### SOIL MICROARTHROPODS: A PRELIMINARY STUDY OF THEIR POTENTIAL USE AS BIOINDICATORS IN ECOLOGICAL RESTORATION

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#### Abstract:

This study introduced students to the use of the soil microarthropods as bioindicators of soil health by using population distribution data to evaluate and compare ecological health at selected reclaimed sites in the Sudbury area. The area has undergone extensive ecological degradation due to anthropogenic impacts such as forestry and smelting, followed by decades of revegetation treatments. The treatments included the application of dolomitic limestone and fertilizer, seeding with grasses and legumes followed by tree planting and, most recently, the selective placement of imported forest-floor transplant plots on selected regreened sites. The morphology of collected Humus Forms from both untreated and treated sites were described in detail, with soil arthropods being then extracted using a series of Berlese-Tullgren funnels for estimates of population membership and diversity which were then interpreted as indicators of ecological health status. The results from this small preliminary study indicated that the transplant plots have greater humus form profile development, an observation suggestive of higher levels of arthropod activity and species diversity producing more organic detritus promoting higher microbiological decomposition rates. The lack of extensive sample replication, coupled with identification of the arthropods mainly to the order level, limit any definitive conclusions from being drawn from this study, although it most certainly served as a valuable learning experience.

**Key Words:** forest floor transplants, Berlese-Tullgren extraction, ecosystem restoration, Sudbury

#### **Introduction**

Soils are the very basis for the majority of life on earth, and the importance of the diverse array of organisms which inhabit these ecosystems underfoot cannot therefore be underestimated (Orgiazzi *et al.* 2016). As key decomposers (Latif, 2013), soil dwelling arthropods are included amongst these critical soil inhabitants, and as their populations are often seen as indicative of ecosystem health they are termed bioindicators by many (Bardgett 2002). Crucial as they are in the functioning of soils, observing these arthropods is not however straightforward, with specialized techniques and tools required for their extraction and examination (Orgiazzi *et al.* 2016). The application of these techniques vary and, although a subject of debate within the

scientific community, researchers agree that the observation method selected depends on the ecology and behaviour of the target species (Yi *et al.* 2012).

Reviewed studies which conducted arthropod sampling in order to assess their abundance, richness and diversity when evaluating the health of an ecosystem used some variation of the Berlese – Tullgren funnel, with a few employing a modified version, such as one which used an additional chemical gradient (Freedman and Hutchinson, 1980), and another which employed Murphy's split funnel, as well as a simple funnel without heat in order to tease out acarina and collembola, and nematodes and enchytraeids respectively (Marshall, 1974). The Berlese-Tullgren funnel did, however, appear to be known for being a simple yet biased method as it is unable to obtain a complete picture of the soil arthropods present (Andre 2002).

The depth of the soil cores collected ranged between 5 cm (Andrés and Mateos, 2006) to 15 cm (Marshall, 1974), though 10 cm cores were the most common (St.John et al. 2002; Lindberg and Bengtsson, 2006; Iloba and Ekrakene, 2009). All studies performed multiple replications per plot, ranging from 2 (Creamer et al. 2008; St. John et al. 2002), to 5 (Komulainen and Mikola, 1995), and in one case, 60 (Freedman and Hutchinson, 1980). One of the studies surveyed outlined the techniques which they used to identify, sort, and extract the soil microarthropods, which was by examining the microarthropods suspended in a solution under a dissection microscope, and extracting and sorting them using a pipette (liloba and Ekrakene, 2009).

The study region of Sudbury, Ontario has been subjected to an intensive regreening effort following extensive environmental degradation beginning in the 19th century due to anthropogenic activities such as forestry, mining and smelting (Courtin 1994). As a result of this industrial damage, 20 000 hectares were completely barren and devoid of vegetation, with an 80 000 additional hectares being semi-barren at the initiation of large scale reclamation projects in the 1970s (VETAC 2007). The loss of vegetation due to forestry, fire, and the release of environmental toxins such as copper, nickel, and sulphur dioxide from smelting also promoted severe soil erosion (Freedman and Hutchinson, 1980). However, by 2007, the regreening program, a well-coordinated community initiative operating through multiple partnerships, had led to: (1) the planting of 9.5 million trees, (2) the planting of 280 thousand shrubs, (3) and the liming, seeding, and fertilizing of approximately 3500 hectares of land (VETAC 2015, figure 1).



Figure 1. Map of the Sudbury area, with the location of the historically barren zones (outlined in red) and semi-barren zone (outlined in yellow). The map also indicates (in green) all of the areas to which lime was applied, and/or trees were planted, between the years 1978 and 2008. The three experimental sites have also been highlighted, Kelly Lake (orange), Laurentian University (black), and the Jane Goodall trails (blue) (VETAC, n.d).

Despite all of the regreening efforts, the Sudbury Area Risk Assessment completed in 2007 recognized that the land reclamation project had neither restored woodland understory species (Beckett et al., 2007), nor had terrestrial insect communities recovered to the extent as had plant communities (Babin-Fenske and Anand, 2010). These understory species are normally established as a component of the succession process, and their continued absence is likely due to a combination of unfavourable conditions such as thin, nutrient-poor soils, and lack of a suitable seed-bank (Kozlov and Zevera 2007). In an effort to reintroduce these missing species, including herbaceous plants, mosses and lichens, as well as other taxa such as arthropods, the addition of a new initiative to the regreening program in 2010 was the import of forest floor plots (VETAC 2010). Preliminary observations have indicated that some of these new understory species are surviving and thriving in their new habitats (Santala 2014).

This report outlines preliminary work investigating the soil arthropod communities within these transplanted materials as soil arthropods are often measured as bioindicators when evaluating site health, especially when assessing site recovery following environmental stressor disruptions such as drought (Lindberg and Bengtsson, 2006),

pesticide use (Latif, 2013; liloba and Ekrakene, 2009), and mining activities (Creamer et al., 2008; St.John et al., 2002; Andrés and Mateos, 2006). For example, a study by Southwood et al. (1982) which found that (1) arthropod richness was correlated with tree abundance, and (2) arthropod abundance increased with community species richness, suggests that, if the transplant plots increase understory vegetation species richness, the abundance of arthropods should also increase.

Surface humus form samples were collected from several sites within the region, including regreened, transplant, and untreated plots, to assess the diversity of soil arthropods present in these plots, if they may serve as indicators of ecological health, and especially whether the transplant plots hosted the most diverse communities. A detailed description of the soil humus forms at the sites was completed to provide an understanding of the humus development rates as an indicator of soil organism plant degradation activity, an additional simple visual characteristic to evaluate site health.

#### **Methods**

#### **Study Sites**

The study region within the City of Greater Sudbury, Ontario, Canada is located within the northern region of the Great Lakes – St. Lawrence forest zone which experiences hot summers and cold winters, with temperatures ranging from above 30 °C to below - 30°C, and a mean annual precipitation of approximately 870 mm. Tree species, either planted or volunteer colonizers, include White Pine, *Pinus strobus*, Red Pine, *Pinus resinosa*, Jack Pine, *Pinus banksiana*, White Spruce, *Picea glauca*, Black Spruce, *Picea mariana*, White Birch, *Betula papyrifera*, Trembling Aspen, *Populus tremuloides*, and Red Maple, *Acer rubrum* (SARA group 2004).

Samples were collected from three sites throughout the Sudbury area, two located in historically barren areas, Kelly Lake (KL) and the Jane Goodall trails (JG), while the third, from Laurentian University (LU), was located within the historically semi-barren zone. Four separate vegetation zones were sampled at the KL site, an oak-poplar, stand, a White Pine stand, a White Birch stand, and a barren area. The birch stand the barren zone had not been limed as a part of the re-greening program. The vegetation at both the JG site and the LU site was dominated by the typical Sudbury mixed forest species.

#### **Transplant Plots**

Forest floor mats, measuring 0.64m long, 0.54m wide, and 0.10m thick were taken from undisturbed donor sites approximately 50km South of Sudbury and placed at disturbed, recipient sites as 4m by 4m experimental plots to encourage the survival of vegetation even if the edges of the plots dried out (Santala 2014). The mats were 0.10m thick to allow for the shallow root systems within the humus forms to be harvested and, thus, replanted (Santala 2014).

**Sampling and Analysis** 

Soil samples were taken in the form of soil cores, which were extracted using soil corers fashioned from 10 cm sections of 10 cm diameter aluminium pipe. Six soil samples were collected at the KL site, two were collected at the LU site, and three were collected at the JG site. At the KL site, two samples were collected from both the regreened oak-poplar and the white pine stands, with each having a sample taken from both a transplant and a control plot. A single sample was taken at the KL untreated, barren zone birch stand. At the regreened LU site, one sample was taken from a transplant plot, while another was taken from a control plot. At the JG site, two samples were taken from transplant plots, one of which was introduced in 2010 and the other in 2015, while the third sample was from a control plot.

The soil arthropods were extracted from the cores using a Berlese-Tullgren funnel installed below a 60w incandescent lightbulb to establish a temperature gradient. The arthropods were then stored suspended in a sugar-alcohol-water solution prior to identification to Order level on examination under a dissection microscope of a thin film of the organism-rich solution into a petri dish. Humus form samples were also taken to enable detailed pedological descriptions. The Shannon – Wiener index of diversity was used to quantify the diversity of arthropod orders found in each sample.

#### **Results**

Of all of the arthropod orders, acarina was the most abundant. The proportion of total arthropods counted in the sample which were acarina ranged from 23% in the KL White pine sample to 84% in the KL barren sample, with the abundance of acarina ranging between 6 individuals in the JG 2010 transplant to 482 in the KL barren sample. Collembola were the only other order found in all ten of the samples analyzed. There were 14 orders of arthropods identified at the KL site (Figure 2), 11 at the JG site (Figure 3), and 7 at the LU site (Figure 4).

Figure 2. Abundance of all arthropod orders identified in the samples collected at the Kelly Lake sites; two from the oak-poplar stand (Control (C), Transplant (T5)), one from the unlimed birch stand, one from the unlimed barren zone, two from a white pine stand (Control (C), Transplant (T4)).

Count

Figure 3. Abundance of the arthropod orders identified in the samples collected from the Jane Goodall trails site; one from a transplant established in 2010, the other from a control plot.

(J.G) 2010 (J.G) C Figure 4. Abundance of each of the arthropod orders identified in the samples collected at the Laurentian University site; both from a birch stand, one from a control (C) plot, one from a transplant (T) plot.



The diversity index values for arthropod orders found in these samples ranged from 0.6, for the KL barron completed 1.25 for the LL birch stand transplant complet (Table 1)

Sample Name	Horizon Name	Classification
(J.G) 2010 Transplant	Ln	Lignomoder
	Fa	
	Hgw	
(J.G) 2015 Transplant	Ln	Lignomor
	Fr	
	Hc	
(J.G) Control	Ln	Lignomor
	Fr	1.5
	Hew	
(L.U) Birch Control	L (<1 cm)	Rhizomor or Lamimor
	Fm (1 cm)	
	Fr (1.5 cm)	
	Hc (1 cm)	
(L.U) Birch Transplant	L (<0.5 cm)	Leptomoder
	Fz (<0.5 cm)	and a summer and
	Hc (3 cm)	
(K.L) Oak-Poplar Control	Ly (<0.5 cm)	Lamimoder
	Frm (1-1.5 cm)	
	Ah (3 cm)	
(K.L) Oak-Poplar Transplant 5	L/F (transplant) (1.5cm) Ah (3 cm)	Mullmoder

Table 2. Humus form profile descriptions for seven soil samples, three from the Jane Goodall trails site (J.G), two of which were from transplant plots, while the third was a control. Two samples were taken from a birch, *Betula papyrifera*, stand at the Laurentian University site (LU), one of which was a transplant, while the other was a control. Two samples were from an Oak-Poplar, *Quercus* sp. *Populus* sp., stand at the Kelly Lake site (KL).

#### **Discussion**

At both the LU and the JG sites the arthropod abundance for the samples taken from the transplant plots had higher indices of diversity than their respective control plots. The results were slightly more complicated at the KL site, as neither the oak-poplar, nor the white pine transplant plots had higher diversity indices than their respective controls, though that of the barren zone was the lowest of all samples analyzed. In the case of the oak-poplar and white pine samples at KL the differences in diversity indices were relatively minor, at 0.77 to 0.74 and 0.99 to 1.05 respectively. For the LU and JG samples, however, the differences were much greater, being 1.29 to 0.96 and 1.35 to 0.93 respectively.

The results obtained for the unlimed KL barren zone sample were somewhat expected, as the acidic soils of the area support very little vegetation cover (SARA Group, 2004). Given this combination of environmental factors, the soils at the barren site may not have been favourable to a wide range of species (Creamer *et al.* 2008). Interestingly, however, the stunted birch covered barren zone sample did have the highest

abundance of acarina of all of the samples analyzed. This high abundance is not necessarily correlated with high diversity of acarina species, as they were not identified to that level, and therefore may have been a case of only a few colonizing species dominating an area, as it is acknowledged to occur in many instances of stressed ecosystems, such as those polluted by metals (Creamer *et al.* 2008).

Nematodes, normally exceedingly common in soils worldwide, were only identified in low abundances in five samples. This observation may be due to multiple factors, including high metal concentrations, such as copper, being observed as limiting nematode abundance (Creamer *et al.* 2008). There is also the possibility that nematodes were not properly extracted from the soil samples because of a high drying and temperature gradient during the extraction phase, leading to few organisms for identification under the dissection microscopes.

Colonization of polluted areas by microarthropods has been demonstrated to be limited to only a few species, even if there are revegetated areas nearby, as they disperse very slowly (St.John et al. 2002). This limited dispersal potential, along with the hypothesis that the degraded Sudbury soils may be less habitable to many species, may explain why diversity was greater in the transplant plots than in the control plots at the LU and JG sites. Studies previously conducted on revegetated mine tailings in Sudbury demonstrated that the species richness and diversity of acarina was less on tailings, even on sites revegetated 40 years previously, compared to nearby control plots (St.John et al. 2002). The tailings study also found the density of mite species to be similar in the old tailings plots and control plots, with species compositional diversity indicating the plots to be no more than 60% similar (St. John et al. 2002). In this latter study, the abundance was high in the low diversity stressed system, an observation similar to that found in this study. However, direct comparisons between the two studies cannot be conclusively drawn as that study identified mites to the species level, specifically, whereas this broad study identified only arthropod orders. The results, based on the parameters measured, in the current study cannot conclude whether the soil microarthropods from the transplant plots have migrated outwards. However, as soil microarthropods migrate exceedingly slowly (Creamer et al. 2008), the five year period between the import of the transplants to the Sudbury regreeening plots and the sampling period in the Fall of 2015 may have been insufficient to allow for significant migration.

The litter layer (L) on the soil profiles at the LU site was similar. The regreened control plot humus form had two distinct fermentation layers (F), neither of which was zoogenous in nature as observed for the transplant plot. In the transplant plot, the litter layer (L) and the fermentation layer(s) (F) were thicker in the control plot than in the transplant plot. These observations may support the hypothesis of a thriving and active microarthropod community in the transplant plot speeding decomposition (Latif, 2013), thinning the litter and fermentation layer, and leaving abundant fecal matter in the form of a zoogenous fermentation layer (Klinka *et al.* 1981). The JG site had two transplant plots, one installed in 2010, and the other in 2015 only months before the sample was taken. The results of the JG soil profiles did not point towards a clear pattern in soil

arthropod activity, with the younger 2015 transplant being more similar to the regreened control than the 2010 transplant. The 2010 transplant was the only one of the three to have a humus layer (H) composed primarily of faunal droppings. Both the control and transplant profiles from the KL oak-poplar site had less than 2 cm of litter and fermentation materials, with no clear humic layer (H). In contrast, the thin organic layer was over an organo-mineral layer enriched with humic materials (Ah). Thus the limed control plot was different from the transplant plot, with the fermentation layer being distinguished from the litter layer (L), whereas the transplant had a surface layer which was a mix of litter and fermenting (F) materials. These results may, as with those for the LU sites, support the hypothesis that the transplant plots contained a more active microarthropod community which aided in the decomposition of the annual litter contribution from the understorey and canopy vegetation.

The use of the simple Berlese – Tullgren funnel was well suited to this preliminary study. Similar studies surveyed also appear to use a variant of the technique, with the simplicity of the method for this preliminary class-based study being critical in spite of the potential lack of reproducibility and accuracy. The collection of 10 cm deep soil samples was suitable as the studied soils are commonly shallow, often with less than a 10 cm surface humus form, a depth described in the literature as containing the majority of soil microarthropods (St.John *et al.* 2002; Marshall, 1974). There may have been several sources of error in the identification and enumeration of these microarthropods, with the identification being completed by several students, with potential discrepancies in observation and use of the systems for identification. The lack of extensive sample replication also limits the strength of conclusions drawn from the results which fail to truly account for variation, and therefore may not present a complete picture as to the number and diversity of microarthropods present at each site.

#### **Conclusion**

Although the results of this study were inconclusive, the preliminary findings appear to support the hypothesis that the transplant plots introduced into the area may host a greater diversity of microarthropod orders. The barren site at KL had the least diverse microarthropod community, strongly supporting the hypothesis that microarthropod diversity is potentially indicative of ecosystem health. The transplant plots had humus form profiles with thinner litter and fermentation layers, as well as more evident faunal droppings, than their respective regreened control plots, indicative of greater, more productive, or more diverse microarthropod communities in those plots.

In future studies, the systematic errors in this study might be mitigated by increasing the replication, and by having a team of researchers more comparable in their methods. The impact of these transplant plots could be more thoroughly understood by determining with a greater degree of precision which microarthropod taxa inhabit these transplants, which groups disperse from the transplants, and at what rate this dispersal occurs. This detailed faunal studies could be completed by sampling on a gradient over a series of years, and identifying the microarthropods found to the genus or even species level.

The Sudbury Story was once only a cautionary tale, an example of the harm which we could wreak on our environment as a consequence of unmanaged industrial releases to the host environment. The continued successes of the Sudbury regreening program however, further supported by these preliminary findings, have since expanded that cautionary tale into one of inspiration for all the world to see.

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