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M049 Effects of Compaction and Soil Moisture on American Burying Beetles

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16. Abstract

Silphid beetles in the genus *Nicrophorus* bury themselves during periods of inactivity, however, the influence of soil characteristics on burial behavior remains unclear. We examined soil preferences of the federally endangered *Nicrophorus americanus*, as well as of *N. carolinus*, *N. marginatus*, *N. obscurus*, and *N. orbicollis*, in a series of experiments. Differences in burial depth by sex were found for *N. carolinus, N. marginatus,* and *N. obscurus,* which are diurnal species. Both sexes of *N. americanus* bury to a mean depth of 18 cm. Soil preferences tested included sand versus sandy loam versus sandy loess, degrees of soil moisture, and soil cover (cut vegetation) versus bare soil. *Nicrophorus marginatus* preferred moist sandy loam with cut vegetation, *N. orbicollis* preferred moist sandy loam with cut vegetation, *N. carolinus* preferred sand with cut vegetation, and *N. americanus* only had a significant preference for moisture. Regarding the degree of soil moisture, *N. americanus* and *N. orbicollis* preferred extremely moist soils (0.4 wfv), *N. marginatus* did not have a preference, and *N. carolinus* preferred dry soils (0.02 wfv) over moist soils. Soil preferences during reproductive burials were equivalent to those of non-reproductive burials.

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Summary

The American burying beetle, *Nicrophorus americanus* Olivier, is the largest North American member of the Silphidae. It was declared federally endangered in 1989 and many efforts to prevent this species from going extinct are ongoing. The *Nicrophorus* beetles bury small carcasses for reproductive purposes. They also reside in the soil during times of daily and seasonal inactivity. To better understand why the American burying beetle is in decline, the importance of soil texture, moisture, vegetation, and gravel, the burial depth, and the effect of compaction on their burying behavior was examined.

Limited research was performed on *N. americanus* and more detailed research was performed on four other *Nicrophorus* species. All tested species preferred moist soils with *N. orbicollis* having a significant preference for wet ($p<0.01$) sandy loam ($p<0.05$) soil with daily burial depths to 20cm. The diurnal *N. marginatus* had a significant preference for wet, sandy loam soil with cut vegetative cover ($p<0.001$) and buried to depths of 18cm while *N. carolinus* had a significant preference for wet, sandy soil with vegetative cover ($p<0.001$) and can bury to depths of 60 cm. The American burying beetle preferred moist sandy loam soil with cut vegetative cover and buried to a depth of 20 cm.

All tested species preferred loose over compact soil when given a choice ($p<0.001$) and the presence of cut vegetation influenced the compaction preference. When exposed to compaction from moving standard pickup trucks, 95% of buried beetles survived in sand, sandy loam, and silt loam soils. Compaction limitations were determined where survival was lowered at a compaction of 3.0 kg/cm^2. The results showed that soil compaction caused by normal off-road vehicles is well below the 3.0 kg/cm^2 threshold and it is therefore unlikely to harm buried *Nicrophorus* beetles, including the endangered *N. americanus*. Compaction potentially can be used as a management practice, as well as removing cut vegetative cover, because the beetles are likely to avoid the altered habitat when locating their next period of inactivity.

Response of Burying Beetles to Soil Type, Moisture, and Cut Vegetation

Abstract

Silphid beetles in the genus *Nicrophorus* bury themselves during periods of inactivity, however, the influence of soil characteristics on burial behavior remains unclear. We examined soil preferences of the federally endangered *Nicrophorus americanus,* as well as of *N. carolinus, N. marginatus, N. obscurus,* and *N. orbicollis,* in a series of experiments. Differences in burial depth by sex were found for *N. carolinus, N. marginatus,* and *N. obscurus,* which are diurnal species. Both sexes of *N. americanus* bury to a mean depth of 18 cm. Soil preferences tested included sand versus sandy loam versus sandy loess, degrees of soil moisture, and soil cover (cut vegetation) versus bare soil. *Nicrophorus marginatus* preferred moist sandy loam with cut vegetation, *N. orbicollis* preferred moist sandy loam with cut vegetation, *N. carolinus* preferred sand with cut vegetation, and *N. americanus* only had a significant preference for moisture. Regarding the degree of soil moisture, *N. americanus* and *N. orbicollis* preferred extremely moist soils (0.4 wfv), *N. marginatus* did not have a preference, and *N. carolinus* preferred dry soils (0.02 wfv) over moist soils. Soil preferences during reproductive burials were equivalent to those of non-reproductive burials.

Introduction

Beetles within the Silphid genus *Nicrophorus* have a unique reproductive behavior, where males and females work together to bury the carcass of a small vertebrates by removing the soil underneath, causing the carcass to sink into the ground (Milne and Milne 1944). The beetles display bi-parental care where the parents prepare a brood ball and feed regurgitated carcass to the larvae until they are old enough to feed themselves (Anderson and Peck 1985; Ratcliffe 1996). Soil plays an important role in the reproduction of burying beetles. By burying a carcass quickly, these beetles can avoid inter- and intraspecific competition. The speed by which a carcass can be buried is affected by several factors, especially soil structure, humidity, and soil moisture (Muths 1991). Appropriate soil is also required to create a strong burial chamber that will not collapse and produce a stable environment in terms of temperature and moisture (Milne and Milne 1976; Jakubec and Růžička 2015). The correct type of soil can therefore result in a more successful reproduction.

Although variation exists among *Nicrophorus* species, most have a one-year life cycle where parents die in the late summer or fall, and teneral adults search the environment for food and an overwintering site (Anderson and Peck 1985). The beetles of this new generation overwinter underground for approximately eight months and emerge when spring temperatures rise (Ratcliffe 1996; Schnell et al. 2008).

Nicrophorus beetles do not only bury themselves for reproductive purposes, but they also spend a large portion of their adult lives buried in the soil during daily and seasonal periods of inactivity. Presumably this non-reproductive burial behavior is associated with predator avoidance and possibly escape from unfavorable environments. For instance, desiccation is a potential mortality with *N. marginatus*, and likely other *Nicrophorus* beetles, which exhibit high rates of water loss (Bedick et al. 2006).

Soil characteristics are also likely important for the endangered American burying beetle, *N. americanus*. The American burying beetle was once found in 35 states and three Canadian provinces, but has disappeared from over 90% of its historic range during the last century (Bedick et al. 1999). As the largest Silphid species in the Western Hemisphere (20-35 mm), *N. americanus* have correspondingly larger larvae and require larger carrion resources (Scott 1998). Larger carcasses may attract more and bigger scavengers, forcing beetles to bury carrion even deeper to avoid competition. Larger carcasses are also more difficult to manipulate and take more time to bury (Anderson 1982; Lomolino and Creighton

1995). Thus, having loose soils where carrion can be buried quickly and efficiently is likely necessary (Creighton et al. 1993).

General observations of Silphid beetles and their habitat have been made (Anderson 1982; Kozol et al. 1988; Creighton et al. 1993; Lomolino et al. 1995; Lomolino and Creighton 1995; Bishop 2002; Bedick et al. 2006; Jurzenski et al. 2011). Traps are being set out and beetles within a 7 km radius will be prone to capture (Jurzenski et al. 2011). This will give an idea of the whereabouts of these beetles. However, this does not give any information on where they bury and which soil characteristics they prefer. Given the lack of information on how Silphid behavior is influenced by soil characteristics and the significance of burying behavior (both biologically and in beetle conservation), we conducted a series of experiments to better describe the relationship between soil and burying beetle behavior. Specifically, we evaluated the effects of soil texture, presence of cut vegetation, gravel, and soil moisture on burial behavior of *N. orbicollis*, *N. carolinus*, *N. marginatus*, *N. obscurus* and *N. americanus* during daily inactivity. This information should improve the determination of burying beetle occurrence and could be helpful when selecting appropriate conservation or reintroduction sites for the endangered *N. americanus.*

Materials and Methods

Beetles. *Nicrophorus* beetles were collected following the methods recommended by the United States Fish and Wildlife Service (USFWS 2008); using baited pitfall traps (Bedick et al. 2004). Burying beetles including *N. orbicollis*, *N. marginatus*, *N. carolinus*, *N. obscurus*, and *N. americanus* were collected under permit # TE045150-1 between June and September of 2012 – 2014 from various locations in central Nebraska. About 3,000 *Nicrophorus* beetles were used for the experiments with each individual only used once. At the end of each study, the beetles were released in the area from which they were collected. In subsequent seasons and years burying beetles were collected at the same locations, suggesting that impacts to populations were minimal.

All soils used for experiments were collected in Nebraska. The sand used for this research was collected at Cotton Mill Park, Kearney, sandy loam was collected at the Bassway Strip State Wildlife Management Area near Kearney, and silt loam came from the canyons south of the Platte River in Lincoln County. All soils were sifted through an 8mm Newark N°10 sieve and larger particles (>0.5 mm) were removed to ensure similar consistency among soil types. The moisture level was kept constant for all experiments at a level of 0.10 water fraction per volume (wfv), with the exception of experiments that compared moisture effects.

Single factor experiments. Ten containers were made of plywood (33 cm long, 18 cm wide and 31 cm high). In the middle of each cage a smaller piece of plywood (3 cm thick and 25 cm high) was placed to divide the cage into two equal compartments. Each compartment represents the experimental unit where conditions (presence of gravel, moisture, or cut vegetation) can be altered (so beetles had a choice between the two experimental units (=two different treatments) within a container).

Two beetles, one male and one female of a species were used per trial. The beetles were placed on the middle plank and given a whole night (nocturnal) or day (diurnal) to be active prior to finding where the beetles were buried during their period of inactivity. *N. carolinus, N. marginatus, and N. obscurus* are diurnal, while *N. orbicollis* and *N. americanus* are nocturnal. After every trial both sides were excavated to recover the beetles.

Gravel. The potential influence of gravel mixed with soil on burial behavior was examined. The experimental unit was a container of soil with or without gravel mixed through it. Therefore, the treatment was the presence or absence of gravel. The gravel was collected from the roadside in Kearney County (1-6 cm in diameter). Twelve trials were conducted using different beetles. A total of 192 *N. orbicollis* and 28 *N. americanus* were tested.

Moisture. To determine moisture preference, beetles were provided the same type of soil that had different moisture levels. An experimental unit was a container of soil with one of four moisture levels.

Our treatments consisted of a factorial treatment arrangement of moisture x soil type. We examined four moisture levels: dry (0.02 wfv), moist (0.10 wfv), very moist (0.25 wfv) and extremely moist (0.40 wfv); and three soil types: silt loam, sandy loam, and sand. Because we had limited numbers of beetles and containers, we could not test all treatment combination and all potential paired choices (within a container) (the complete design would have required 12 treatments (4 moisture x 3 soils) and for paired comparisons of treatments we would have needed 66 containers per replicate to accommodate all possible combinations. Consequently, we only looked at selected combinations (determined *a priori*) that we anticipated would be of greatest biological interest. For all combinations of treatments, we use 5 replications. Additionally, we conducted separate experiments for each of three beetle species. The moisture level was maintained by measuring a container without beetles to have an idea of moisture level without interfering with the experiment. If necessary, water was added through the use of a spray bottle.

The preference of *N. marginatus* and *N. carolinus* was assessed during the night, while *N. orbicollis* preferences were determined during the day. A total of 90 *N. orbicollis,* 40 *N. marginatus*, and 80 *N. carolinus* were tested. The preference of *N. americanus* was not assessed with these methods.

Cut Vegetation Cover. The effect of cut vegetation on the surface was tested by giving beetles the choice between bare and covered soil. Each experimental unit was one container in the wooden chamber with or without vegetation coverage and had at least five replicates. Therefore, the treatment was the presence or absence of vegetation cover. The influence of vegetation was tested for *N. orbicollis* using leaf litter or cut grass, while *N. carolinus* and *N. marginatus* were tested only with mixed vegetation coverage. The cut vegetation layer was kept at approximately 2 cm in thickness. Sand was the only soil used in this experiment and was kept at a constant moisture level of 0.10 wfv. A total of 10 *N. orbicollis*, 20 *N. marginatus*, and 30 *N. carolinus* were tested.

Burial Depth. To determine how deep *Nicrophorus* sp. bury for non-reproductive purposes polyvinylchloride (PVC) tubes (=experimental unit) were used. Each PVC tube was 1.2 m in length and 10 cm in diameter. Tubes were cut in half to produce two, 5cm deep troughs and were then rejoined by using duct tape. This allowed for easy extraction of the beetles. The tubes were filled with 9.4 L of moist sandy loam soil. One male and female were added on top of the soil and the tube was capped. The nocturnal beetles were left overnight and checked in the morning (10:00), while the diurnal beetles were checked during the night (22:00). All beetles were kept in the tube for at least 12 hours. Twenty males and 20 females were tested for *N. carolinus, N. marginatus,* and *N. orbicollis* (N=40 for each species). Six males and 6 females were tested for *N. americanus* (N=12) and 16 males and 14 females were tested for *N. obscurus*.

Tent and Multiple Factor Experiments. Tests of multiple factors were conducted inside tents (3.5 m length x 3.0 m width x 2 m height). Within each tent, cat litter boxes (44 cm length x 30 cm width x 12 cm height) with carboard ramps for beetle access were used as experimental units. The tents were housed in a windowless room maintained at 24 °C with fluorescent lighting set to a 16:8 light: dark cycle.

Soil preferences. To test *Nicrophorus* preference for soil condition, an eight choice test with factorial treatment arrangement was used. The factors were soil texture (sand or sandy loam) x moisture [high (0.25 wfv) or low (0.02 wfv)] x detritus cover (present or absent). The experimental unit was a cat litter box inside a tent (the blocked replicate). Each tent contained 8 cat litter boxes each randomly distributed with the factorial treatment. The experiment was replicated depending on available beetles with 5 replicates of approximately 100 individuals each for *N. orbicollis*, 16 replicates for *N. marginatus*, and 12 replicates for *N. carolinus*. Because of permit limitations only 5 replicates using 12 *N. americanus* beetles were conducted. Beetles were excavated during their periods of inactivity (around 10:00 for night-active species and 02:00 for day-active species).

Moisture. Each tent contained 6 connected cat litter boxes with sandy loam of which two had a moisture level of 0.02 wfv, two between 0.10-0.25 wfv, and two of 0.40 wfv. A total sample size of 54 *N. americanus* was used. The first tent contained 8 males and 13 females, the second tent contained 10 males and 8 females, and the third tent contained 5 males and 10 females. The beetles were left for approximately 28 hours and excavated during the next time of inactivity.

Cut vegetation cover. The tent experiments had different types of cut vegetation (grass cuttings, forbs, and leaves) that were added to the soil surface of cat litter boxes with moist sandy-loam with 0%, 25-50%, and 75-100% coverage. The choice tests regarding degree of vegetative cover versus bare soil included three replicates for *N. americanus* (N=54) and twenty-four replicates for *N orbicollis* (N= 494).

Statistical analysis. Sigma Stat, SAS (SAS Institute 2013), and JMP (11.0 SAS Institute 2013) were used for statistical analysis. For Chi-Square test an online calculator was used (Preacher 2001).

Single factor experiments. The single factor choice tests were analyzed using chi-square goodness of fit tests to determine if gravel, moisture, or vegetation cover affected burial choice within a species. Differences between burial depths for males and females of a species were analyzed using a Mann-Whitney U or Student's t-test.

Multiple factor experiments. As described, the experimental design was suited for an analysis of variance to identify main effects and interactions. However, in practice, some treatment combinations were not possible because of limitations in availability of beetles. Consequently, generalized linear models were used for unbalanced datasets and as alternatives to analysis of variance to indicate treatment differences and trends where treatments represented levels of a factor (for example, levels of moisture in soil).

The data from multifactor experiments in tents were normalized using arcsine transformation. This technique uses the proportional, binomial data and makes the distribution normal. Afterwards the transformed data were analyzed by using analysis of variance (ANOVA) to detect differences in mean response. Beetles that did not bury during the trial were excluded from analysis.

For some experiments, a series of individual choice trials were combined to determine if there was a significant difference among levels of a treatment (for example, three beetle species or three soil types). For these tests, a nominal logistic model or general linear model (GLM) in JMP was used to test whether different levels of the treatment (e.g. individual beetle species) differed in their preference. Both methods allow the user to compare different levels of a treatment where the data have a binomial distribution, as was the case in these choice experiments. The nominal logistics model is the default approach in JMP, but GLM is used when unbalanced data or low sample sizes introduce bias. GLM in JMP corrects for these limitations using Firth Adjusted Maxima. The resulting Chi-square values test whether the mean responses for the treatment combinations differ significantly from each other.

Results

Gravel. Across all soil types *N. orbicollis* buried in soil without gravel about twice as often. Out of a sample size of 53 *N. orbicollis*, 36 beetles were buried in the soil without gravel and 17 in the side with gravel. In all soil types and in the aggregate, soils without gravel were preferred by *N. orbicollis*, $\chi^2(1, N = 53) = 6.81$, $P < 0.01$. In contrast, out of 28 *N. americanus* tested approximately equal numbers were found in soil containing gravel (N = 14) and in plain soil (N = 12). Two beetles did not bury. In both silt loam and sandy loam, one more *N. americanus* beetle was found in the soil containing gravel; which was not significantly different from bare soil $\chi^2(1, N = 26) = 0.154$, $P = 0.69$.

Moisture. There was a significant difference in moisture preference among species, $\chi^2(2, N = 190) = 25.87$, $P < 0.0001$. The sample size for each *Nicrophorus* species was 10. All *N. orbicollis* were found in the extremely moist soil (= 0.40 wfv), half the *N. marginatus* were found in the dry soil (=0.02), while the other 5 remaining beetles were found in the extremely moist soil, and all *N. carolinus* beetles were found in the dry soil. Among species tested, *N. orbicollis* is more attracted to very moist soil, *N. marginatus* does not seem to have a preference between dry and very moist, and *N. carolinus* prefers dry soil.

Using the tents to test three soil moistures with 54 *N. americanus* beetles, all were found in the sandy loam soil containing the highest moisture level (0.40 wfv). The data show *N. americanus* significantly prefers very moist soils over regular over moist or dry sandy loam, $\chi^2(2, N = 54) = 108$, $P < 0.0001$ (Table. 1).

Cut Vegetation Cover. Seven out of 10 *N. orbicollis*, 19 out of 30 *N. carolinus*, and 17 out of 18 *N. marginatus* preferred the side covered with vegetation; *N. marginatus* $\chi^2(1, N = 18) = 14.22$, $P <0.001$ showed a significant preference for the side covered with vegetation while *N. carolinus* $\chi^2(1, N = 30) = 2.13$, $P = 0.14$ and *N. orbicollis* $\chi^2(1, N = 1.6) = 25.87$, $P = 0.21$ did not have a significant preference.

Using the tents to test soils of the same moisture level but with no vegetation, moderate vegetation, or heavy vegetation coverage, *N. americanus* selected heavy coverage, $\chi^2(3, N = 54) = 162$, $P <0.0001$. The closely-related species, *N. orbicollis* was found in leaf litter covered soils but was also found in soils with less litter; no beetles were found in the bare soil χ^2(3, N = 54) = 638.11, P <0.0001 (Fig. 1).

Burial Depth. For diurnal species females buried significantly deeper than males across all species. For nocturnal species no differences in burial depth by sex were observed (Fig. 2). For diurnal species, females buried deepest *N. carolinus* (U = 82.5; P = 0.012), followed by *N. obscurus* (T = 2.44; df = 16.42; P = 0.026), and then *N. marginatus* (U = 76.0; P = 0.012). Both sexes of nocturnal *N. americanus* (T = 0.75; df = 20; P = 0.463) and *N. orbicollis* (T = 1.12; df = 32; P = 0.272) buried to similar depths of about 18cm.

Moisture, Vegetation and Soil Type. Among 5 trials using 12 *N. americanus*, most selected the wet sandy loam with detritus. To a lesser extent they also buried in sandy loam without organic matter, and moist sand with and without organic detritus (Fig. 3a). A small number of tested beetles did not bury and none were found in dry conditions. A significant preference for moisture (F = 9.52; df = 1; P < 0.01) was found, but there was no significant preference for soil type (F = 1.17; df = 1; P = 0.2879) or leaf cover (F = 0.01; df = 4; P = 0.9996).

The results for *N. marginatus*, showed significant influences of soil (sand vs. sandy loam) (F = 52.03; df = 1, 56; P < 0.0001), moisture (wet vs. dry) (F = 299.55; df =1, 56; P < 0.0001) and leaf cover (with vs. without) (F = 12.17; df = 1, 56; P < 0.001). Specifically, these results demonstrate that *N. marginatus* preferred sandy loam over sandy soil, moist over dry soil, and leaf-covered soil over bare soil. Moisture was the most important factor, then texture, and less important is the presence of vegetation cover (Fig. 3b).

For *N. orbicollis*, the data showed significant preference for soil type (F = 5.57; df =1, 21; P > 0.05) and moisture (F = 11.33, df = 1, 21; P < 0.01). Leaf-cover was not significant (F = 3.36; df = 1, 21;

P = 0.08) (Table 2). Sandy loam and wet soils were preferred by *N. orbicollis* (Fig. 3c). For *N. carolinus*, significant influences of soil (F = 12.88; df = 1, 83; P < 0.001), moisture (F = 82.54.98; df = 1, 83; P < 0.001), and leaf cover (F = 56.38; df= 1, 83; P < 0.0001) were observed. The two way interactions were not significant. These results show that generally *N. carolinus* prefers sandy soils that are moist and leaf-covered soils (Fig. 3d).

Discussion

Gravel. Most of the tested *N. orbicollis* avoided soil mixed with gravel, perhaps because soil without gravel is easier to dig into and does not require as much energy. In addition, *N. orbicollis* is most common in forested areas where gravel is likely not as prevalent as in other areas (Ratcliffe 1996). For *N. americanus*, approximately equal numbers were found in plain soil and soil containing gravel. This beetle is the biggest *Nicrophorus* species in North America and is either able to move past gravel or may use the gravel to aid in digging. Characterized as a habitat generalist, *N. americanus* may be more likely to come in contact with many combinations of soil during activity, while reproductive burials are likely to be limited to loose soils (Anderson 1982; Kozol et al. 1988; Creighton et al. 1993; Lomolino et al. 1995; Lomolino and Creighton 1995)

Moisture. Moisture strongly influenced beetle choices during inactivity. When *N. carolinus* was given the choice between dry (<0.02 $\pm$ 0.01 wfv) and moist (0.10 ± 0.01 wfv) soil, more individuals chose the moist soil, but when water was added to the moist soil to create an extremely moist soil (0.40 ± 0.01 wfv) all the individuals chose the dry soil (these were separate trials with different individuals). This implies that *N. carolinus* prefers a moist soil when possible, most likely to prevent desiccation (Bedick et al. 2006), but soils that are too wet are avoided (Table 1).

Soil preference in this study confirms that *N. marginatus* is a generalist and did not distinguish among tested moisture levels (Bishop 2002; Bedick et al. 2006). Similarly, *N. americanus* is a habitat generalist and has been recorded in partially forested loess canyons, grasslands, oak-hickory forests, wet meadows and shrub land (Anderson 1982; Kozol et al. 1988; Creighton et al. 1993; Lomolino et al. 1995; Lomolino and Creighton 1995; Bishop et al. 2002; Jurzenski et al. 2011). However, observations indicate that *N. americanus* in Nebraska and South Dakota is most likely to be found in habitat with sandy loam soils, scattered trees, a water source, and a large expanse of unfragmented habitat (Hoback, unpublished data). Our study supports the observation that *N. americanus* uses multiple soil types and has a strong preference for high moisture levels (>0.25 wfv).

Cut Vegetation Cover. My results indicate that cover can influence habitat choice for during periods of inactivity (Fig. 1). Observations of the beetles after they were released in the experimental unit, revealed that most immediately crawled and hid underneath the leaves/grass. Handling the beetles is a threat to them and may alter their immediate reaction but the beetles were left overnight (nocturnal) or for a whole day (diurnal), and thus, a choice was made by the beetles on where to bury after the activity period.

Burial Depth. Data show that diurnal species bury more deeply than nocturnal species and females bury more deeply than males for these species (Fig. 2). Burial depth is likely to be influenced by abiotic factors including temperature and soil moisture. Bedick and his colleagues (2006) showed that in low humidity conditions, *N. marginatus* loses 1-5% of its body mass per hour leading 50% of the beetles to die within 7-16 hour at 16-28 ˚C (Bedick et al. 2006). In Nebraska, the day temperatures average 29 ˚C in June and 31 ˚C in August. On some summer days, temperatures can reach over 43 ˚C (UNL 2014). It is clear that during the summer, diurnal *Nicrophorus* must find moist or lower temperature refuges to prevent lethal desiccation levels. Bedick et al. (2006) showed that *N. marginatus* has reduced activity when temperatures are high.

Diurnal species are buried at night when heating should be less. Niche partitioning is a possible explanation of differences in burial depths. Bishop et al. (2002) noted that *N. orbicollis* is collected more often in pitfall traps near forests and rivers while *N. marginatus* and *N. carolinus* are found in higher numbers near rangeland. If *N. orbicollis* favors moist habitats, they would likely not need to bury as deep as the other beetles to prevent desiccation. Behaviorally *N. marginatus* and *N. carolinus* may dig deeper, because of associations with drier conditions. However, males and females would be expected to bury to the same depth as a result of niches. Burial depth could also be explained by biotic factors. Predators of *Nicrophorus* may play a role in burial depth as soil-dwelling mammals are active at night (Jurzenski and Hoback 2011). Nocturnally active burying beetle species would avoid these soil-dwelling nocturnal predators.

Moisture, Vegetation, and Soil Type. Among tested *Nicrophorus* species, a similar preference for wet sandy loam with organic matter was observed, with the exception of *N. carolinus* and *N. americanus* (Table 2). These findings support previous observations by Anderson and Peck (1984), Scott (1998), Trumbo and Bloch (2000), and Bishop et al. (2002). These authors found *N. carolinus* to be associated with sandy soil. According to Bishop et al. (2002) *N. marginatus* can be found in sand, sandy loam and silt loam soil. In our experiments the highest percentage of *N. marginatus* beetles were found in sandy loam, followed by sandy soils (Fig. 3).

Surprisingly, *N. carolinus* utilized the most variety of soil conditions. In our trials, *N. carolinus* chose sandy loam and sand, wet and dry soils and soil with and without organic material. This finding does not agree with the literature that describes *N. marginatus* as the strongest generalist (Bishop et al. 2002; Bedick et al. 2006), while *N. carolinus* is most likely to be found in sandy rangeland where water is more scarce. These data suggest that habitat associations observed in the field may be a result of biotic interactions or differences in physiology as suggested by Jacques et al. (2009) who found antimicrobial activity to be temperature sensitive for *N. carolinus*.

Water molecules are more strongly connected to fine particle sizes. Of the soils tested sand has the largest particle sizes (2.00 – 0.05 mm), silt has particle sizes between 0.05 – 0.002 mm and clay has the smallest particle sizes below 0.002 mm (Gee and Bauder 1986). Sandy soil will therefore retain the least amount of water. The results of these experiments suggest that *N. carolinus* has a slight preference for soils with 0.25 wfv. The other beetles preferred wetter soils. No *N. orbicollis* were ever found in the dry soil treatment which coincides with their association with moist, forest soils near water resources (Wilson et al. 1984).

Scott (1998) argues that during reproduction, smaller species prefer damp soils with organic material that is easier to dig in, whereas larger species, such as *N. carolinus*, can manage to dig in dry, sandy soils. Considering the broad geographic range formerly occupied by *N. americanus*, it is unlikely that vegetation or soil type were historically limiting. Habitats in Nebraska where these beetles have been recently found include grassland prairie, forest edge, and scrubland (Jurzenski et al. 2011). Our results show that *N. americanus* is more likely found in moist sandy loam soil with organic matter, but has no significant preference among sand and loam soils.

It is important to recognize that these experiments were performed in the laboratory and the beetles were given choices between discrete types of soil in an artificial setting. The lab trials did not include predators or competitors that, in nature, could affect their choice (Scott 1984; Wilson et al. 1984; Eggert and Müller 1997; Ratcliffe 1996; Bedick et al. 1999; Bishop et al. 2002;), nor were beetles allowed to choose among mixed soils.

Jakubec and Růžička (2015) argued that the presence of food and limited mobility coupled with the adaptation to local conditions are the main factors influencing long range habitat selection. If there are so many factors influencing their spatial structure, trapping observations might be misleading. Our experiments show the preferred soil characteristics and may improve conservation site selection.

Conclusions. The presence of gravel deterred *N. orbicollis*, while *N. americanus* did not distinguish between the two conditions. *N. carolinus*, and *N. marginatus* showed a preference for leaf litter, but no significant preference was found for *N. orbicollis* and *N. americanus.* Both *N. americanus* and *N. orbicollis* preferred very moist soil (0.4 wfv), while *N. marginatus* showed less preference for soil moisture and *N. carolinus* preferred drier soils. Both *N. americanus* and *N. carolinus* appear to be more of a generalist in soil choice.

During periods of rest, diurnal species buried more deeply than nocturnal species and females buried significantly deeper than males for these species. The American burying beetle burrowed to approximately 18 cm when inactive in sandy loam soil. It is most likely found in soil covered with leaf litter and with a high moisture level. Sandy loam soil was preferred over sand and silt loam in our experiments.

Soil and habitat preference for non-reproductive burial is equivalent to the soil and habitat preference for reproductive burial (Ratcliffe 1996; Amaral et al. 1997; Scott 1998; Trumbo and Bloch 2000; Bishop et al. 2002; Walker and Hoback 2007). We can conclude that the *Nicrophorus* beetles bury in similar habitat to where they search for carcasses.

In summary, the endangered *N. americanus* is likely found 18 cm deep in the soil, is a generalist when it comes to gravel, leaf litter, and soil type, but has a strong preference for moist soils (> 0.40 wfv). When locating appropriate conservation or reintroduction sites, moisture should be the primary consideration. We can conclude that soil characteristics are not a contributing factor to the endangered status of these beetles.

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Tables

Table 1. Soil moisture preferences of *N. orbicollis*, *N. marginatus*, and *N. carolinus* with chi-square test results.

	N. orbicollis	*N. marginatus*	*N. carolinus*
D vs. M	Moist	NS	NS
	$\chi^2(1, N = 10) = 10, P < 0.01$	$\chi^2(1, N = 9) = 2.78, P = 0.096$	$\chi^2(1, N = 10) = 3.6, p = 0.058$
D vs. EM	Extremely moist	NS	Dry
	$\chi^2(1, N = 10) = 10, P < 0.01$	$\chi^2(1, N = 10) = 0, P = 1$	$\chi^2(1, N = 10) = 10, p < 0.01$
M vs. VM	NS	NS	NS
	$\chi^2(1, N = 36) = 2.78, P = 0.096$	$\chi^2(1, N = 10) = 0.4, P = 0.52$	$\chi^2(1, N = 20) = 0.2, p = 0.65$
M vs. EM	NS	NS	Moist
	$\chi^2(1, N = 20) = 0.05, P = 0.82$	$\chi^2(1, N = 10) = 2.6, P = 0.11$	$\chi^2(1, N = 20) = 7.2, p < 0.01$

D= Dry, moisture level of 0.02 wfv. M= moist, moisture level of 0.10 wfv. VM= very moist, moisture level of 0.25 wfv. EM= extremely moist, moisture level of 0.40 wfv. NS = Not significant. Choice tests were analyzed using chi-square goodness of fit tests.

Table 2. Soil preference of *N. americanus* (N= 12, 5 trials), *N. orbicollis* (N= 40, 5 trials), *N. marginatus* (N= 90, 10 trials) and *N. carolinus* (N= 70, 12 trials).

Table 2. Soil preference of *N. americanus* (N= 12, 5 trials), *N. orbicollis* (N= 40, 5 trials), *N. marginatus* (N= 90, 10 trials) and *N. carolinus* (N= 70, 12 trials).

	N. americanus	***N. orbicollis***	***N. marginatus***	***N. carolinus***
Soil	NS*	Sandy loam	Sandy loam	Sand
	(F= 1.17; df= 1; P= 0.2879)	(F = 5.57; df =1, 21; P > 0.05)	(F = 52.03; df = 1, 56; P < 0.0001)	(F = 12.88; df = 1, 83; P < 0.001)
Moisture	Wet	Wet	Wet	Wet
	(F= 9.52; df= 1; P < 0.01)	(F = 11.33, df = 1, 21; P < 0.01)	(F = 299.55; df =1, 56; P < 0.0001)	(F = 82.54.98; df = 1, 83; P < 0.001)
Cut vegetation	NS*	NS*	With	With
	(F= 0.01; df=4; P= 0.9996)	(F = 3.36; df = 1, 21; P = 0.08)	(F = 12.17; df = 1, 56 ; P < 0.001)	(F = 56.38; df= 1, 83; P < 0.0001)

Data were normalized using arcsine transformation. The transformed data were analyzed by using analysis of variance (ANOVA). NS= Not significant.

Figures

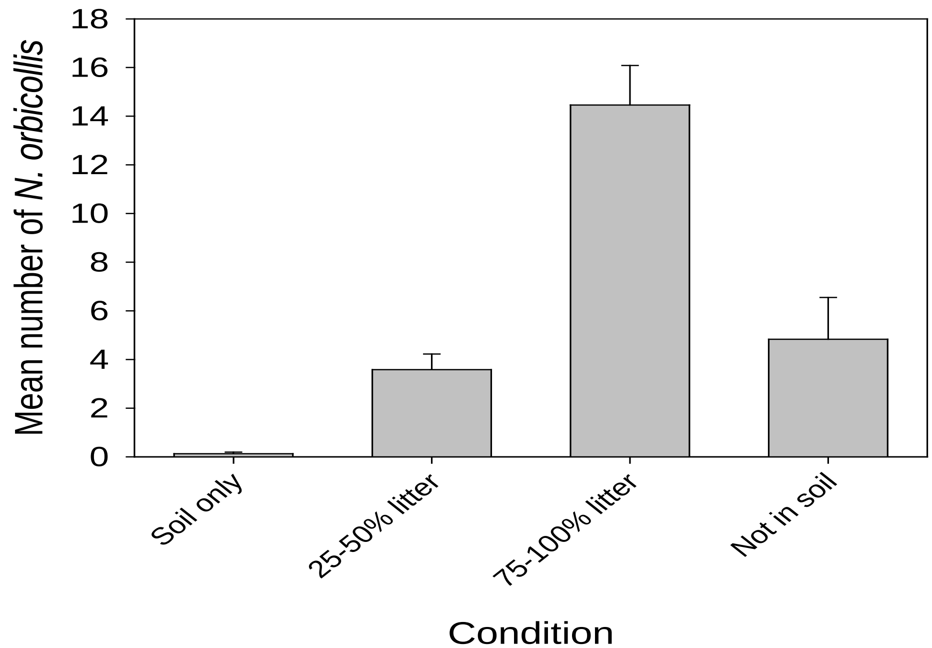

Fig. 1. The number of *Nicrophorus orbicollis* found in loam soil covered with different percentages of cut vegetation.

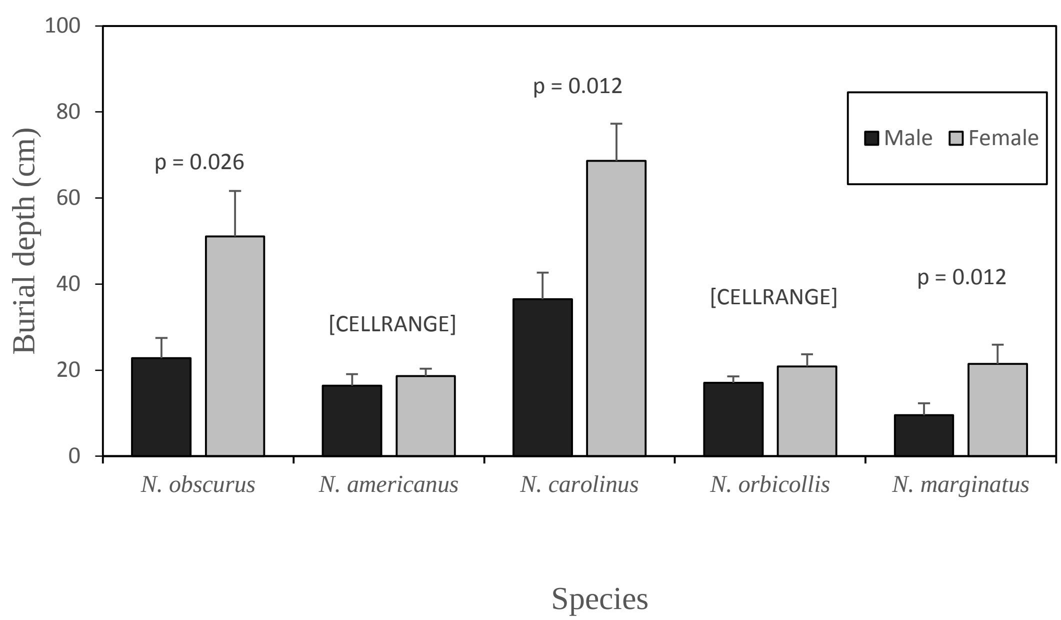

Fig. 2. Mean burial depth (cm ± SE) of both sexes of *Nicrophorus* beetle species in moist sandy loam soil.

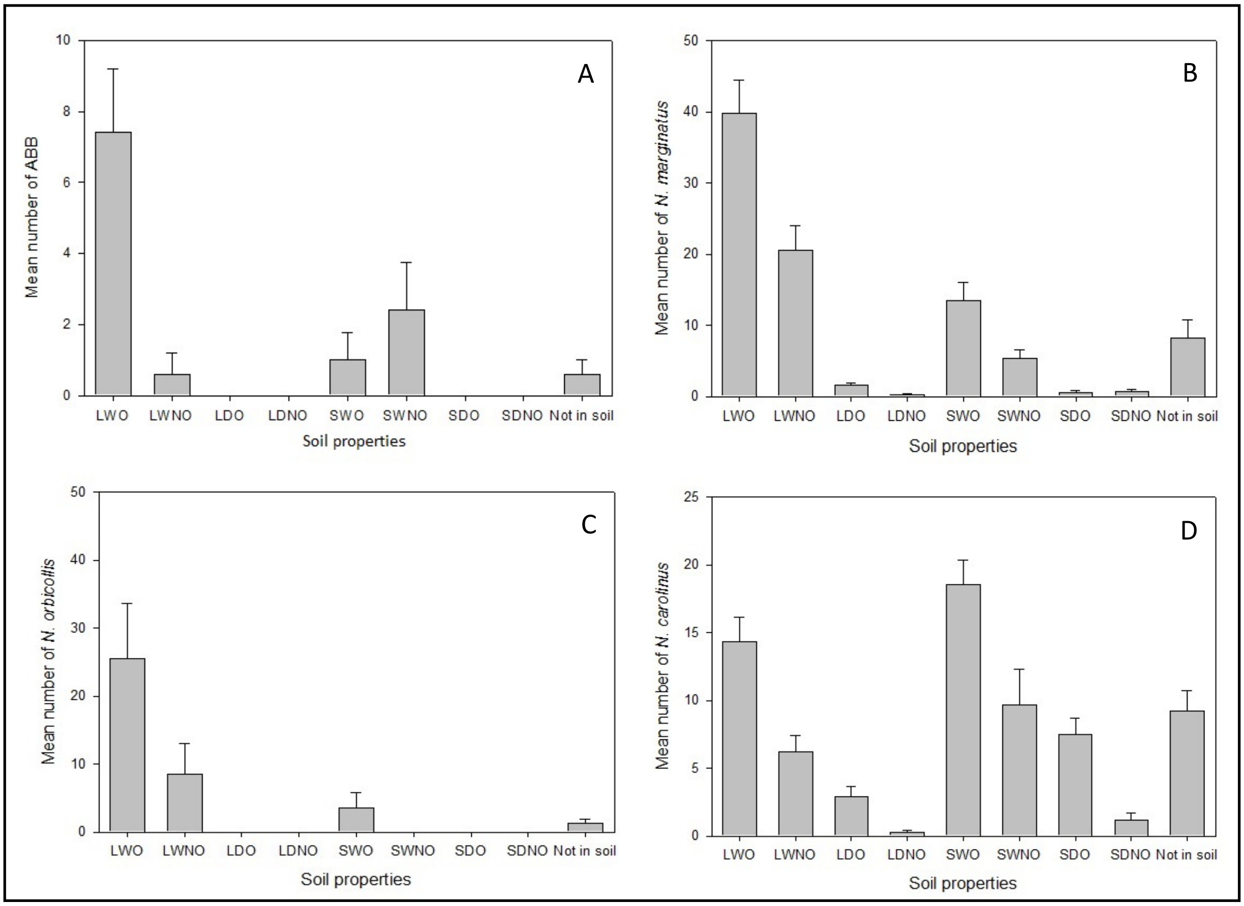

Fig. 3. Soil preference of *N. americanus* (A), *N. marginatus* (B), *N. orbicollis* (C), *N. carolinus* (D). Mean number of beetles differs between species, so the graphs cannot be directly compared. LWO= Wet sandy loam with organic material, LWNO= Wet sandy loam without organic material, LDO= Dry sandy loam with organic material, LDNO = Dry sandy loam without organic material, SWO= Wet sand with organic material, SWNO= Wet sand without organic material, SDO= Dry sand with organic material, SDNO= Dry sand without organic material (mean ± SE).

Response of Burying Beetles to Soil Compaction

Abstract

Burying beetles (Silphidae: *Nicrophorus*) take their common name from the behaviors associated with interring small vertebrate carcasses and using them for reproduction. In addition to this unique reproductive behavior, burying beetles spend a large portion of their adult lives buried in the soil during daily and seasonal periods of inactivity. While buried in the soil, these beetles are potentially affected by compaction from human activities such as the use of off-road vehicles, construction equipment, mowing, and haying. In addition to these potential sources of compaction, cattle grazing within fenced pastures can also generate substantial soil compaction. I conducted a series of experiments to test *Nicrophorus* survival of compaction while buried and behavioral responses to compacted soils. While buried, greater than 95% survival was observed for beetles buried in sand, loam, and silt loam soils that were exposed to compaction from moving standard pickup trucks. When vehicles were parked on top of buried *Nicrophorus* survival declined with 17.5%. Laboratory trials revealed that beetles avoided compact soil when provided a choice of loose soil except during burial of carcasses. Cut vegetation on the surface changed preferences in some trials. Based on these results, burying beetles, including *Nicrophorus americanus* are likely to survive compaction <3.0 kg/ cm^2 from normal off-road vehicle traffic and represents a temporary impact that is unlikely to result in harm but will cause them to avoid the habitat.

Introduction

Beetles in the family Silphidae are an important group of detritivores that recycle decaying materials into the ecosystem. Within this group, beetles in the subfamily Nicrophorinae are known as “burying beetles” because they bury small vertebrate carcasses in the soil which they use for reproduction. These beetles also spend large portions of their adult lives buried in the soil during periods of daily inactivity, and seasonal aestivation (Muths, 1991; Milne & Milne, 1976; Ratcliffe, 1996; Gibbs & Stanton, 2001).

In North America, the largest member of the Nicrophorinae is the American burying beetle, *Nicrophorus americanus* Olivier, (Anderson & Peck, 1985). This beetle was listed as an endangered species by the U.S. Fish and Wildlife Service in 1989 because it has been eliminated from more than 90% of its historic range. Many factors have been hypothesized to contribute to the decline of this species but the reasons for its decline across most of its range but persistence at the edges has yet to be explained (Anderson, 1982; Kozol et al., 1988; Sikes & Raithel, 2002; Jurzenski et al., 2014; Lomolino et al., 1995; Lomolino & Creighton, 1995).

One factor that may contribute to local declines is soil compaction associated with vehicle traffic. According to Riley (1984) road construction and shoulder maintenance causes the soil to compact 200 times compared to undisturbed areas. For construction project in Nebraska where N. americanus occur, the U.S. Fish and Wildlife Service and Nebraska Game and Parks Commission require that construction zones be mowed, vegetation removed, and that road kill be removed daily from these zones, prior to construction. Each of these actions requires that vehicles such as pick-up trucks and tractors be driven and parked in the grassy road shoulders (US FWS, 1991; Panella, 2013).

Previous studies have shown that moving vehicles over open ground can compact the soil, create depressions and can, in some cases, kill organisms in the soil (Knisley & Hill, 1992; Althoff et al., 2006; Meadows et al., 2008; Cornelisse et al., 2009; Lull, 1959). Meadows and his colleges (2008) showed that all-terrain vehicle traffic also cause the soil to compact, reducing infiltration and causing runoff that removes sediment and organic material and affects waterways. Cornelisse et al., (2009) found that repeated compaction caused by human disturbance reduced the occurrence of Cicindela tiger beetle species that inhabit the soil as larvae. Unfortunately, even after human use is discontinued in a construction zone, road, or agricultural land, soil compaction can take decades to recover and the subsoil compaction (30 cm and deeper) may even be permanent (Arvidsson et al., 2001; Trombulak & Frissel, 2000).

Conversion of land to agriculture has likely reduced the range of occurrence of *N. americanus*, although the species is associated with lands used for hay and for cattle grazing in Nebraska (Jurzenski et al. 2014). In areas used for having of cattle, compaction may be a factor that contributes to the decline of *N. americanus*. Lull (1959) measured the ground pressures created by different types of tractors and found that the ground pressure (kg/cm^2) is influenced by the number of tires, the inflation pressure, vehicle widths, loads, frequency of travel, and the soil type (Table 2, chapter 1). Cattle also increase soil compaction and reduce soil infiltration rates (Mulholland & Fullen, 1991) with observable compaction in field topsoil (0 - 5cm) within 15 days and effects observed to a depth more than 10 cm (Mulholland, 1991).

Although compaction has an effect on soil dwelling organisms, no studies to date have examined the effects of compaction on burying beetles. In this study I tested surrogate species (*N. orbicollis*, *N. carolinus* and *N. marginatus*) to examine compaction limitations (survival, and effects on burial and ability to dig out), behavioral response to compaction and how cut vegetation affects use, and how vehicle traffic and cattle affect soil compaction.

Materials and Methods

Experimental animals

Adult burying beetles were collected as needed from roadside areas in central Nebraska using pitfall traps baited with previously frozen laboratory rats that were aged approximately 4 days. Species were sorted, sexed and housed in the laboratory with moist soil, water soaked cotton, and ground beef until used in the experiments. All individuals were tested only once.

Measuring compaction

Two kinds of bulk density measurements were used for the experiments: Surface compaction was measured using a penetrometer (Test Mark Industries, SA-0240) that can measure up to 4.5 kg/cm^2 (Humboldt, 2015). For loose soils, an adapter foot (SA-0241) was added to the penetrometer, allowing measures lower than 0.01 kg/cm^2. A second measure of soil compaction was used in some experiments to assess changes to bulk density. A metal cylinder (10.2 cm in height and 5.2 cm in diameter) with a volume of 216.62 cm^3 was pushed into the soil to take a sample. The soil was then oven dried for 48 hours at 105°C, and weighed. Bulk density (g/cm^3) was calculated by dividing the dry weight of the soil by the volume of the soil. Increasing compaction reduces pore space and thus has more soil per volume with higher values representing a higher compaction level for the same soil types (Blake & Hartge, 1986).

Effects of Compaction on Behavior

A two choice test was used to assess burying beetle preferences for loose or compact soil when seeking an area for daily inactivity. In the laboratory, three species, *N. orbicollis*, *N. carolinus*, and *N. marginatus* were tested with three soil types, sandy loam, silt loam, and sand. Wooden containers (33l x 18w x 31h cm) were built with a partition in the middle separating loose soil (0.03 kg/cm^2) and compacted soil (0.5 kg/cm^2) of the same type which was made compact using a hammer and measured with a penetrometer prior to introducing one male and one female burying beetles. Because *N. orbicollis* is nocturnal, they were left overnight and assessed for preference in the morning, while the diurnal species *N. carolinus* and *N. marginatus* were introduced to the chamber during the day and assessed at 20:00. All species were tested with a minimum of 10 replicates while *N. orbicollis* were tested 50 times in sand and 15 in silt loam.

The influence of compaction on reproductive burial behavior was also tested, using one male and two females along with a thawed dead mouse that was placed on the divider in the experimental chamber. The beetles were left in the cages for three days to allow enough time for the burial of the mouse. Reproductive trials of *N. orbicollis* were replicated 30 times in sand and 5 times in silt loam while *N. carolinus* was tested 5 times in sand, 5 in silt loam, and 15 times in sandy loam and *N. marginatus* was tested 5 times in sand, 5 in silt loam and 10 times in sandy loam.

Cut Vegetation and Compaction Combined

Previous research revealed that N. orbicollis and N. carolinus have a slight preference for soils with cut vegetation, while N. marginatus has a strong significant preference. Seven trials were conducted using the experimental container where one side was randomly chosen to be made compact and one side was randomly chosen to be covered with vegetation. In some trials both sides had the same compaction with alternating vegetation coverage or both sides had vegetation coverage but the compaction differed.

All possible combinations were tested. Loose soil had a compaction level of 0.03 kg/cm^2 and compact soil had a compaction level of 0.5 kg/cm^2. The cut vegetation layer was approximately 2 cm thick and the soil was maintained at a moisture level of 0.10 wfv to avoid desiccation.

Compaction Limitations

The effects of compaction on burying beetles, were tested for three aspects: 1) ability to dig into compact soil where soil was compacted and beetles were added; 2) survival of compaction after beetles had buried; 3) ability to dig out of compacted soil where soil was compacted and beetles were placed at the bottom of the tube. The surface was then monitored for emergence holes. In all trials, a PVC tube with an outside diameter of 6.7 cm, inside = 5.2 cm, and length of 30 cm was used. Two soils, silt loam and sandy loam, were moistened to 0.10 water fraction volume (wfv) and sifted prior to the experiments.

Compaction was created using a hammer and wooden board and compaction levels were determined using a penetrometer. Five different compaction levels were tested during each trial, 1.25, 1.50, 1.75, 2.00, and 2.25 kg/cm^2 tested. Once the beetles were added a metal, mesh screen was placed over the tubes and held in place with zip ties.

Vehicle Impact on Buried Beetles

Parked vehicle

The experimental unit (N = 4) was one mesh bag with the dimensions of 33 cm h X 33 cm w X 33 cm filled with a soil "block" which was excavated from a meadow at the Fort Kearny Wildlife Management Area. Burying beetle species were tested individually using five male and five female beetles which were placed into each mesh bag and given 30 minutes to burrow. The bags were dug into the ground so that the tops were level with the surface and spaced so that each tire of a 1997 Ford F150 pickup truck could be parked on top of the bags. Soil compaction was measured with a pocket penetrometer in five locations in each bag following a diagonal line across the surface before and after each trial. Soil moisture levels were tested using a Field Scout moisture meter. Each trial began at approximately 22:00h and concluded at 06:00h. At the end of each trial, the truck was removed from the bags and the number of living and dead males, and females was recorded.

Five trials were conducted for both *N. marginatus* and *N. carolinus*. The mesh bag experiments were also tested in a single pass driving trial. Each replicate consisted of 4 mesh bags containing 11 male and 11 female *N. marginatus*.

Driving tests

Two rectangular metal open-topped boxes were constructed with two metal ramps attached to allow the truck to drive in and out of the box to test survival of beetles in a test where a standard F-150 pick-up truck could be driven over soil containing buried beetles. The boxes were filled with sifted sand until 6 cm from the top. Twenty-five male and twenty-five female burying beetles were placed in each box, for a total of 100 beetles per trial. The beetles were then covered with a 6 cm layer of sod, so it was level with the top of the boxes. One experimental unit of one box containing 50 beetles was replicated 10 times for each species (*N. carolinus* and *N. marginatus*). After driving the truck over the boxes, the grass was removed, the sand was sifted, and the number of beetles that survived was recorded. Each beetle's body size was determined by measuring its pronotum width and by weighing it to the nearest 0.01 g. A third set of experiments was created to specifically examine how beetle burial depth in loose soil affected survival. Burying beetles were marked with dots of acrylic paint and placed either near the bottom of the box or near the surface. The two boxes were filled with a bottom layer of 8 cm of sand and 10 N, marginatus were added. As quickly as possible, more sand was added until it reached approximately 6 cm below the surface.

Adult *N. marginatus* were added and the boxes were filled with sand until level to the surface. The experiment was repeated using *N. carolinus* and loose sandy loam soil. Instead of immediately digging out the beetles, they were left for two days to see whether or not they were able to escape. After two days the boxes were carefully excavated to find crushed or trapped beetles.

Environmental Compaction Measures

Changes in bulk density were measured in response to compaction from a Nebraska Public Power District line truck that weighed approximately 30,000 kg and had 14 tires. The experiment was conducted in two locations, near Elm Creek, NE. The first location was in a sandy area and the second location was in sandy loam soil. Ten soil samples from undisturbed soil were taken in the middle of the field using metal cylinders. The truck was then guided over the soil and 10 additional samples were taken. The samples were dried in an oven at 105°C for 48 hours and the dry weight was measured.

Cattle trampling

The effect of cattle on soil compaction was measured near the town of Shelton, NE. A total of 25 samples were taken at different locations. Ten samples were taken from the area outside of the cow pasture (NC) that was considered to contain soil that had not been exposed to cattle trampling. Five samples were taken from the area in the field with grass (F) that had been grazed. Ten samples were taken from the area on the cow path (C) without grass near the fence. Samples were collected with the metal cylinder and were taken in a line with about 0.6 meters between each sample.

Statistical Analysis

Excel (Microsoft Office 2013) was used for the statistical analysis. Chi-square goodness of fit tests were used to determine if soil compaction affected burial choice within a species. Specifically it tells us whether or not the observed responses differed significantly from the random expectation, in other words, an equal chance of the beetles burying in the loose and compact soil. Students' t-tests were used to determine if compaction increased before and after a certain pressure was applied (Pick-up truck, NPPD truck, cattle, etc). After performing these tests I can tell if the means differ from each other or not.

Results

Compaction preference

In trials where burying beetles were presented bare compact and loose soil of the same type, more than 90% of individuals chose to dig into the loose soil for the daily period of inactivity. Out of 60 *N. orbicollis* tested initially, 26 escaped and 34 chose to bury in the loose sand side. After initial trials with *N. orbicollis*, different wooden cages were constructed and fewer burying beetles escaped. For trials in sandy soil, all (N = 10) *N. marginatus* and *N. carolinus* were found in loose and. For trials using silt loam soil, all (N = 20) *N. orbicollis* and *N. carolinus* beetles were found in the loose soil, while 18 of 20 *N. marginatus* were found in the loose soil, one was found in the compact side, and one escaped . Similarly, for sandy loam soil, all (N = 10) *N. orbicollis* beetles, *N. marginatus*, and 9 of 10 *N. carolinus* were found in loose soil while one *N. carolinus* escaped (Table 3).

For reproductive trials, the side into which the mouse was interred was interpreted as preference because the burial of the mouse was associated with two burying beetles, one male and one female. The other female was often found on the other side of the partition, likely as a result of losing the competitive interaction. Across trials, the mouse was buried more frequently on the loose side for all species and all soils (Table 4). For trials in sand, the mouse was found on the loose soil side in 100%, 90% and 90% of trials for *N. orbicollis*, *N. marginatus*, and *N. carolinus* respectively. For sandy loam trials, the mouse was buried in loose soil in 60% of trials with *N. marginatus* and equally in the loose and compacted side for trials with *N. carolinus*. In silt loam soils, the mouse was buried in the loose soil 100%, 80% and 60% of the time by *N. orbicollis*, *N. marginatus* and *N. carolinus* respectively (Figure 4).

Cut vegetation and compaction combined

Seven trials were performed to test soil compaction and cut vegetation. When *Nicrophorus* chose between a compact side with cut vegetation and a loose side without cut vegetation, 9 out of 10 selected the compact side with leaves (table 5). For the reproductive trials, the mouse was buried in the compact soil on the side with leaves in 9 out of 15 trials. When given the choice between both loose sides but one with and one without cut vegetation, 8 out of 10 would choose the cut vegetation side and 2 would choose the side without cut vegetation. For the reproducing trials a less profound result is found, 8 out of 15 chose the cut vegetation side and 7 chose the side without cut vegetation. There does not seem to be a preference when both soil types are loose (Chi-squared: resp. 3.6 and 0.067). When both sides are compact and one side has cut vegetation and one side does not, 9 out of 10 beetles chose the cut vegetation side and only 1 chose the side without cut vegetation. When reproducing 10 out of 15 chose the cut vegetation side and 4 chose the side without any cut vegetation (Table 6).

When given the choice between compact soil with cut vegetation and loose soil without cut vegetation, 3 mice were buried in the compact soil and 2 were buried in the loose soil. When one side was loose with cut vegetation and the other side was also loose but without any cut vegetation, 4 mice were buried in the loose side with cut vegetation while only 1 mouse was buried in the side without cut vegetation. When they could choose between a compact side with cut vegetation and a compact side without cut vegetation, 3 mice were buried in the side with cut vegetation and 2 were buried in the side without any cut vegetation (Table 7).

Compaction limitations

To determine maximum soil compaction that burying beetles could dig into, *N. orbicollis, N. carolinus* and *N. marginatus* were placed on previously compacted soil and allowed to bury. The maximum compaction level that *N. orbicollis* could bury into was 2 kg/cm^2 in both silt loam and sandy loam soils while *N. marginatus* was able to dig into silt loam at 1.75 kg/cm^2 and 2.25 kg/cm^2 in sandy loam. The larger *N. carolinus* could dig into silt loam soil compacted to 3.5 kg/cm^2 and sandy loam soil compacted to 4 kg/cm^2. The results were variable and not all beetles could dig into a tested compaction level.

To determine compaction levels from which *N. orbicollis, N. carolinus* and *N. marginatus* can escape, soil was added to the tubes, the beetles were placed in the middle, more soil was added and then compacted. A single beetle was able to escape at 0.25 kg/cm^2. All other beetles were found immobilized but alive. An alteration was made to the experiment by putting a small container (± 5 cm diameter x 3.5 cm height) at the bottom before compacting the soil in the chamber. After compaction, the container was removed and the beetle was placed in the hole, allowing room to remove the compact soil. With space to move, all species were able to escape maximum compacted > 4.5 kg/cm^2 soils.

For survival of compaction, beetles were placed in the middle of a PVC tube with silt loam or sandy loam soil and the soil was then manually compacted. All tested *N. marginatus* and *N. carolinus* survived a compaction higher >4.5 kg/cm^2. For *N. orbicollis* an individual died at 3.0 kg/cm^2 in sandy loam soil, bot survived at 3.5 kg/cm^2. One beetle died and one survived at compaction level, both beetles survived at compaction level 3.5 kg/cm^2, and all tested beetles died at compaction levels of 4 and higher (Table 7).

Human impact on soil

Parked vehicle

Out of 200 *Nicrophorus marginatus* used in 5 trials (4 experimental units per trial), 26 males and 25 females were crushed by the parked truck. During the time that the truck was parked on the mesh bags each night, a total of 22 male and 32 female *N. marginatus* escaped. The mean ($\pm$ 1 SE) soil compaction before parking the truck on the bags was 1.02 $\pm$ 0.06 kg/cm^2 and the average soil compaction after the truck was removed was 1.98 $\pm$ 0.08 kg/cm^2 which was a significant increase (Students t-test; P<0.01) (Table 8). The average percent survival for *N. marginatus* was 74.5 $\pm$ 4.74% and was not different for males and females. In comparison, 85.5 $\pm$ 2.29% of tested *N. carolinus* died in the parking trials which was significantly greater than for *N. marginatus* (Table 17). Soil compaction measures showed that the soil was more compact at the beginning (1.66 ± 0.06 kg/cm^2) and end of the trials (2.6 ± 0.05 kg/cm^2) with *N. carolinus* (Table 8) compared to *N marginatus* ($p<0.05$). The size of the beetles did not affect survival ($p>0.05$).

Driving tests- Ford F-150

The mean (± SD) soil compaction level of the soil before a truck was driven over it was 0.84 ± 0.44 kg/cm^2. After the Ford F-150 passed over the soil the compaction significantly increased to 1.14 kg/cm^2 (Table 8).

For *N. marginatus* the mean mortality rate for males was 1.6% ± 0.76 and 1.0% ± 0.67 for females which was not significantly different (Mann-Whitney U=27.0, p=0.645) (Table 20). For *N. carolinus*, mean mortality rates were significantly higher for males with 3.67% dying compared to 0.88% for female (Mann-Whitney U=24.5, p=0.034) (Table 21). Despite differences in survival between the bigger *N. carolinus* and the smaller *N. marginatus*, the size of the beetle did not influence survival (Table 12). For the second set of experiments the sand had an initial compaction level of 0.03 ± 0.008 kg/cm^2 which increased to 0.120 ± 0.008 kg/cm^2 after the truck was driven onto the container (Table 8). Each box contained 20 *N. marginatus* beetles and after the Ford F-150 was driven over the soil, the boxes were left outside for two days. Of the tested beetles, four that were buried in the upper sand died and 36 survived (16 in the upper sand and all 20 in the lower sand). For the trials with *N. carolinus* in sandy loam, 21 were able to escape and 19 died.

NPPD truck. The sandy soil had a mean compaction level of 1.13 $\pm$ 0.14 g/cm^3 before the NPPD truck was driven across and compaction increased to 1.29 $\pm$ 0.17 g/cm^3 which was not significant. Prior to compaction the sandy loam soil was 1.39 $\pm$ 0.06 g/cm^3 and increased to 1.45 $\pm$ 0.04 g/cm^3 afterwards which was not a significant change (Table 8).

Cattle trampling. Samples taken from the area outside of the cattle field had a mean bulk density of 1.14 $\pm$ 0.02 g/cm^3. The samples on the cow path had a significantly greater bulk density of 1.60 $\pm$ 0.03 g/cm^3. The samples taken from the pasture showed that cattle had impacted soil compaction and had a mean bulk density of 1.54 $\pm$ 0.06 g/cm^3 (Figure 6).

Discussion

Laboratory trials revealed that while above ground, *N. orbicollis* select loose bare sand over compacted bare sand for their daily inactive period. Similar results were observed for breeding trails with beetles interring a mouse carcass in loose soil in 90% of trials. The daily burial trials for *N orbicollis, N. marginatus* and *N. carolinus* on silt loam and sandy loam soil show a similar trend where loose soils were preferred to compacted soils. The reproductive burial in silt loam and sandy loam soil for the three beetle species were less consistent. During daily periods of inactivity, beetles likely attempt to minimize energy expenditures. For reproductive burial, beetles must balance energy expenditures with the need for a structured brood chamber which may explain the reason for more beetles to bury the mouse on the compact side. However, these results should be interpreted with caution because burying beetles are in constant competition with their own species and different species for the carcass and therefore have to bury their claimed carcass as quickly as possible (Scott et al., 1987).

The presence of cut vegetation altered beetle response to compact soils with beetles resting beneath leaves on either loose or compact soil. In trials where beetles were allowed to choose between loose soil and loose soil with cut vegetation, beetles rested under the leaves or buried into the loose soil with similar frequencies. When provided with a carcass, cut vegetation did not strongly influence where the mouse was buried although compact soil with cut vegetation was chosen over loose soil without cut vegetation in many of the trials (Table 5). This suggests that cut vegetation is a more important factor than compaction during a reproductive bout, possibly because beetles are extremely prone to moisture loss (Bedick et al., 2006).

Another factor in these trials is the habitat associations of the species tested. For example, *N. orbicolis* is associated with forested areas which would contain leaf litter (Ratcliffe, 1996; Walker & Hoback; 2007). Previously, Scott et al. (1987) documented burying beetles covering the area where a mouse is interred to not leave signs of burial (Scott et al., 1987). In my trials, some beetles did not bury the carcass but hid it under the leaf litter while others that buried the mouse in the open side and moved leaf litter over the place of burial. Vegetation coverage reduces evaporation, causing the soil to remain moist for a longer time (Russel, 1940). Vegetation coverage could reduce the risk of soil drying around the brood chamber and may therefore be preferred (Bedick et al., 2006).

For every experiment, the compaction significantly increased after a truck was driven over or parked on the soil. Compaction levels were 1.98, 2.58, 1.14, 0.09 and 0.16 kg/cm^2 on simplified moist soils, without any vegetation imbedded (Arvidsson et al., 2001). From these data compaction levels and mortality rates for parked trucks were higher than those of a truck driving over the soil. This is likely because the weight of the truck has a longer time to affect the soil. The longer a weight affects the soil, the more air can escape from the pore spaces and will therefore result in more soil per volume (Blake & Hartge, 1986; Defossez & Richard, 2002). Compaction limitation trials show that in silt loam and sandy loam, *N. orbicollis* and *N. marginatus* can dig into soils compacted to 2kg/cm^2 and *N. carolinus* can dig into soils compacted to 4 kg/cm^2.

In laboratory trials beetles were immobilized at compaction level 0.25 kg/cm^2 but were able to escape in the more natural trials. The beetles are most likely to escape because of being able to move in uncompacted soil. When a hole was left at the bottom during laboratory trials the beetles could dig out of soil at the highest compaction level that could be achieved (>4.5 kg/cm^2). In trials where beetles were artificially placed under only 8 cm of soil, mortality was much greater than for those beetles buried deeper. The beetles near the bottom (± 22cm below the surface) survived which is consistent with the depth that *N. americanus* buries.

In both silt loam and sandy loam all beetles survived compaction levels over 4.5 kg/cm^2 except for one *N. orbicollis* that died in sandy loam soil with compaction 3kg/cm^2. The maximum level the penetrometer could measure was 4.5 kg/cm^2. To be conservative, the compaction level of 3 kg/cm^2 is recommended when considering risks to buried *N. americanus* beetles.

Arvidsson and Keller (2007) argue that there are two ways to reduce soil compaction: 1) By increasing the contact area and 2) By reducing the load. The contact area between soil and tire can be increased by lowering the tire pressure or by using bigger tires. The tire pressure/contact area relationship depends on tire characteristics. Their research showed that changes in tire inflation have the most influence on compaction at 10cm depth and that wheel load had a large influence on subsoil compaction (Arvidsson & Keller, 2007). Subsoil compaction could be avoided if limits for inflation pressures and wheel loads are given depending on the subsoil's mechanical properties. However, little standardization is possible for mechanical properties of subsoil. This makes it challenging to prepare a generally acceptable model for predicting the compaction under field conditions (Arvidsson et al., 2001).

Socomo (Soil Compaction Model) is an analytical model developed to calculate soil stress under different wheel loads. It is not yet perfected but can be used to assess the wheel load-load-carrying capacity. I hope to use this model in the future to assess how heavy trucks can be before they harm *Nicrophorus* beetles, or any soil dwelling organism in fact (Van den Akker, 2004). Once perfected, this could be a powerful tool for any threatened small soil dwelling animal.

Lull et al. (1959) present a summary can be found of crawler tractor weight, length, and tracks and the ground pressure resulting from it. A tractor of 3434 kg with a wide track and extended length results in a ground pressure of 0.19 kg/cm^2, while a tractor of 18405 kg with a standard track and standard track will result in a ground pressure of 0.62 kg/cm^2 (Table 6). Agricultural tractors had an average ground pressure of 1.4 kg/cm^2. When comparing the probability of direct mortality (> 3kg/cm^2) to the compaction caused by farm vehicles it leads me to conclude that compaction caused by the tractors is not sufficient to harm the endangered American burying beetle unless it is parked directly on a spot where *N. americanus* is buried for a long duration.

Pick-up trucks on the other hand form more of a threat where depending on wheel size and load, the ground pressures range anywhere between 3.5 – 7 kg/cm^2. *N. orbicollis* is likely to get crushed by a heavy loaded truck with small tires. However, my data did not show as high of compaction levels. Lull and his associates (1959) did not mention which soil types were used and perhaps soils with a high clay content caused these high numbers. The *N. americanus* beetle is bigger than *N. orbicollis* and more likely to survive high compaction levels. The equipment available was not capable of measuring compaction higher than 4.5 kg/cm^2. To be certain of *N. americanus* survival, direct tests with better equipment on a variety of soils are recommended. This might be a future project.

The compaction caused by cattle and the NPPD truck were measure using the core method which measures the bulk-density in g/cm^3. Because these results are factor 3 and the penetrometer results were factor 2, it is impossible to compare. For future reference, it would have been better to keep using the penetrometer. Nevertheless, I can conclude that the NPPD truck does not cause high compaction levels and is not likely to harm the beetles. Cattle causes a significant increase on soil compaction, but this happens over a long time period and only to a depth of 10 cm. Because of the long duration and because beetles usually dig deeper than 10 cm deep, it is unlikely that cattle compaction will harm the beetles by instant compaction.

Compaction may be useful as a pre-construction activity because it will not immobilize or kill the *N. americanus*, but they will avoid burying there again the next day. This measure is especially appropriate in areas where the soil is likely to recover as a result of seasonal cycles, plant growth and microbial activity (Wortmann, 2009).

This information is not only applicable to *Nicrophorus* beetles but to many other soil dwelling animals. The eastern beach tiger beetle, *Cicindela dorsalis*, is a good example of this. It used to occur on beaches between New Jersey and Cape Cod but now can only be found in well protected or private beaches that barely have any traffic. Compaction of soil is most likely the cause of its decline (Knisley & Hill, 1992). Not only insects, but any small animal that uses soil in some part of their lifecycle can be affected by compaction. The giant kangaroo rat (*Dipodomys ingens*) for instance, is endemic to California and occurs on semi-arid slopes in barren shrubless areas containing sandy loam soils. They are often found in heavily grazed areas by sheep or cattle and can be found underground in burrows when they are inactive (IUCNredlist, 2015). Compaction from off-road vehicles may form a threat for these species because of potential collapse of burrowing chambers causing the young to die. Some of the smallest ground using mammals might even struggle with making burrowing chambers in compacted soil.

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Tables

Table 3. Results of trials in which burying beetles had a choice between burial in loose (0.03 kg/cm^2) or compact (0.5 kg/cm^2) soil for daily inactivity or for reproduction. Data are presented as percent of total number of beetles buried in loose soil (L) compact soil (C) and not buried (NB).

Daily inactivity

	Sand			Sandy loam			Silt loam		
N. orbicollis	100	0	0	100	0	0	100	0	0
N. marginatus	90	0	10	100	0	0	100	0	0
N. carolinus	90	10	0	90	0	10	100	0	0

Reproductive burial

	Soil Type								
	Sand			Sandy loam			Silt loam		
N. orbicollis	90	10	0	-	-	-	100	0	0
N. marginatus	60	20	20	60	40	0	80	20	0
N. carolinus	80	20	0	40	40	20	60	40	0

Table 4. The amount of beetles found in the compact side with cut vegetation (C cv), the compact side with no vegetation (C), the loose side with cut vegetation (L cv) and/or the loose side with no vegetation (L). Investigation of which factor is more important; compaction or cut vegetation. In the self- burial trials 10 beetles were added (1 male and 1 female per cage) while in the reproductive burial trials 15 beetles were added (1 male and 2 females per cage).

	Compact cv	Compact	Loose cv	Loose
Self-burial C vs. L	9	/	/	1
Self-burial L cv vs. L	/	/	8	2
Self-burial C cv vs. C	9	1	/	/
Self-burial C vs. L cv	/	0	10	/
Reproductive C vs. L	9	/	/	5
Reproductive L vs. L cv	/	/	7	8
Reproductive C cv vs. C	10	4	/	/

Table 5: The amount of mice found in the compact side with cut vegetation (C cv), the compact side with no vegetation (C), the loose side with cut vegetation (L cv) and/or the loose side with no vegetation (L). Investigation of which factor is more important; compaction or cut vegetation.

	Compact cv	Compact	Loose cv	Loose
Reproductive C vs. L	3	/	/	2
Reproductive L cv vs. L	/	/	4	1
Reproductive C cv vs. C	3	2	/	/

Table 6: Compaction limitations of *N. orbicollis*, *N. carolinus* and *N. marginatus* in silt loam and sandy loam soil. Units are given in kg/cm^2 and all error margins are 0.124 kg/cm^2. The penetrometer could only measure up to 4.5 kg/cm^2.

	Silt loam			Sandy loam		
	N. orbicollis	*N. carolinus*	*N. marginatus*	*N. orbicollis*	*N. carolinus*	*N. marginatus*
Able to dig in soil with compaction (kg/cm^2)	2	3.5	1.75	2	4	2.25
Able to dig out of soil with compaction (kg/cm^2)	Maximum	Maximum	Maximum	Maximum	Maximum	Maximum
Die at compaction level (kg/cm2)	> 4.5	> 4.5	> 4.5	3	> 4.5	> 4.5

Table 7: Summarization of all the field compaction trials and its effect on beetle survival. Parked field= Truck parks 24 h on mesh bag containing *N. marginatus* or *N. carolinus*. Driving field= Truck drives once over mesh bag containing *N. marginatus* or *N. carolinus*. Ramp= Truck drives over ramp containing sand or sandy loam and *N. marginatus* or *N. carolinus*.

	Parked Field *N. marginatus*	Parked Field *N. carolinus*	Driving Field *N. marginatus*	Driving Field *N. carolinus*	Ramp Sand *N. marginatus*	Ramp Sandy loam *N. carolinus*
Compaction before truck (kg/cm^2)	1.02	1.66	0.84	0.84	0.03	0
Compaction after truck (kg/cm^2)	1.98	2.58	1.14	1.14	0.12	0.16
Beetle survival (%)	74.5	85.5	98.7	96.3	90	55

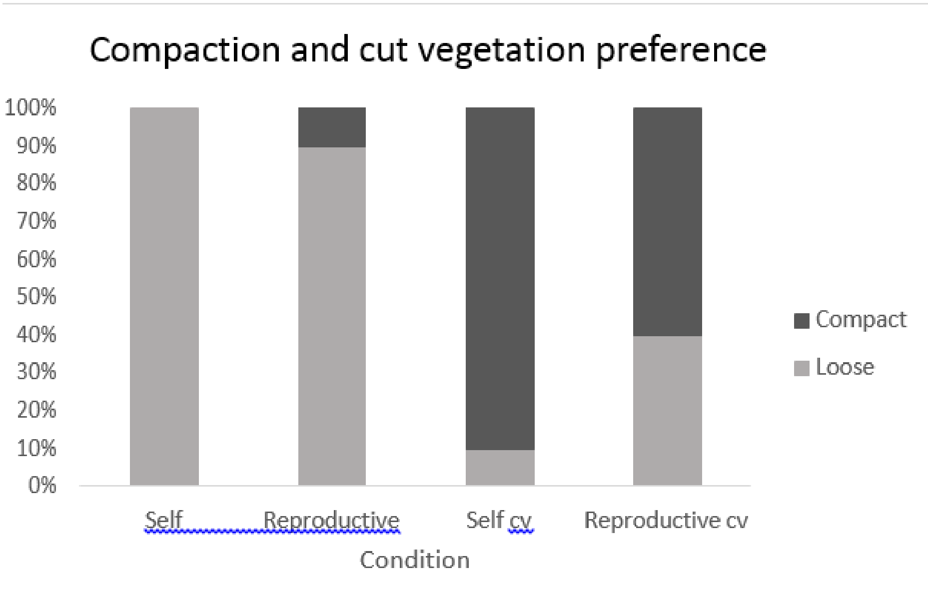

Fig. 4. The effect of cut vegetation on compaction preference.

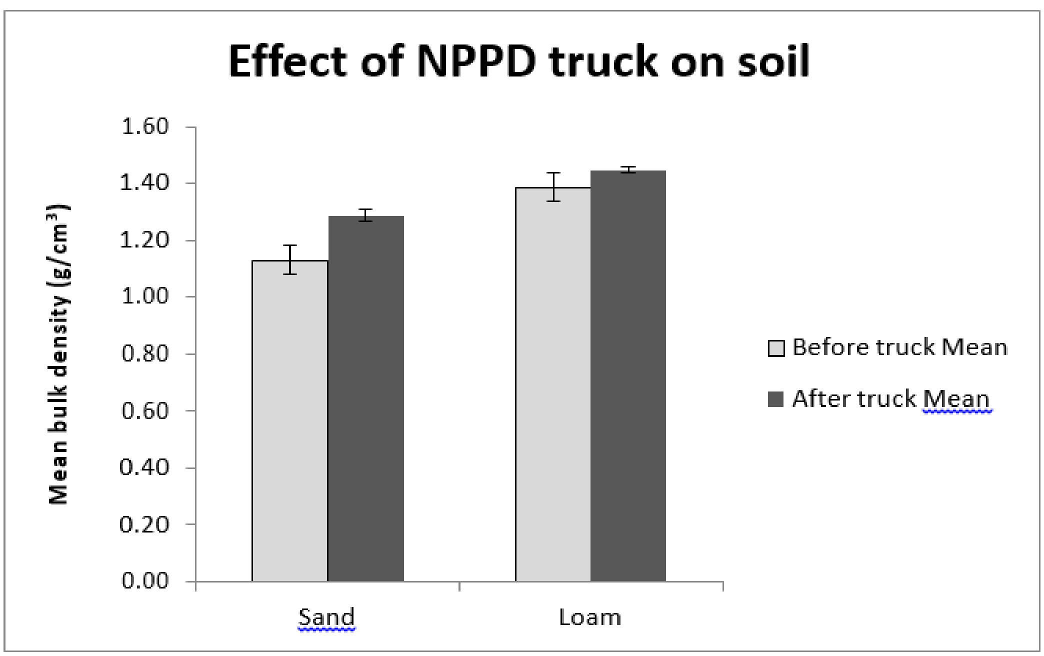

Fig. 5. The mean bulk density (g/cm^3) of sand and sandy loam soil before and after a NPPD truck (30,000 kg) drove over it.

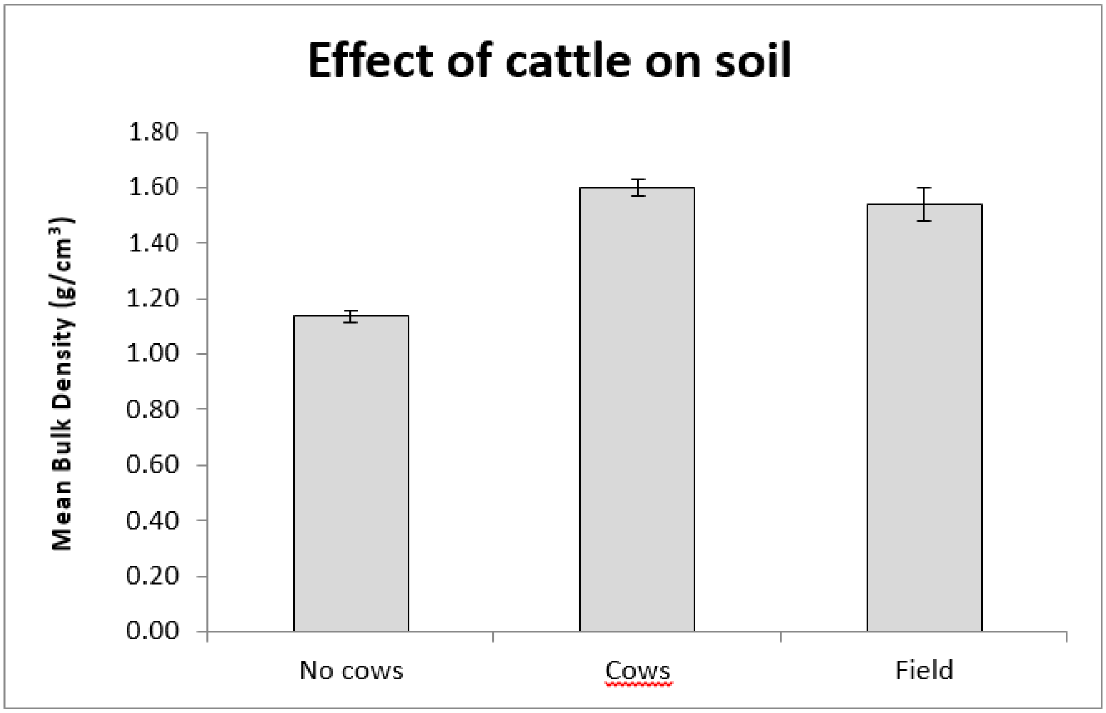

Figure 6. The effect of cattle on the mean bulk density (g/cm3) of soil. Measurements from an area without cows (No cows), a cow path (Cows) and a field containing cows (Field).

Management Implications for Burying Beetles Based on Results of Soil Preference and Compaction

Most research to date on the conservation of American burying beetle, *Nicrophorus americanus*, have focused on attraction of beetles to baited traps, while much less is known about where they bury during daily and seasonal periods of inactivity (Wilson et al., 1984; Backlund, 1997; Bedick et al., 1999; Trumbo & Bloch, 2000, Bishop et al., 2002, Bedick et al., 2004). The objectives of this research were to examine the soil preferences during times of inactivity and breeding for burying beetles in the genus *Nicrophorus.* The research included direct tests of burial behavior and soil selection of *N. americanus*, and used surrogate species to test the effects of compaction. The surrogate species, *N. orbicollis, N. carolinus, N. marginatus,* and *N. obscurus* were used to determine preference for moisture, soil type, presence of cut vegetation, gravel, soil compaction, and to determine burial depth when inactive. Among these species, *N. orbicollis* is most similar ecologically and phylogenetically (Sikes & Venables, 2013), while *N. carolinus* and *N. obscurus* are most similar to *N. americanus* in size.

By documenting soil preferences of burying beetles conservation during construction projects will be more-efficient, reducing impacts to the species and reducing costs associated with mitigation when less suitable habitats are disturbed. Studies of the effects of compaction on buried beetles allow additional decisions to be made concerning the impacts of construction access and pre-construction activities and point to a possible method to prepare a construction zone ahead of the work. By determining the compaction levels that impact survival and behavior of *Nicrophorus* beetles, and comparing this to the level of compaction caused by a vehicle, informed decisions about the conservation of *N. americanus* can be made. Together these data indicate that 1) Alterations to habitats (e.g. mowing, vegetation removal, and light compaction) make *N. americanus* less likely to bury in construction zones, 2) provide work limitations for which buried *N. americanus* are unlikely to be harmed.

Nicrophorus americanus, *N. carolinus* and *N. marginatus* show a strong preference for soils covered with cut vegetation. The conservation measure of mowing and removing vegetation from a construction zone as a pre-work conservation measure should reduce the number of *N. americanus* beetles present. In addition, removal of vegetation exposes the soil to sunlight and wind causing it to dry. Because soils containing high moisture levels (75-100%) are preferred by *N. americanus* and *N. orbicollis,* drier soils will limit burying beetle occurrence. However, care should be taken to restore both vegetation and surface moistures because the potential competitors *N. marginatus* and *N. carolinus* either do not distinguish habitat based on moisture or prefer drier conditions. During drier periods, watering of an area near a construction zone may further benefit conservation of *N. americanus* by providing attractive habitat. Reintroduction efforts could also benefit by ensuring that areas with moist soils are selected for reintroduction attempts.

In these studies, the *N. americanus* preferred sandy loam soil over sand soil, but other soil textures and combinations of different soils were not tested. The data support the results of previous studies that have found *N. americanus* to be generalists (Trumbo & Blotch 2000). The compaction data show that all surrogate species, *N. orbicollis, N. carolinus,* and *N. marginatus,* bury in loose soil when given the choice between loose and compact soil. These data suggest the *N. americanus* will also bury in loose soil when both conditions are present. Despite the preference for loose soil, *Nicrophorus* beetles were able to bury into densely compacted soil (1.75 kg/cm^2). Larger beetles are likely to be able to dig into higher soil densities and the larger *N. carolinus,* were able to dig into silt loam soils with compaction levels of 3.5 kg/cm^2 and sandy loam soils with compaction levels of 4kg/cm^2. Because *N. americanus* beetles are even larger than *N. carolinus* it is likely that they can bury into similar or greater compaction levels.

Driving over soil in areas that may be occupied by buried *N. americanus* beetles appears to present little risk as *N. americanus* beetles burrowed to 20 cm in laboratory trials and all tested species were able to dig out of highly compacted (>4.5 kg/cm^2) soils as long as there was an area of uncompacted soil around the beetle. Thus, the nocturnal *N. americanus* should be able to escape the following night from areas that receive daytime compaction from most normal-sized vehicles (pickup truck, tractor). All surrogate species, except for *N. orbicollis* in sandy loam, survived compaction levels >3.0 kg/cm^2, which is greater than compaction generated from travel. Beetles were more likely to be crushed by instant compaction when close to the surface (<10 cm) and parked vehicles resulted in greater mortality. It is therefore recommended that parking areas in construction zones be designated and prepared ahead of time through vegetation removal and compaction from driving over the area before parking is required.

Because soil compaction is a result of a vehicle's weight and the amount of surface area the vehicle applies to the ground, smaller vehicles with less ground contact are likely to generate greater pressure. Heavier vehicles often have larger tires and more tires and thus, generate less compaction than smaller vehicles and thus, a fully loaded pickup is likely to create greater compaction than a tractor that weighs more and tracked vehicles are likely to generate much less pressure than wheeled vehicles. Lull et al. (1959) measured the ground pressure caused by tractors of different weights, different tracks, and different lengths. A tractor weighing 18,405 kg with a standard track resulted in a ground pressure of only 0.62 kg/cm^2, well below the amount that caused mortality in burying beetles. Therefore, ground pressures caused by farm vehicles are not likely to be high enough to harm the endangered American burying beetle, and in fact they occur in hay meadows and range areas grazed by cattle. Because higher soil moisture is preferred by *N. americanus* and because moist soils become more compact, driving over wet soils should be avoided when possible.

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