NDOR American Burying Beetle Research Final Report

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Introduction

The American burying beetle, (*Nicrophorus americanus*) is a member of the carrion beetle family Silphidae, an important group of detritivores that recycle decaying materials into the ecosystem. The American burying beetle is the largest carrion-feeding insect in North America reaching a length of about 4 centimeters and a weight of 3 grams. Although it has historically been recorded from at least 150 counties in 35 states in the eastern and central United States, it declined from the 1920s to the 1960s and is currently only found at the peripheries of its former range. In 1983 the American burying beetle was included as an endangered species in the Invertebrate Red Book published by the International Union for the Conservation of Nature. In the United States, it was placed on the state and federal endangered species lists in August, 1989.

Considering the broad geographic range formerly occupied by the American burying beetle, 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. Unlike other members of the *Nicrophorus* genus, no strong correlation with soil type or land use seems to exist (Bishop et al. 2002; Bishop and Hoback unpublished), however, adequate soil moisture levels appear critical (Hoback et al. unpublished). Within its remaining range in Nebraska, there is a large population (>500 individuals) in the southern loess hills and another significantly larger population in northern Nebraska and southern South Dakota (Hoback and Snethen unpublished).

Like other carrion beetles, American burying beetles search the environment for fresh carcasses which they use for feeding and rearing of offspring. Because carrion is a typically limited resource, the discovery of a carcass often occurs within two days, but has been reported to occur as quickly as 35 minutes post death (Ratcliffe 1996). Usually, multiple individuals comprising several species discover the carcass. As the beetles arrive at the carcass a fierce competition erupts. This competition can lead to damage to beetles including loss of legs, antennae, and even mortality (Bedick et al. 1999).

If the carcass is fresh and is of appropriate size, competition ensues until there is only a single beetle pair occupying the carcass. This pair is generally the largest individuals of the largest species that discovered the carcass with the other beetles either being driven away or killed by the victorious pair (Wilson et al 1984). The victorious pair will then work cooperatively to quickly entomb the acquired carcass. This behavior seems to have evolved out of necessity to remove the carcass from the realm of discovery by other invertebrate necrephores as well as vertebrate scavengers. Studies have demonstrated that there is an intense competition between flies and ants for the resources present in the carcass (Scott 1998). If flies discover and reproduce on the carcass prior to Nicrophorinae beetles, the developing dipteran larvae can quickly consume all the nutrients within the carcass effectively eliminating the carcass as a reproductive resource for the beetles. If discovered by ants adult beetles must fend away the ants and sometimes become victims of aggressive ant colonies (Ratcliffe 1996).

After finding a suitable burial locality, the parental beetles will begin plowing under the carcass creating a compacted depression, that will become the final resting place for the carcass. As the carcass falls into the depression through the action of gravity, it is forced into a tight ball by the beetles. The carcass is further molded into a tight ball as the beetles move over the carcass and remove the fur or feathers from the carcass (Ratcliffe 1996).

With the federal listing of the American burying beetle in 1989, a recovery plan was created and a trapping protocol was established to allow surveys for this beetle. However, the protocol was created based on information concerning the beetle in Connecticut where the population is restricted to an island. Bedick et al. 1999 and 2004 reviewed trapping protocols and found that small cup traps where beetles do not have access to bait increases contact between beetles in the Genus *Nicrophorus* and frequently results in mortality. Thus, Nebraska has adopted a modified protocol where larger traps (5 gallon buckets), and open bait (previously rotted laboratory rat carcasses) are used (Hoback 2007).

As research and surveying has expanded the known range of the American burying beetle, methods to prevent harm to beetle populations (conservation measures) have been developed. In areas which will be disturbed through construction, the U.S. Fish and Wildlife Service advocates either "bait-away" or "trap-and-relocate" procedures.

Because "bait-away" is designed to attract beetles to an area away from construction, it does not require a Federal permit and is conducted by placing whole carrion onto a structure some distance from the impacted habitat. The carrion is monitored and replaced frequently. However, this protocol impacts beetles including the American burying beetle by attracting them to a bait which cannot be used as a breeding material and by increasing the competition among attracted beetles. In 2006, mortality exceeding 30% was observed in association with field bait-away stations in Nebraska (Snethen and Hoback unpublished).

The alternative to "bait-away" is "trap-and-relocate". This protocol is used extensively by the Oklahoma Department of Transportation (M. Pajoh pers. comm.) and seeks to remove American burying beetles from potentially impacted areas. Attracted beetles are collected and moved a distance (usually greater than two miles). Several factors are currently unknown which would aid in implementing conservation measures. First, the microhabitat requirements for beetles when they are in the environment during periods of inactivity are unknown because technology to track the beetles is not yet available. Second, the effect of rocks and gravels on beetle burial during periods of inactivity is unknown.

We performed a series of laboratory experiments and field measures to assess soil conditions selected by beetles during periods of inactivity. We also measured soil characteristics from four types of soil in central Nebraska.

Materials and Methods

Laboratory: Soil Moisture

Three enclosed tents (8 wide x 10 long x 6.5 tall) were used inside of a research building maintained at 75 degrees F. The windows of the building were completely covered to exclude natural light. Overhead florescent lights were turned on at normal daylight hours. Eight kitty litter boxes (1 foot and 4 ½ inches length, 1 foot wide and 5 inches deep) were placed in each tent and were secured against the back wall of the tent by industrial strength, double sided velcro. The rough side was used to secure the sides of the kitty litter boxes and the back of them to the tent. The soft side of the velcro was used to secure a cardboard ramp to the front of the kitty litter boxes. The cardboard ramp was placed at a 22 degree angle to allow the burying beetles to reach the soil. The sides and bottom of the ramp were also secured to the tent.

The containers were filled with 5 gallons of either loam or sand in a randomized complete block design. There were 8 experimental conditions with one per container. 4 containers of soil received water until saturation. The remaining four containers remained dry. Soil moisture was measured using a Fieldscout TDR 300 meter (Spectrum Technologies) and moisture was adjusted prior to each experiment. The average soil moistures were 11 % for the wet sand and 1.5 % for the dry sand. Soil moisture was an average of 30% for wet loam and 5% for dry loam. One half of each soil moisture condition received the addition of deciduous leaves on the surface of the soil. We tested 100 *N. marginatus* and 70 *N. carolinus* in each tent for a total of 4 days. Because these beetles are day active, their distribution was assessed each day between 01:00 and 04:00. Next we tested 40 *N. orbicollis* in each tent for 4 days. Finally we tested *N. americanus*. Because of permit regulations, we used 10 *N. americanus* in one tent for each trial, and used the same beetle no more than twice, returning tested beetles to the field site near O'Neil Nebraska and bringing new beetles back to the laboratory.

Laboratory Study: Effect of Gravel

There are no known studies regarding carrion beetle choice during periods of inactivity for soil containing various amounts of gravel. To study this, we devised an laboratory study using 2.5 gallon fish tanks (N = 16). We divided each tank in half by placing a piece of plastic lawn edging in the center. Each tank had only one type of soil added. Soil without gravel was added to one side of all the tanks. The soil with the gravel was added to the other. The plastic divider was level with the soil.

Within sets of these tanks, four different types of soil were used (loam, loess, clay, and sand). The four different soils types were sifted to ensure uniformity of particle size prior to placing it in the tanks and any particles larger than 0.5 mm were discarded. The soils were checked for moisture content and were made uniform among tanks of each soil type. The moisture levels used were 28-30% for loam, 24-26% for loess, 7-9% for sand, and 32-34% for clay. For tests, we mixed roadside gravel (1-6 cm diameter) into half of each soil type.

We conducted 12 trials for *N. orbicollis* and *N. americanus*. For the trials we collected beetles from the field and brought them into the lab. They were stored in 5 gallon buckets with moist soil, fresh air, and raw ground beef. Each night we used 16 different beetles. Only one was placed in a tank at a time and trials were started at 20:00. The beetles were placed directly in the middle of the tank parallel to the center divider. They were then checked the next morning to determine preference for soil with or without gravel. All beetles were used only once. A new beetle was then placed in the tank and the process was repeated. A total of 192 *N. orbicollis* 48 *N. americanus* were tested.

Field Measures of Roadside Characteristics

Three sites were selected for monitoring moisture and soil composition under different soil conditions. An area predominated by loam soil was measured near Gibbon Nebraska (40 45'31.49'N 98 51'18.08'W), a sandy area was monitored west of Arnold (41 25'26.88'N 100 16'13.84'W) and a loess soil dominated area south of Gothenberg (40 45'28.44 'N 100 10'24.59'W). These sites were chosen based on soil characteristics to represent loam, sandy, and loess soils respectively. None of the roadsides were in areas that had been recently disturbed by construction activities.

Each site was visited once per month between June and August. For each site in June and July, moisture was measured gravimetrically in the laboratory. Soil samples were collected in and then sealed. Upon arrival at the laboratory, soil samples were weighed. They were then dried for 48 hours at 60 C and reweighed. In August, soil moisture was measured using the Fieldscout TDR 300 by Spectrum Technologies, Inc. Soil compaction was determined using a Dickey John Soil Compaction meter. At each site location (roadside, ditch and fence), 3 measures were made and then averaged. These readings were recorded in PSI (lbs per square inch)

Soil was collected from the edge of the road, from the ditch, and from the area close to the fence in four spots. The soil was transported to the laboratory for further analysis.

Laboratory Soil Determinations

Samples from the field were place in soil bags for drying and further analysis. Soil bags were placed in a drying oven for 48hrs at 110C to ensure no soil moisture. Large soil clods were then broken and a total dry weight was determined. Soil was then passed through a 4 mm sieve to determine gravel content and the percentage of gravel greater that 4 mm in size was then determined.

After gravel was removed, forty grams of dry soil was broken using a mortar and pestle. The hydrometer method was used (Laboratory Manual for Soil Science, 8th edition, Ed. Thien and Graveel) to determine particle size composition, soil type, and percent sand, silt, and clay.

Results

Laboratory: Soil Moisture

American burying beetles sought moist soils during periods of inactivity (Figure 1). Approximately 70% of tested beetles were found in moist loam soil with the vast majority also being found associated with leaf litter. Approximately 20% of tested beetles were found in wet sand with the majority being found not associated with leaf litter.

In comparison to American burying beetles, *Nicrophorus marginatus* was similar with the majority of individuals being found in moist soils (Figure 2). Again, loam was more attractive than sandy soils and the presence of leaf litter for both loam and sand increased its attractiveness.

Nicrophorus orbicollis which is active at night like American burying beetles was found primarily associated with moist loam (Figure 3). Interestingly, this species was found in concentrated groupings of up to 60 individuals.

In contrast to the other species tested, *Nicrophorus carolinus* was found in most soil types and did not strongly avoid drier soils (Figure 4). When found in drier soils, the presence of organic matter increased its occurrence. These results support the use of *N. carolinus* as an indicator of conditions that are less favorable to American burying beetles.

Laboratory Study: Effect of Gravel

Approximately equal numbers of American burying beetles were found in soil containing high percentages of gravel and soils without gravel regardless of the type of soil used (Figure 5). In both loam and loess, slightly more American burying beetles were found in soils containing gravel. The beetles were buried to a depth of approximately 6 to 8 cms.

In contrast to American burying beetles, *Nicrophorus orbicollis* were found more often in soil that did not have gravel (Figure 6). Under no soil condition were more beetles found in the soil containing gravel; however, differences were not significant.

Field Measures of Roadside Characteristics

Measures of roadside compaction were similar across soil types, ranging from around 50 to more than 300 psi. Depending on time of year, soil compaction was greater in the ditch or at the fence (figures 7-9). In June, areas closest to the roadway had the highest soil compaction measures for loess and sandy soils. Interestingly for both loam and loess, soil compaction steadily increased from the road shoulder to the fence in August when American burying beetles would be active. Potentially, the use of shoulder areas would be greater than use of areas further from the road in these soil types. The differences observed in soil compaction may be related to heterogeneity of the roadside areas or to experimental error associated with the Dickey John soil compaction meter. In the laboratory, drier soils measure at higher compaction readings and soil moisture may also in part explain the results.

Laboratory Soil Determinations

Percentage of gravel was similar among roadside soils ranging between 6 and 10% for loam and sandy soil respectively. As expected, the percentage of gravel in samples fell quickly from the roadside shoulder to the fence line. In loess soil, the percentage at the roadside was highest (22%) and for loam and sand it was similar (12.2 and 14.6 respectively).

Summary and Conclusions

Laboratory and field measures reveal that burying beetles including the American burying beetle seek moist soils during periods of inactivity. Presence of leaf litter increased the use of soils by some species but not others. Burying beetles have high water loss rates and likely seek moist microhabitats to avoid desiccation (Bedick et al. 2007). Conforming to observations in the field, *Nicrophorus carolinus* used dry soils more than other species studied. In paired choice tests, American burying beetles did not avoid any soil type based on the presence of gravel. Thus, earlier speculation that burying beetles will avoid graveled areas seems to be incorrect. Field measures revealed that soil compaction changes seasonally but is similar among soil types. Presence of gravel is not great enough to serve as a deterrent to beetles even at the road shoulder. Additional studies should be conducted to further pinpoint moisture requirements of beetles and to determine if gravel deters the use of road shoulders for carcass burial.

References

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Appendix

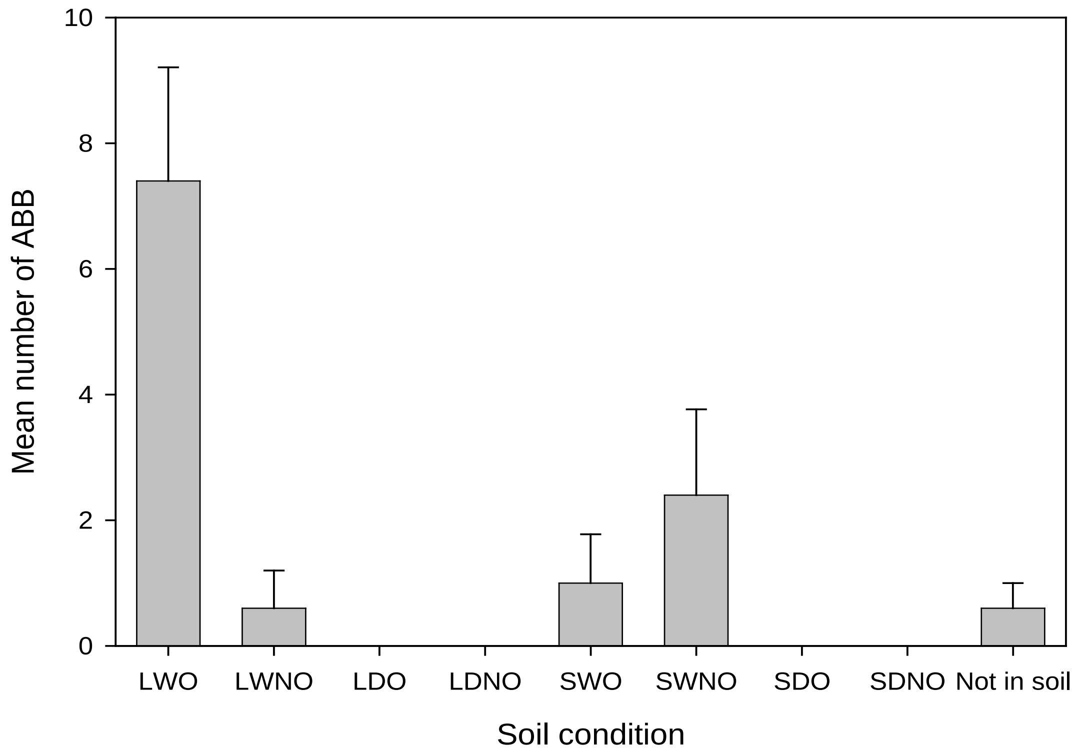
Tents were used to allow beetles to be active and to choose soils during periods of inactivity.

There were 8 experimental conditions. Beetles could choose between wet or dry loam, wet or dry sand and with or without organic matter.

Beetle choice of soils was determined by sifting all experimental soils.

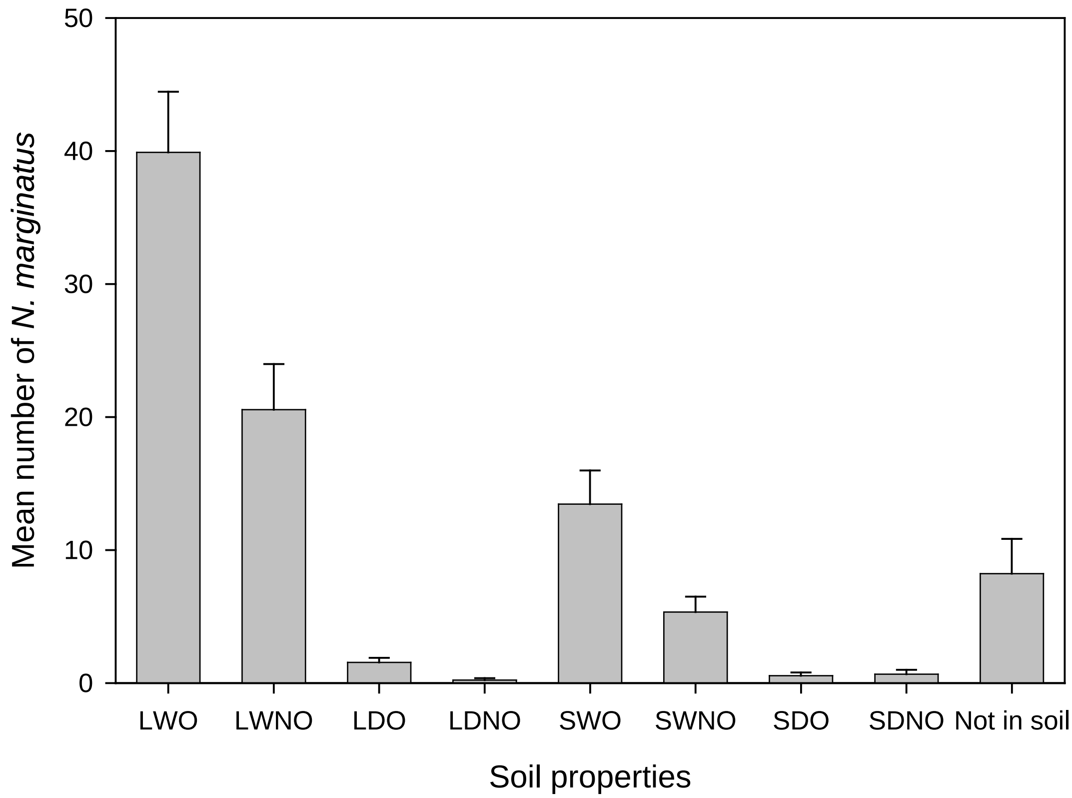

Tests to determine beetle avoidance of gravel substrate.

Figure 1. Mean ±1 S.E. number of American burying beetle, *Nicrophorus americanus* (N= 10 per trial, 5 trials).



LWO = Wet loam with organic material
LWNO = Wet loam without organic material
LDO = Dry loam with organic material
LDNO = Dry 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

Figure 2. Mean ±1 S.E. number of *N. marginatus* (N= 90 per trial, 10 trials).



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LDNO = Dry loam without organic material
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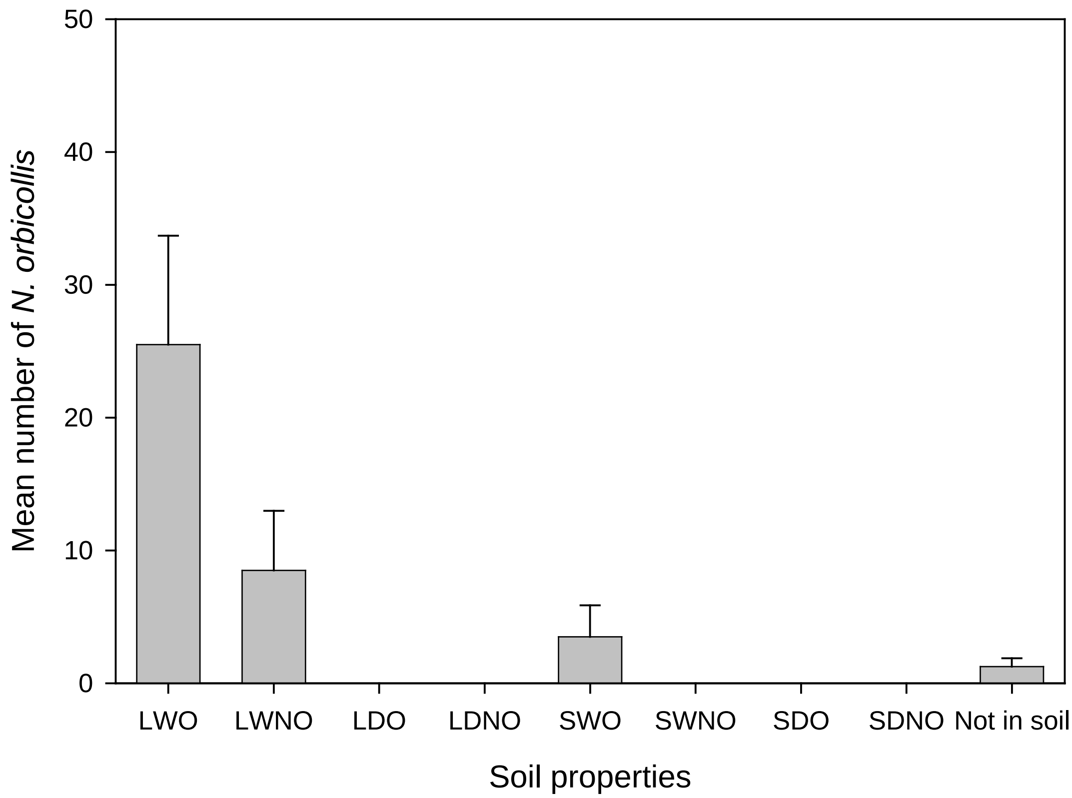
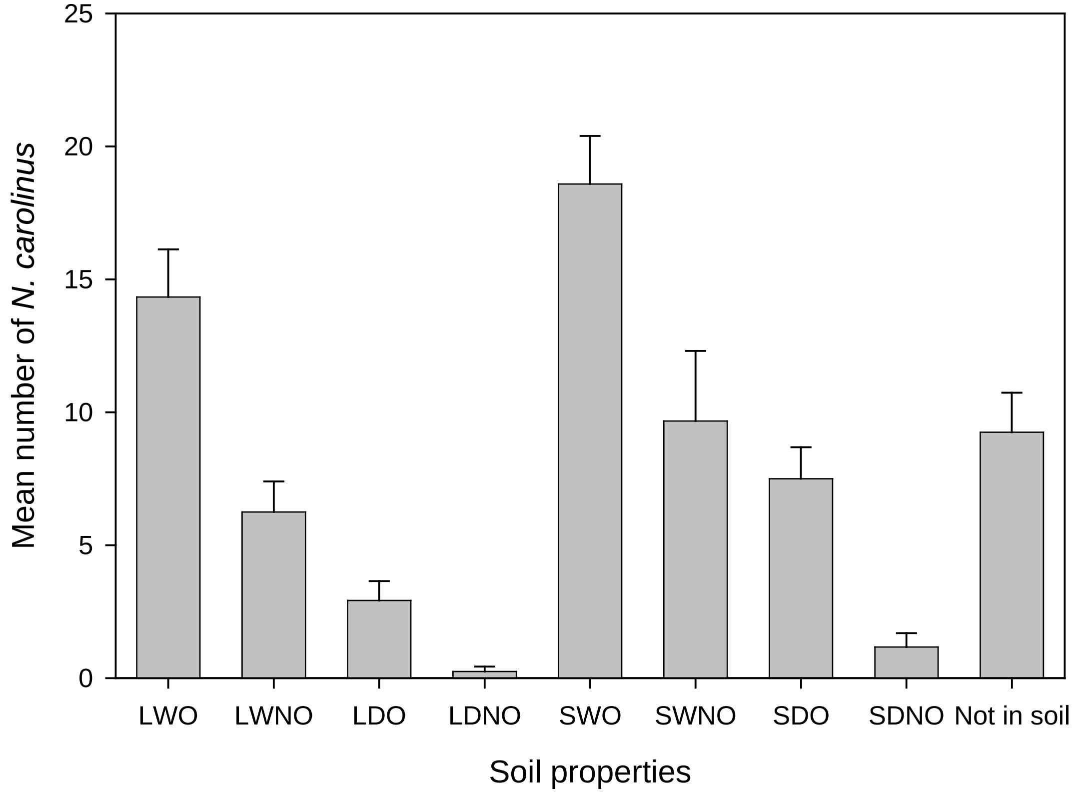
SWNO = Wet sand without organic material
SDO = Dry sand with organic material
SDNO = Dry sand without organic material

Figure 3. Mean $\pm$1 S.E. number of *Nicrophorus orbicollis* (N = 40 per trial, 5 trials).



LWO = Wet loam with organic material
LWNO = Wet loam without organic material
LDO = Dry loam with organic material
LDNO = Dry 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

Figure 4. Mean ±1 S.E. number of *Nicrophorus carolinus* (N = 70 per trial, 12 trials).



LWO = Wet loam with organic material
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LDNO = Dry 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

Figure 5. Effect of gravel on soil preference of American burying beetle.

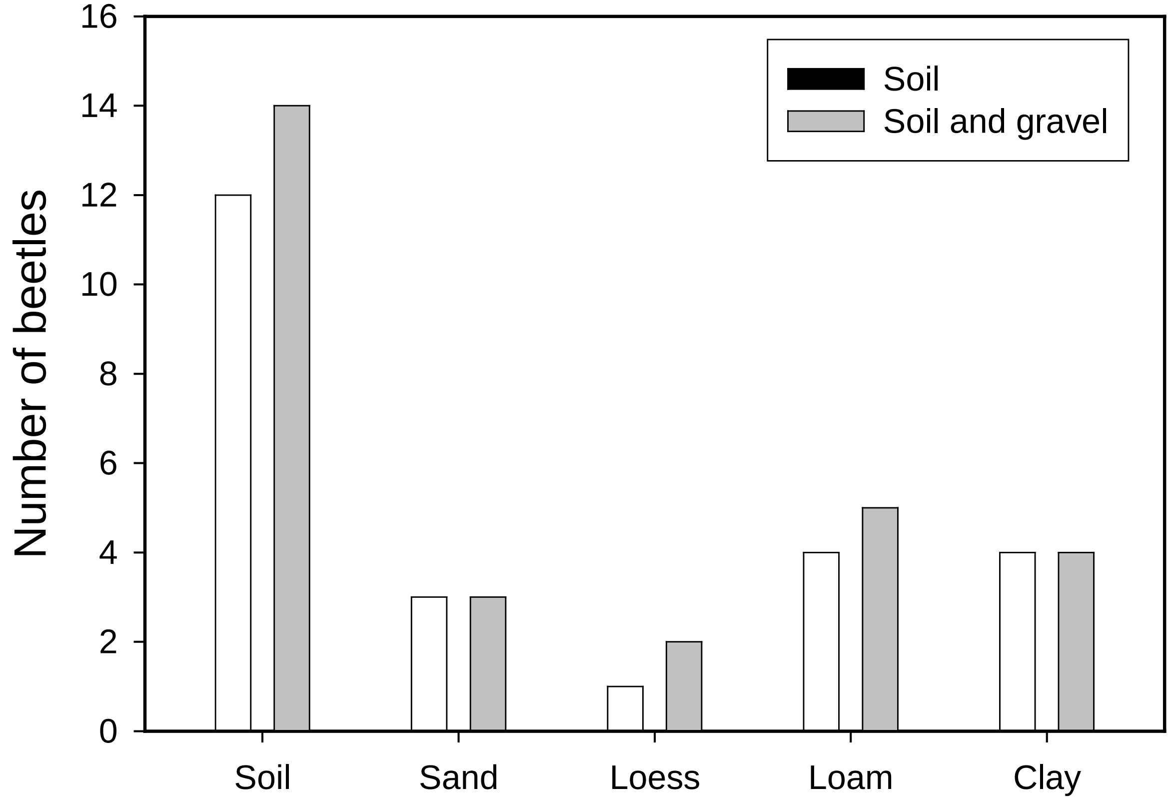

Figure 6. Effect of gravel on soil preference of *Nicrophorus orbicollis*.

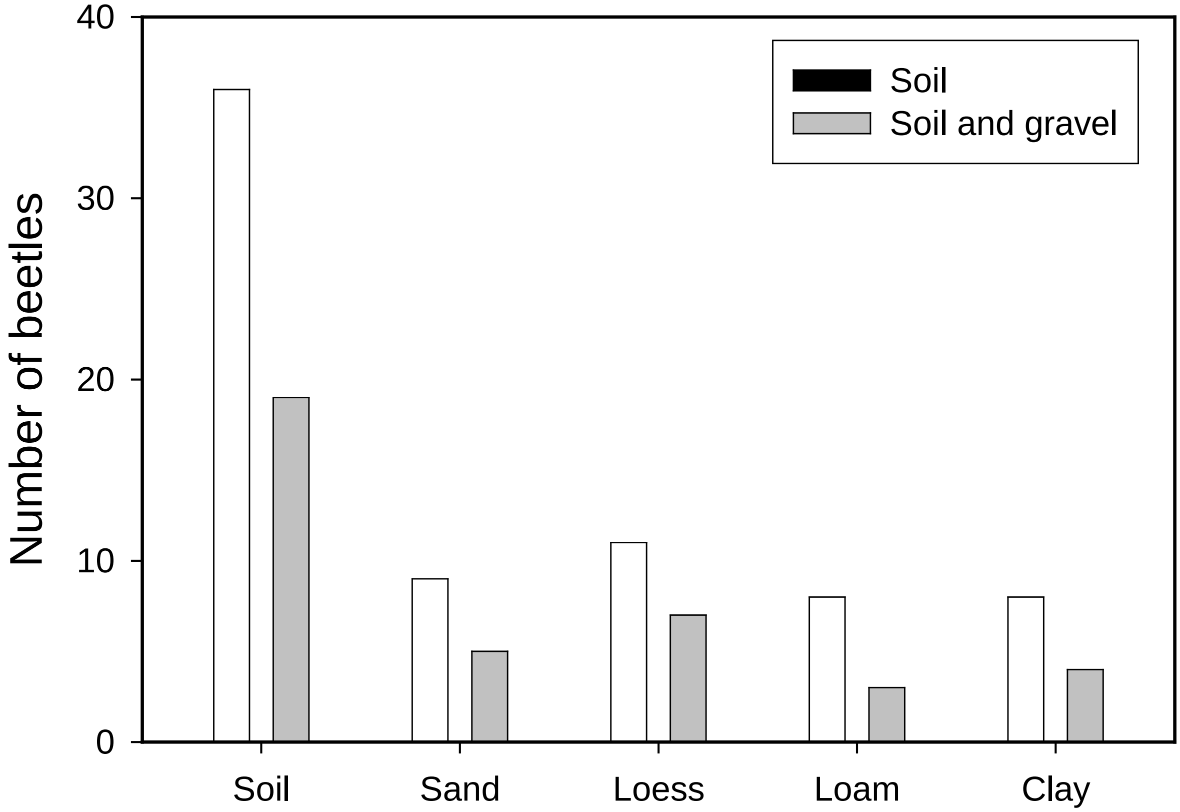

Figure 7. Mean + compaction of soil at the roadside, ditch, and fence areas for loam soil.

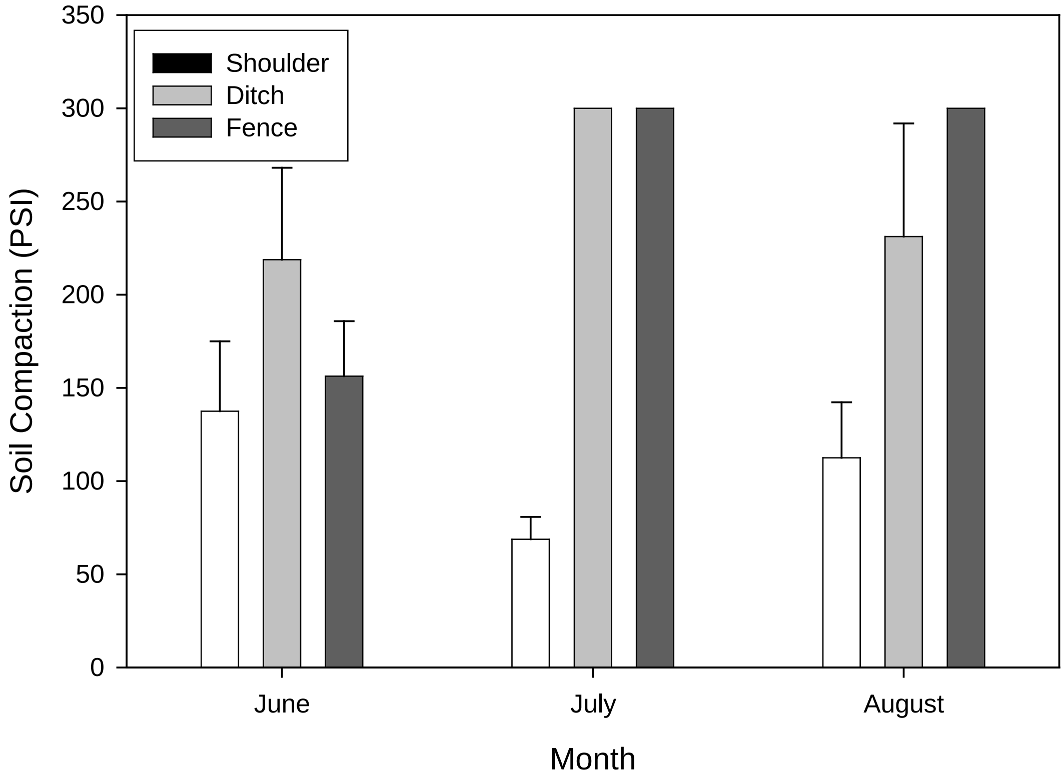

Figure 8. Mean ± compaction of soil at the roadside, ditch, and fence areas for loess soil.

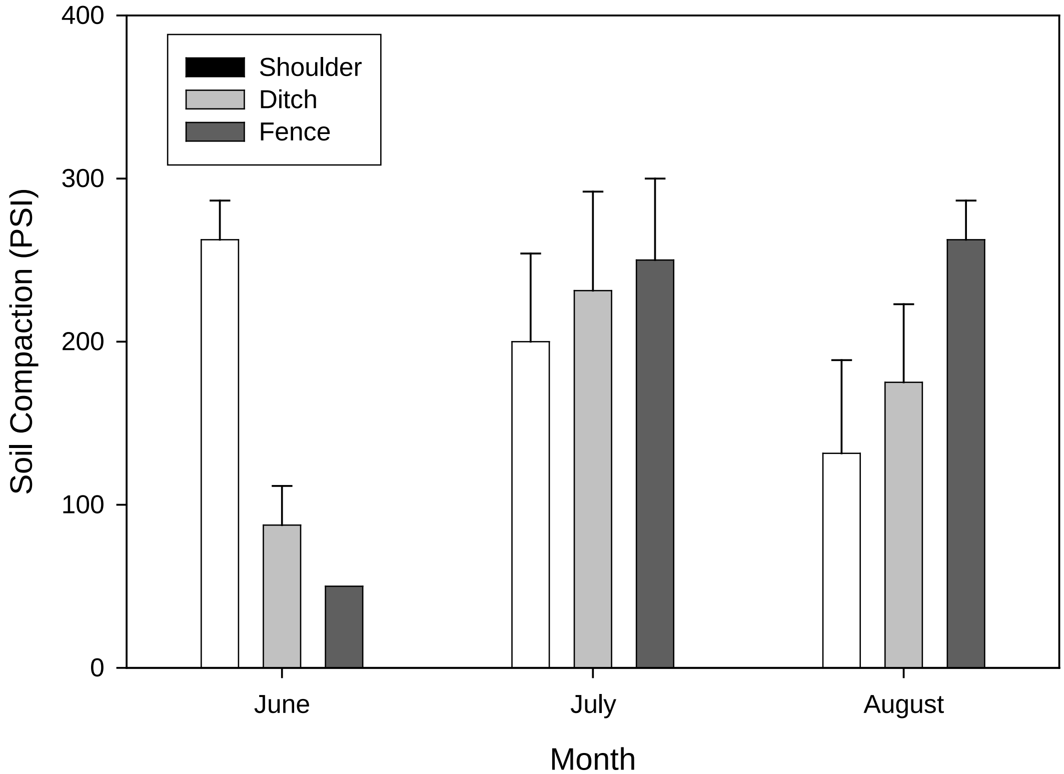

Figure 9. Mean + compaction of soil at the roadside, ditch, and fence areas for sandy soil.