $P < 0.001$), but not in plots with wood addition ($F_{1,24} = 0.49$, $P = 0.49$). Total N, total P, pH, and soil organic matter in plots with straw or wood addition did not differ from control plots (contrasts in ANOVA, $P > 0.05$). Sowing mid-successional plant species reduced NO_3 and NH_4 ($F_{1,24} = 16.28$, $P < 0.001$ and $F_{1,24} = 22.20$, $P < 0.001$, respectively), but no other soil parameters.

3.2. *Microcosm experiment*

Shoot biomass production was strongly affected by wood and straw addition and by top soil removal (Fig. 3). Shoot biomass production was significantly affected by soil treatment ($F_{3,16} = 471.16$, $P < 0.001$) and by harvest ($F_{3,48} = 16.91$, $P < 0.001$), and also their interaction term was highly significant (harvest × treatment: $F_{3,48} = 50.14$, $P < 0.001$). The interaction was due to the short-term effects of wood, which reduced shoot biomass by approximately 65% relative to the control, and straw, which caused >80% shoot biomass reduction. These effects had disappeared after the first harvest. However, the short-term effects of straw and wood addition on biomass production had longer-term effects on plant community composition through different responses of the individual plant species (data not shown). Final root biomass was highest in pots with straw (Tukey hsd test, $P < 0.05$). Soil from TSR produced low shoot and root biomass.

Effects of straw and wood addition on numbers of nematodes differed markedly among feeding groups (Table 2). Numbers of bacterial-feeders and root hair-feeders were significantly higher in straw and wood treatments than in the control. Fungal-feeders were more dominant in soil with wood than with straw. The fungal-feeder *Tylencholaimus* sp. was exclusively found in wood treatments. Omni-carnivores were not affected by straw and wood addition. Root hair-feeders (*Tylenchidae*) were significantly enhanced by straw and wood addition; densities were particularly high in straw treatments. Overall, there were low numbers of nematodes in TSR soil.

We did not find direct evidence for immobilisation of plant-available nutrients by adding straw or wood. Levels of NH_4 and NO_3 were low in all treatments (<2.4 and <1.5 mg kg^{-1}, respectively). Overall, NH_4 content was higher in soil with wood and straw than in control soil (contrasts in ANOVA, $P > 0.05$). NO_3 was higher in soil with straw than with wood and control soil, but only at the first harvest (Tukey hsd tests, $P < 0.05$). NH_4 and NO_3 content in TSR soil were (close to) zero. Available P did not differ among soils (contrasts in ANOVA, $P > 0.05$), except for TSR, where it was 13-fold lower.

4. Discussion

The effectiveness of introducing later-successional plant species in reducing the dominance of fast-growing annual weeds and tall forbs may rely upon (temporal) reduction of soil fertility. We had expected that sowing later-successional species would be more successful with than without fertility reduction measures, however, the sown plant species established successfully and independently of carbon amendments. TSR even reduced the success of sowing. This strongly suggests that initial plant community development on these sandy soils is more seriously limited by seed dispersal than by high soil fertility. Lack of differences in sowing efficiency between control and carbon addition treatments could have been due to the high rates of sowing. Possibly, carbon amendments could have affected the initial establishment of later-successional species if they were sown at low rates, although effects of sowing rate in grassland restoration generally persist only for a short-term (1–3 years) (Pywell et al., 1994; Stevenson et al., 1995). Noteworthy, mid-successional plant species showed species-specific responses to the straw treatment under controlled conditions in the microcosm experiment, resulting in lasting effects on plant community composition. Similar plant species-specific effects have been found in response to easily available carbon sources in a field experiment by Eschen et al. (2006). Such species-specific effects could be decisive if restoration is aimed at establishment of particular (red list) species. However, further research is needed before specific recommendations can be made.

As expected, removal of the organic top soil layer resulted in long-lasting (>3 years) reduction of plant-available nutrients

and plant biomass. Hence, top soil removal can reduce competition from fast-growing pioneer species (Walker et al., 2007). Indeed, early-successional weeds were almost completely absent. However, after three years, both unsown and sown TSR plots were sparsely covered and far from mid- or late-successional, species-rich grasslands. While top soil removal can create favorable conditions for establishment of later-successional grassland species at moist or wet soils (Klimkowska et al., 2007; Kardol et al., in press), in our study, the dry, mineral soil turned out to be inhospitable for seed germination and inhibited plant species establishment. The low plant species cover in TSR plots, therefore, was less due to the eradication of the seed bank (e.g., Verhagen et al., 2001) than to removing soil organic matter, which contains nutrients and influences soil abiotic growth conditions, particularly water holding capacity. Under constant levels of soil moisture content, seedlings of mid-successional species successfully established in the TSR treatment in the microcosm experiment, although biomass production was extremely low. These results from the greenhouse experiment suggest that the low plant cover in TSR treatments in the field experiment was most likely due to strong fluctuation in soil moisture content as a result of the low water holding capacity of the mineral subsoil.

We could not demonstrate microbial nutrient immobilisation, probably because we did not determine N-immobilisation or mineralization in the absence of living plants (Van der Wal et al., 2006a). However, reduced biomass in treatments with straw and wood in the early phase of the field experiment, strongly suggests a short-term (<1 year) decrease in plant-available nutrients due to carbon addition. Similarly, biomass production was reduced in the straw and wood treatment in the microcosm experiment, but only at the first harvest. This further confirms the truly short-term effect of microbial N-immobilisation, probably due to the immobilization of carbon in fast-growing, opportunistic micro-organisms that are dominating the microbial community in ex-arable soils (Van der Wal et al., 2006a). Although we did not observe severe herbivore damage in the field experiment, we cannot exclude effects of low intensive grazing by deer or rabbits on standing shoot biomass at the time of harvest. However, initial reduction of biomass production in straw and wood treatments in the microcosm experiment could only be attributed to microbial N-immobilization (or to other inhibitory effects of the carbon substrates).

Effectiveness of labile substrates, such as sugar and sawdust, has been shown to range from a few days (Bjarnason, 1987), a few months (Reever Morghan and Seastedt, 1999; Huddleston and Young, 2005) to over one year (Blumenthal et al., 2003; Eschen et al., 2007). In our study, we used more recalcitrant straw and wood substrates, because they are inexpensive and easily available for nature managers. The short-term reduction in plant biomass in straw and wood treatments probably reflected microbial responses to easily available carbon sources on the surface of the wood and straw fragments (Van der Wal et al., 2007). Addition of straw resulted in initial suppression of the dominant weed *C. canadensis*, while addition of wood did not do so. Probably, straw provided more microbial-available carbon sources than the recalcitrant wood fragments. This may have been reflected by stronger initial biomass reduction by straw than by wood. To note, more than two years after applying the treatments, large fragments of straw and wood were still present in the field soil. The actual breakdown of lignin present in wood and straw may take many years and after utilisation of the easy available fraction, the remaining recalcitrant carbon may not be readily accessible for opportunistic soil micro-organisms, and can only be decomposed by slow-growing rot fungi (Van der Wal et al., 2006a). This slow breakdown probably does not induce a substantial peak of nutrient immobilisation.

An increase in microbial biomass would be reflected in higher numbers (or biomass) of their consumers. Higher numbers of bacterial-feeding nematodes in straw and wood treatments under controlled conditions in the microcosm experiment indeed suggested a correlation between primary decomposers and their consumers. However, contrary to our prediction and to a previous study (Wardle et al., 1995), the soil nematode community in the field did not reveal longer lasting changes in the soil food-web structure. Hence, we did not find evidence that changes in microbial biomass trickle-up to higher levels of the soil food-web. The increase of root hair-feeding nematodes in soil with wood, and particularly with straw addition in the microcosm experiment suggested that root architecture was influenced by these soil treatments, or that the root hair-feeders have been feeding on fungal hyphae (Okada et al., 2002).

In conclusion, temporal soil fertility reduction by microbial N-immobilisation is ineffective in restoring species-rich grasslands when vegetation succession is strongly recruitment limited. Top soil removal reduces soil fertility and suppresses weeds for a longer period. However, our results show that top soil removal constrains the establishment of later-successional plant species, at least for three years after application. Therefore, on ex-arable sandy soils, artificial introduction of later-successional species immediately after land abandonment appears to be highly effective for short-term restoration of species-rich plant communities, when sowing is allowed. Once established, priority effects may prevail and prevent replacement of later-successional plant communities by early-successional weeds (e.g., Van der Wal et al., in press). To reduce costs, later-successional plants can be seeded in small 'focal patches', which may serve as source from which they can colonise larger areas over time (Pywell et al., 2007). As alternative to seeding, later-successional species can be introduced by transferring seed containing hay (Donath et al., 2007; Kardol et al., in press). Initial weed suppression can be enhanced by straw addition. Therefore, it would be interesting to start land abandonment on sandy soils after harvesting a cereal crop and leaving the straw widespread and disked in. Wood addition did not effectively influence plant community development. Reducing soil fertility by top soil removal will lead to a completely different successional trajectory and probably only meets management goals on a much longer-term than covered by our study (3 years).

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biocon.2008.06.011.

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