

On the road to depletion: road impact on soil fauna in Sugar

Maple (*Acer saccharum*) forests at UNDERC.

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Abstract

Invertebrate soil fauna holds important ecosystem processes and it is sensitive to changes in land use. They have been used as bioindicators of the ecological quality of systems, specially Coleoptera assemblages. The species richness and abundance has been reported to change due to habitat disturbance. The University of Notre Dame Environmental Research Centre UNDERC locates in this region with a long history of anthropogenic disturbances. Here, I assessed if in protected areas as UNDERC, road disturbance leads into changes in the invertebrate assemblages diminishing the abundance, richness and diversity of soil invertebrates and Coleoptera. We selected 10 Sugar Maple (*Acer saccharum*) dominated forests in UNDERC and set transect divided in three strata: near (0-4.99m), far (5-9.99m) and farthest (10-14.99m) from the road and set pitfall traps for seven days. We analysed the abundance, richness and diversity of the assemblages. We did not find statistically significant differences in greater taxonomic classifications along the transect (Abundance: $F_{(.05,2,27)} = 1.813$, $p=0.183$). However, we found a decreasing abundance ($F_{(.05,2,27)} = 3.354$, $F_{obs}=3.852$, $p=0.0337$) and species richness of Coleoptera with the proximity to the road ($F_{(.05,2,27)} = 3.354$, $F_{obs}=3.531$, $p=0.043$). This effect disappeared when removing the exotic Coleoptera present. This could be explained due to restricted flight of these organisms. Finally, the changes in invertebrate assemblages may be responding to other factors than the road itself.

Introduction

Invertebrate soil fauna holds important ecosystem processes such as water supply, soil formation, nutrient cycle, primary production, decomposition, pollination, erosion control and climate regulation (Lavelle et al. 2006). They can act as predators, herbivores, detritivores, omnivores and parasites (Gerlach et al. 2013). They also play a key role on providing diverse ecosystem goods and services (Barrios, 2007). Unfortunately, they have been poorly considered for forest management decisions (Westby-Gibson, 2017).

Soil fauna is sensitive to changes in land use. The ecological complexity of epigeal communities tends to be higher in native forest than monoculture plantations, like the case of the Caatinga biome in Brazil (Lima et al. 2017) and the Mediterranean biome in Chile (Cifuentes, 2018). Furthermore, not only the structure of the communities, but also the functions and processes they hold are altered (Gómez-Cifuentes et al. 2017). Assessments on tree plantations and mature forest revealed that natural habitats present higher rates of decomposition than monocultures in Amazonas (Höfer et al. 2001), showing that this process is strongly driven by the structure of communities. Assessments on tree plantations and mature forest reveal the effects of invertebrates in decomposition process.

Hence, it is proposed that invertebrate assemblages can function as efficient bioindicators of the ecological quality of systems (Maleque et al. 2009). Beetles (Order: Coleoptera) have been described as good bioindicators (Pearce and Veiner, 2006), since species number and abundance has been reported to change due to habitat alterations (Rainio and Niëmela, 2003). These trends are useful for assessing the quality of certain environments, as well as the effectiveness of restoration and management initiatives.

However, forests are constantly exposed to multiple natural disturbances (Lorimer and White, 2003) such as fires or strong winds that can remove trees. On the other hand, anthropogenic activity is also a critical driver of forest change (Rab, 2004), altering the

resilience and resistance of the ecosystems. The systems affected by constant and intense disturbances have been reported to present slow recovery rates of the forest structure and function (Rab, 2004). Assessing the effect of disturbances is critical, as the impacts can drive the systems into unstable dynamics and sometimes irreversible change.

Most of the eastern North America underwent intensive logging during the last century (Pimm, 1995). This practice has been shown to cause fragmentation and edge effects as immediate impact (Broadbent et al. 2008), as well as long term effects on the depletion of soil fauna richness and abundance (Haskell, 2000). Roads represent a major disturbance as well, as they cause fragmentation and alter the microclimate conditions of the area (Lugo and Gucinski 2000). They have been considered as the primary mechanism of landscape fragmentation in the Northwoods (Saunders et al. 2002). However, some of these areas were closed to the public access during the last century to reduce the human pressures and prohibit the extensive logging and road building.

The University of Notre Dame Environmental Research Centre UNDERC locates in this region with a long history of logging activity. Before the acquisition by Notre Dame in 1943, the site was extensively partial or clear-cut, and the most recent logging activity was dated between 1955-1967 (Holland 2006). However, the maintenance of roads restricted to the UNDERC personnel and researchers represent one of the actual anthropogenic disturbances on property. Therefore, we will assess the question of how these roads affect the soil invertebrate communities in the forest. The specific hypothesis to be tested is that road disturbance leads into changes in the invertebrate assemblages as it alters the habitat structure and function, diminishing the abundance, richness and diversity of soil invertebrates and Coleoptera, in protected areas as UNDERC.

Methods

Study site. The study was conducted at UNDERC, located between northern Wisconsin and the Upper Peninsula of Michigan (46° 13' N, 89° 32' W). The zone comprises 3035 Ha (Holland, 2006). It is characterized by a warm summer humid continental type of climate (Kottek 2006). The most prevalent forest tree species are sugar maple (*Acer saccharum*), hemlock (*Tsuga canadensis*) among mixed hardwood-conifer forests. Soils have a poor drainage and a low cation exchange capacity.

Sampling. To analyse the macroinvertebrate communities along a disturbance gradient, we chose 10 forest dominated by sugar maple (*Acer saccharum*) near roads (n = 10) (Figure 1). In each site, we set a transect of 15m from the road into the forest, leaving a 5m buffer zone between the road and the beginning of the forest. For each transect we performed a random stratified sampling. We broke up the transect in three strata: near (0 - 4.99m), far (5 - 9.99m) and farthest (10 - 14.99m) from the road. For each stratum we selected a random distance in which install the pitfall traps.

To assess the epigeal soil fauna, we set pitfall traps made of 900 mL plastic cups, filled with 300 mL of neutral dish washer solution. Each pitfall had a plastic lid of 20 cm X 20 cm to prevent the capture of non-target animals (i.e. vertebrates). The traps were set for seven days. The samples were preserved with 70% ethanol in glass jars.

The identification of specimens was performed using field guides of insects (Borror and White 1970) and beetles (White 1983). I observed the samples under a Leica dissecting microscope. I identified the Orders and Families present in the sample for the Insecta and Entognatha (other classes were identified until order or suborder level). I identified the Genus and, in most cases, species of Coleoptera. Following identification, I measured the absolute abundance, richness and diversity of the invertebrate community and the coleoptera assemblages. I used Shannon's H-index (equation 1) for assessing the diversity.

equation 1.

$$H = -\sum_{i=1}^n p_i \ln(p_i)$$

H: Shannon index of diversity. n: total number of families present; pi: proportion of individuals of species i.

Statistical Analysis. Statistical analysis was performed in the Software R i398 3.3.3 (R Core Team, 2017). I used a confidence interval of 95%. I first tested for normality using a Shapiro-Wilk test and a Bartlett test for the homoscedasticity. Even when not all the variables presented a normal distribution, we ran an ANOVA test given the homoscedasticity of the variances that all response variables presented (See Table A1 in Appendix 1). I ran 6 different ANOVA tests, to assess the effect of the roads on the absolute abundance; species, families and orders richness and coleoptera's abundance, family and species richness and species diversity. I also ran a separately ANOVA test to assess the effect of roads on the native Coleoptera assemblages. Post-hoc analysis was made with a TukeyHSD test. Graphs were obtained from the statistical platform SYSTAT (SYSTAT, 2009).

Results

Community composition

Invertebrate community was composed by specimens from three Phyla: Arthropoda, Mollusca and Annelidae. Within the Phylum Arthropoda, we identified 470 specimens from the Class Insecta, 51 Entognatha, 1 Diplopoda, 10 Malacostraca and 109 Arachnida (Table 1). The Class Insecta presented the higher richness of orders, as we found specimens belonging to the Orders Coleoptera, Diptera, Hymenoptera, Lepidoptera, Orthoptera and Hemiptera. However, not all the taxa were found in every point sampled.

The presence of certain exotic taxa was important. The 54.8% of a 317 Coleoptera specimens were detected as exotic species. We identified five species of Curculionidae, each of them introduced to the United States. The strawberry-root weevil (*Otiorynchus ovatus*), the apple weevil (*Otiorynchus cribricollis*) and the rough strawberry root weevil (*Otiorynchus rugosostriatus*) are some of the exotic species found.

Taxonomic breakdown

The absolute abundance was measured as the total number of individuals present in each point of the transect. This measure tended to be higher in samples farthest from the roads (29.9 ± 20.65) than in the far (20.3 ± 16.22) and near zone (16.3 ± 10.91). However, these differences were not statistically significant ($F_{(.05,2,27)} = 1.813$, $P = 0.183$).

We measured the invertebrate order richness in each distance from the road. Again, we observed a higher number of orders present in the farthest zone but the difference was not statistically significant (near: 5.5 ± 1.27 ; far: 5.1 ± 2.64 ; farthest: 6.7 ± 2.21 ; $F_{(.05,2,27)} = 1.541$, $P = 0.232$). The same trend was observed with the invertebrate Family richness, as it increased with the distance from the road, but no statistically significant difference was found (near: 8.4 ± 2.55 ; far: 8.1 ± 4.82 ; farthest: 9.8 ± 3.36 ; $F_{(.05,2,27)} = 0.603$, $P = 0.555$). Finally, the same

pattern was found in the invertebrate species richness along the transect, as it tended to be higher in the farthest distance from the road, and decreased with the proximity to it (near: 9.3 ± 3.19 ; far: 9.4 ± 5.97 ; farthest: 11.9 ± 4.53 ; $F(.05, 2, 27) = 0.981$, $P = 0.388$).

Coleoptera assemblages

We measured the same parameters, specifically for the specimens belonging to the Order Coleoptera. The abundance tended to be higher in the farthest zone (16.5 ± 11.41) than in the far (8.8 ± 6.92) and the near zone (6.5 ± 5.95). This difference was found to be statistically significant ($F(.05, 2, 27) = 3.852$, $P = 0.0337$, Figure 2) and the farthest zone was statistically different from the near zone (diff=10, $P = 0.034$), but not different from the far (diff=7.7, $P = 0.122$) and the near was not different from the far (diff=-2.3, $P = 0.816$). However, no significant difference was found in the family richness (near: 2.2 ± 0.789 ; far: 2.4 ± 1.07 ; farthest: 3.3 ± 1.42 ; $F(.05, 2, 27) = 3.354$, $F_{obs} = 2.788$, $P = 0.084$).

We also measured the species richness of Coleoptera. We found a decreasing number of species, from the farthest zone (5 ± 2.58), to the far (3.3 ± 1.77) and the near zone (2.8 ± 1.23). This pattern was statistically significant ($F(.05, 2, 27) = 3.531$, $p = 0.0434$, Figure 3). The farthest zone was statistically different from the near zone (diff=2.2, $P = 0.044$), but not different from the far (diff=1.7, $P = 0.142$) and the far was not different from the near (diff= 0.5, $P = 0.834$). However, when we removed the exotic species counts neither the abundance ($F(.05, 2, 27) = 1.779$, $p = 0.188$) or the species richness ($F(.05, 2, 27) = 2.518$, $P = 0.0993$) showed any statistically significant trend.

For the coleoptera diversity, we found but no statistically significant difference (near: 0.79 ± 0.43 ; far: 0.476 ± 1.07 ; farthest: 1.08 ± 0.51 ; $F(.05, 2, 27) = 1.129$, $P = 0.338$).

Discussion

Community composition

Invertebrate epigeal fauna was composed by diverse taxa. The greater proportion of specimens in the samples belonged to the Class Insecta. The identification was restricted until the Family or Order taxonomic level due to the scarce taxonomic works in this area (Giller, 1996). This limitation balks the understanding of the specific functions held by the present organisms. However, some general ideas can be shaped from the identified Orders. For example, we detected the presence of herbivores as Orthoptera, Hemiptera, and the larvae of Lepidoptera and Coleoptera. We also found few predators from the Order Araneae. Parasitic taxa were also detected with individuals from Hymenoptera and mutualistic associations between Acari (*Poecilochirus sp.*) and Coleoptera (*Nicrophorus marginatus*). Detritivores as Coleoptera were also identified.

The presence of exotic taxa was another interesting issue. More than half of Coleoptera collected were not native from this geographic range. All the weevils found (Family: Curculionidae) were exotic. Some species as the strawberry-root weevil (*Otiorynchus ovatus*) and the rough strawberry root weevil (*Otiorynchus rugosostriatus*) belong to Europe and it is known that they have been established since the arrival of the first Europeans into North America (Wheeler, 1999). The larvae of these species have been reported to feed on the roots of strawberries and other crops and cause damage to agricultural production (Fisher, 2006). The high prevalence detected could be a sign of successful establishment and a symptom of the displacement of native Curculionidae. In fact, the root feeding assemblages in the Northwoods are composed by almost 99% of exotic weevils (Coyle et al, 2010), as native specimens have been dramatically displaced.

Taxonomic breakdown

We detected a greater abundance of invertebrates in the farthest zone from the road than near it. This difference, however, was not statistically significant ($F(.05,2,27) = 3.354$, $F_{obs} = 1.813$, $p=0.183$, Figure 2). The same trend was observed in the order richness ($F_{obs} = 1.541$, $p=0.232$), Family richness ($F(.05,2,27) = 3.354$, $F_{obs} = 0.603$, $p=0.555$) and species richness ($F(.05,2,27) = 3.354$, $F_{obs} = 0.981$, $p=0.388$). These results show that the roads have no significant effect over the richness and abundance of Order, Family or Species of the invertebrate assemblages. Nevertheless, this appears to be contradictory to the literature. Some authors have suggested that the roads impact negatively the community structure and composition, even if the lanes do not have a great size (Haskell 2000). The abundance tends to be decreased, as well as other related variables, as leaf-litter depth. However, Haskell (2000) showed that the edge effect of roads can persist up to 100m, so it is possible that all the points measured along the transect have been equally disturbed.

On the other hand, the effects of disturbances strongly rely on the taxa physiology, behavior and ecology (Westby-Gibson et al, 2017). The response of the organisms to the disturbance seemed to be more evident at lower taxonomic levels, such as Genus or Species. In that sense, no significant variation was reported in the abundance or richness of Orders and Families in harvested areas than mature forests (Westby-Gibson et al. 2017). Is it possible that the same phenomenon has been detected in UNDERC.

Coleoptera assemblages

I detected significant a trend when we measured abundance and richness specifically for the specimens belonging to the Order Coleoptera. The abundance tended to be higher in the farthest zone than zones closest to the roads. This difference was found to be statistically significant ($F(.05,2,27) = 3.354$, $F_{obs} = 3.852$, $p=0.0337$, Figure 3). However, family

richness differences seemed not to be statistically significant ($F_{(.05,2,27)} = 3.354$, $F_{obs} = 2.788$, $p = 0.084$). The species richness of Coleoptera presented a statistically significant difference ($F_{(.05,2,27)} = 3.354$, $F_{obs} = 3.531$, $p = 0.0434$, Figure 4), as the richness increased with the distance from the road. This response depends on the ecological traits of the taxa (Driscoll and Weil, 2005), as flightless or underground species are more sensitive to habitat fragmentation.

Nevertheless, when removing the exotic species, this trend observed in abundance ($F_{(.05,2,27)} = 1.779$, $p = 0.188$) and species richness ($F_{(.05,2,27)} = 2.518$, $p = 0.0993$) was no longer significant. In this sense, the effects of the disturbance are not to be predicted by itself, but they must be considered because of the interactions with the biota. This does not mean that native fauna is not sensitive to disturbance. The overall numbers reflect a lower abundance of native specimens and they might be affected primarily by the presence of the invasive species rather than the edge effect of the roads.

Additionally, it has been reported that *Otiorhynchus* adults are flightless and the larvae lives underground (Van Tol et al, 2004). *Maladera castanea*, another exotic species found only flies under specific temperature conditions (between 18.3–21.1°C) (Held and Ray, 2009) and *Bradycellus harpalinus* presents short wings (Den Bieman, 1990). This means that all exotic species reported presented flight restrictions and hence, a greater sensitivity to fragmentation disturbances.

In conclusion, we found that road disturbance leads into changes in the invertebrate assemblages of Coleoptera, diminishing the abundance and richness. The impacts are species-dependent, so they cannot be easily predicted without knowing the biological features of the taxa. Hence, no effects of the roads were observed when the whole community was analysed. However, most of the variation in Coleopteran assemblages may be attributable to the biological features of the species reported, as all the 7 exotic species found presented

restriction to flight or a life cycle that involves an underground phase. These characteristics might lead to an increased sensitivity to habitat fragmentation and edge effects of the road. This may explain why the statistically significant differences are seen because of these exotic taxa.

Finally, it is important to mention the limitations of these study. The samples may not reflect the actual abundance and diversity of groups present in the ecosystem, since the time the pitfall traps were set was short. The usual set-up time is between 14 or 21 days (Yekwayo et al, 2015), rather than seven as in this study. The shortening of the time responded to ethical concerns about non-target species that kept being caught in the traps. However, we present a short glimpse about the composition and structure of the invertebrate soil assemblages and their variation along a disturbance gradient. Future researchers must take care of this point, making sure that the collection methods meet the needs of the ethical concerns.

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Table 1. Taxonomic breakdown of specimens found in Sugar Maple (*Acer saccharum*) Forests at UNDERC.

Phylum	Class	Order	Family	Species	Number of individuals	%Family within Order
Arthropoda					649	
	Insecta				470	
		Coleoptera			317	
			Scarabaeidae		2	0,63
				<i>Maladera castanea</i> (i)	1	
				<i>Geotrupes</i> sp.	1	
			Carabidae*		78	24,61
				<i>Agonum gracile</i>	49	
				<i>Bradycellus harpalinus</i> (i)	9	
				<i>Carabus</i> sp.	13	
				<i>Tachys</i> sp.	3	
			Cantharidae	<i>Rhagonycha</i> sp.	29	9,15
			Curculionidae		164	51,74
				<i>Otiorhynchus cribricolis</i> (i)	64	
				<i>Otiorhynchus ovatus</i> (i)	54	
				<i>Otiorhynchus rugostriatus</i> (i)	40	
				<i>Otiorhynchus uncinatus</i> (i)	1	
				<i>Phyllobius oblongus</i> (i)	5	
			Silphidae		26	8,20
				<i>Nicrophorus marginatus</i>	22	
				<i>Nicrophorus defodiens</i>	2	
				<i>Necrophila americana</i>	2	
			Elatridae	<i>ME1</i>	1	0,32
			Mycetophagidae	<i>Typhaea</i> sp.	1	0,32
			Lucanidae	<i>Nicagus</i> sp.	1	0,32
			Staphilinidae	<i>Dinerea</i> sp.	12	3,79
			Chrysomelidae	<i>Neocrepidodera</i> sp.	3	0,95
		Diptera**			53	
			Mycetophiliidae	<i>ME2</i>	12	22,64
			Tipulidae	<i>ME3</i>	1	1,89
			Rhagonidae	<i>ME4</i>	1	1,89
			Anthotomidae	<i>ME5</i>	1	1,89
			Culicidae	<i>ME6</i>	7	13,21
			Sacrophagidae	<i>ME7</i>	8	15,09
			Lonchopteridae	<i>ME8</i>	14	26,42
			Muscidae	<i>ME9</i>	7	13,21
		Hymenoptera			69	
			Pompiilidae	<i>ME10</i>	2	2,90

		Encyrtidae	ME11	1	1,45
		Chalcidae	ME12	4	5,80
		Platygastridae	ME13	2	2,90
		Proctopuridae	ME14	4	5,80
		Brachonidae	ME15	1	1,45
		Eupelmidae	ME16	14	20,29
		Formicidae		41	59,42
			ME17	35	
			<i>Camponotus sp.</i>	6	
		Lepidoptera		6	
		Geometriidae	ME18	3	50,00
		Noctuididae	ME19	2	33,33
		Tineidae	ME20	1	16,67
		Orthoptera	Tettigoniidae	ME21	9 100,00
		Hemiptera		16	
		Delphicidae	ME22	10	62,50
		Ligaeidae	ME23	3	18,75
		Cercopidae	ME24	1	6,25
		Miridae	ME25	2	12,50
Entognatha	Colembola			51	
		Entomobryidae	ME26	47	92,16
		Poduridae	ME27	1	1,96
		Sminthuridae	ME28	3	5,88
Arachnida				109	
	Araneae			22	
		Lycosiidae	ME29	6	27,27
		n/i	ME30	11	50,00
		n/i	ME31	5	22,73
	Opiliones	Sclerostomatidae		29	100,00
			ME32	23	
			ME33	6	
	Mesostigmata			58	
		Parasitidae	<i>Poecilochirus sp.</i>	20	34,48
		Laelapidae	ME34	38	65,52
Diplopoda	Spirobolida	n/i	ME35	1	100,00
Malacostraca	Isopoda	n/i	ME36	10	100,00
Mollusca	Gastropoda	Panpulmonata		9	
		Helicidae	ME37	2	22,22
		Agriolimacidae	ME38	7	77,78
Annelida	Clitellata	Haplotaxida	Lumbricidae	ME39	1
Total				659	

*4 Carabidae larvae found, excluded from species analysis; **2 Diptera larvae found, excluded from species analysis; n/i: not identified; ME: Morphospecies; (i) Invasive species.

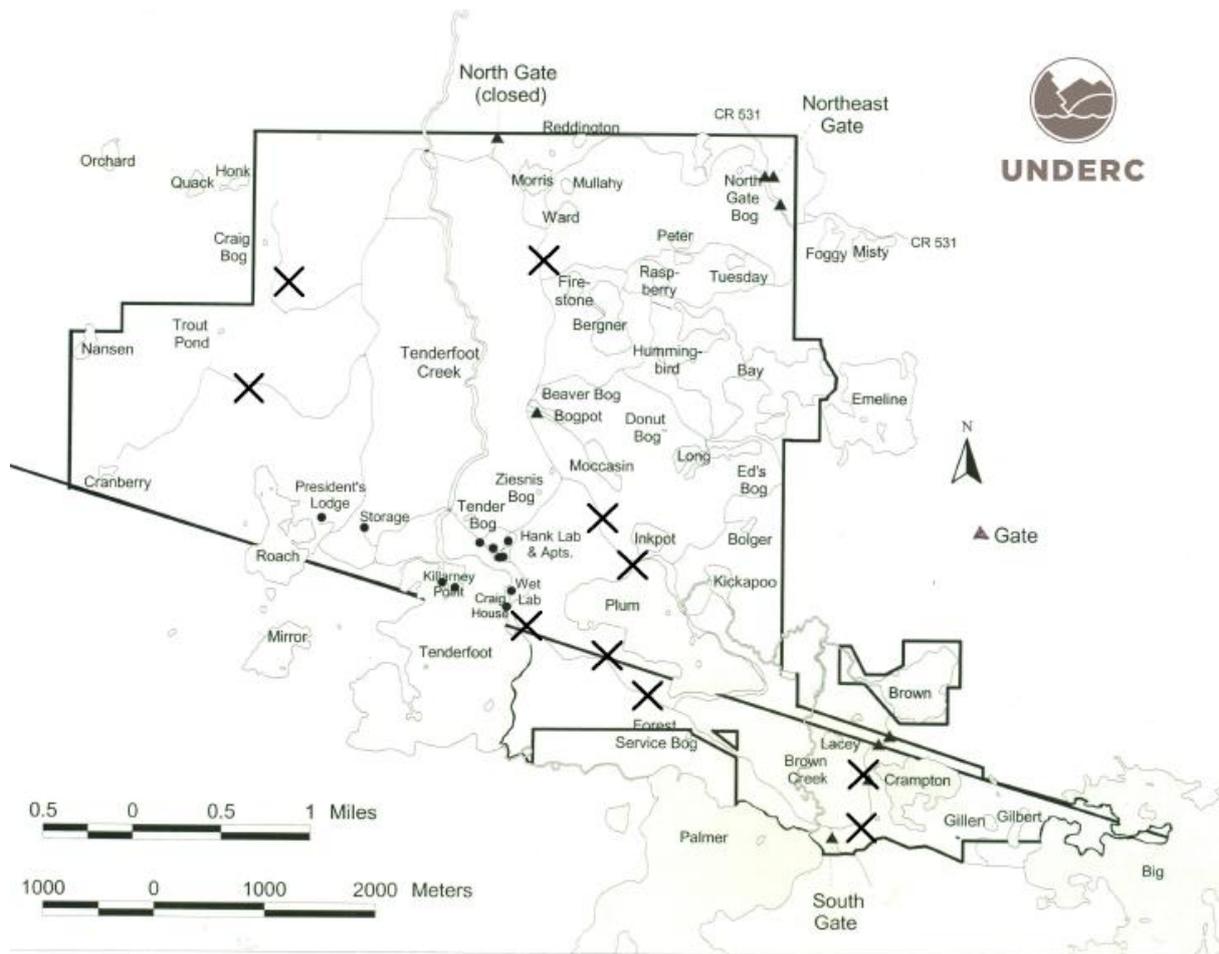


Figure 1. UNDERC map with sites sampled, indicated with X marks. (n=10)

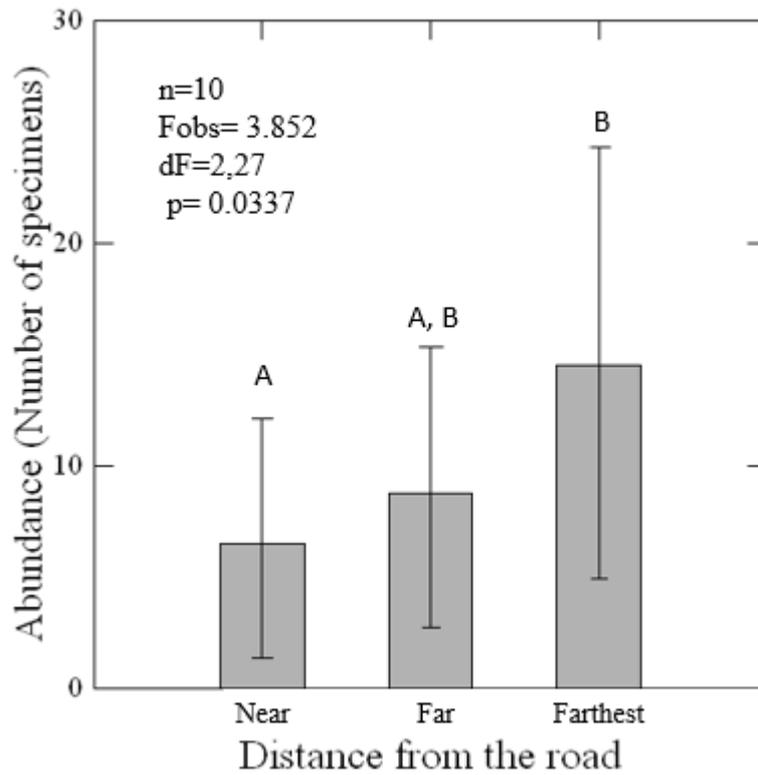


Figure 2. Coleoptera abundance by distance from the road. The abundance tended to be higher in the farthest zone (16.5 ± 11.41) than in the far (8.8 ± 6.92) and the near zone (6.5 ± 5.95). This difference was found to be statistically significant ($F_{(.05, 2, 27)} = 3.852$, $P = 0.0337$) and the farthest zone was statistically different from the near zone (diff=10, $P = 0.034$), but not different from the far (diff=7.7, $P = 0.122$) and the far was not different from the near (diff=2.3, $P = 0.816$).

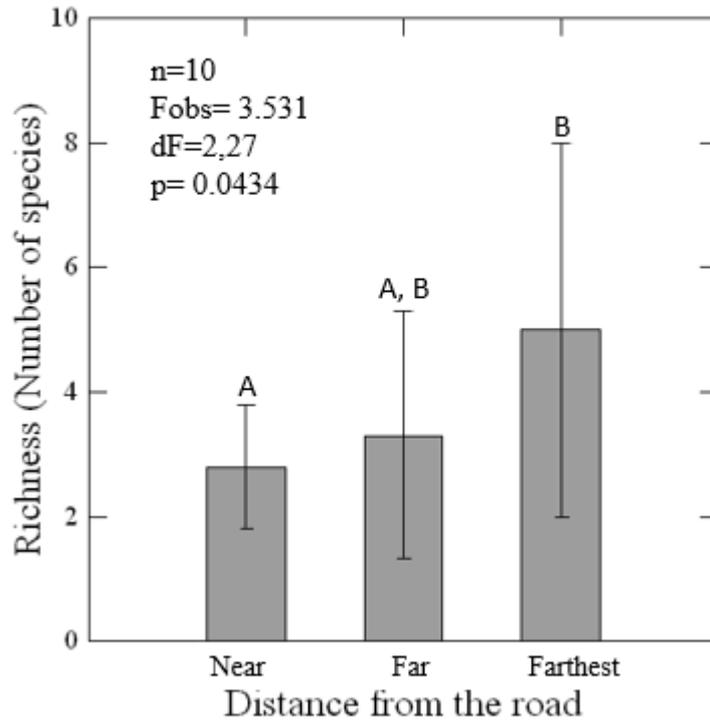


Figure 3. Coleoptera species richness by distance from the road. We found a decreasing number of species, from the farthest zone (5 ± 2.58), to the far (3.3 ± 1.77) and the near zone (2.8 ± 1.23), and it was statistically significant ($F_{(.05, 2, 27)} = 3.531$, $P=0.0434$, Figure 4). The farthest zone was statistically different from the near zone (diff=2.2, $P=0.044$), but not different from the far (diff=1.7, $P=0.142$) and the far was not different from the near (diff=0.5, $P=0.834$).

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Appendix 1

Table A1. Normality and Homoscedasticity values obtained.

Data	Normality test		Homocedasticity	
	W-value	P value	Bartlet K-squared (dF=2)	P value
Total abundance	0.88916	0.004609	32.761	0.1944
Order richness	0.97085	0.5627	43.033	0.1163
Family Richness	0.97135	0.5766	3.478	0.1757
Species Richness	0.9705	0.553	31.704	0.2049
Coleoptera Species Richness	0.91458	0.01945	45.461	0.103
Coleoptera Families	0.88902	0.004573	28.437	0.2413
Coleoptera Abundance	0.88082	0.002944	42.063	0.1221
Coleoptera Diversity	0.9306	0.05092	0.23613	0.8886