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Soil Preferences of Nicrophorus Beetles and the Effects of Compaction on Burying Behavior

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SOIL PREFERENCES OF NICROPHORUS BEETLES AND THE EFFECTS OF
COMPACTION ON BURYING BEHAVIOR

By

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SOIL PREFERENCES OF NICROPHORUS BEETLES AND THE EFFECTS OF COMPACTION ON BURYING BEHAVIOR

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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 . My 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.

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Chapter one:

INTRODUCTION TO THE *NICROPHORUS* BEETLES AND THE
ENDANGERED AMERICAN BURYING BEETLE, *NICROPHORUS*
AMERICANUS OLIVIER.

INTRODUCTION

Silphidae

Beetles that belong to the family Silphidae are known as carrion beetles. Worldwide there are 13 genera and 208 species of silphids. Nebraska has 6 genera and 18 species (Ratcliffe 1998). Silphid beetles use vertebrate carrion as a food or reproduction resource and therefore play an important role in nutrient cycling and the reduction of flies that also breed in carcasses. The family is divided into two subfamilies: Silphinae and Nicrophorinae. Silphinae beetles search for carrion in the mid-stage of decay and lay fertilized eggs in the top layer of the soil, close to the carcass. Adults then abandon their eggs and the offspring will hatch after 4 or 5 days. These larvae consume maggots as the main food source (Anderson and Peck, 1985; Hoback et al., 2003).

Beetles that belong to the subfamily Nicrophorinae exhibit unique behaviors of burying carcasses, leading to the common names burying beetles or sexton beetles. After the carcass is buried, they exhibit bi-parental care and have been researched extensively by naturalists and ethologists. The earliest studies that describe burial behavior were written in the early eighteenth century. Today *Nicrophorus* beetles have become model organisms for research on competition, parental care and cooperation research (Eggert and Müller, 1997; Scott, 1998). They also have become models for investigations of physiology because of their use of oral/anal antimicrobial secretions for carcass preservation (Hoback et al., 2003; Jacques et al., 2009).

Parental care in insects

Invertebrates typically exhibit far less developed behaviors compared with mammals, birds, reptiles and other vertebrates. Although the eusocial behaviors of Hymenoptera and Isoptera are well-documented, parental care in insects is relatively rare. For example, maternal care can be found in thrips (Thysanoptera), true bugs (Hemiptera), hydrophilid beetles (Coleoptera) and webspinners (Embiopoda), where mothers guard the eggs against parasites and predators (Tallamy, 1984). The wolf spiders (Lycosidae) construct egg sacs to carry their young around with their spinnerets, providing protection during their development (Montgomery, 1903). One of the best described cases of paternal care in insects is found in the giant water bugs (Belostomatidae). Females cement their eggs to the backs of males for protection and aeration. The male will only allow this if he can copulate with her before and during oviposition, ensuring paternity (Tallamy, 1984).

Despite examples of parental care in the insects, biparental care is rare in insects. Beetles of the genus *Nicrophorus* display elaborate biparental care (Eggert and Muller, 1997). Both male and female independently search for carrion, they both bury the carcass, provide protection against intra- and interspecific competition and provide food for their offspring until they larvae are old enough to feed themselves (Eggert and Muller, 1997). More detailed information will be given in the following sections.

Life history

Detecting carrion

Nicrophorus beetles feed on carrion but also use the carcasses for reproduction purposes. They seem to prefer smaller size carcasses such as small birds, small prairie dogs, rats, mice, among others. The preferred size of carrion depends on each species. The largest *Nicrophorus* beetle in North-America, *Nicrophorus americanus*, searches for large carrion that can weigh up to 300 grams. These carcasses represent important nutritional resources for numerous scavengers including crows, raccoons, opossums, ants, flies, fungi and many other organisms which also feed on carcasses.

Nicrophorus beetles had to develop mechanisms to quickly detect dead animals. They have the ability to find an animal that died within an hour of its death and a number of field studies show that most carcasses are found in less than a day (Ratcliffe, 1996; Wilson, 1984; Lomolino & Creighton, 1996; Sikes & Raithel, 2002). *Nicrophorus* use the olfactory organs on their antennae to detect carcasses from distances of more than 3.22 km. For shorter distances they use olfactory organs residing in the palpi. It is thought that the sensillae on both organs are able to detect hydrogen sulfide and cyclic carbon compounds that are released shortly after an animal dies (Dethier, 1947).

Bedick and his team (1999) found a *Nicrophorus americanus* individual that had travelled up to 6 km. Jurzenski and her associates (2011) found travel distances of 7.24 km in one night and one individual even made it to an incredible 29.19km with wind-support. This last individual is considered an exception and wasn't used for average distance calculations (Jurzenski et al., 2011).

Carrion is a scarce and unpredictable resource, which makes competition intense (Bishop et al., 2002; Kozarec, 2001). When *Nicrophorus* beetles find a carcass they treat it as if it is their only chance at reproducing. They face strong inter- and intraspecific competition. Usually the size of the beetle influences the outcome and the biggest individual male and female will most likely win. The fight goes on until only one pair remains (Scott, 1998; U.S. FWS, 1991; Wilson et al., 1984). This competition can lead to severe damage including loss of legs, antennae, and even mortality (Bedick et al., 1999). The discovery of a carcass often occurs within two days, but has been reported to occur as quickly as 35 minutes post death (Milne and Milne, 1976). Carrion flies also detect bodies soon after death and will oviposit their eggs within minutes of carcass discovery. The maggots will then feed upon the carcass and deplete the resource. To prevent this from happening, the *Nicrophorus* beetles limit competition from flies by burying the carcass, removing the fur or feathers and by transporting symbiotic phoretic mites which feed on fly eggs on the carcass (Anderson and Peck, 1985). It is argued that one of the reasons why burying beetles don't occur in the tropics is because they are outcompeted by specialized carrion ants and flies. Ants at southern temperatures have more species diversity and are more abundant (Scott et al., 1987).

Resource and niche partitioning

As a result of intraspecific competition, *Nicrophorus* beetles exhibit resource and niche partitioning (Bishop et al., 2002). They vary in seasonal and temporal patterns, like different emergence times and patterns of sexual maturity (univoltine/multivoltine). Early emerging beetles that are able to be active at cool

temperatures will have the chance to find a carcass and bury it before other beetles emerge. For example, *Nicrophorus tomentosus* are able to breed during cold weather in both early spring and again in fall; their young will overwinter as prepupae instead of adults (Scott, 1984; Eggert & Müller, 1997). Wilson et al (1984) suggested that *N. defodiens* uses a temporal refuge against the bigger and therefore more competitive *N. orbicollis*. The night active *N. orbicollis* is able to find most experimental carcasses during relatively warm nights, while *N. defodiens* can win carcasses during nights with lower temperatures. Some beetles like *N. americanus* and *N. orbicollis* are nocturnal and search for carcasses during the night, while other beetles such as *N. carolinus* and *N. marginatus* are active during the day (Ratcliffe, 1996, Scott, 1984), although *N. marginatus* avoids the hottest parts of the day (Bedick et al. 1999). Limited research has investigated habitat partitioning, however, soil, vegetation, temperature, and moisture are factors in habitat associations (Scott, 1984; Bishop et al., 2002).

Ten species of *Nicrophorus* have been recorded from Nebraska with *N. carolinus*, *N. americanus*, *N. tomentosus*, *N. marginatus*, *N. obscurus*, *N. orbicollis*, and *N. postulator* most-commonly collected (Ratcliffe, 1996). The characteristics of the *Nicrophorus* beetles I used for my research are shown in table 1.

Burial process

The *Nicrophorus* beetles are called sexton beetles or burying beetles because of their behavior of burying appropriate sized carcasses for use in reproduction. Both males and females search for carrion. When a male is first to find and successfully

claim a carcass, he will excrete a sex pheromone to attract a receptive female. When the female arrives they will both assess the carcass (Ratcliffe, 1996; Anderson & Peck, 1985). They do this by crawling underneath the carrion and trying to lift it (McPherron, 2011). The beetles will then determine if the soil is loose enough for burial but stable enough to keep a burial chamber intact. If the ground is too hard, the beetles can move the carcass about one meter per hour until a suitable burial site is found (Ratcliffe, 1996; Muths, 1991). The beetles then use their head and legs to remove the soil from underneath the carcass causing it to move downwards. When the carcass does this, it rolls into a ball (Ratcliffe, 1996). Anderson (1982) found that while most *Nicrophorus* beetles bury carrion around 7 cm deep, *N. germanicus*, a large European species, buried it 20 cm deep in the soil. Another study done by Scott (1998) found that the carcass can be buried up to 60 cm underground. After it is completely buried, the beetle pair will construct a burial chamber and remove the fur or feathers of the carcass, to remove fly eggs and to gain access to the skin. The remaining ball is then covered with anal and oral secretions to prevent decay and contamination (USFWS, 1991). It is thought that the secretions contain antimicrobial secretions that inhibit bacterial growth (Bishop et al., 2002; Degenkolb et al., 2011; Hoback et al. 2002; Scott, 1998; Jacques et al., 2009; Hall et al., 2011 & Ratcliffe, 1996). Hoback and his team (2002) actually tested this and found that most *Nicrophorus* species have antimicrobial properties in their secretions and the oral secretions contain most of the active substances. These findings aren't true for every *Nicrophorus* species and there may be more mechanisms to be discovered. They also expect *Nicrophorus* beetles to have a combination of proline-rich, glycerine-rich,

cercopins and defensins proteins (Hoback et al. 2002). In a more recent study done by Hall et al. (2011) supportive evidence was found. The antimicrobial components are proteins with fragments ranging from 20 to 40 kDa in size. A detailed list of metabolites found in these secretions can be found in the paper by Degenkolb et al. (2011).

Scott and her associates showed that while a female beetle is assessing and burying the carcass, her ovaries mature rapidly (Scott et al., 1987). A brood usually consists of 10-30 eggs. After the eggs are laid, both parents construct a conical depression on top of the carcass and regurgitate droplets of partly digested food to their offspring. The larvae are directly fed by the regurgitation of the parents. After molting to second instar, the larvae are big enough to feed themselves and eat from the prepared carcass. The male remains with the carcass to protect it from potential competitors. Both parents continuously watch over their brood and remove fungi. After about a week the carcass will be nothing more than bones and the male will leave. The larva will pupate and it takes them 1 to 2 months to emerge as adults (Ratcliffe, 1996; Anderson & Peck, 1985). The burial process is shown in figure 1.

The American Burying Beetle

The American Burying Beetle is the largest carrion beetle of North America with a length between 20-35mm. They can easily be distinguished from other carrion beetles by a large orange spot on their pronotum. Males and females can be distinguished by the shape of the spot on the clypeus. Males have a big, orange rectangle while females have a small orange triangle (Ratcliffe, 1996). Adults of *N.*

americanus only live one year and during most of their lifespan they reside in the soil. They overwinter underground and become active when temperatures rise above 15°C (Kozol et al., 1988). In Nebraska, adults become active in early June and remain in the environment until the end of September.

Beginning in 1976, the American Burying Beetle gained increased interest because of their rapid decline in numbers and range (Anderson, 1982). Before 1980, their historic range decreased more than 90 percent (Lomolino et al., 1995). They have been recorded historically from 35 American states in East of the Rocky Mountains and from 3 provinces in Canada (Figure 2). When listed as federally endangered, only two natural populations were known, one located on Block Island, Rhode Island, and the other found in eastern Oklahoma. After 1980 *N. americanus* was also observed in southwestern Missouri and near the Platte-River Valley in west-central Nebraska. In 1989 the American Burying Beetle was declared federally endangered and the U.S. Fish and Wildlife Service developed a recovery plan to prevent the extinction of this species. Research has led to the discovery of populations in seven different states: South-Dakota, Nebraska, Kansas, Oklahoma, Texas, Arkansas and Rhode Island (U.S. FWS, 1991; U.S FWS, 2004 and Jurzenski et al., 2011) although the population in Texas appears to have been extirpated.

Although the American Burying occurs in a greater range than known at the time of listing, numerous re-introduction attempts have failed in Ohio, Vermont, and Missouri and much of its biology remains unknown. A number of hypotheses have been proposed to explain the decline in American burying beetles: (1) DDT/pesticide use (2) Artificial lighting affecting nocturnal populations and (3) Pathogens. Habitat

alterations can be split up in: (4) Old growth specialist (5) Prairie specialist (6) Vertebrate competition (7) Loss of ideal carrion and (8) Congener competition (Anderson, 1982; Kozol et al., 1988; Sikes & Raithel, 2002; U.S. FWS, 2013; UNSM, 2013; Jurzenski et al., 2014; Lomolino et al., 1995; Lomolino & Creighton, 1995). However, none of these explanations appears to fully explain the reduction in American burying beetle while other *Nicrophorus* beetles do not appear to have similar declines. It is most likely that not one effect, but a combination of several effects, has caused the species to decline as much as they did (Sikes & Raithel, 2002).

Human influence on the environment

Roads can impact the environment in at least 6 ways: “(1) habitat loss and modification with accompanying effect on populations; (2) intrusion of the edge effect into the core of natural areas; (3) subdivision and isolation of populations by acting as a barrier; (4) a source of disturbance to wildlife; (5) increased road-kills; and (6) increased human access with undesirable impacts on undisturbed areas” (Andrews, 1990).

Road construction, which includes clearing, leveling, cutting and filling procedures, will cause modifications and the destruction of vegetation. The compaction caused by the road-construction vehicles will change the hydrological patterns of the soil and re-vegetation. The change of vegetation can negatively influence animals that solely utilize that specific plant species and it can give the opportunity for invasive weeds to grow.

Species that can't disperse far will be most affected by roads. If their movement from one area to another is hindered it is likely to cause a reduction in population size. If movement becomes impossible and the road becomes a barrier, the habitat can become an "island" and create edge effects. (Andrews, 1990).

The edge effect will cause an abrupt change of two habitat types instead of a natural intergradation. This will change the whole dynamics between species known to occur in the edges, more mixed habitat and core habitat. When the habitat becomes too fragmented the core species will bear the consequences (Andrews, 1990; Sikes & Raithel, 2002).

The noise, danger, unnatural look of, and human activity on roads also cause animals to avoid high road density areas. Even though the road doesn't form a barrier, it does alter their occurrence (Andrews, 1990).

Extinction of species as a result of human activities has increased. Not only the construction and use of roads have been responsible for this but also hunting, deforestation and other habitat changes, pesticides, introduction of exotic species, and pollution contribute to losses of diversity (Lubchenco et al., 1991; Goudie, 2013). Human population growth has increased pressure on the environment and has been of great concern of ecologist and conservation biologists (Lubchenco et al, 1991).

Although large cities with abundant industrial, residential and commercial land-use drastically change local environments, agricultural conversion has altered the most amount of habitat. Increases in agriculture between the 1940s and the present have caused change, destruction and isolation of preferred *Nicrophorus* habitat (for

types of habitat, see table 1). Klein (1989) and Gibbs & Stanton (2001) observed dramatic declines in carrion beetle species richness in fragmented forests. Habitat alteration can cause a change in carrion species and the increased edge habitat can harbor more native vertebrate competitors for carrion. This makes the already scarce food/reproductive resources more difficult to obtain (U.S. FWS, 2013; UNSM, 2013; Trumbo & Bloch, 2000; Sikes and Raithel, 2002). A study done by Jurzenski and Hoback (2011) revealed that some opportunistic vertebrates like opossums and leopard frogs eat the larger/nocturnal silphid beetles. Several reports have been made of them feeding on the endangered American burying beetle. The increase of these opportunistic vertebrates due to habitat fragmentation may be another explanation of the decline of *N. americanus* (Jurzenski & Hoback, 2011).

Urban and suburban fragments may also contain higher levels of heavy metals and this can cause a reduced development of the carrion beetle larvae (Gibbs & Stanton, 2001). Fragmented forests experiences soil changes, including compaction and reduced organic matter, which can make it difficult for *Nicrophorus* beetles to bury carcasses (Lomolino & Creighton, 1996; Lull, 1959). If carcasses can't be buried deeply enough, scavengers or other competitors can more easily find and take the carrion (Gibbs & Stanton, 2001).

Soil compaction

Gibbs and Stanton (2001) suggest that soil quality is likely to affect carrion beetle abundance. Soil can be directly impacted through pollution and deforestation but there are also indirect factors to be considered. Agricultural land use can be

characterized as pasture and cropland, although there is overlap with many farmers that use cattle to graze field stubble after harvest. Cattle hooves create high stress on soil by increasing compaction and reducing soil infiltration rates (Mulholland & Fullen, 1991). Mulholland (1991) showed that when cattle are introduced to a pasture, significant compaction in field topsoil (0 - 5cm) occurs within 15 days and effects are observed to a depth of 10.5 cm. The mean dry bulk density increased 21.6% from a low-trampled area to a high-trampled area. Uncompacted pastures had a mean of 31.4% pore space, while after trampling the pore space was reduced to 12.6% (Mulholland & Fullen, 1991). Trampling by horses causes similar effects. For example, a horse causes a pressure of 1,400 to 4,000 g/cm² (24 to 57 lb/in²). Lull (1959) presents compaction values for animals and farm equipment (Table 2). The pressures increase during movement because the body weight is distributed over a smaller bearing surface.

Lull (1959) found that compaction from grazing was mostly found in the 2.5 cm (1in) surface layer. Different types of pasture soil (clay loam & sandy loam) were affected similarly with observed bulk densities from heavily grazed sites ranging between 1.54 and 1.91 compared with ungrazed sites that had bulk densities between 1.09 and 1.51. Lull reviewed a paper by Chandler (1940) that showed the bulk densities of grazed and ungrazed sites in second-growth hardwood stands in New York. Heavily grazed sites had a bulk density of 1.15 and the ungrazed sites had a bulk density of 0.92 (Lull, 1959).

Not only animals cause compaction of the soil. Tractors, pivots, trucks, and all-terrain vehicles drive over crop and rangeland to plant, harvest, water, spray

pesticides, etc. These wheeled vehicles often weigh much more than farm animals but also have a wider bearing surface (Lull, 1959). The ground pressures created by different types of tractors can be found in table 2. The ground pressure (lb/in²) is influenced by the number of tires, the inflation pressure, widths, loads, the soil type and frequency of travel.

In a study conducted by the USDA it was clear that all-terrain vehicles affect the soil and their natural resources. Only 20 to 40 passes are needed to change a low disturbance to a medium disturbance zone. Forty to 120 passes suffice to change a medium disturbance to a high disturbance zone. The vehicles cause the soil to compact, reduce infiltration and cause run off that removes sediment and organic material and affects the natural resources (Meadows et al., 2008). Not only the soil is affected by all-terrain vehicles, but soil dwelling arthropods are suffering as well. Knisley and Hill (1992) looked at the effects of human impacts on *Cicindela dorsalis*, also known as the eastern beach tiger beetle. This species had a disjunct historic range from central New Jersey to Cape Cod and also occurred on eastern and western shores in Chesapeake Bay. It is federally listed as threatened and now only occurs in inaccessible, private or well protected beaches. Beaches with high human foot and vehicle traffic contain low amounts of no *C. dorsalis* at all. Knisley and Hill (1992) suspect that human impact interferes with adult mating, ovipositioning and larval feeding. Larval burrows are disrupted and compacted and it can mix up the soil in such a way that moisture is affected, causing the desiccation of larvae. Cornelisse and Hafernik (2009) collected data that supported this hypothesis. They looked at the effects of variations in moisture, salinity, grain size and, pH on *C. hirticollis* and *C.*

oregona. More importantly, their study showed that repeated human compaction of sand causes a significant decrease in the amount of larval burrows (Cornelisse & Hafernik, 2009). The previous studies support our hypothesis that soil dwelling arthropods are affected by soil compaction caused by human activities.

Objectives

Because *Nicrophorus* beetles spend most of their life buried underground, soil is a critical factor in their life cycle. However, few studies have focused on the soil rather than habitat preference and the effects of fragmentation based on results of baited pitfall trap surveys (Anderson, 1982; Bishop et al., 2002; Lomolino & Creighton, 1996; Muths, 1991; Klein, 1989). Compaction is likely important because of its effects on burying carcasses. Compaction causes increased soil density because the pore space is reduced (Lull, 1959). Additionally, soil with a higher bulk-density does not hold as much moisture and the risk for desiccation in *Nicrophorus* beetles will be higher (Bedick et al., 2006).

Because soil is important to all aspects of the life cycle of burying beetles, it is necessary to examine soil preferences (moisture and presence of cut vegetation) and the effects of compaction on burying beetle behavior. Surrogate species, *N. orbicollis*, *N. marginatus* and *N. carolinus*, were used to determine potential effects of compaction on the endangered *N. americanus*. By determining the level of compaction that impacts survival or behavior of burying beetles, and determining the amount of compaction a vehicle with a certain weight, tires, and width will cause, informed conservation decisions can be made.

The specific objectives of my thesis are: 1) Determine the influence of moisture, cut vegetation, gravel, and soil compaction on burying beetle preferences during periods of inactivity and breeding; 2) Determine consequences of compaction on *Nicrophorus* beetle survival and behavior and compare these levels to compaction generated by various animals and vehicles.

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Tables

Table 1: Characteristics of *Nicrophorus* beetles used in this research

	<i>N. americanus</i>	<i>N. orbicollis</i>	<i>N. carolinus</i>	<i>N. marginatus</i>
Body size (mm)	20.0 – 35.0	14.8 – 23.0	13.8 – 26.6	13.9 – 22.0
Habitat	Generalist	Forest	Open Fields	Generalist
- Soil	Sandy loam	Alluvial	Sand	Loess/sand
- Land use	No data	Riverine	Range	Range
Activity	Nocturnal	Nocturnal	Diurnal	Diurnal
Reproductive period	June – September	May – October	March – October	February – October
Generation time	Univoltine	± Univoltine	Univoltine	Univoltine

(Amaral et al., 1997; Bishop et al., 2002; Ratcliffe, 1996; Scott, 1998; Trumbo & Bloch, 2000; Walker & Hoback, 2007)

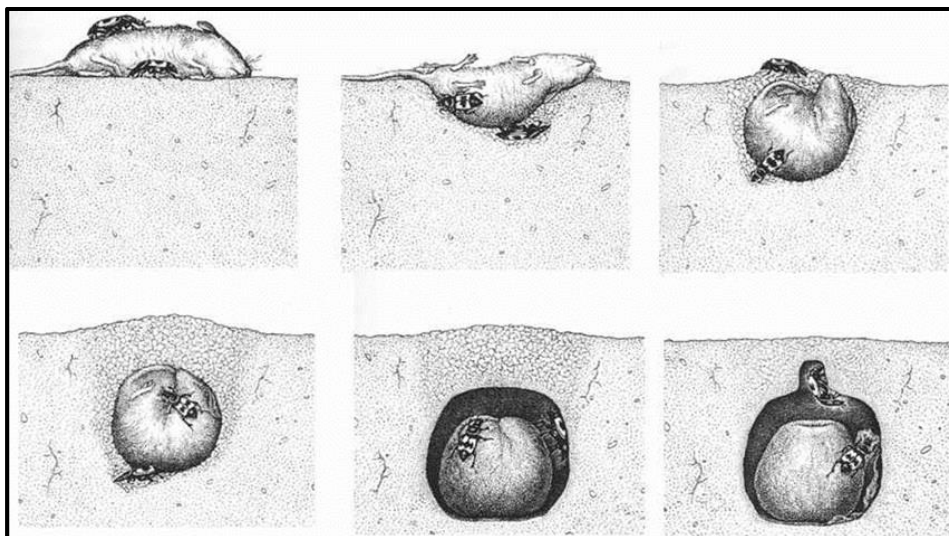
Table 2: Farm animal and tractor pressures on soil.

	Average bearing area (square inches)	Average weight (pounds)	Ground pressure (pounds per square inch)
Sheep.....	13.0	120	9.2
Cattle.....	56.4	1,350	23.9

	Standard track		Wide track	
	Standard length (pounds per square inch)	Extended length (pounds per square inch)	Standard length (pounds per square inch)	Extended length (pounds per square inch)
Tractor weight (pounds):				
40,575.....	8.82	7.86	6.93	6.18
18,170.....	7.24	6.36	4.83	4.24
10,930.....	6.66	5.76	3.61	3.12
7,570.....	5.41	4.49	3.25	2.69

(Tables from Lull, 1959)

Figures

Figure 1: Burial process of *Necrophorus* beetles. A mouse is buried for reproduction purposes (Ratcliffe, 1996).

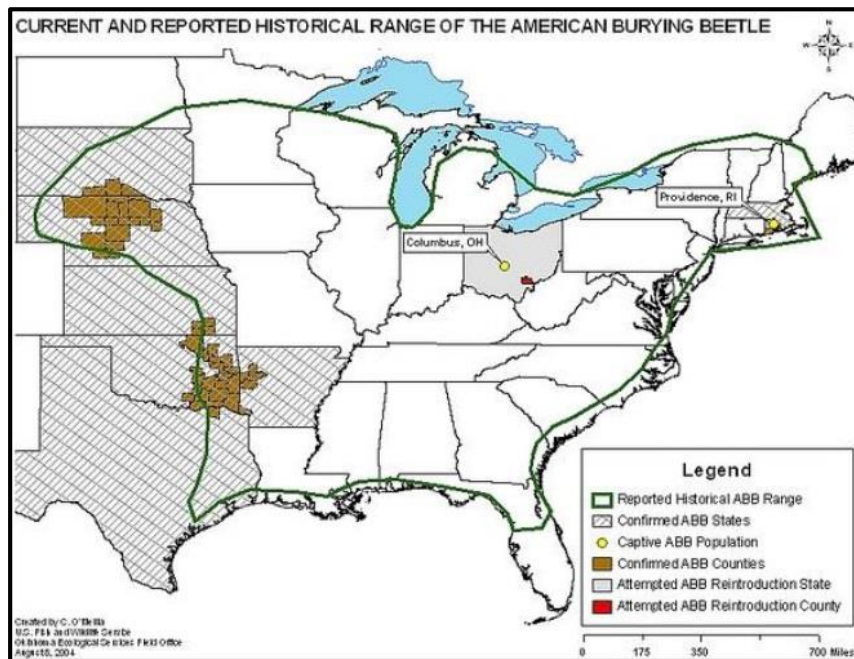


Figure 2: Current and reported historical range of the American burying beetle (U.S. FWS, 2004).

Chapter two:

SOIL PREFERENCES OF NICROPHORUS BEETLES DURING TIMES OF
INACTIVITY

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ABSTRACT

Burying beetles (*Nicrophorus*) are so named because of their characteristic of burying small carcasses for reproduction. Not only do they use the soil for reproductive purposes, but they bury themselves in it during daily and seasonal periods of inactivity. One member of this family, the American burying beetle (*N. americanus*), was declared federally endangered in 1989 and as a result management and conservation efforts have increased. The species is characterized as a habitat generalist which is surveyed by using baited pitfall traps, where little information about habitat preferences can be gained. It is therefore also important to know where they are most likely to occur during times of inactivity, but no studies to date have examined their soil preferences and conditions while inactive. The soil preferences of five *Nicrophorus* beetles were tested in this study. The American burying beetle is most likely to be found in moist sandy loam soil with cut vegetation coverage at a depth of approximately 20 cm. Similar preference was found for *N. marginatus* and *N. orbicollis*, but not for *N. carolinus*. These results provide important insights for surveys, habitat conservation, and reintroduction activities.

INTRODUCTION

Once found in 35 states and three Canadian provinces, the American burying beetle, *Nicrophorus americanus* Olivier, has disappeared from over 90% of its historic range during the last century (Bedick et al., 1999). *Nicrophorus americanus* was declared federally endangered in 1989 and a recovery plan was developed by the U.S. Fish and Wildlife Service in 1991. Populations of *N. americanus* have been

recently reported in seven states (U.S. FWS, 1991; U.S FWS, 2004; Jurzenski et al., 2011; Godwin & Minich, 2005) and Nebraska is estimated to be home to at least a fifth of the remaining population of the *N. americanus* (Bedick et al., 1999; USFWS, 2008).

Beetles within the genus *Nicrophorus* have a unique reproductive behavior, where males and females display biparental care and work together to bury a carcass by removing the soil underneath causing the carcass to sink into the ground (Milne and Milne, 1944). The female will lay her eggs in a separate chamber near the carcass, and the larvae will hatch a few days later. The male and female will then feed regurgitated carcass to the larvae until they are old enough to feed themselves (Anderson & Peck, 1985; Ratcliffe, 1996). Although the life cycle varies among species, most have a one year life cycle where parents will die at the end of the year, while the newly emerged tenax search the environment for food and for an area in which to overwinter. These adult beetles remain underground for approximately eight months and emerge when spring temperatures rise and the cycle continues (Ratcliffe, 1996).

Soil plays an important role in the lifecycle of burying beetles, because they spend most of their time underground and use soil for reproduction purposes. Additionally, by burying a carcass quickly, burying 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, therefore, have a higher chance of a successful reproduction (Milne & Milne, 1976).

Potentially soil is even more important for the endangered *Nicrophorus americanus*. As the largest silphid species of the Western Hemisphere (20-35 mm), *N. americanus* has bigger larvae but the same brood size as other silphid species, so they need bigger carrion/food resources. The larger carcasses 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, & Creighton, 1996). Thus, having loose soils where carrion can be buried fast and efficiently is likely necessary.

Previous research on carrion beetles has identified habitat association for some species, for example, *N. orbicollis* is most likely to be found in alluvial soils, *N. carolinus* in sand and *N. marginatus* in sand/loess (Scott, 1998; Ratcliffe, 1996; Trumbo & Bloch, 2000; Bishop et al., 2002) (Table 1, chapter 1). These associations are likely related to reducing intraspecific competition through habitat partitioning (Bishop et al., 2002; Scott, 1984). The most common habitat of *N. orbicollis* is forested habitat with alluvial soil and a nearby water source. These types of soil contain a higher moisture level because of a decreased evaporation, smaller soil particle sizes and nearby water sources which create a constant moisture flow. The most common habitat of *N. carolinus* is open sandy prairie with no water sources nearby. This type of habitat has more evaporation and larger soil particle sizes (Bedick et al., 2006; Bishop et al., 2002; Ratcliffe, 1996; Scott, 1998; Trumbo & Bloch, 2000; Wilson et al., 1984).

In contrast, *N. americanus* is characterized as a habitat generalist and has been recorded in partially forested loess canyons, grasslands, oak-hickory forests, wet meadows and shrub land (Anderson, 1982; Bishop et al., 2002; Creighton et al., 1993; Jurzenski et al., 2011; Kozol et al., 1988; Lomolino et al., 1995; Lomolino & Creighton, 1996). This generalistic behavior makes it difficult to determine a potential distribution of *N. americanus* and to designate priority conservation and reintroduction areas (Crawford & Hoagland, 2010; Lomolino & Creighton, 1996; Jurzenski et al., 2014). In Nebraska where *N. americanus* prefers open habitats over forested ones (Walker & Hoback, 2007), and is likely associated with a litter layer, and deep, loose soils (Lomolino & Creighton, 1996). Jurzenski et al., (2013) showed that agricultural development diminishes capture rates, and as little as 30% cropland within 5 km is enough to lower capture rates.

At finer scales, soil characteristics likely influence burying beetle behavior, but are more difficult to determine because adults are highly mobile and attracted to baited traps. Burial into the soil during inactive periods (day for nocturnally active, night for diurnally active species) will be designated as ‘self burial’ and is probably used to avoid predation and desiccation or other environmental hazards. During self-burial *Nicrophorus* beetles can choose to bury in the soil or to hide underneath leaf litter, as even during overwintering some beetles choose to stay near the surface underneath leaf litter (Schnell et al., 2008). Reproductive burial is burial of a carcass for egg laying and larval development and may be less subject to beetle choice because of the rarity of vertebrate mortality.

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), I conducted a series of experiments to better describe the relationship between soil and burying behavior. Specifically, I tested the effects of soil texture, coverage by cut vegetation, gravel, and soil moisture, on burial behavior of *N. orbicollis*, *N. carolinus*, *N. marginatus*, *N. obscurus* and *N. americanus*.

MATERIALS AND METHODS

Common Materials

Beetles. *Nicrophorus* beetles were caught following methods recommended by the United States Fish and Wildlife Service (USFWS, 2008), using baited pitfall traps (Bedick et al., 2004). Permission for collecting *N. americanus* was obtained from the U.S. Fish and Wildlife permit number: TE045150-1.

A five-gallon (18.9 liter) bucket is buried into the ground, a small layer of soil with added moisture is placed into the bucket along with bait (Bedick et al., 2006). Previously frozen laboratory rats were obtained from RodentPro (www.RodentPro.com) and were placed outside in the sun for approximately three days before use. After the rat is placed into the bucket, two wooden sticks and a wooden board are placed on top. Soil and vegetation are placed on top of the wooden board to prevent it from moving and for insolation (USFWS, 2008). After one night the traps were checked for beetles, and *N. orbicollis*, *N. carolinus* and *N. marginatus* specimens were collected. *N. americanus* beetles were collected the same way with laboratory experiments following USFWS guidelines for permit TE045150-1.

Senescent beetles were collected and bred in the lab to reduce field take. Both teneral and senescent beetles were used for the experiments. After one experiment the beetles were kept alive in a container with moist sand, a water source and, ground beef. When there was no need to collect more beetles, the remaining ones were released in the areas from which there were collected.

Male and female *N. orbicollis*, *N. marginatus* and, *N. carolinus* were collected between June and September of 2013 and 2014. The beetles were collected from Buffalo, Garfield, Knox, Holt, Lincoln, Hall, Sherman, and Dawson counties in Nebraska. The Bassway Strip State Wildlife Management Area in Kearney and Jones Canyon road in Burwell were primarily used for the collection of *N. orbicollis*. Most of *N. carolinus* beetles were collected North of Brady, Nebraska and on 24th and S road, south of Kearney, Nebraska, and *N. marginatus* were collected in almost every location.

Soil. The sand used for this research was collected at Cotton Mill Park, Kearney and contained no detritus or rocks. The loam was collected at Bassway Strip State Wildlife Management Area in Kearney, and the loess 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 were removed to ensure similar consistency in all. Soil samples were analysed by Ward Laboratories Inc. (Kearney, NE) (table 3). After analysis it was found that the loam soil was actually sandy loam and the loess was silt loam. The silt loam is a typical sub- surface soil in the loess canyons areas of Nebraska, known to harbor many *Nicrophorus* beetles, including the American burying beetle. Unmixed sand, silt loam and sand loam were used to examine the

basic burial behaviors. The sand I used reaches its field wilting point at a volumetric water content of 4%, its field capacity at 11%, and it is completely saturated at 30%. The sandy loam reaches its wilting point at 11% , its field capacity at 22%, and is completely saturated at 44%. The silt loam reaches its wilting point at 11%, its field capacity at 28%, and is completely saturated at 47% .

For most experiments, water was mixed through a soil type to end up with an average moisture level of 0.10 wfv (Water fraction per volume; 100 ml of water per 1 liter of soil). All moisture levels were measured with a Stevens Water Hydra-Probe. Based on this moisture level, sand was between field capacity and wilting point, sandy loam was on the boarder of wilting point and silt loam was below wilting point (table 4) (Saxton et al., 1986).

Single factor experiments. For soil preference experiments, I built cages to use as experimental units (Figure 3). Cages were made of plywood with a length of 33cm, a width of 18 cm and a height of 31cm. In the middle of each cage a smaller piece of plywood (3cm thick and 25 cm high) was placed to divide the cage into two smaller compartments. In preliminary research I found that most beetles will not dig deeper than 15 cm in buckets containing moist sand, so a cage height of 31cm was sufficient to accommodate beetle burial. Each cage had a lid, consisting of a wooden frame and mesh to allow natural light and air flow into the cage.

For self-burial trials, two beetles, one male and one female of a species were used per trial. The beetles were placed on the 3cm wide plank in the middle of the chamber and were given a whole night (nocturnal) or day (diurnal) to choose a

preferred side in which to bury. Beetles were given a choice between the same soil type that differed by one factor (moisture, compaction, cut vegetation, or presence of gravel). After every trial both sides were excavated to find the beetles.

For reproductive experiments, two females and one male of a given species were used. A second female was included to increase the chance of having a female that was ready to mate. A previously frozen laboratory mouse was thawed 24 hours before a trial and placed in the middle of the cage on the 3cm wide plank. The three beetles were placed on the same plank and were given 72 hours to bury the mouse. When needed, water was evenly sprayed on the top of the soil to prevent desiccation of the beetles. After each trial pictures were taken and the side where the mouse was buried was recorded.

Tent and Multiple Factor Experiments. Tests of multiple factors and some single factor experiments were conducted inside tents (3.5m x 3.0m x 2 m high). Within each tent, cat litter boxes (0.44m x 0.30m x 0.12 cm deep) 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.

Experiments

Burial Depth. To determine how deep burying beetles bury in loam 10 polyvinylchloride (PVC) tubes were used. Each PVC tube was 1.2 meter in length and 10 cm in diameter, containing 9.4 liters of soil. Tubes were cut in half to produce two, 5cm diameter troughs and were then rejoined by using duct tape. One male and female were added on top of the soil and the tube was capped. The nocturnal beetles

(*N. orbicollis* and *N. americanus*) were left overnight, while the diurnal beetles (*N. marginatus*, *N. carolinus*, and *N. obscurus*) were checked during the night. Each beetle was kept in the tube for a minimum of 24 hours. Twenty males and 20 females were tested for *N. carolinus*, *N. marginatus*, and *N. orbicollis* experiment (N=40 for each species). Six males and 6 females were tested for *N. americanus* (N=12) and 16 males and 14 females were used for *N. obscurus*.

Soil preferences. To test burying beetle 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 or low) x detritus cover (with and without). The experimental unit consisted of tents (2.5 m width x 3 m length x 2 m high) containing 8 cat litter boxes (44 cm length x 30 cm width x 12 cm depth) each with a unique combination of the factors. The experiment was replicated depending on available beetles with 5 replicates of *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).

Gravel. The potential influence of gravel was examined in aquarium tests. The experimental unit was a 7 liter aquarium divided in half by placing a piece of plastic in the center. Each side of the aquarium contained the same soil, but one side had gravel mixed through it. The gravel was collected from the roadside in Kearney County (1-6cm in diameter). There were a total of 16 aquaria. Silt loam, sandy loam, clay and sand were sifted before tests. Sifting ensured uniformity of particle size and

particles larger than 0.5 mm were discarded. The moisture level was kept constant at a level of 28-30 % for sandy loam, 24-26 % for silt loam, 7-9 % for sand, and 32-34 % for clay. Twelve trials were conducted using different beetles. A total of 192 *N. orbicollis* and 28 *N. americanus* were tested. The beetles were placed on the plastic divider and left overnight. The next morning the test chambers were excavated and beetle location was noted.

Moisture. To determine moisture preference, beetles were provided a two choice test where comparisons are made between different moisture levels of different types of soil. The experimental unit was the wooden chamber with soil of two different moistures in the two compartments. Every experimental unit had 5 replicates. The tested moisture levels were dry (0.02 wfv), moist (0.10 wfv), very moist (0.25 wfv) and extremely moist (0.40 wfv) for silt loam, sandy loam, and sand. The preference of *N. marginatus* and *N. carolinus* were determined during the night, while *N. orbicollis* preferences were determined during the day.

A moisture experiment was also conducted using tents. Each tent contained 6 connected cat litter boxes with sandy loam of which 2 had no moisture, 2 had 25-50% moisture, and 2 had 75-100% moisture. 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 overnight and excavated the next morning.

Cut Vegetation Cover. The effect of cut vegetation was also a two choice test where the choice was given between bare and covered soil. The influence of

vegetation was tested for *N. orbicollis* with both self- and reproduction burial experiments using leaf litter and cut grass, while *N. carolinus* and *N. marginatus* only had a self-burial experiment with vegetation coverage. The cut vegetation layer was kept about 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. Every experimental unit had at least 5 replicates.

To test the influence of the amount of vegetation, a tent experiment also was performed. The tent experiments had different types of cut vegetation (grass cuttings, wildflowers and leaves) that were added to the soil surface of cat litter boxes with 0%, 25-50%, and 75-100% coverage. In separate trials, *N. orbicollis* and *N. americanus* were released and re-collected during periods of inactivity.

Statistical analysis

Excel (Microsoft Office 2013), SAS (SAS Institute, 2013), and JMP (11.0, SAS Institute, 2013) were used for statistical analysis. Chi-square goodness of fit tests were used to determine if soil texture, moisture, vegetation cover, or gravel affected burial choice within a species. For some experiments, a series of individual choice experiments 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 a 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.

Differences in burial depth for males and females of a species were analyzed by using a Student's t-test. The data from the soil conditions experiment were transformed using arcsine transformation. This technique uses the proportional, binomial data and makes the distribution normal. Afterwards the transformed data are analyzed by using analysis of variance (ANOVA) to detect differences in mean response.

RESULTS

Across Experiments

No differences were found between senescent and teneral beetles in any tests where both were used. Similarly, no differences in results were associated with males and females, with the exception of the burial depth experiment. Consequently, age and sex are not considered further in results presented for individual experiments.

Burial Depth

The females buried significantly deeper (mean +1 SE) than males in diurnal species (Figure 13). No significant difference between sexes was found for the nocturnal species. Out of a sample size of 40 for each species, 2 males and 2 females

escaped for *N. carolinus*, 4 males and 2 females for *N. orbicollis*, 3 males for *N. marginatus*. Out of a sample size of 12 *N. americanus*, 3 males and 4 females escaped. Out of 16 male *N. obscurus*, 5 escaped and out of 14 females, 1 escaped. The average burial depth for *N. marginatus* was 18 cm, for *N. carolinus* it was 60cm, for *N. orbicollis* it was 20cm, for *N. americanus* it was 20 cm and for *N. obscurus* it is 35 cm (Figure 13).

Soil conditions

After 5 trials using 12 *N. americanus*, most selected the wet sandy loam with detritus. Although sandy loam without organic matter, and moist sand with and without organic detritus were also used. A small number of tested beetles did not bury and none were found in dry conditions (Figure 4).

. In two choice experiments, burying beetles exhibited different preferences (Table 5). The results for *N. marginatus*, showed significant influences of soil (sand vs. sandy loam), moisture (wet vs. dry) and leaf cover (with vs. without) (ANOVA results in Table 6). Additionally, all two way interactions were significant. Specifically, these results demonstrate that *N. marginatus* preferred sandy loam over sandy soil, moist over dry soil, and leaf-covered soil over uncovered. Similar results were observed with beetles on the surface, and in analyses of total (=surface + buried) beetle preferences. These results show that generally *N. marginatus* prefer sandy loam, which is moist and leaf-covered when given a choice. Also these preferences are largely consistent whether or not *N. marginatus* bury themselves or remain on the

surface. Moisture is the most important factor, then texture, and less important is the presence of vegetation cover.

For *N. carolinus*, the soil condition experiment showed significant influences of soil, moisture, and leaf cover. The two way interactions were not significant. These results show that generally *N. carolinus* prefers sand, wet, and leaf-covered soils when given a choice. For *N. orbicollis*, the data showed significant preference for soil type and moisture. Leaf-cover was not significant ($p=0.08$). Sandy loam and wet soils were preferred by *N. orbicollis*. The only two way interaction that was significant was soil texture and moisture.

Among species, *N. marginatus* does not show as strong of a preference as the *N. americanus* or *N. orbicollis*. The highest number of beetles were found in wet sandy loam with organic matter but they were also found in sandy soils, with or without organic matter and in dry soil. *N. carolinus* appears to be the most generalistic. When found in drier soils, the presence of organic matter increased its occurrence. The *N. americanus* beetles sought only moist soils during times of inactivity. While approximately 70% of the tested beetles were found in moist sandy loam, a vast majority were found associated with leaf litter (Figure 5).

Gravel

Out of a sample size of 192 *N. Orbicollis* an average of 35 beetles were buried in the soil without gravel and 18 in the side with gravel. Although stronger statistical significance would need to be calculated from a larger sample size, the data show that there is a trend toward a preference for soil that does not contain gravel. In all soil

types and in the aggregate, soils without gravel were preferred (Figure 6). Out of 28 *N. americanus* tested 14 were found in the soil containing gravel and 12 in the plain soil leading to the conclusion that *N. americanus* is not affected by the gravel and does not avoid gravel. In both silt loam and sandy loam, slightly more *N. americanus* beetles were found in the soil containing gravel (Figure 7); however, there were no significant differences.

Moisture

Complete data for the moisture experiments can be found in the Appendix, while a summarized table with statistical values is found in table 7. The data indicate that *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 drier soil.

The tent experiment shows that out of 54 *N. americanus* beetles, 54 were found in the sandy loam soil containing 75-100% of moisture. The data shows that *N. americanus* prefers very moist soils over regular or dry soil (Chi-squared=108). No difference in moisture preference was found between males and females.

Cut vegetation Cover

Eighty percent of *N. orbicollis* were found in soil covered with leaf litter regardless of whether it was a self- or reproduction trial (Figure 9), although results were not significant. The sample size for self-burial was 10 and reproductive burial was 5 which gave the tests low power.

Seventy percent of *N. orbicollis*, 60 percent of *N. carolinus* and 100 percent of *N. marginatus* buried in the loose soil when given a choice between loose and compact soils (Figure 10). No statistical difference was found for *N. orbicollis* and *N. carolinus* (Chi-squared < Probability), but *N. carolinus* had a significant preference for soils with cut vegetation (Chi-squared = 10). After performing a nominal logistic model it is clear that the preference of *N. carolinus* differs ($p=0.0156$) from *N. marginatus* and *N. carolinus*.

When given the choice of soils of the same moisture level but with no vegetation, moderate vegetation, or heavy vegetation coverage, *N. americanus* selected heavy coverage 100% of the time (Figure 11) (Chi-squared= 108). The closely related species, *N. orbicollis* was found in leaf litter covered soils but was also found in soils with less litter (Figure 12); no beetles were found in the bare soil.

DISCUSSION

About 3,000 individual *Nicrophorus* beetles were used for my experiments. This raises the question if the beetles are being negatively affected by the annual *Nicrophorus* research. Southwood and Henderson (2000) argued that invertebrate populations cannot be depleted from using baited traps. They came to this conclusion because trapping and removing insects to control insect populations have not been successful in the past. After my study was done, the beetles were released in the area from which they were collected and in subsequent seasons and years, burying beetles were collected at the same locations.

Burial depth

Data show that diurnal species bury more deeply than nocturnal species and females bury more deeply than males for these species. Burial depth is likely to be influenced by abiotic effects including temperature and soil moisture. Bedick and his colleagues showed that in low humidity conditions, *N. marginatus* loses 1-5% of its body mass per hour. Fifty percent of the beetles die within 7-16 hour at 16-28 °C (Bedick et al., 2006). During the day temperatures rise significantly where an average day in June is somewhere around 29 °C and 31 °C in August. On certain summer days, temperatures can reach over 43 °C (UNL, 2014; K. Willemssens personal observation). It is clear that during the summer, burying beetles need to alter their behavior and find moist or lower temperature refuges to prevent lethal desiccation levels. Especially diurnal beetles (*N. marginatus*, *N. carolinus*, *N. obscurus*) have a higher need for water conservation. Bedick showed that *N. marginatus* copes with this by having a reduced activity when temperatures are high and by looking for moist soil in the field and borrowing into it during times of inactivity (Bedick et al., 2006).

However, diurnal species are buried at night when environmental effects should be less. One possible explanation of differences in burial depths could be niche partitioning. Bishop et al (2002) noted that *N. orbicollis* is more easily found in pitfall traps near rivers while *N. marginatus* and *N. carolinus* are found in higher numbers near rangeland (Bishop et al., 2002). If *N. orbicollis* naturally prefers moist soil, they do not have to dig as deep as the other beetles to prevent desiccation. A possibility could be that *N. marginatus* and *N. carolinus* are used to digging deeper, causing a difference in burial depth at the same moisture level. Burial depth could

also be explained by biotic factors. Another possible explanation for this observed difference is soil-dwelling mammal predators such as moles (Jurzenski & Hoback, 2011). Nocturnally active burying beetle species would avoid these soil-dwelling nocturnal predators. These predators may also influence females to bury deeper as they will have developing eggs that may make them more susceptible to predation.

The federally endangered *N. americanus* burrows to approximately 20 cm. This is important for construction activities because surface activities should pose little threat to the species while it is underground. It is important to point out that these conditions are laboratory-based and may alter in the field. As mentioned, the outside temperature, moisture, soil composition and bulk-density can affect the burial depth. Because tracking devices for these beetles are not on the market yet, this data does give us an indication of what these beetles might prefer and if there is a difference between males and females.

Soil Conditions

For the four *Nicrophorus* beetles there seems to be a similar preference for wet sandy loam with organic matter. However, other soil types and combinations were not tested. Even though the most preferable soil might therefore be different for the four species, I can conclude that sandy loam soil is preferred over sandy soil with the exception of *N. carolinus*. These findings support previous studies done by Bishop et al. (2002), Ratcliffe (1996), Scott (1998), Trumbo & Bloch (2000). All papers show that *N. carolinus* is found in sandy soil. It was expected that *N. orbicollis* would choose the sandy loam over the sand because they are common near alluvial soils

(Bishop, 2002). According to Bishop et al. (2002) *N. marginatus* can be found in sand, sandy loam and silt loam soil. In my experiment the highest percentage of *N. marginatus* beetles was found in sandy loam, they were also found in sandy soils which supports Bishop et al.'s (2000) field data.

What does not match up with the literature is the fact that out of all the beetles, *N. carolinus* utilized the most soil conditions. In lab trials, it was found in sandy loam and sand, wet and dry soils and in soil with or without organic material. This finding does not agree with the literature because *N. marginatus* has previously been described as the most generalistic (Bishop, 2002; Bedick et al., 2006). *N. carolinus* is most likely to be found in sandy range land where water is scarce.

Water molecules are more strongly connected to fine particle sizes. Sand has the biggest 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 & Bauder, 1986). Sand will therefore retain the least amount of water. The results of these experiments suggest that *N. carolinus* has a slight preference for the wet soils of 0.30 wfv. The other beetles preferred wet over dry soil which makes sense because a moist soil helps to prevent dessication. No *N. orbicollis* were ever found in the dry soil which coincides with their association with moist, forrest soils near water resources (Wilson et al., 1984).

Scott (1998) argues that smaller species prefer a damp soil with organic material that is easier to dig in, whereas larger species, such as *N. carolinus*, can manage to dig in dry, sandy soils (Scott, 1998). 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 consist of grassland prairie, forest edge, and scrubland. Adequate soil moisture levels appear to be critical for *N. americanus* (Hoback et al. unpublished). My results show that *N. americanus* has a strong preference for moist sandy loam soil with organic matter.

It is important to note that these experiments were performed in the lab and the beetles were given different types of soil in an artificial setting. The lab trials did not include predators or competitors that, in nature, could affect their choice (Bedick et al. 1999; Bishop et al., 2002;; Eggert & Müller, 1997; Ratcliffe, 1996; Scott, 1984; Wilson et al., 1984). It could be that beetles have their ideal soil properties, which they will choose in the lab, but are simply outcompeted by other species and will therefore not choose it in nature.

Gravel

Gravel consists of a combination of crushed stone, sand, silt, and clay. Most of the tested *N. orbicollis* seem to avoid 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 regular soil and soil containing gravel. This beetle is the biggest *Nicrophorus* species in North America and might actually use the gravel to push away the soil as

an aid for digging. Being a generalist, *N. americanus* is more likely to come in contact with all sorts of combinations between crushed stone, sand, silt and clay during periods of inactivity, while reproductive burials are likely to be limited in these situations (Lomolino et al., 1995; Lomolino & Creighton, 1996; Bishop et al., 2002; Anderson, 1982; Creighton et al., 1993; Kozol et al., 1988; Jurzenski et al., 2011; Jurzenski et al., 2014).

Moisture

Data in table 7 and figure 8 support previous findings on moisture preferences associated with *Nicrophorus* species (Scott, 1998; Ratcliffe, 1996; Trumbo & Bloch, 2000; Bishop et al., 2002; Bedick et al., 2006; Wilson et al., 1984). Scott (1998) argues that smaller *Nicrophorus* beetles need soils which are easy to dig into and are usually found in forested areas with damp soils and organic material. Among species tested, *N. orbicollis* is the smallest (14.8 – 23.0 mm), *N. marginatus* is bigger (13.9 – 22.0 mm) and *N. carolinus* is the biggest (13.8 – 26.6 mm). It seems counterintuitive that *N. carolinus*, would search for a different habitat, since being bigger implies better competitiveness and *N. carolinus* should be able to fight off *N. orbicollis* (Scott, 1998; U.S. FWS, 1991; Conley, 2014). However, *N. carolinus* is able to bury in harder soils and *N. orbicollis* is not.

When *N. carolinus* was given the choice between dry (<0.02 wfv) and moist (0.10 ± 0.01 wfv) soil, more individuals chose the moist side, but when water was added to the moist side to create an extremely moist soil (0.40 ± 0.01 wfv) all the individuals chose the dry side (These were 2 separate trials with completely different

individuals). This implies that *N. carolinus* prefers a moist soil when possible, most likely to prevent desiccation (Bedick et al., 2006), but soils can also be too wet. A comparison among species showed a highly significant difference between ($p < 0.0001$) where each species preferred a different moisture level.

The data confirms that *N. marginatus* is a generalist and does not really distinguish between 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 (Lomolino et al., 1995; Lomolino & Creighton, 1996; Bishop et al., 2002; Anderson, 1982; Creighton et al., 1993; Kozol et al., 1988; Jurzenski et al., 2011). However, unpublished data by Hoback and Conley indicate that *N. americanus* 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 & Conley, unpublished; Amaral et al., 1997).

Cut vegetation Cover

Results on *Nicrophorus* preferences (table 9, figure 9) indicate that cover can influence habitat choice. Observations of the beetles after they were released in the experimental unit, revealed that most immediately ran 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) so a choice was made to bury after the activity period. During reproduction trials I observed that after the mouse was buried, the beetles closed the holes so it was

difficult to find where the mouse was buried and that some beetles even dragged leaves or grass from the compact side to cover up the hole on the loose side. Scott and her colleagues argued that burying beetles want to cover up the hole as well as possible so as not to leave any evidence, resulting in reduced (Scott et al., 1987).

I conclude that removing the vegetation out of a construction or high-mortality-risk zone will help reduce the presence of burying beetles. However, when no better site can be found or the carcass is present, the beetles will still bury the carcass.

CONCLUSION

Overall, *N. americanus*, *N. orbicollis*, and *N. marginatus* showed a preference for leaf litter, but no significant preference was found for *N. carolinus*. Both *N. americanus* and *N. orbicollis* preferred very moist soil but *N. marginatus* showed less preference for soil moisture. The presence of gravel deterred *N. orbicollis*, while *N. americanus* did not distinguish between the two conditions.

Diurnal species buried more deeply than nocturnal species and females buried significantly deeper than males for these species. The American burying beetle burrows to approximately 20 cm when inactive. It is most likely found in soil covered with leaf litter and with a high moisture level. Sandy loam soil was preferred over sand, but clay, silt loam, and soil mixtures were not tested.

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Table 3: Characteristics of three soil types used in the experiments (means \pm SD), determined by Ward Laboratory, Kearney, Ne.

	pH	Organic Matter (LOI %)	N (ppm)	K (ppm)	S (ppm)	% Sand	% Silt	% Clay	Texture
Loam	7.70 \pm 0.10	2.67 \pm 0.12	34.33 \pm 2.55	339.33 \pm 30.66	10.33 \pm 0.57	58.33 \pm 1.15	26.00 \pm 2.00	15.67 \pm 2.31	Sandy loam
Loess	7.93 \pm 0.15	0.53 \pm 0.06	120.83 \pm 0.06	447.33 \pm 7.23	23.33 \pm 1.53	24.67 \pm 3.06	59.67 \pm 3.06	15.67 \pm 1.15	Silt loam
Sand	7.47 \pm 0.15	0.27 \pm 0.06	3.53 \pm 0.06	21.33 \pm 7.23	2.50 \pm 1.53	92.00 \pm 2.00	6.00 \pm 2.00	2.00 \pm 0.00	Sand

Table 4: Soil wilting points and field capacity in relation to standardized water fraction per volume used in the experiments.

Wp= Wilting point (Minimal moisture level so plants do not wilt in that soiltype), Fc= Field capacity

Treatment	%volH ₂ O	Sandy loam	Silt loam	Sand
Dry	2	2<<Wp	<<Wp	Wp
Moist	10	Wp	Wp	Fc
Very moist	25	fc<25<<Sat	Wp<<25<Fc	Fc<<25<Sat
Extreme	40	40<Sat	40<<Sat	Sat<<40

(Moisture present in soil after excess water has drained), Sat= Saturation (Maximum level of water content is reached).

Table 5: 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.s.= Not significant.

	<i>N. americanus</i>	<i>N. orbicollis</i>	<i>N. marginatus</i>	<i>N. carolinus</i>
Soil	Sandy loam	Sandy loam	Sandy loam	Sand
	-	<0.05	<0.001	<0.001
Moisture	Wet	Wet	Wet	Wet
	-	<0.01	<0.001	<0.001
Cut vegetation	With	N.s.	With	With
	-	> 0.05	<0.01	<0.001

Table 6: Analysis of Variance (ANOVA) results for arcsine-transformed proportion of buried *Nicrophorus marginatus* in preference test. The experiment had a 2x2x2 factorial treatment arrangement of soil (sandy vs. silt loam), moisture (dry vs. wet), and cover (no cover vs. vegetation cover).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	15	5.848258	0.389884	28.3	<.0001
Error	56	0.771383	0.013775		
Corrected Total	71	6.619641			
blk	8	0.013946	0.001743	0.13	0.9979
soil	1	0.716743	0.716743	52.03	<.0001
moist	1	4.126178	4.126178	299.55	<.0001
cover	1	0.167689	0.167689	12.17	0.001
soil*moist	1	0.616971	0.616971	44.79	<.0001
soil*cover	1	0.055177	0.055177	4.01	0.0502
moist*cover	1	0.12136	0.12136	8.81	0.0044
soil*moist*cover	1	0.030195	0.030195	2.19	0.144

Table 7: Soil moisture preferences with chi square to determine significance.

	<i>N. orbicollis</i>	<i>N. marginatus</i>	<i>N. carolinus</i>
D vs. M	Moist	Inconclusive	Inconclusive
	<0.001	>0.05	=0.0578
D vs. EM	Extremely moist	Inconclusive	Dry
	<0.01	>0.05	<0.01
M vs. VM	Inconclusive	Inconclusive	Inconclusive
	>0.05	>0.05	>0.05
M vs. EM	Inconclusive	Inconclusive	Moist
	>0.05	>0.05	<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.

Figures



Figure 3: A) Concrete cages set-up B) Wooden cages set-up.

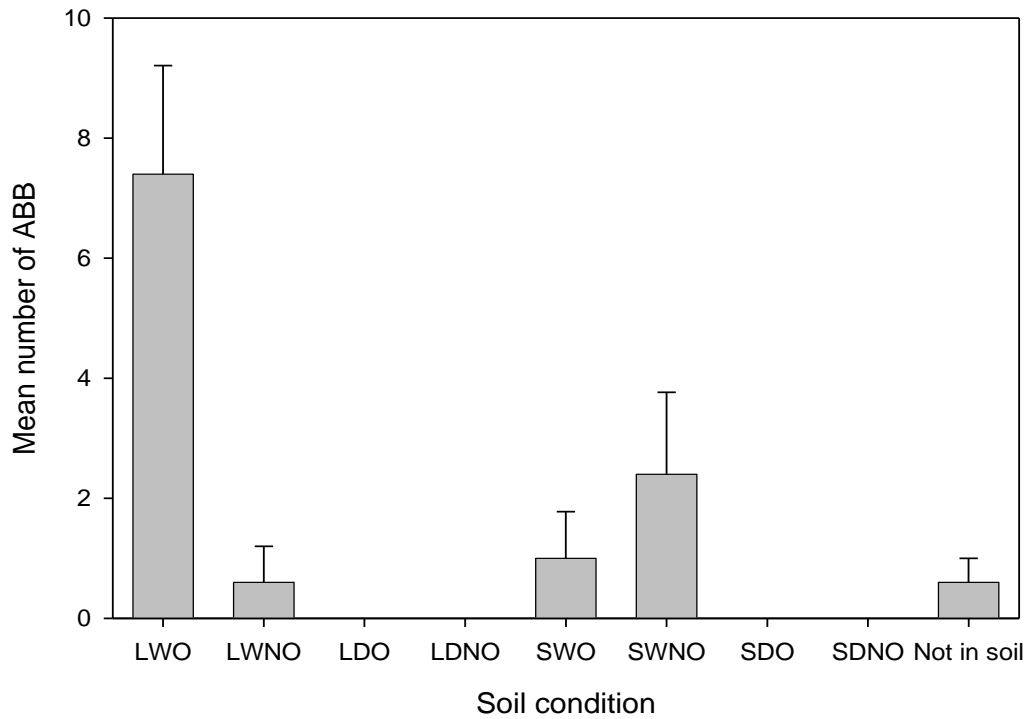


Figure 4: Soil preferences of the American burying beetle, *Nicrophorus americanus* (N= 1 tent, 5 replicates) LWO= Wet sandy loam with organic material, LWNO= Wet sandy loam without organic material, LDO= Dry sandy loam with organic material, LDNO = Dry sandy loam without organic material, SWO= Wet sand with organic material, SWNO= Wet sand without organic material, SDO= Dry sand with organic material, SDNO= Dry sand without organic material (mean \pm SE).

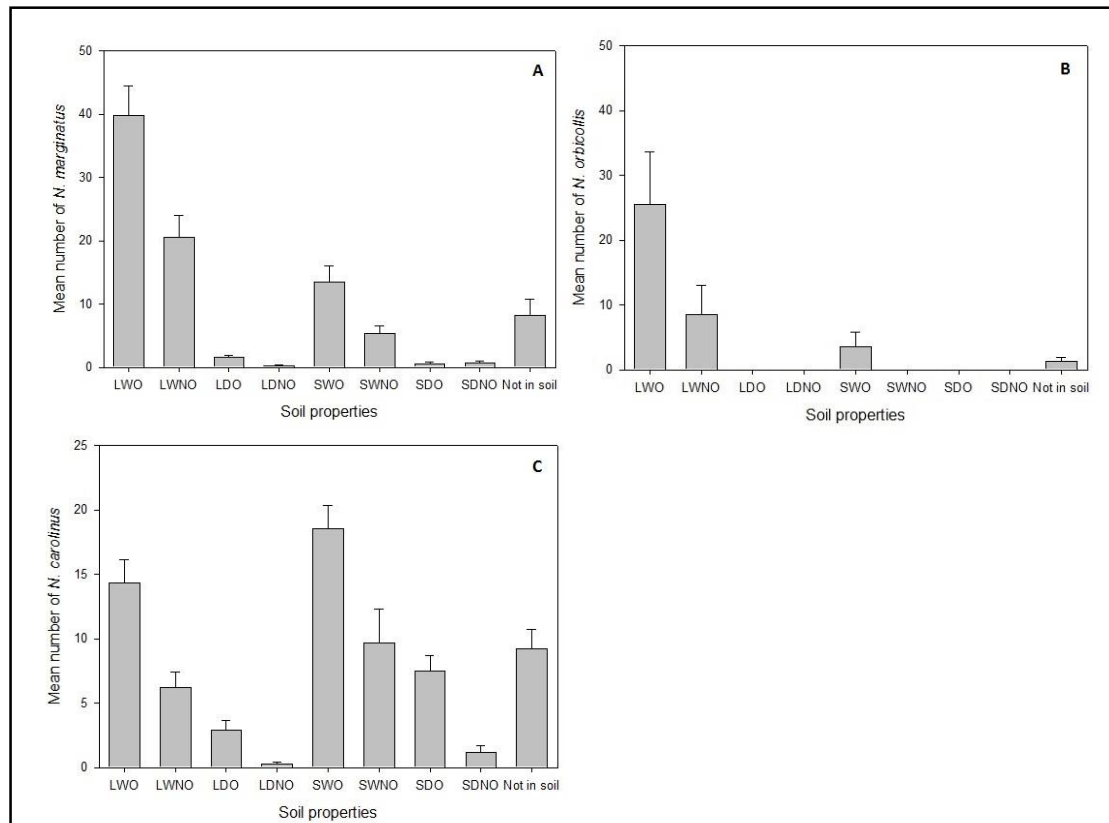


Figure 5: Soil preference of *N. marginatus* (A), *N. orbicollis* (B), and *N. carolinus* (C). Mean number of beetles differs between species, so the graphs cannot be directly compared. LWO= Wet sandy loam with organic material, LWNO= Wet sandy loam without organic material, LDO= Dry sandy loam with 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 \pm SE).

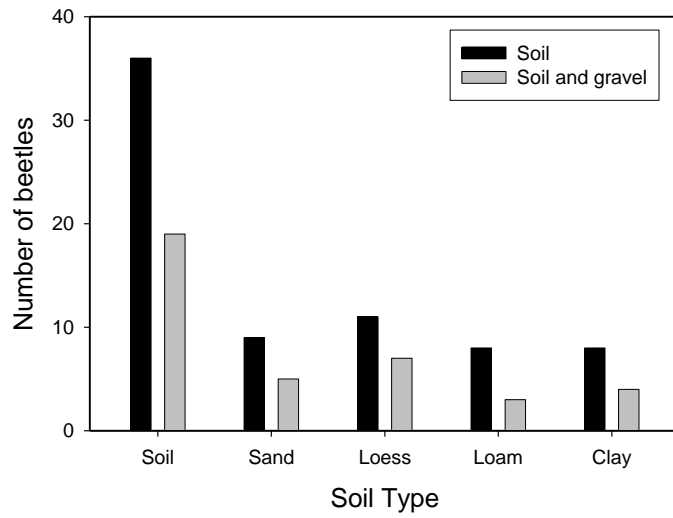


Figure 6: Choice of *N. orbicollis* for soil types without or containing gravel.

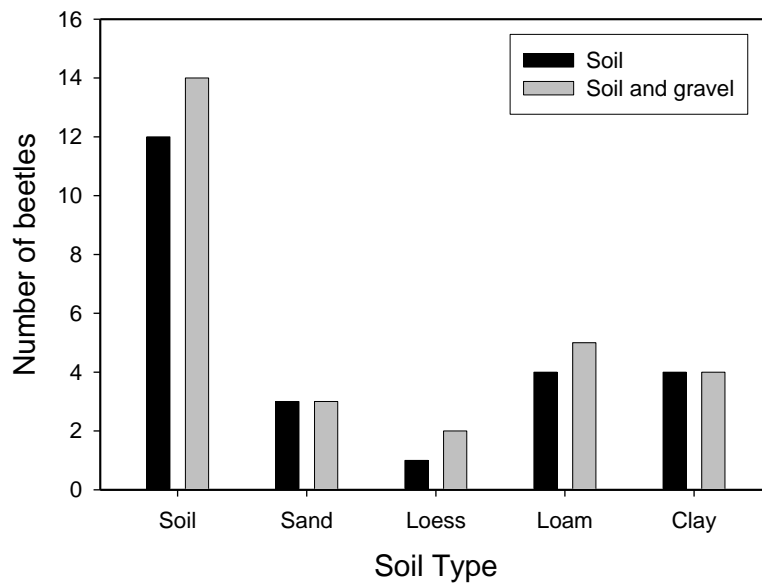


Figure 7: Choice of *N. americanus* with soil types without or containing gravel.

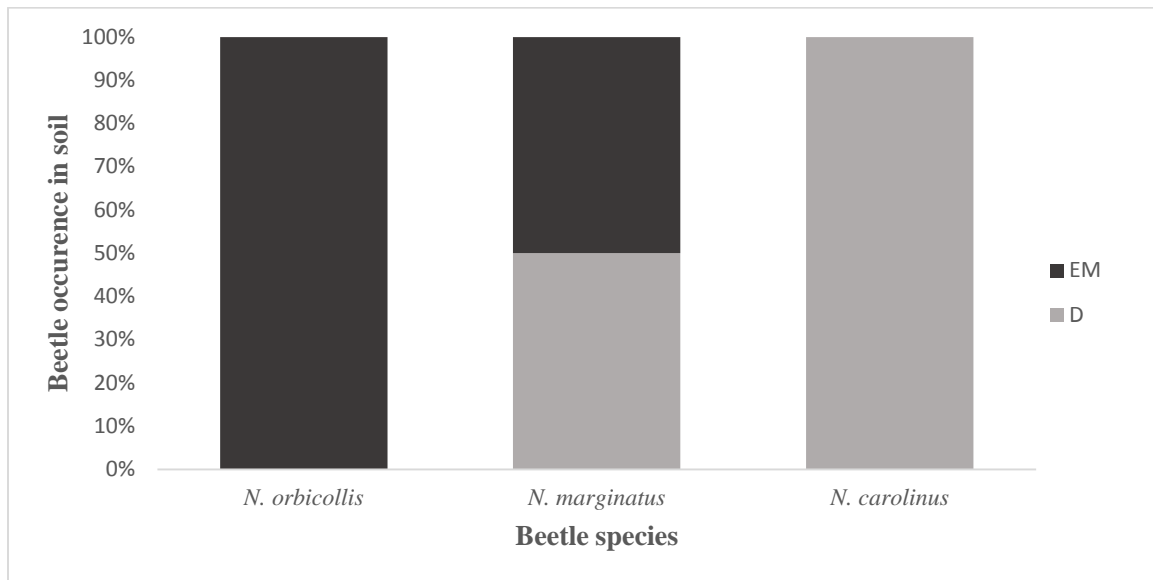


Figure 8: *N. orbicollis*, *N. marginatus* and *N. carolinus* occurrence in dry (=D) and extremely moist (=EM) soil. D has a wfv of 0.02, while EM has a wfv of 0.40.

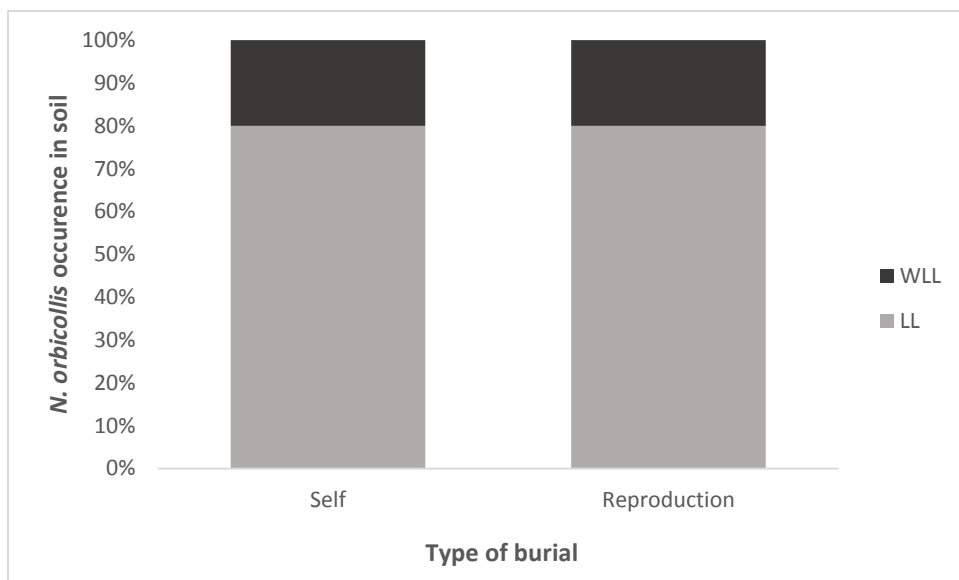


Figure 9: Effect of leaf litter on *N. orbicollis* burial. WLL= Without leaf litter, LL= Leaf litter. Self- burial= When not reproducing (N= 10). Reproducing= While burying a carcass for reproduction (N= 5).

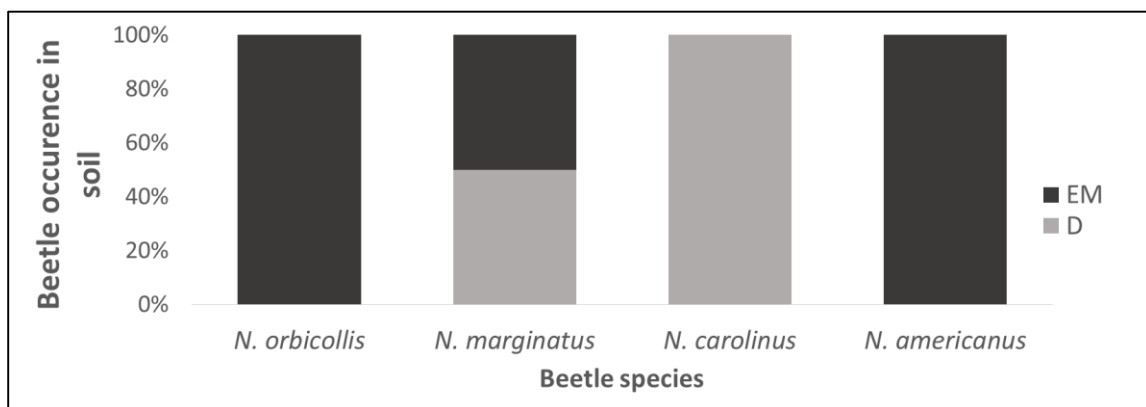


Figure 10: Effect of grass coverage on *N. orbicollis*, *N. marginatus*, *N. carolinus*, and *N. americanus* burial. N= 10 for each species.

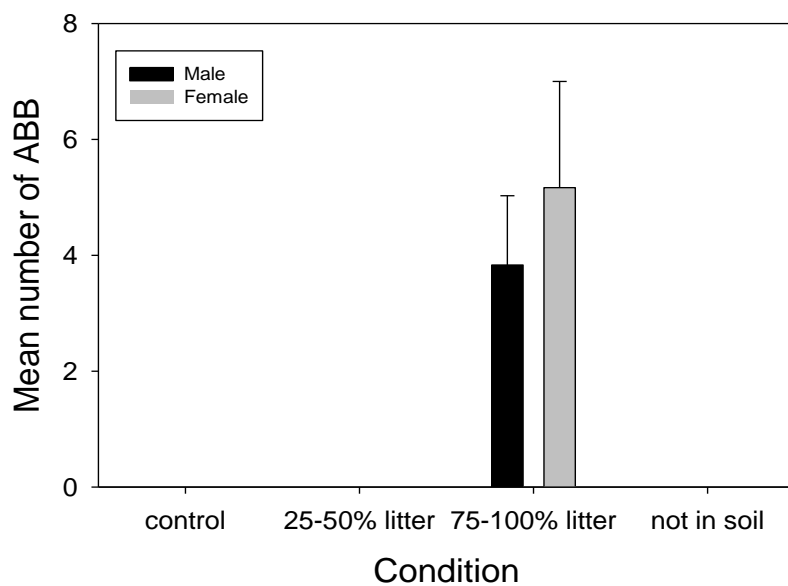


Figure 11: Response of *Nicrophorus americanus* found in sandy loam soil with different percentages of cut vegetation.

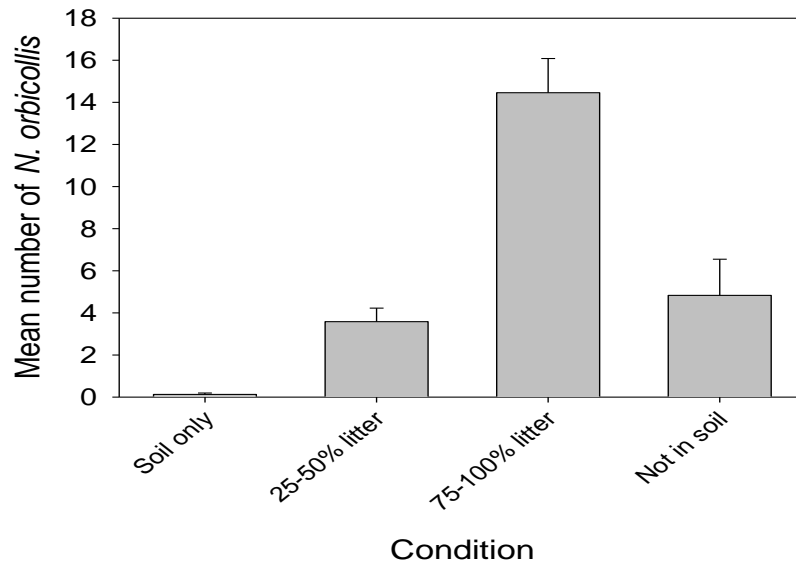


Figure 12: The amount of *Nicrophorus orbicollis* found in loam soil with different percentages of cut vegetation.

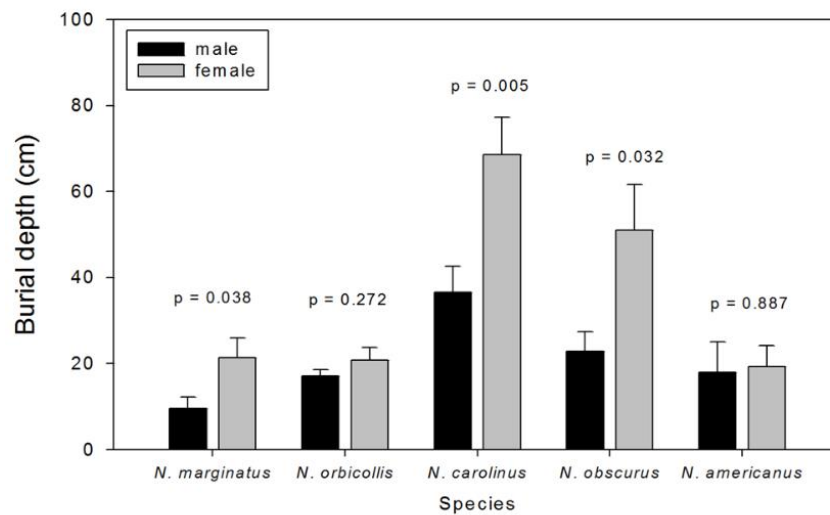


Figure 13: Mean burial depth (cm) of both sexes of carrion beetle species in moist sandy loam soil.

Chapter three:

RESPONSE OF BURYING BEETLES (NICROPHORUS SP.) TO SOIL
COMPACTION

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W. Wyatt Hoback

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 $<3.0 \text{ kg/ cm}^2$ 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/cm²) 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/cm² (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 (Figure 14). 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).

Compaction preference

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/cm² 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 was 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. 10

N. marginatus beetles 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 about 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 meter between each sample (Figure 17).

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 8).

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 9). 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 17).

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 8). 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 9).

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 10).

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/cm² in both silt loam and sandy loam soils while *N. marginatus* was able to dig into silt loam at 1.75 kg/cm² and 2.25 kg/cm² in sandy loam. The larger *N. carolinus* could dig into

silt loam soil compacted to 3.5 kg/cm² and sandy loam soil compacted to 4 kg/cm². 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/cm². All other beetles were found immobilized but alive. An alteration was made to the experiment by putting a small container (\pm 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/cm² 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/cm². For *N. orbicollis* an individual died at 3.0 kg/cm² in sandy loam soil, but survived at 3.5 kg/cm². One beetle died and one survived at compaction level, both beetles survived at compaction level 3.5 kg/cm², and all tested beetles died at compaction levels of 4 and higher (Table 11).

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 (± 1 SE) soil compaction before parking the truck on the bags was 1.02 ± 0.06 kg/cm² and the average soil compaction after the truck was removed was 1.98 ± 0.08 kg/cm² which was a significant increase (Students t-test; $P < 0.01$) (Table 12, Appendix). 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/cm²) and end of the trials (2.6 ± 0.05 kg/cm²) with *N. carolinus* (Table 16) compared to *N. marginatus* ($p < 0.05$). The size of the beetles did not affect survival ($p > 0.05$) (Table 12, Appendix).

Driving tests

Ford F-150

The mean (\pm SD) soil compaction level of the soil before a truck was driven over it was 0.84 ± 0.44 kg/cm². After the Ford F-150 passed over the soil the compaction significantly increased to 1.14 kg/cm² (Table 12, Appendix).

For *Nicrophorus marginatus* the mean mortality rate for males was $1.6\% \pm 0.76$ and $1.0\% \pm 0.67$ 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, Appendix).

For the second set of experiments the sand had an initial compaction level of $0.03 \pm 0.008 \text{ kg/cm}^2$ which increased to $0.120 \pm 0.008 \text{ kg/cm}^2$ after the truck was driven onto the container (Table 23). 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 $1.13 \pm 0.14 \text{ g/cm}^3$ before the NPPD truck was driven across and compaction increased to $1.29 \pm 0.17 \text{ g/cm}^3$ which was not significant. Prior to compaction the sandy loam soil was $1.39 \pm 0.06 \text{ g/cm}^3$ and increased to $1.45 \pm 0.04 \text{ g/cm}^3$ afterwards which was not a significant change (Table 12, Appendix).

Cattle trampling

Samples taken from the area outside of the cattle field had a mean bulk density of $1.14 \pm 0.02 \text{ g/cm}^3$. The samples on the cow path had a significantly greater bulk density of $1.60 \pm 0.03 \text{ g/cm}^3$. The samples taken from the pasture showed that cattle had impacted soil compaction and had a mean bulk density of $1.54 \pm 0.06 \text{ g/cm}^3$ (Figure 17).

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 trials 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 10). 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. orbicollis* 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/cm² 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; Defosse & 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/cm² and *N. carolinus* can dig into soils compacted to 4 kg/cm².

In laboratory trials beetles were immobilized at compaction level 0.25 kg/cm² 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 ($>4.5 \text{ kg/cm}^2$). 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 ($\pm 22\text{cm}$ 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 3 kg/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.

In the paper written by Lull et al. (1959) 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/cm², while a tractor of 18405 kg with a standard track and standard track will result in a ground pressure of 0.62 kg/cm² (Table 2). Agricultural tractors had an average ground pressure of 1.4 kg/cm². When comparing the probability of direct mortality (> 3kg/cm²) 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/cm². *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).

I have noticed an interesting relationship when I compared the historical range of *N. americanus* to the 100th meridian (Figure 18) and noticed that they almost exactly overlap. The 100th meridian is a longitudinal line that divides North America into two halves. It goes through North-Dakota, South-Dakota, Nebraska, Kansas, Oklahoma, all the way through Texas. The east side of the line has more average precipitation than the west side (Borchert, 1950). It is interesting that *N. americanus*, which has a strong preference for moist soil, almost exclusively occurred on the east side of the 100th meridian and now is mostly found near the border of the 100th meridian (U.S. FWS, 2004). Because of the difference in moisture the East side contains mostly cropland, while the West side contains more pasture land. As mentioned, *N. americanus* numbers decline near agricultural developments. This might be an explanation for the decline of *N. americanus* from the center of its range to the outside borders (Jurzenski et al., 2013). However, this phenomenon makes me wonder what will happen if climate change changes the moisture levels in the United States. A review study by Dai (2011) showed that most climate models project an increase in draught over most of the United States. The drought will be shifting upwards so I predict that *Nicrophorus* beetles will have to move to the North in order to survive. If the barriers are too big and the beetles can not cross them to move towards wetter habitat, I predict that a lot of *Nicrophorus* species will decline in numbers and struggle. I also predict that *Nicrophorus carolinus* is most likely to stabilize or adjust to climate change in the United States of America.

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 8. 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									
	Soil Type								
	Sand			Sandy loam			Silt loam		
	L	C	NB	L	C	NB	L	C	NB
<i>N. orbicollis</i>	100	0	0	100	0	0	100	0	0
<i>N. marginatus</i>	90	0	10	100	0	0	100	0	0
<i>N. carolinus</i>	90	10	0	90	0	10	100	0	0

Reproductive burial									
	Soil Type								
	Sand			Sandy loam			Silt loam		
	L	C	NB	L	C	NB	L	C	NB
<i>N. orbicollis</i>	90	10	0	-	-	-	100	0	0
<i>N. marginatus</i>	60	20	20	60	40	0	80	20	0
<i>N. carolinus</i>	80	20	0	40	40	20	60	40	0

Table 9. 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 10: 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 11: Compaction limitations of *N. orbicollis*, *N. carolinus* and *N. marginatus* in silt loam and sandy loam soil. Units are given in kg/cm² and all error margins are 0.124 kg/cm². The penetrometer could only measure up to 4.5 kg/cm².

	Silt loam			Sandy loam		
	<i>N. orbicollis</i>	<i>N. carolinus</i>	<i>N. marginatus</i>	<i>N. orbicollis</i>	<i>N. carolinus</i>	<i>N. marginatus</i>
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/cm ²)	> 4.5	> 4.5	> 4.5	3	> 4.5	> 4.5

Table 12: Summarizing table 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 <i>N.</i> <i>marginatus</i>	Parked Field <i>N.</i> <i>carolinus</i>	Driving Field <i>N.</i> <i>marginatus</i>	Driving Field <i>N.</i> <i>carolinus</i>	Ramp Sand <i>N.</i> <i>marginatus</i>	Ramp Sandy loam <i>N. carolinus</i>
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

Figures



Figure 14: The metal cylinder used to collect soil samples to measure bulk density. A penny was included to provide a point of reference. Although outside might look uneven, the inside measurements were exactly the same at several measurement points, providing a volume with a small error margin. Dents are present because of hammer use.

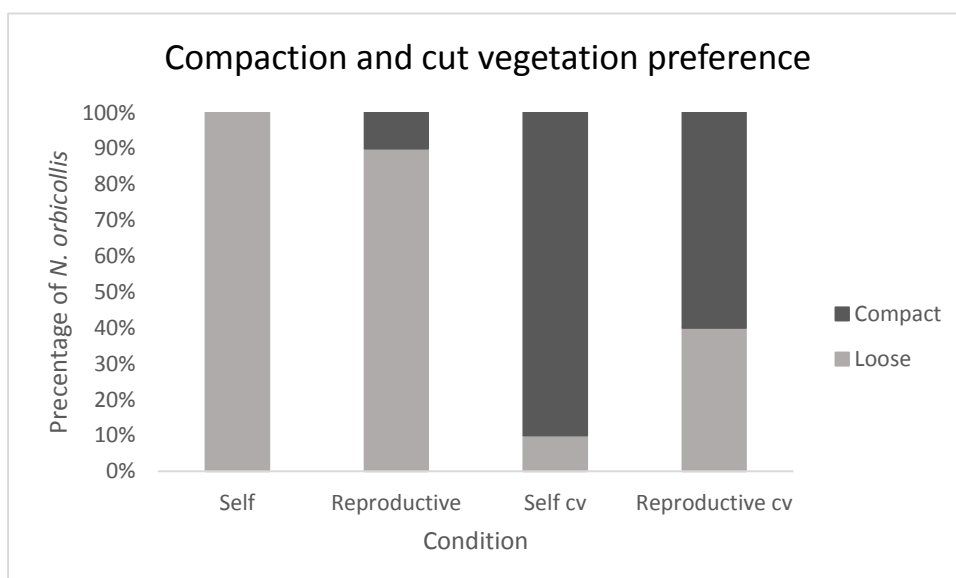


Fig 15: The effect of cut vegetation on compaction preference.

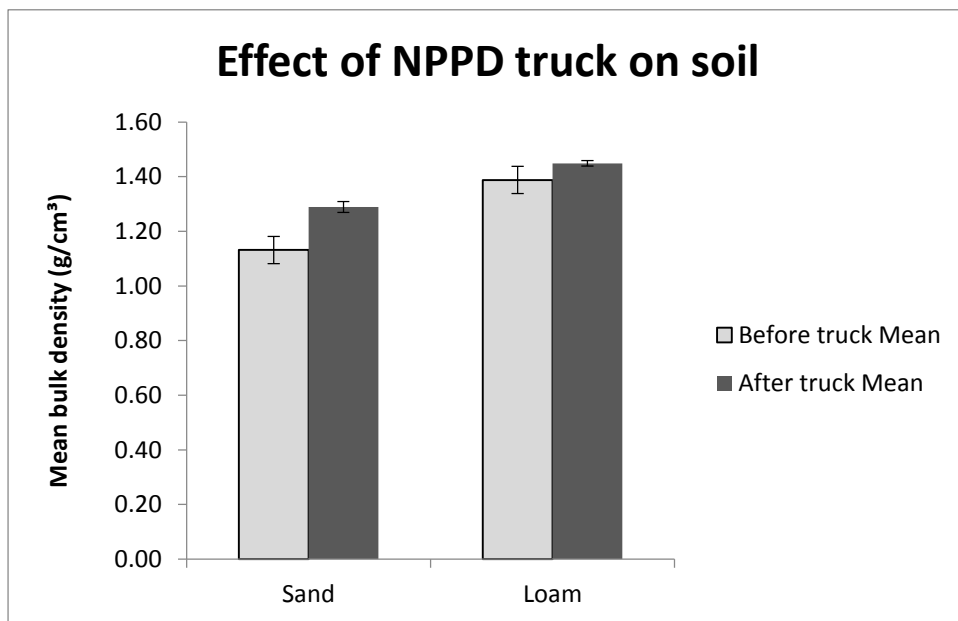


Figure 16: The mean bulk density (g/cm³) of sand and sandy loam soil before and after a NPPD truck (30,000 kg) drove over it.

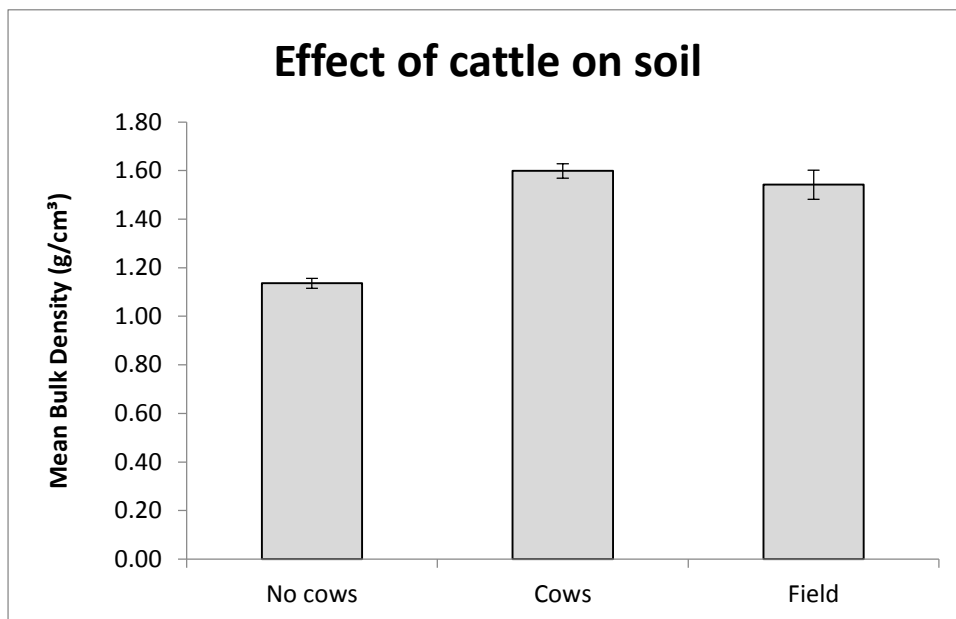


Figure 17: The effect of cattle on the mean bulk density (g/cm³) of soil. Measurements from an area without cows (No cows), a cow path (Cows) and a field containing cows (Field).

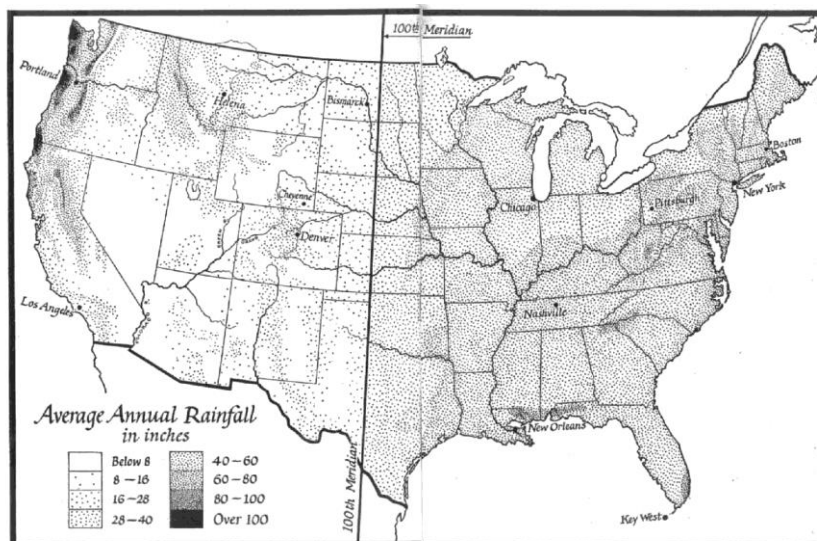


Figure 18: Average annual rainfall in the United States and the 100th meridian (Source: <https://greatplainstrail.files.wordpress.com/2011/11/rain.jpg>).

Chapter four

MANAGEMENT IMPLICATIONS FOR *NICROPHORUS* FROM RESULTS OF SOIL PREFERENCE TESTS AND COMPACTION

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Management implications

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 4 kg/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 ($>4.5 \text{ kg/cm}^2$) 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 $>3.0 \text{ kg/cm}^2$, which is greater than compaction generated from travel. Beetles were more likely to be crushed by instant compaction when close to the surface ($<10 \text{ cm}$) 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/cm², 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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Table: Moisture preferences of *N. orbicollis*, *N. marginatus*, and *N. carolinus* in different soil types.

	<i>N. orbicollis</i>			<i>N. marginatus</i>			<i>N. carolinus</i>		
	Silt loam	Sandy loam	Sand	Silt loam	Sandy loam	Sand	Silt loam	Sandy loam	Sand
D vs. M		0 – 10	0 – 10		2 – 7			2 – 8	
D vs. EM		0 – 10			5 – 5			10 – 0	
M vs. VM	0 – 10		4 – 6 3 - 6 8 – 1	6 – 4			7 – 3		4 - 6
M vs. EM	0 – 10	9 – 0		7 – 2			6 – 4	10 – 0	

Table: The average soil compaction (kg/cm²) before and after each of the five parked vehicle trials with *N. marginatus*, along with the standard errors (p<0.01).

Trial	Average soil compaction before (kg/cm ²)	Average soil compaction after (kg/cm ²)
1	0.75	1.41
2	0.5	2.01
3	1.14	2.22
4	1.12	1.96
5	1.57	2.31
Average \pm SE ^a	1.02 \pm 0.06	1.98 \pm 0.08

^a SE= Standard Error

Table: The percent survival of male and female *N. marginatus* during each of the parked compaction trials, along with the averages and standard deviations.

Trial	Percent Survival of Males for Parked Compaction	Percent Survival of Females for Parked Compaction
1	75	80
2	60	55
3	80	85
4	95	95
5	60	60
Average \pm SE ^a	74.00 \pm 6.60	75.00 \pm 7.58

^a SE= Standard Deviation.

Table: The average soil compaction (kg/cm²) before and after each of the three parked vehicle trials with *N. carolinus*, along with the standard errors (p<0.01).

Trial	Average Soil Compaction Before (kg/cm ²)	Average Soil Compaction After (kg/cm ²)
1	1.85	2.73
2	1.45	2.46
3	1.68	2.54
Average \pm SE ^a	1.66 \pm 0.06	2.58 \pm 0.05

^a SE= Standard error.

Table: The percent survival of male and female *N. carolinus* during each of the parked compaction trials, along with the averages and standard deviations.

Trial	Percent survival of males for parked compaction	Percent survival of females for parked compaction
1	80	90
2	90	100
3	90	85
4	80	75
5	85	80
Average ± SE ^a	85.00 ± 2.24	86.00 ± 4.30

Table: Percentage of beetles that escaped and died after a Ford F-150 drove over sand containing *N. marginatus* and sandy loam containing *N. carolinus*.

	Percentage beetles escaped (%)	Percentage beetles died (%)
Sand	90	10
Sandy loam	55	47.5

^a SE= Standard error

Table: The average soil compaction level before and after a truck drove over the soil.

	Before truck	After truck
Average soil compaction (kg/cm ²) ± SE*	0.84 ± 0.10	1.14 ± 0.07

* SE= Standard error

Table: Percent survival of male and female *N. marginatus* beetles after a Ford F-150 truck drove over soil containing them.

Mean of mortality (%) \pm SD		
	<i>N. marginatus</i>	<i>N. carolinus</i>
Male	1.56 \pm 0.764	3.67 \pm 0.953
Female	1.02 \pm 0.668	0.88 \pm 0.587

Table: Percentage of mortality for *Nicrophorus marginatus* and *Nicrophorus carolinus* \pm standard deviation.

<i>Nicrophorus carolinus</i>	Pronotum (mm)	Weight (g)
Dead Male	6.811 \pm 0.323	0.74 \pm 0.0936
Dead Female	6.683 \pm 0.0869	0.69 \pm 0.0738
Alive Male	7.005 \pm 0.0652	0.766 \pm 0.0242
Alive Female	7.113 \pm 0.0547	0.776 \pm 0.0159

Table: Results from ramp experiment. Sand and sandy loam compaction level before and after a Ford F-150 drove over it.

	Sand (kg/cm ²) \pm SE*	Sandy loam (kg/cm ²) \pm SE*
Before truck	0.030 \pm 0.008	0 \pm 0.008
After truck	0.120 \pm 0.008	0.160 \pm 0.008
Compaction increase	0.090 \pm 0.016	0.160 \pm 0.016

* SE= Standard error

Table: Sand and sandy loam compaction level before and after a NPPD truck drove over it.

		Sand (g/cm ³)	Sandy loam (g/cm ³)
Before truck	Mean	1.13	1.39
	SE*	0.05	0.02
After truck	Mean	1.29	1.45
	SE*	0.05	0.01

* SE=Standard error



Figure: Process of catching *Nicrophorus* beetles. 1) Dig hole 2) Place bucket with dead rat and moist soil 3) Cover up but leave room for beetles to crawl into bucket 4) Collect beetles.