

Use of shrub willows (*Salix* spp.) to develop soil communities during coal mine restoration

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Abstract: Afforestation or reforestation in highly degraded environments (e.g., surface mines) is often complicated by the total removal of vegetation and severe soil degradation that occurs during mining operations, necessitating revegetation to be undertaken in tandem with the re-establishment of soil developmental processes. Shrub willows (*Salix* spp.) are effective as colonizer species initiating revegetation dynamics; however, it is unclear if they also serve as nurse plants facilitating the establishment of soil communities such as those of nematodes. We established a study in a former coal mine site in New Brunswick, Canada, to assess whether the presence of willows on otherwise bare, poorly developed soil contributed to nematode community development and to what degree landform design (e.g., slope) may influence these dynamics. Our results demonstrate that willows can facilitate nematode communities at this site, but that slope strongly influences these effects, likely as a consequence of hydrology and overland water flow. These results confirm the beneficial role that willows can play in reforestation of highly degraded environments both for revegetation and for the re-initiation of soil ecosystem processes.

Key words: nurse plant, facilitation, reforestation, plant–soil interactions, nematodes.

Résumé : Le boisement ou la reforestation dans les milieux fortement dégradés (p. ex. les mines à ciel ouvert) est souvent compliqué par l'enlèvement complet de la végétation et la grave dégradation du sol qui surviennent durant l'exploitation minière, ce qui nécessite d'entreprendre la revégétalisation en même temps que le rétablissement des processus de formation du sol. Les saules arbustifs (*Salix* spp.) sont des espèces pionnières efficaces pour initier la dynamique de la revégétalisation. Cependant, il n'est pas certain si ces saules servent aussi de plantes compagnes qui facilitent l'établissement des communautés édaphiques telles que celles des nématodes. Nous avons initié une étude sur le site d'une ancienne mine de charbon du Nouveau-Brunswick, au Canada, pour déterminer si la présence de saules sur un sol autrement nu et peu développé contribue au développement de la communauté de nématodes et dans quelle mesure la configuration du terrain (p. ex. pente) peut influencer ces dynamiques. Nos résultats démontrent que les saules peuvent promouvoir les communautés de nématodes à cet endroit mais que la pente influence beaucoup ces effets, probablement à cause de l'impact de l'hydrologie et du ruissellement. Ces résultats confirment le rôle bénéfique que peuvent avoir les saules dans la reforestation des milieux fortement dégradés tant dans la revégétalisation que la ré-initiation des processus de l'écosystème. [Traduit par la Rédaction]

Mots-clés : plantes compagnes, facilitation, reforestation, interactions entre les plantes et le sol, nématodes.

Introduction

The last several centuries have seen the loss or degradation of up to 50% of forested areas globally (Minnemayer et al. 2011), highlighting the important challenges of afforesting or reforesting highly degraded habitats such as surface mines and sites used for fossil fuel extraction. The removal of vegetation and extreme restructuring of soil horizons or sometimes complete destruction of developed soil during surface-mining operations makes restoration of these sites particularly challenging (Herath et al. 2009). In many cases, restoring the local plant community is only one challenge to reforestation and must be conducted in tandem with restoring soil processes involved in soil formation and development to facilitate colonization by later successional plant species.

Willows (*Salix* spp.) are attractive early successional plants for revegetation because they are easily propagated vegetatively, grow quickly on low-fertility sites, are relatively short-lived, and can rapidly increase their cover area even on marginal habitats (Kuzovkina and Quigley 2005). The high diversity of the genus

allows for the selection of candidate species specialized for growth under a variety of habitat conditions, from wetland or riparian areas to drier upland soils (Kuzovkina and Volk 2009). Once planted, their rapid growth helps to stabilize soil structure through root development, minimizing soil erosion and beginning the process of aggregate formation and incorporation of organic material into mineral soil (Kuzovkina and Volk 2009). As a consequence of these properties, several studies have demonstrated success using willows in mine site reclamation, reporting high willow survival rates on soil of varying depths and qualities (e.g., topsoil or waste rock) and high aboveground biomass (Bissonnette et al. 2010; Larchevêque et al. 2014; Mosseler et al. 2014a).

An important question in the successful restoration and reforestation of degraded habitats by willows is to what degree they may be able to serve as nurse plants, facilitating colonization by later successional species. Much of the nurse plant literature focuses on the role that they play in facilitating other plant species in the formation of “islands of fertility” by creating favorable

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microclimatic conditions and concentrating resources through litter deposition below plant canopies (e.g., Hook et al. 1991), but there has been increased interest in examining how nurse plants facilitate the development of soil communities, especially with regard to how these soil communities, in turn, contribute to soil development processes and then assist in driving later successional plant communities (van der Putten 2009; Rodríguez-Echeverría et al. 2016). Soil organisms are seldom added during reclamation activities (Lawrence et al. 2013); however, they provide numerous services to ecosystems, including decomposition, nutrient cycling, and the incorporation of soil organic matter, and have also demonstrated contributions to plant community dynamics by influencing plant community composition and community resilience to disturbance (see Sylvain and Wall 2011). Nematodes, owing to their high abundance and species diversity within ecosystems and the number of trophic roles that they fill within soil food webs (e.g., root herbivores, bacterivores, fungivores, omnivores, and predators), are an ecologically important group that serves as an ideal taxon for study in the context of ecosystem development during restoration. As a consequence of their feeding preferences or requirements, different nematode trophic groups may be more sensitive to certain environmental factors or disturbances. Root herbivores, for example, depend on the presence of plant roots for the acquisition of carbon and nutrients, and so the larger concentration of active roots under willows would provide much greater habitat for these organisms than would be found in bare soil patches with low root densities. Similarly, bacterivorous nematodes are typically considered to be a colonizer group capable of establishing on marginal habitats early in ecosystem development (Bongers and Ferris 1999), and high abundance of these nematodes relative to other groups is an indicator of disturbed environments, especially compared with fungivorous nematodes, which depend on networks of fungal hyphae that typically develop in more stable ecosystems. Plant diversity has also been shown to positively influence soil community abundance and diversity (Bardgett and Wardle 2010), and as willows facilitate herbaceous recolonization of sites under reclamation, the increase in plant diversity (and associated increases to belowground production and diversity of both root exudates and litter quality) should drive increases in soil organism abundance and diversity (Bardgett and Wardle 2010).

Nematodes are aquatic organisms that require close association with soil material and can be passively transported across a landscape through soil erosion driven by overland water flow (Baxter et al. 2013). This provides an opportunity for nematodes to be captured and retained by the stem and root systems of willows planted to reduce soil cover losses in degraded habitats, as well as by those of herbaceous plants present in later successional areas after capture by and establishment below willows. Despite their ecological importance and interactions with plant communities, few studies have examined nematode community recovery following restoration of former coal mine sites. Recovery rates appear to differ by vegetation cover, with nematode communities under deciduous cover recovering more successfully than under coniferous cover (Háněl 2008) and more rapidly in shortgrass systems with simpler initial communities than in more complex systems such as tallgrass prairie or forest (Frouz et al. 2013). Treatment of the topsoil also appears to contribute to recovery speed and success; topsoil stockpiling and spreading has been demonstrated to reduce soil biota (Schuman 2002; Menta 2012), and studies have shown more rapid recovery of nematode communities in systems in which topsoil is translocated from new mine locations to reclaim former mine areas without stockpiling (Chen et al. 2014) as opposed to much longer recovery times necessary for sites where topsoil was stockpiled and spread during reclamation (Frouz et al. 2013) or where communities were left to develop on colliery spoils without topsoil (Háněl 2008).

Our objective was to determine to what degree shrub willows facilitate the establishment of nematode communities on poorly developed soils of a former coal mine. At this site, willows are being grown as part of ongoing studies investigating the role that they can play in reforesting former coal mine sites, as well as their suitability for use as a biofuel feedstock. We sampled two locations in two different years (2014 and 2015) and examined nematode communities related to willow cover, bare soil, herbaceous cover and a mix of willow and herbaceous cover, and landscape orientation (slope aspect and overland water flow). We investigated the following hypotheses. (1) Total nematode abundances and the abundances of individual nematode trophic groups would be greater under willows than in bare soil but would be lower than samples collected from under herbaceous cover. We expect nematode abundances to increase with increasing root biomass and organic matter inputs to the rhizosphere, both of which should be greater under herbaceous plant cover, intermediate under willow cover, and lowest in bare soil. (2) Nematode communities collected from a sloping, ephemeral stream channel would be less abundant and have lower abundances of individual trophic groups than those collected from a hillslope, and that the hillslope would, in turn, have lower abundances than a forest located at the top of the hillslope. Given the small body size and close relationship that nematodes have with the surface of soil particles, we anticipate that slopes should have greater passive transport of nematodes away from these sites due to erosion and that this effect should be greater in the stream channel where water flow is greatest. After sampling and initial analyses to address our first two hypotheses, we became interested in whether the facilitation effect of willows on nematode communities may interact with slope positioning. To address this, we developed an additional a posteriori hypothesis to test using a subset of our 2014 and 2015 sampling as preliminary data: (3) nematode community composition and structure would differ by willow presence or absence and slope position, as more complex communities should assemble as nematode abundances increase under willow roots (i.e., a greater abundance of primary consumers should support more individuals from higher trophic levels), and bare soils less capable of capturing and maintaining nematode populations should support low nematode abundances characterized by microbial grazers.

Methods

This study was carried out at the Salmon Harbour coal mine near Minto, New Brunswick (NB), Canada (46°07'N, 66°05'W). Mean annual precipitation at the site is 987 mm, and mean annual temperature is 5.7 °C. Soils are relatively undeveloped sandy loams with a high incidence of rocks (>40%), low organic matter content (<0.3%), low nitrogen (<0.2%), and a pH of 7.5. We performed two observational studies at two separate locations within the coal mine site, one in November 2014 and one in September 2015. These two sites are approximately 2 km apart on the same former coal mine site (Fig. 1). The 2014 site is flat lowland with willows (*Salix eriophylla* Michx. and *Salix discolor* Muhl.) planted in 2008 as part of a three-site common garden experiment (for planting design, see Mosseler et al. 2014b) and with herbaceous cover located along part of the sampling area (for plant species and cover, see Table 1). The 2015 site comprised an upland forest (for plant species and cover, see Table 2) at the summit of an initial slope proceeding to a perpendicularly sloping stream channel (both slopes ~14% grade and between 100 and 120 m from slope top to bottomland) with naturally established willows (*Salix bebbiana* Sarg. and *S. discolor*); samples at this site were collected from the forest edge and along both slopes.

Treatments and replication differed between sampling years. In 2014, at the lowland site, four replicate samples were collected from bare soil, soil with willow cover only, soil with herbaceous cover only, and soil with willow and herbaceous cover (see Table 1

Fig. 1. Satellite map of 2014 and 2015 sampling locations showing site relief (3 m contour lines) and ground cover. LiDAR data generated in 2015 by the New Brunswick Department of Energy and Resource Development.



for herbaceous species and their associated cover). In 2015, at the forest and slope site, five replicate samples were collected from the forest margin, along the broad slope from both bare soil and soil under willow cover ~54 m downslope from the forest margin, and then along the stream channel from both bare soil and soil under willow cover ~70 m from the midslope location; for all sampling locations, samples were collected along 50 m transects and from under *S. discolor*. In 2014, samples were collected every 5 m directly under willow stems (willow and willow + herbaceous treatments) and 4 m away (perpendicular to the transect) from samples collected under willow stems (bare and herbaceous treatments), with two “blocks” along the transect, one containing willow and bare sites separated by approximately 20 m from the second, which contained herbaceous and willow + herbaceous treatments. The 2015 samples were collected from the forest margin and then from either directly under willow stems or from bare

soil 4 m away located at the same height along the slope face, with samples being taken along the face of the slope proceeding from upslope to downslope. Soil samples were collected using a trowel to remove 6 cm diameter cores to a depth of 15 cm (i.e., the biologically active layer for most soils), placed into coolers, and transported to the laboratory for extraction and soil moisture determination. Soil moisture was determined gravimetrically, with a 50 g subsample heated to 105 °C for 48 h in an oven and water mass loss due to evaporation determined (Barrett et al. 2008). Nematodes were extracted by Baermann funnel (Baermann 1917) over 72 h using a 50 g subsample of fresh soil; they were counted and identified to trophic groups (see Yeates et al. 1993) while live using an Olympus CKX41 inverted microscope. Remaining soil was sent to the Laboratory for Forest Soils and Environmental Quality at the University of New Brunswick for determination of pH, percent organic matter, and percent total nitrogen.

Table 1. Data on herbaceous vegetation sampled from 20 plots (1 m × 1 m square) along a 100 m transect at the Salmon Harbour mine site sampled in 2014 for nematode populations associated with native willows established for land reclamation purposes.

Species		Presence across 20 sampled plots		
Common name	Latin name	No. of plots	% Plots	% Cover
Red clover	<i>Trifolium pratense</i>	19	95	26
Creeping bush clover	<i>Lespedeza</i> sp.	7	35	12
Hawkweed	<i>Hieracium</i> spp.	15	75	5.6
Mosses	Unidentified	4	20	2.5
Low hop clover	<i>Trifolium procumbens</i>	9	45	2.3
Grasses	Unidentified	5	25	2.2
Wild strawberry	<i>Fragaria virginiana</i>	6	30	1.7
Coltsfoot	<i>Tussilago farfara</i>	6	30	0.9
Goldenrod	<i>Solidago</i> spp.	3	15	0.8
Purple vetch	<i>Vicia</i> sp.	3	15	0.6
Fall dandelion	<i>Leontodon autumnalis</i>	1	5	0.2
Calico aster	<i>Aster lateriflorus</i>	1	5	0.2

Note: Mean herbaceous vegetation coverage over all 20 plots was 58.5% (overall, 42% of plots were not covered by any herbaceous vegetation).

All analyses were conducted using R version 3.2.4 (R Core Team 2016). Two-way ANOVA testing was conducted using the “aov” command in the base R package and $\log(x + 1)$ -transformed abundance data standardized to individuals per kilogram of dry soil, with models relating nematode abundances to vegetation cover (bare, forest, herbaceous, willow, or willow + herbaceous) and slope position (bottom, slope, or stream channel). Data from each year were analyzed separately to determine effects of vegetation cover. Final models for analyses were (1) nematode abundance ~ vegetation cover type (2014 sampling) and (2) nematode abundance ~ willow presence or absence × slope position (2015 sampling); models for each trophic group initially included interaction terms and were then simplified using Akaike information criterion (AIC) comparisons and backward selection, using $\alpha = 0.05$ for the threshold of significance. All reported treatment effects were determined using post hoc testing with Tukey’s honest significant difference (HSD) (“TukeyHSD” command in the base R package) to correct for family-wise error rate from multiple comparisons. We also performed correlations using the “cor” command in the base R package to determine Pearson correlation coefficients for the relationships between nematode trophic group abundances and soil moisture, soil organic matter, soil pH, and percent total nitrogen in the soil. For our correlation analyses, we excluded the forested sites sampled in 2015 as they had outsized influence on the strength of the correlations, in some cases altering the direction of the relationship.

After sampling and analyses to address our first two hypotheses were complete, we became interested in exploring whether the facilitation effect of shrub willows on nematode community development may be influenced by slope position. We tested a third model, nematode abundance ~ willow presence or absence × slope position, using our 2014 and 2015 sampling data to provide preliminary results; this cross-year comparison excluded samples from 2014 collected under herbaceous or willow + herbaceous cover and samples from 2015 collected under forest soils. As each site within the mine location was sampled only once (i.e., lowland location in 2014, slope sites in 2015), interannual variability was confounded with slope positioning and could not be assessed for models comparing vegetation and slope positioning between years. These analyses to address hypothesis 3 are therefore of secondary interest and represent preliminary results derived from exploratory data analysis. However, we feel that comparisons of slope positioning remain valid, as Háněl (2008) reported no significant interannual effects of year for nematode popula-

tions sampled from colliery spoils in the Czech Republic and de Goede and Bongers (1994) noted the greater importance of site characteristics relative to annual demographic variation in driving nematode community dynamics. To further explore the potential influence of willows and slope position on nematode communities, nonmetric multidimensional scaling (NMDS) ordination was carried out on untransformed nematode community data using the “metaMDS” command and Bray–Curtis distance measure in the vegan package (version 2.4-0; Oksanen et al. 2016) to determine dissimilarities between nematode trophic structuring for assemblages sampled from different vegetation cover types and slope aspects. PERMANOVA analysis was carried out using the “Adonis” command (also in vegan) to test pairwise differences between nematode trophic assemblages by treatment by comparing treatment centroids and their associated variance.

Results

In 2014, a total of 1854 nematodes were collected and identified to trophic group, with 878 nematodes collected and identified in 2015; Table 3 lists mean nematode abundances (standardized to per kilogram of dry soil) and selected soil characteristics by treatment for both sampling years. Correlations between soil characteristics (moisture, pH, organic matter, and percent total nitrogen) and nematode trophic groups and total nematode abundances showed that pH (negative) and organic matter content (positive) showed the strongest (albeit moderate) relationships with nematode groups (Table 4), whereas only weak relationships were observed for soil moisture (negative) and percent total nitrogen (positive) (Table 4). There were significant effects of vegetation cover from the 2014 study for predatory nematode abundance ($P = 0.042$, $F_{[3,12]} = 3.7$) and total nematode abundance ($P = 0.045$, $F_{[3,12]} = 3.6$), with both having significantly higher abundances in herbaceous cover relative to bare ground, and willow and willow + herbaceous cover intermediate between (and not significantly different from) herbaceous cover and bare ground. Vegetation cover also showed significant differences for percent total nitrogen ($P = 0.0003$, $F_{[3,12]} = 13.76$), soil organic matter ($P = 0.002$, $F_{[3,12]} = 8.91$), and soil moisture ($P = 0.001$, $F_{[3,12]} = 9.9$) (Table 3). The effects of vegetation cover in 2014 were also suggestive for fungivorous nematodes ($P = 0.068$) and omnivorous nematodes ($P = 0.067$). Vegetation cover had an even stronger effect in 2015, with assemblages of root herbivorous nematodes ($P = 0.0009$, $F_{[1,18]} = 15.9$), bacterivorous nematodes ($P = 0.006$, $F_{[1,18]} = 9.6$), and total nematode abundances ($P = 0.01$, $F_{[1,18]} = 8.2$) having higher abundances under willows than in bare soil and with soil under willows also having greater percent total nitrogen than under bare ground ($P = 0.0008$, $F_{[1,18]} = 16.4$). Slope had a significant effect for predatory nematodes ($P = 0.03$, $F_{[1,18]} = 5.8$), with higher numbers collected from the stream channel than the slope face.

Significant effects of willow cover presence and slope positioning were observed between years, although there were no interactions between the two factors observed for any nematode group. Slope was important for all nematode groups (total nematode abundance $P = 0.0002$, $F_{[1,24]} = 10.4$; all individual trophic groups $P < 0.0001$), with the flat area sampled in 2014 always supporting higher abundances than either of the two slope locations sampled in 2015, which did not differ significantly from each other. The presence of willows had a significant, positive effect on the abundances of the total nematode community ($P = 0.004$, $F_{[1,24]} = 10.4$), bacterivorous nematodes ($P = 0.003$, $F_{[1,24]} = 10.9$), and root herbivorous nematodes ($P = 0.0002$, $F_{[1,24]} = 19.7$). The NMDS ordination revealed a clear gradient of treatments along the first ordination axis, with forest sites clustering largely alone to one side, samples from the 2014 lowland area clustering on the left half of the figure, and those from the 2015 slope sites clustering together on the right (Fig. 2); PERMANOVA analyses revealed further differences between communities from different topograph-

Table 2. Dominant woody vegetation on 2015 study sites used to characterize nematode populations.

Study site	Woody species	No. of stems	% Cover	Height (m)	Site description
Channel (toe of slope)	<i>Salix cordata</i> Michx.	79	77	1–2	Willow field test established on gravel outwash at 2 m × 2 m spacing in 2013
	<i>Salix interior</i> Rowlee	17	17	1–2	
	<i>Salix discolor</i> Muhl.	6	6	1–2	
Slope (midslope)	<i>Populus tremuloides</i> Michx.	40	56	0.5–1	Slope of shale overburden, landscaped in 2010 following cessation of mining operations; sparsely populated by colonizing hardwood shrub and tree species
	<i>Betula</i> spp.*	18	25	0.5–1.5	
	<i>Salix bebbiana</i> Sarg.	9	13	0.5–1.5	
	<i>Salix discolor</i> Muhl.	2	3	0.5–1	
	<i>Salix lucida</i> Muhl. ssp. <i>lucida</i>	2	3	0.5–1	
Forest edge (top of slope)	<i>Picea mariana</i> (Mill.) BSP	59	54	6–14	Edge of natural forest cover type that existed prior to coal mining operations
	<i>Betula</i> spp.*	31	28	2–6	
	<i>Alnus incana</i> Du Roi	10	9	2–3	
	<i>Acer saccharinum</i> L.	6	6	10–16	
	<i>Populus tremuloides</i> L.	2	2	4–5	
	<i>Larix laricina</i> (Du Roi) K. Koch	1	1	16	

*Birch population includes local native birches: *Betula papyrifera* Marsh. and *B. populifolia* Marsh.

Table 3. Nematodes collected per treatment (means ± standard errors) from 2014 and 2015 sampling locations.

	Nematode abundances (per kg dry soil)						Soil characteristics			
	Total	Bacterivores	Fungivores	Root herbivores	Omnivores	Predators	pH	% Organic matter	% Soil moisture	% Total N
2014 sampling										
Bare	1158±255b	451±108	227±20	294±123	39±6	91±38b	7.7±0.1	0.93±0.05b	18.7±1.4a	0.07c
Willow	2953±317ab	943±85	646±117	1111±140	104±38	94±19ab	6.6±0.3	1.13±0.14b	13.7±0.25b	0.09±0.01bc
Willow + Herb	3193±1353ab	512±186	100±59	2029±1320	50±43	465±240ab	5.7±0.7	1.32±0.1ab	12.9±0.44b	0.1ab
Herbaceous	4894±1746a	2105±1171	789±442	826±120	328±169	703±230a	5.4±0.4	1.65±0.12a	15.1±0.6b	0.11a
2015 sampling										
Forest	19 667±12 787	10 289±5659	4029±3775	2098±1377	637±387	2086±1358	3.9±0.04	42.7±6.9	92.2±4	1.25±0.21
Slope (Bare)	25±13b	8±5b	8±8	0b	4±4	0b	7.4±0.4	0.14±0.03	6.7±1.3	0.16±0.01b
Slope (Willows)	780±551a	105±42a	170±118	460±393a	4±4	0b	7±0.2	0.5±0.13	8.8±0.72	0.21±0.01a
Channel (Bare)	154±92b	100±80b	34±14	0b	0	17±8a	7.4±0.2	0.37±0.13	7.8±0.86	0.18b
Channel (Willows)	167±45a	63±28a	21±9	55±30a	4±4	12±12a	7.5±0.2	0.36±0.08	7.5±0.27	0.21±0.01a

Note: Total nematodes include those unable to be sorted to trophic groups and so are greater in number than adding individual trophic groups. Bold text and letters indicate significant differences and treatment groupings identified using post hoc Tukey HSD tests (see text). Note that for the 2015 sampling, nematode abundances from forest sites were always significantly higher, and so test results reflect differences between the four slope and channel treatments only.

Table 4. Correlation coefficients for relationships between soil characteristics and nematode total and trophic group abundances.

	pH	% Organic matter	% Soil moisture	% Total N
Bacterivores	-0.514	0.504	-0.38	0.395
Fungivores	-0.431	0.513	-0.364	0.39
Root herbivores	-0.551	0.442	-0.271	0.25
Omnivores	-0.577	0.482	-0.336	0.329
Predators	-0.41	0.599	-0.341	0.387
Total nematodes	-0.684	0.669	-0.447	0.46

Note: Correlations exclude data from forested sites sampled in 2015 due to their outsized influence on the resultant relationships. Italics denote correlation coefficients stronger than 0.5 or -0.5.

ical treatments, with forested sites different from all other site types and lowland sites from 2014 differing from the slope sites sampled in 2015 (which were both similar to one another; Table 5).

Discussion

Our study demonstrates that planting shrub willows can be an effective method to initiate colonization of abandoned mine sites by soil organisms such as nematodes, although herbaceous cover may be more effective where its establishment is possible. Our preliminary findings addressing our third hypothesis also highlight the potentially important role that landscape engineering may play in the reclamation process. These results complement

the existing body of literature on nurse plants and reclamation of abandoned mine habitats by showing that the benefits of nurse plants extend to soil communities and contribute to the development of complex, functioning ecosystems with a greater diversity of biotic interactions.

Willows as nurse plants for soil communities

Our data confirm that shrub willows can act as nurse plants to facilitate the development of soil nematode communities in otherwise bare and poorly developed soils of a former coal mine. Whereas data from 2014 show that only herbaceous cover significantly affected nematode community abundances relative to bare soil, data from 2015 and our preliminary comparisons of communities from under willows and bare soil between the 2 years demonstrate that willows contribute to increases in root herbivorous and bacterivorous nematode trophic groups, as well as total nematode abundances. These results provide partial support for our first hypothesis, that herbaceous cover should have greater nematode abundances than communities found under willows, which should in turn possess more abundant communities than bare soil. The greater abundance of nematodes under herbaceous cover (2014 sampling) and under willows relative to bare ground (2015 sampling) suggests that plant cover does successfully capture and maintain nematode populations, likely increasing in capacity as plant diversity and (or) cover increases. The pattern of nematode abundances that we observed, with populations greatest under herbaceous cover, intermediate under willows (and willows + herbaceous), and lowest under bare ground, is

Fig. 2. NMDS ordination of nematode trophic group abundances by vegetation cover and landscape location. Symbols correspond to sampling site and year, with solid symbols denoting treatments containing willows: from the 2014 lowland site are bare soil (Bare), soil from under herbaceous cover (Herb), soil from under willow cover (Willow), and soil from under mixed willow and herbaceous cover (Willow + Herb); from the 2015 hillslope sites are bare soil from the stream channel (Channel (Bare)), soil from under willows in the stream channel (Channel (Willows)), forest soil (Forest), bare soil from along the hill slope (Slope (Bare)), and soil from under willows along the hill slope (Slope (Willows)).

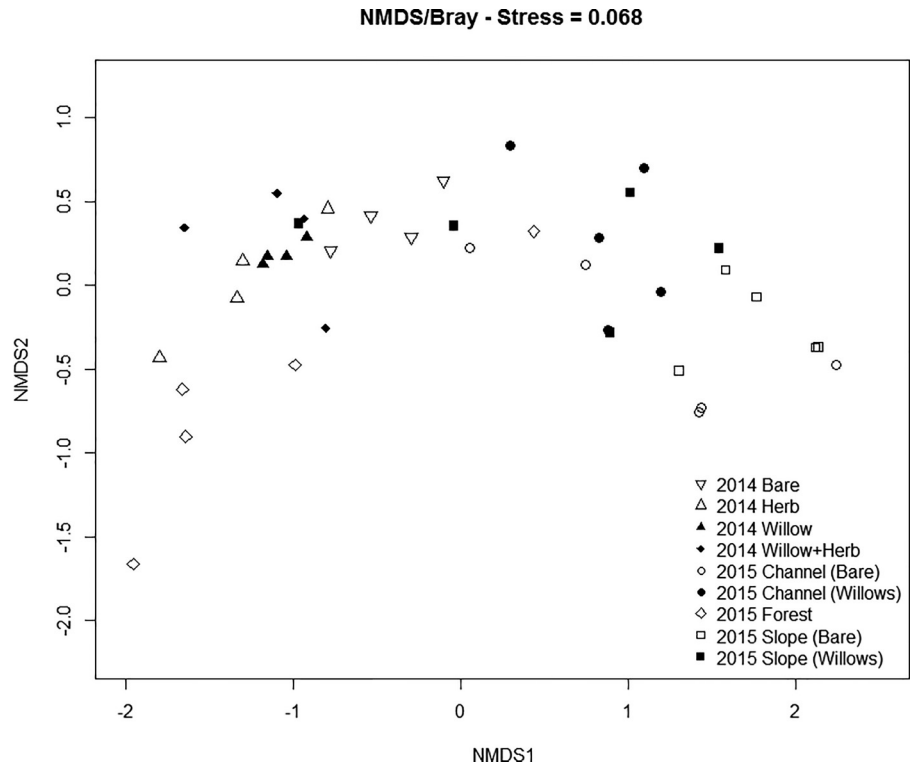


Table 5. PERMANOVA comparisons between topographical treatments, with associated *F* statistics and *P* values.

Comparison	<i>F</i> statistic	<i>P</i> value
Forest vs. lowland	3.88	0.024
Forest vs. channel	5.10	0.012
Forest vs. slope	5.32	0.018
Lowland vs. channel	14.56	0.006
Lowland vs. slope	15.51	0.006
Channel vs. slope	2.02	0.40

Note: Bold text indicates a significant difference between treatments.

mirrored by observed differences in percent organic matter and percent nitrogen in our 2014 sampling and for willows as compared with bare ground in our 2015 sampling. These two factors have been shown to increase with greater plant cover and diversity and are likely to drive interactions with the nematode community as organic matter and available nitrogen should stimulate microbial communities on which they feed (Bardgett and Wardle 2010); the opposite trend that we observed for soil moisture is possibly due to evapotranspiration and the use of water by plants for photosynthesis, processes not present on bare soil. Additionally, the weak but negative correlations that we observed between nematode abundances and available soil moisture match findings of Sylvain et al. (2014), who found that nematode abundances for several groups declined with increasing soil moisture within a variety of desert and grassland ecosystems, which also are characterized by localized plant communities interspersed with gaps of varying sizes.

Willows at the 2015 site are only recently established relative to when nematode sampling was conducted, and the two trophic groups (bacterivores and root herbivores) that showed increased abundances represent groups that would be expected to show early responses given the presence of roots (directly fed upon by root herbivores) and their associated exudates (which would stimulate bacterial colony growth for bacterivores to graze upon). The ability of nurse plants to capture and develop soil communities, especially with respect to microbial communities, may be particularly important in arid or degraded ecosystems where nurse plants may act as an environmental filter favoring microbial communities that facilitate recruitment of other plant species (Hortal et al. 2013, 2015). More abundant nematode populations have also been observed under shrub cover relative to bare ground in arid environments (Wall-Freckman and Huang 1998), and our results suggest that if willows in our system are able to also capture seeds of herbaceous plants, as the plant diversity in these areas increases, more numerous soil communities with more diverse trophic groups should develop as the plant community increases the amount and variety of organic inputs into soil (Bardgett and Wardle 2010), a trend that we observed in the correlations between percent organic matter and most nematode groups in our study (Table 4).

Influences of slope on willow activity as nurse plants

This study shows that although vegetation cover was important in structuring nematode communities, data from 2015 and preliminary analyses using data across both sampling years suggest that the strongest influence on these communities may be related to position along hillslopes, likely due to effects of hydrology. In the 2015 sample period, nematodes from forested sites were significantly more abundant than those collected from slopes (in

some cases, nearly two orders of magnitude greater); only predatory nematodes appeared to respond to the different slope positions within the study, with a significantly higher abundance found in the stream channel locations than along the hill slope face. These results provide limited support for our second hypothesis, and it is possible that the two slope positions show few differences due to their similar degree of slope, and the potential for greater volume of water flow in the channel may not be as important as we had hypothesized.

In contrast, the preliminary comparison between years showed that all nematode groups, as well as total nematode abundances, were strongly influenced by slope position. The lowland area sampled in 2014 always had higher abundances than the slope and stream channel areas sampled in 2015. Predators may have been greater in abundance within the stream channel after being washed down from along the hill slope and aggregating along the base of the willows in the stream channel, although it is unclear why this would not also be the case for other groups. Between years, it is possible that the more level lowland area sampled in 2014 provides both a habitat that maintains soil moisture (necessary for nematode activity and survival) over longer periods of time and one in which less erosion (and subsequently fewer nematode translocations) is likely to occur as topographic conditions are less favorable for overland water flow and the transport of propagules and sediment relative to the sloping sites sampled in 2015. Additionally, the lowland sampling location in 2014 is also likely to encourage establishment of herbaceous plants given the lower likelihood of seeds being carried away by overland water flow. The closer proximity of these sites to vegetated habitat, coupled with increased soil moisture (which could improve nematode migration through the soil substrate), possibly explain why communities were so much greater in abundance at this site than at the 2015 sites, as well as why nematode communities under willows, willows + herbaceous cover, and bare soil were so similar (Fig. 2). These findings thus suggest that our third hypothesis may be correct and that slope position and vegetation cover may interact to drive nematode community structuring; these results highlight the importance that further experiments designed specifically to explore these possible interactions may have for better informing future restoration methods in these landscapes.

Topography and landform design are an integral component of reclamation efforts (Toy and Chuse 2005), especially with respect to how this design contributes to hydrological factors (Macdonald et al. 2015). Badía et al. (2007) observed significant decreases to woody plant (pine, juniper, and mastic) growth with increasing slope at a mine reclamation site in Spain and suggested that these responses may have been due to soil water availability. A coal mine restoration study near Houston, Texas, supports the importance that water availability plays in woody plant establishment, as irrigation was found to promote seedling survival in the first 2 years after planting (Messina and Duncan 1993). Additionally, Espigares et al. (2011) showed that vegetation recovery is dramatically constrained by soil erosion rates on constructed slopes, with losses of soil to water movement also very likely leading to translocation of nutrients and other propagules away from restoration locations. This suggests that it is important to consider the use of cover materials (especially woody debris and topsoil additions) to influence hydrologic conditions and initiate nutrient cycling (Macdonald et al. 2015). At our site, the surface soil layer from the hill slope sites sampled in 2015 is composed of shale overburden with minimal organic material, and vegetation is largely limited to naturally established willows with only minor amounts of herbaceous cover. These factors, coupled with the unbroken slope, likely contribute to the low nematode abundances collected from under willows at the 2015 sampling locations. The presence of small rills across the slope face suggests that erosive losses of surface material are likely a factor inhibiting soil community and possibly herbaceous establishment at these sites. Our results dem-

onstrate that shrub willows may serve as nurse plants during coal mine reforestation and that topography is critical to the ability of willows to facilitate the establishment of herbaceous plants and nematode communities, furthering the progress of restoration efforts.

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