Sod cutting and soil biota effects on seedling performance

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ABSTRACT Sod cutting (i.e. top soil removal) is a restoration management option for enhancing seedling establishment and for lowering the nutrient concentration in eutrophicated soils of nutrient-poor species-rich grasslands. Removal of the upper soil changes not only abiotic soil properties but may also affect the resident soil community. We investigated the effects of sod cutting on the establishment and performance of two endangered plant species (Cirsium dissectum and Succisa pratensis) while simultaneously manipulating the interaction between seedlings and soil biota. In intact grassland and sod-cut areas at two localities, seedlings were grown in plastic tubes. Half of the tubes had a filter that excluded roots but allowed entry of fungal hyphae and soil microorganisms. The other tubes were closed (i.e. no contact with the surrounding soil). In a greenhouse experiment we studied the effect of soil solutions (with or without fungal tissue) from three grasslands and three sod-cut areas on seedling growth. Sod cutting had a positive net effect on seedling growth for S. pratensis. Access to (mycorrhizal) fungi and other soil biota resulted in a negative impact on seedling growth of both plant species, both in grassland and sod-cut areas. The greenhouse experiment confirmed that the soil biota in these meadows reduced seedling growth. Although sod cutting did not mitigate negative plant-soil feedback, it enhanced seedling growth, presumably by decreasing competition for light. Sod cutting is therefore very useful when seedling establishment needs to be stimulated.

Keywords: mycorrhizas; plant-soil feedback; seedling establishment; soil biota; turf stripping.

1. Introduction

Eutrophication often leads to biodiversity losses in species-rich grasslands as only a few, competitive plant species become dominant and exclude many subordinate species (Tilman 1982; Grime 2001; Harpole and Tilman 2007). Consequently, restoration management in former species-rich grasslands often focuses on reducing nutrient availability by grazing, mowing and sod cutting (Bakker and Berendse 1999; Tallowin and Smith 2001). These measures also tend to increase microsite environments that are suitable for seedling establishment (Grubb 1977). For instance, by removing the vegetation and the sod (usually the top 10 to 40 cm of the soil) windows of opportunity are created for seedling establishment (Eriksson and Fröborg 1996). Furthermore, competition for light and nutrients is temporarily reduced, which may benefit the seedlings of many plant species.
Plant-soil feedback has been found to control plant diversity and succession (Mills and Bever 1998; Klironomos 2002; Kardol et al. 2006). Top soil removal may change this feedback by removing soil pathogens and uncovering subsequent soil layers in which a microbial community characteristic of plant root zones has not yet established (van der Putten et al. 1993; Blomqvist et al. 2000; Olff et al. 2000). However, sod cutting may also have a negative impact on seedling establishment given that arbuscular mycorrhizal fungi (AMF) may be removed as well. AMF have been found to improve survival and growth rates of many plant species, in particular by contributing to P uptake (e.g. Smith et al. 2004; van der Heijden 2004). The majority of the mycorrhizal inoculum is situated in the topsoil (Neville et al. 2002) and recolonization of AMF from spores after sod cutting may be a very slow process (Vergeer et al. 2006). Since many herbaceous seedlings depend on mycorrhizal colonization, the presence of AMF should be taken into account in restoration projects (Richter and Stutz 2002).

The potential positive and negative effects of sod cutting on establishment of seedlings of target species are not mutually exclusive, and their net outcome is unclear. Most studies have focused on only one or a few aspects of sod cutting (e.g. nutrient and ammonium levels, hydrological processes, pH, seed banks) or investigated the effects of specific groups of soil biota on seedling establishment (Diemont 1990; Tallowin and Smith 2001; Isselstein et al. 2002; Matus et al. 2003; Dorland et al. 2005; Vergeer et al. 2006; van der Hoek and Heijmans 2007). For restoration management to be successful it is important to take all aspects into account to assess the overall outcome of sod cutting.

The aim of this study is to separate the aboveground effects of sod cutting (less above-ground competition due to decreased vegetation biomass) and the belowground effects (removal of soil biota, including AMF) on the establishment and performance of seedlings, while focusing on two endangered plant species characteristic of wet, nutrient-poor Cirsio-Molinietum grasslands: *Cirsium dissectum* and *Succisa pratensis*. We hypothesized that the positive effects of decreased competition for light and of the loss of pathogens are partly balanced by negative effects of the loss of predominantly positive interactions in the soil after sod cutting. We studied this balance by conducting a field experiment in which we tested whether seedlings performed better when grown in sod-cut sites than in grasslands. In a greenhouse we tested whether the positive effect of the absence of pathogens outweighs the negative effect of the absence of AMF by comparing seedlings grown with fractions of field soil biota from intact grassland and sod-cut sites.

### 2. Materials and methods

*Cirsium dissectum* (L.) Hill (*Asteraceae*) and *Succisa pratensis* Moench (*Dipsacaceae*) are native perennials. The latter has long-lived rosettes whereas the former forms new clonal ramets at the end of rhizomes (Adams 1955; Jongejans et al. 2006a; de Vere 2007). We collected seeds from several natural areas in the central-eastern part of the Netherlands (52°N, 6°E). Seeds were germinated on filter paper, and the seedlings were transplanted into autoclaved soil (1:5 agricultural soil:white sand; see Table 1 for soil characteristics). This “standard” soil was used
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As potting medium both in the field and greenhouse experiment to maintain similar edaphic characteristics (nitrogen and phosphorus) for all plants.

For this study, we selected three nature reserves in the central-eastern part of the Netherlands (Konijnendijk (52° 02' N, 6° 23' E), Koolmansdijk (52° 01' N, 6° 26' E) and Veerslootlanden (52° 36' N, 6° 08' E)), each consisting of a wet nutrient-poor grassland habitat. These nutrient-poor grasslands, where *C. dissectum* and *S. pratensis* naturally occur, have been impacted by nutrient deposition. To counteract the eutrophication in these fields, the top soil layer (approximately 10 to 25 cm) of Konijnendijk, Koolmansdijk and Veerslootlanden was removed in some areas in the winter of 1996/1997, 2000/2001 and 1999/2000, respectively to restore nutrient-poor conditions (Table 1). The research sites were therefore each divided into an undisturbed grassland area and a sod-cut area.

### 2.1 Field experiment

To study the effects of soil biota and sod cutting we performed a field experiment only at Konijnendijk and Koolmansdijk. These locations, in contrast to Veerslootlanden, were located in the same region and had similar edaphic conditions. To prevent below-ground root competition, we planted the seedlings in 30 cm long plastic tubes (32 mm inside diameter, 2 mm rim) that were open at the top and bottom, and were filled with sterilized standard soil. These ‘closed tubes’ excluded the contact with the soil biota except at the bottom. In contrast, similar tubes but with two vertical grooves (10x1.6 cm from the top that were covered with a 22.4 µm polyamide filter (“filter tubes”) allowed contact with the microbial soil life (including colonization by fungal hyphae) but excluded plant roots (modified from Johnson et al., 2001). On the 15th of April 2003, the tubes, each containing one two-week old seedling, were planted in the field. First, soil cores were taken to remove a volume of soil that corresponded to the size of the tubes. Then, the tubes were inserted into the hole in the soil. In total, two plant species by two tube types by two soil conditions (sod-cut or undisturbed grassland control) by 10 replicated blocks were placed into each location, resulting in 160 tubes. Blocks were 1 m apart, and within the blocks the individual tubes were placed at 15 cm intervals. Vegetation height was measured at the location of each tube, and was indeed higher in the grasslands than in the sod cut areas (Table 1).

### 2.2 Greenhouse experiment

This experiment was set up to study the effect of soil biota from field soil on seedling growth under controlled climatic and edaphic conditions. We used two types of solutions derived from soil from the three locations to study the effect of sod cutting on soil biota. To achieve this we collected ten random soil samples (soil cores 4x15 cm) from each of six areas: from an intact grassland and a sod-cut area at the localities Konijnendijk, Koolmansdijk and Veerslootlanden. The 10 soil samples per area were mixed and stored at 4°C for later use: the determination of chemical soil properties (Table 1) and the preparation of soil solutions. Solutions were made in one litre bottles that were filled for 1/3 with soil from one of the six sites, filled up to 1 L with water and put on a shaking machine for one hour. The suspension was allowed to settle for 45 min. after which only the top solution was poured into a new beaker for further usage. Half of each solution was used as a soil biota solution (“soil solution”), containing bacteria, fungi, nematodes and other small soil
organisms. The other half of the solution was centrifuged for 6 min. at 1000 RPM to remove clay particles and filtered through Whatman No. 1 filters (particle retention level 11 μm) to exclude hyphae, spores and nematodes but not all bacteria. This filtered solution was used as the control treatment (“soil filtrate”). All solutions were stored at 4°C before further use.

Four weeks after transplanting seedlings from the filter paper to 15 cm long bottom closed plastic tubes the experiment started by adding the solutions to the soil of the tubes. Twelve different treatments (3 locations x 2 soil types (intact grassland or sod cut) x 2 solutions, (soil filtrate or soil solution) were added to 8 replicates of both C. dissectum and S. pratensis seedlings, which adds up to a total of 192 plants.

2.3 Measurements

After 10 and 11 weeks of growth in the field and greenhouse, respectively, all surviving seedlings were harvested and their fresh weights of roots and shoots were determined. We did not use dry biomass in our analyses because fresh root samples were needed for determining the colonization by fungi. Significant proportions of the small seedling root systems were therefore lost for potential dry weight measurements. However, the fresh and dry weight of the shoots proved to be highly correlated in the field (n=166, correlation coefficient=0.78) and in the greenhouse (n=180, correlation coefficient=0.94). The root-subsamples (or the entire root system of very small plants) were stained with Trypan blue using lactoglycerol (Phillips and Hayman 1970) and ten pieces of 1 cm were mounted on a slide to assess mycorrhizal colonization (modified from the slide method described by Giovannetti and Mosse (1980)). Colonization assessment was based on visual observations (10 to 40 times magnification) of hyphae, arbuscules and vesicles, and divided into 4 classes: no colonization, < 5%, < 25% and > 25%. We used the abundance of vesicles (a sign of colonization) in the statistical analyses because vesicles are easily recognized as mycorrhizal structures, in contrast to hyphae, and because vesicles were relatively abundant in the root samples.

2.4 Statistics

Survival analyses were used to test for treatment and species differences in amount and timing of seedling mortality. Biomass was log-transformed before applying linear mixed-effects models with normal error-distributions and with block as random factor nested within vegetation types nested within localities. The percentage root colonization by vesicles was analyzed with generalized linear models with quasibinomial error-distributions. All analyses were performed in R (R Development Core Team, 2008).

3. Results

3.1 Field experiment

Thirty of the 160 seedlings died during the field experiment. Survival analysis did not show any significant differences in mortality between the two species (z=0.89, p=0.376), vegetation types (intact grasslands and sod-cut sites) (z=0.556, p=0.578), tube treatments (z=1.58, p=0.114) or locations (z=0.85, p=0.395). Fresh weight (shoots and roots) of the surviving seedlings was larger in closed tubes than those in filter tubes (p<0.001) for both plant species (Fig 1). S. pratensis responded mostly to sod-cutting, while the effect of locality was significant in C. dissectum. Especially S. pratensis plants had a higher fresh weight at harvest in sod-cut sites than in
grassland: 0.50±0.066s.e. vs 0.32±0.053 g. This biomass increase in sod-cut areas compared to grasslands was smaller in *C. dissectum*: 0.71±0.063 vs 0.64±0.076 g. Plants were on average larger in Koolmansdijk than in Konijnendijk: 0.75±0.066 vs 0.60±0.071 g for *C. dissectum* and 0.46±0.073 vs 0.36±0.050 g for *S. pratensis*.

The tubes were highly successful in excluding hyphae. In the ‘filter’ tubes seedlings were colonized (14%±2.4%s.e. in *C. dissectum* and 12%±2.7% in *S. pratensis*), while hardly any colonization was found in seedling roots from the closed tubes (0.076%±0.073% in *C. dissectum* and 0.39%±0.16% in *S. pratensis*). Roots of *S. pratensis* in the grassland site of Konijnendijk had higher colonization than in the other sites (Fig. 2). Interestingly, the mycorrhizal colonization of the seedlings in sod-cut areas was not substantially lower than that in the grassland areas, except for *S. pratensis*, which showed higher colonization in the grassland of Konijnendijk (Fig. 2). When we considered only the filter tubes, we found a significant negative relationship between the number of vesicles and fresh weight (-0.013±0.006s.e. ln(g) per vesicle for *C. dissectum*, P=0.039; -0.024±0.011 for *S. pratensis*, P=0.046), which was not affected by sod cutting or plant size at the start of the experiment.

### 3.2 Greenhouse experiment

In the greenhouse experiment, mortality differed per species (p< 0.01). Average mortality was 20% for *S. pratensis*, but only 5% in *C. dissectum*. Survival was not affected by soil-filtrate or solution. No more plants died during the experiment after the first 50 days. The mycorrhizal inoculum was not very effective: less than 3% of the seedlings were colonized in any of the treatments.

Addition of field soil life significantly affected plant biomass. Seedlings had lower fresh weights in the soil solution treatment as compared to the soil filtrate treatment (Fig. 3). The origin of the solution (locality and grassland or sod-cut area) did not influence seedling biomass in the greenhouse.

### 4 Discussion

Sod cutting changes soil characteristics and belowground soil communities, which in turn can have direct and indirect, and positive or negative effects on seedling establishment and performance. In this study we found a predominance of a negative soil-plant feedback that was independent of sod-cutting. The seedlings did, however, perform better in sod-cut areas (where light availability was higher).

#### 4.1 Sod cutting

Sod cutting removes both the top layer of the soil and the associated vegetation, and may create new “windows of opportunity” for seedling establishment (Grubb 1977; Herben et al. 2006). Indeed, both vegetation height (see table 1) and vegetation cover (Jongejans et al. 2008: 44% vs 91%) were lower in sod cut sites than in grasslands. In our study, especially *Succisa pratensis* seedlings performed better (as based on their biomass) in the sod-cut sites with higher light availability than in the grasslands. Canopy presence can reduce seedling survival (Isselstein et al. 2002), which has also been found for the grassland perennials *Centaurea jacea* and *Hypochaeris radicata* (Soons et al. 2005; Jongejans et al. 2006b). These studies showed slightly higher establishment rates for *Cirsium dissectum* in sod-cut sites and no significant difference for *S. pratensis*, but these studies did not measure seedling biomass.
Sod cutting may lower pH and increase NH$_4^+$ levels, which in poorly buffered soils decreases germination, plant growth and survival rates of both S. pratensis and C. dissectum (de Graaf et al. 1998; Dorland et al. 2003; van den Berg et al. 2005). However, high NH$_4^+$ concentrations have been found to persist for only one year after sod cutting (Dorland et al. 2003), and the two sites that we used in our field experiment had been sod-cut two and five years before we started our experiment. Moreover, we did not find any difference in soil pH between sod-cut and grassland sites.

4.2 Mycorrhizas

Sod cutting not only changes plant species composition, plant density and soil chemistry, it also removes a substantial part of the biota in the soil. While the physical removal of pathogens may result in improved plant growth, AMF removal may negatively influence plants that are dependent on the mycorrhizal fungal community. Lovera & Cuenca (1996), Vergeer et al. (2006) and Boerner et al. (1996) found a negative effect of soil disturbance on mycorrhizal colonization percentage. Interestingly, in the present study sod cutting did not influence mycorrhizal colonization percentage much. This suggests that in the two or five years between the sod-cutting and our field experiment at least part of the mycorrhizal community may have recolonized the soil (Gould et al. 1996). However, even though the colonization percentage was similar, the fungal species or strain diversity and activity may still have differed (Lovera and Cuenca 1996).

Despite the consensus that mycorrhizas usually benefit plant growth, we found reduced growth in those tubes that allowed contact with the surrounding soils and especially in those tubes with higher mycorrhizal colonization. Mycorrhizas can in fact occupy various positions along the continuum from parasitism to mutualism (Jones and Smith 2004) depending on the specific plant and fungal genotypes, their abiotic and biotic environments, and life stages (Marler et al. 1999; Klironomos 2003; Kytöviita et al. 2003).

No significant mycorrhizal colonization was found in the greenhouse experiment. In the greenhouse the inoculum potential is lower than in the field where seedlings are easily incorporated into the existing mycorrhizal networks (Johnson et al. 2001). Since less than 3% mycorrhizal colonization was found in the greenhouse experiment, the similar plant growth reductions in the field experiment should likely also be attributed to other soil organisms.

4.3 Soil biota

Sod cutting uncovers “new” soil that may contain less pathogens and a different microbial soil community. Plant species that are prone to a quick accumulation of a specialized pathogen community may benefit and successfully colonize this “new” habitat (van der Putten et al. 1993; Mills and Bever 1998; Klironomos 2002). However, results from our greenhouse study suggest a negative impact of soil biota (other than mycorrhizal fungi) on growth of S. pratensis and C. dissectum seedlings, independent of sod cutting. Apparently the pathogens were present in both the grassland and the sod-cut soil and sod cutting could not be used as a management tool to free seedlings from pathogens when a larger time frame (e.g. several years as in this study) after sod cutting is considered. Perhaps sod cutting did not remove all the pathogens, or the organisms recolonized the newly uncovered soil relatively
quickly. Other explanations like potential differences in nutrient availability of the solutions are less likely because the unfiltered solution (which might have contained more clay particles) resulted in lower plant biomass.

Various groups of soil organisms can cause a negative plant-soil response as we found in both our field and greenhouse experiments. In our study, nematodes were most likely filtered out in the soil filtrates while remaining in the soil solution treatments. Plant parasitic nematodes may have been responsible for the negative plant-soil feedback (Olff et al. 2000; van Ruijven et al. 2005). Van der Stoel et al. (2002) found that negative plant-soil feedbacks were initially caused by plant-parasitic nematodes, but later by other organisms. Therefore, the negative effects of soil biota in our experiments may have been caused by more than one type of organisms.

4.4 Conclusions

Removal of the top soil may provide only a brief escape from soil pathogens. In this study the microbial soil life, including mycorrhizal fungi, had a negative impact on seedlings. However, further study is needed on the presence and the effects of key-pathogens (e.g. bacteria and plant parasitic nematodes) and mycorrhizas to clarify their role in the various life-stages of plants. Although sod cutting did not mitigate the negative plant-soil feedback, seedling growth increased, probably due to lowered above-ground competition for light. Therefore, sod cutting is still a useful restoration tool to promote seedling establishment and persistence of endangered species in previously eutrophicated grasslands.

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Table 1.

Soil characteristics of 3 localities and from soil used as potting medium (standard soil). pH = pH (H2O), SWC = soil water content %, N = total nitrogen mg/g soil, P = total phosphorus mg/g soil. Also included is the vegetation height (VH) in cm for 4 sites. SWC was determined by weighing wet soil, drying at 65°C overnight and reweighing. Total N and P were determined following Novozamsky et al. (1984). See text for further descriptions of locations.

<table>
<thead>
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<th>Soil Sample</th>
<th>pH</th>
<th>SWC</th>
<th>N</th>
<th>P</th>
<th>VH</th>
</tr>
</thead>
<tbody>
<tr>
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<td>42.0</td>
<td>3.16</td>
<td>0.23</td>
<td>10.2</td>
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<tr>
<td>Sod cut</td>
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<td>29.4</td>
<td>0.62</td>
<td>0.07</td>
<td>8.5</td>
</tr>
<tr>
<td>Koolmansdijk grassland</td>
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<td>29.9</td>
<td>2.27</td>
<td>0.13</td>
<td>17.6</td>
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<tr>
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<td>16.9</td>
<td>0.28</td>
<td>0.04</td>
<td>9.8</td>
</tr>
<tr>
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<td>-</td>
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<tr>
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<td>0.28</td>
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</table>
Figure 1. Back-transformed means of total wet biomass (g) per plant species (C. dissectum left and S. pratensis right) for each tube type (closed and filter tube). *** = p<0.001, n = 128.

Figure 2. Proportional abundance of vesicles on roots from S. pratensis plants grown in filter tubes. n = Konijnendijk; o = Koolmansdijk; gra = grassland; cut = sod cut. Error bars represent ± SE. Letters above the bars designate significant (p<0.05) differences within panels.
**Figure 3.** Total wet biomass (g) of *C. dissectum* and *S. pratensis* for the treatments: Soil filtrate = filtrate from soil solution with reduced field soil life, Soil solution = solution with unfiltered field soil life. Error bars represent ± SE. The difference between treatments is significant for both *C. dissectum* (*p*=0.017) and *S. pratensis* (*p*=0.0015).