Biodiversity of Soil Macro-invertebrate Communities as Influenced by Invasive *Lonicera x bella*

BIOS 569: Practicum in Field Biology

Kassia Rudd

Advisor: Prof. Jessica Hellmann

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Abstract:

Soil invertebrates play a fundamental role in the cycling of nutrients and the decomposition of organic matter, and as such, influence the composition and health of surface vegetal communities. A small decrease in invertebrate biodiversity has a disproportionate effect upon an ecosystem. As such, maximum retention of invertebrate diversity is preferable. The purpose of this study was to observe and record soil macro-invertebrate diversity in sites containing invasive honeysuckle (*Lonicera x bella*) and vegetated sites without invasive honeysuckle. This study measured soil invertebrate diversity using pitfall traps and core samples. Results of this work indicated no relationship between presence of *L. x bella* and diversity or invertebrate abundance. Results indicate that *L. x bella*, though invasive, exerts no influence over the diversity of soil invertebrate communities. The study did identify pH as a factor controlling diversity; however, the study was unable to pinpoint the pH determining factor.

Introduction:

Soil macro-invertebrates play a fundamental role in soil processes. They are integral to the cycling of organic matter and associated nutrients, and physically alter soil structure through movement (Belnap 2001, Jouquet et al 2006, Wolfe 2005). Loss of invertebrate diversity can adversely affect soil processes, ultimately altering the availability and composition of nutrients within the soil (Ehrenfeld, Jouquet et al 2006, Lavelle 1996, Belnap 2001). Soil

invertebrates also contribute to the succession of above ground vegetal communities through provision of resources, as well as selective consumption of available resources (Deyn 2003). Thus, maximum retention of invertebrate biodiversity is beneficial to biotic communities above and below the soil surface. The function of soil invertebrates within their communities, and their effects upon the environment, are often disproportionate to their abundance (Lavelle 1996). As a result, a seemingly small decrease in diversity can have a disproportionately large effect upon an ecosystem.

Soil communities are intricately tied to surface vegetal communities. Soil invertebrates break down and utilize organic matter and compounds deposited by surface vegetation, as well as compounds released as root exudates below ground. Alteration of above ground communities can result in a shift in the timing and abundance of organic matter and compound deposition that can negatively impact soil invertebrate diversity (Wolfe 2005).

In response to the relationship between above and below ground plant and invertebrate communities, published studies so far have focused on diversity loss. For example, Brent and Belnap (2001) examined responses of invertebrate diversity to the introduction of the invasive grass species *Bromus tectorum*. The study found that the introduction of *Bromus* resulted in a seasonal difference in the deposition of leaf litter—a change largely responsible for the subsequent decrease in invertebrate biodiversity (Brent and Belnap 2001). Additionally, although the introduction of an invasive results in a decrease in invertebrate

diversity overall, individual species have been shown to increase in abundance (Samways 1996; Wolfe 2005).

Lonicera x bella is an invasive hybrid of two Eurasian honeysuckles: *L. tartarica*, and *L. morrowii*. Indigenous to Asia, both parent species were introduced for ornamental purposes, and have since invaded central and eastern North America (Figure 1). *L. tartarica* prefers dry, cool, semi-desert conditions, while *L. morrowii* is often found in riparian areas and disturbed sites (Love 2006). *Lonicera x bella* is able to tolerate a wide range of climates and substrate that is a composite of its genetic parents, contributing to its success as an invasive (Barnes 1974) (Figure 2). The plant appears on the *IPAW Working List of the Invasive Plants of Wisconsin*, and is rated as having an impact level of 8.9, and a disturbance level of 12.3. Plants on the list feature impact values ranging from 0.0-9.9, and disturbance values ranging from 4.0-12.7. Thus, *L. x bella* is one of the most destructive invasives in Wisconsin (Invasive Plants Association of Wisconsin 2003).

This study attempted to elucidate the relationship between invasive *L. x bella* and soil macro-invertebrates and to determine if soil macro-invertebrate communities experience an overall decrease in biodiversity and species richness where *L. x bella* is present. If this is the case, do some macro-invertebrates experience an increase in abundance? To answer these questions, pitfall traps and core samples were used to assess the biodiversity, species richness, and abundance of soil macro-invertebrates in sites with and without invasive *L. x bella*.

Hypothesis 1: The presence of *L. x bella* will result in an overall decrease in biodiversity.

Hypothesis 2: Despite this difference in diversity, both sites will host a similar number of macro-invertebrates.

Hypothesis 3: This similar number of invertebrates will be the result of an increase in the abundance of invasion tolerant macro-invertebrates, and a decrease in the abundance of invasion vulnerable macro-invertebrates.

Methods:

Two sites on the University of Notre Dame's Environmental Research Center (UNDERC) containing areas where *L. x bella* was both present (Invaded Area) and absent (Vegetated Area) were observed during the course of this study (Figure 3). Invaded and vegetated transect areas were situated within half a kilometer of each other in order to increase control over variation in substrate, shade, and general plant community composition. Uninvaded areas were at least 10 meters away from an invaded area. Additional control over substrate composition was achieved through identification of the dominant abiotic soil component at each site: silt, clay, or sand. The following classes were determined and assigned numbers: Silt, Clay, Sand, Silt/Clay, Silt/Sand, and Clay/Sand. Sites were constructed along a 10 meter transect, marked at the meter and half meter.

To study the macro-invertebrate community at each site, two methods of collection were employed: the pit fall trap, and the core. Each site contained 10

replicates of each collection type. The length of the transect allowed for half a meter of space between replicates of either collection type, and a meter of space between replicates of the same collection type.

Pit fall traps were buried at the half-meter, cores taken at the meter. Pit fall traps consisted of two parts: a glass jar and a plastic cover. The cover was constructed from a 7 cm by 7 cm sheet of plastic, edged by four 5 cm pegs. The glass jar was buried so that its lip was flush with the ground. The cover was placed over the mouth of the jar, pegs inserted 2 cm into the ground, allowing surface invertebrates to enter the jar, but limiting the jar's exposure to the elements (Luff 1975, Edwards 1990). The purpose of the pitfall traps was to collect surface invertebrates bound to the ground by their anatomy, including but not limited to spiders, centipedes, millipedes, and slugs (Edwards 1990). Each pitfall trap was filled with 50 mL of soapy water to prevent invertebrate escape. They were buried between June 14th and 21st, filled with 50 mL of soapy water on June 21st, and collected at Site 1 on June 28th, and at Site 2 on June 30th.

Soil cores were collected using a split core. Light penetration at the surface of the soil was measured using a photometer upon collection of each core replicate. Additionally, temperature was recorded at the 5 meter mark of each transect line. Invertebrates were separated from each core through floatation, a standard method that has been used in previous studies (Anderson 1959, Edwards 1990). A sugar solution with a specific gravity greater than most soil invertebrates, but below most organic debris was prepared. The sugar solution reduced invertebrate fluid loss, and was prepared by mixing 290 g of sugar with a

litre of water. 60 oz of soil were taken from 2 cores (total=120 oz), and placed in a 12 by 17 inch white enamel pan, and covered with 1 litre of sugar solution. The debris were stirred and evenly distributed along the bottom of the pan. Invertebrates were allowed to float to the surface and were then removed from the solution using forceps. After invertebrates ceased to float to the surface, the sample was stirred again. Any invertebrates released to the surface were removed. If more than 10 invertebrates were recovered, the sugar solution was decanted through a sieve, and the sample was again covered with a litre of water. The sample was then examined for invertebrates. This method facilitated the collection of soil dwelling invertebrates, such as earthworms, springtails, and beetles in multiple life stages (McBrayer 1971). Three pairs of core samples were examined for each transect. Floatation was conducted on three separate occasions.

For both the pitfall traps and soil cores, invertebrates collected from each site were classified by morphospecies and stored in 75% ethanol (Oliver 1996). Diversity was calculated separately by transect, treatment, and site. Simpson's Diversity Index,

$$D = \Sigma (n / N)2$$

where n equals the total number of individuals in a species and N equals the total number of all individuals of all species, was used to determine a numeric ranking for diversity between 0 and 1. 0 indicates infinite diversity, while 1 indicates no diversity. Simpson's Diversity Index was selected for this project because it accounts for both species richness and abundance. Sorenson's Similarity index,

$$QS = \frac{2 \cdot C}{A + B}$$

where QS is the index, C is the number of species shared by the two samples, and A and B are the number of species in the two samples, was used to compare diversity between uninvaded and invaded sites. Both indices were calculated using an excel spreadsheet. Increases in abundance were examined on a species by species basis. To analyze the impact of honeysuckle and location on species diversity, a two-way ANOVA was performed with treatment and location as the factors and with diversity index as the dependent variable, A one-way ANOVA was performed with soil type as the factor and diversity index as the dependent variable to determine if soil type affected diversity. In order to analyze if the abundance of organisms was affected by the presence of honeysuckle, a one-way ANOVA was performed by group (location) with treatment type as the factor and total abundance of organisms as the response variable. In order to determine if the difference in collection time had an effect, a one-way ANOVA was performed by group (treatment) with collection day as the factor and species diversity as the response variable. Two regressions were performed to determine the relationship between pH and light with diversity. Inclusion of site level factors, such as pH and light penetration, allowed for control over environmental variability, and revealed the significance of invasive *L. x bella* on the soil macro-invertebrate community. All statistics were calculated using SYSTAT 12.

Results:

Eighty-nine different morphospecies were identified from samples collected from pitfall traps at all locations (Table 1). The average diversity index at Site 1 for *L. x bella* transects was 0.35, while the average diversity index for vegetated transects was 0.36, indicating a higher diversity level at *L. x bella* transects than at vegetated transects. At Site 2, the average diversity index for invaded transects was 0.22, and the average diversity index for vegetated transects was 0.18, indicating a higher level of diversity at vegetated transects than at *L. x bella* transects. Despite the variance in diversity indices, statistically all diversity index results were similar (f=.379, df=1, p=.539). Although there was no statistically significant difference between diversity levels of invaded transects and vegetated transects, there was a significant difference in diversity between the two sites (f=11.009, df=1, p=.001), as well as a significant difference in diversity between between *L. x bella* transects at Site 1, collected after 7 days, and *L. x bella* transects at Site 2, collected after 9 days (f=6.233, df=1, p=.016). There was also a significant difference in diversity between between vegetated transects at Sites 1 and 2, again collected after 7 and 9 days respectively (f=4.832, df=1, p=.032). Core sample data was collected on three separate occasions. Only live specimens were recovered using the floatation method. On the final occasion, few invertebrates were found, most likely resulting from invertebrate death (Anderson 1959). As a result, core results were not representative, and core data could not be used. No interstitial diversity levels were calculated.

At Site 1, 61% of species increased in abundance with the addition of *L. x bella*, and 39% decreased in abundance. At Site 2, 51% of species increased in

abundance with the addition of *L. x bella*, and 49% decreased in abundance. This variance, accounted for by Simpson's Diversity Index, was not significant (f=.379, df=1, p=.539), nor was the difference in total abundance of organisms between treatment types (Site 1: f=.391, df=1, p=.534; Site 2: f=.170, df=1, p=.682). Sorensøn Similarity Index for Site 1 was 0.5, indicating moderate similarity between *L. x bella* transects and vegetated transects. At Site 2 the index was 0.7, indicating a high level of similarity.

Relationships between invertebrate diversity and environmental factors such as soil type, pH, and light penetration were also examined. All environmental factors varied within and between sites. Soil type showed no relationship with invertebrate diversity (f=.679, df=3, p=.567). Light penetration showed similar results (R= .061) (f=.421, df=1, p=.581). As pH approached neutral, species diversity increased (Figure 4). The regression examining the relationship between pH and invertebrate diversity delivered a small R value (R=.06), however the value was found to be significant (f=7.162, df=1, p=.009).

Discussion:

Although the results of Simpson's Diversity Index appear to indicate a higher level of diversity at *L. x bella* transects at Site 1, and a higher level of diversity at vegetated transects at Site 2, these results were not significant (f=.379, df=1, p=.539). As a result, it was necessary to reject the first alternative hypothesis—that there would be greater diversity at vegetated areas than at invaded areas—and to accept the first null hypothesis—that there would be no

difference in diversity between invaded and vegetated sites. The null hypothesis was further supported by the Sorensøn Similarity Index, which indicated a high level of similarity in diversity between treatment types at both sites (Site 1: 0.5; Site 2: 0.7). Rejection of the first alternative hypothesis also necessitated rejection of the third alternative hypothesis—that invasion tolerant invertebrates would increase in abundance while invasion vulnerable invertebrates would decrease in abundance. Differences in total abundance of organisms between treatment types were not significant (Site 1: f=.391, df=1, p=.534; Site 2: f=.170, df=1, p=.682).

In order to examine changes in abundance by species, the number of species that increased in abundance with the presence of *L. x bella* was calculated as a percent (Site 1: 69%; Site 2: 51%), as was the number of species that decreased in abundance (Site 1: 31%; Site 2: 49%). Results did not indicate increases and decreases in a small number of species, rather, they indicated a complete shift in community composition. Because this study did not record the composition of above ground vegetal communities, and because *L. x bella* did not exert a significant influence over diversity and abundance, it is not possible to interpret *L. x bella* as the cause of shifts in abundance of individual species. Results did, however, support the second hypothesis—that both sites would host a similar number of macro-invertebrates.

Results of this study indicate that the invasion of *L. x bella* has had no significant impact upon the invaded soil community. Although soil communities greatly contribute to the availability of nutrients, and to the structure of the soil,

their contributions are contingent upon the above ground vegetal community. Surface vegetation supplies soil invertebrates with the organic matter and compounds that the invertebrates return to the environment in a broken down form, and that are necessary for invertebrates to function (Belnap 2001, Jouquet 2006, Wolfe 2000). If *L. x bella* is not affecting invertebrate biodiversity and function, it is possible to infer that its presence is either failing to alter the deposition of organic matter and compounds, or is not doing so in a way detrimental to soil invertebrates. Another possibility remains: other individuals in the above ground vegetal community. Because the composition of above ground communities was not assessed for either site, it is not possible to determine whether or not a specific plant species, or group of plant species, exerted a significant influence over macro-invertebrate diversity.

In addition to examining the relationship between the presence of *L. x bella* and invertebrate abundance and diversity, this study examined the relationship between invertebrates and environmental factors. Of the environmental factors assessed, only pH was significant. Species diversity increased as pH approached neutral. No basic pH readings were recorded, so it is not possible to state that a neutral pH was the desired pH of collected invertebrates. It is possible, however, to infer a preference for non-acidic conditions. Soil invertebrates inhabit a substrate that is more resistant to drought than most terrestrial habitats. Permeable ectodermal tissue allows them to take advantage of their habitat, while also increasing their vulnerability to shifts in the ion content of their surroundings. Some organisms have adapted to acidic

conditions, however, many more organisms thrive in a neutral habitat, causing an increase in diversity as pH approaches neutral (Godbold, 1994).

Although identification of a relationship between pH and species diversity was possible, it was not possible to determine the cause of shifting pH. Changes in pH did not correspond with soil type, or with the presence or absence of *L. x bella*. If the composition of above ground vegetal communities had been identified and recorded, a relationship between pH and a plant, or group of plants, might have been possible.

This study was unable to determine a relationship between the presence of *L. x bella* and species diversity. Although the results appear conclusive, they are only able to account for soil invertebrates found on the surface of the soil. An attempt was made to identify and quantify below ground soil communities, unfortunately, too much time passed between analysis of the various core samples, and the results were not tenable. Because invertebrates within the soil have a direct effect on soil processes, it is likely that they would be immediately affected by shifts in the above ground vegetal community (Jouquet 2006). Organic matter does not instantaneously enter the soil. As a result, shifts in the deposition of organic matter would affect the above ground community before the soil community. Shifts in root exudates, however, would affect the below ground community quickly (Wolfe 2000). Core analysis would have allowed for insight into the influence of *L. x bella* on interstitial communities, and might have produced significant results

This study also did not determine the relationship of the abundance and diversity of soil invertebrates to the presence of native honeysuckle. There is one species of a native true honeysuckle on UNDERC—*Lonicera canadensis*—that this study could have included. Future analysis of the impact of *L. x bella* on soil invertebrate communities should not only account for the composition of above ground vegetal communities, but should also examine both interstitial and surface invertebrate communities below *L. canadensis*. Such analysis would allow variance in the deposition of organic matter and in root exudates to be examined between species. It is possible that *L. x bella* deposits organic matter similarly to *L. canadensis*, resulting in similar abundances and diversities of soil invertebrates in vegetated and invaded transects.

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Table 1: Lists and describes the morphospecies identified in pit fall trap samples.

Morphospecies	Description
1	Beetle: Black body yellow head.
2	Slug: Yellow.
3	Worm: White lines denote segments.
4	Isopod: Long antennae.
5	Spider: Red. Long legs.
6	Fly: Long black banded wings.
7	Spider: Tan. Long abdomen. Small segments. Pinchlike section at end of abdomen.
8	Isopod: Pink.
9	Ant: Black.
10	Spider: Long legs. Multi-colored pattern on torso.
11	Spider: Tan. Large abdomen. Segmented legs.
12	Beetle: Black. Large, round abdomen.
13	Spider: Widely spread legs.
14	Spider: Grey. Thick legs.
15	Isopod: Grey.
16	Spider: Pink. Miniscule segments.
17	Flying Insect: Small wings. Disproportionately large striped body.
18	Worm: Segmented. Pink.
19	Spider: Pink. Pinched at end of abdomen.
20	Spider: Pink.
21	Spider: Pink. Small segments.
22	Wasp: Black. Disproportionately large wings.
23	Spider: Black. Thin.
24	Insect: Black. Many segments. Winged.
25	Spider: Pink. Prominent mandibles.
26	Inchworm: Legs at front, middle, and behind.
27	Spider: Pink. Large segments. Antennae with round ends.
28	Springtail
29	Worm: Pink
30	Spider: Tan. Stripe on thorax. Small segments.
31	Insect: Silver. Many legged. Small antennae. Many small segments.
32	Arachnid: Pink. Long fore and aft legs.
33	Spider: Tan. Long segmented legs.
34	Spider: Brown. Thick legs. Prominent mandibles.
35	Spider: Pink.
36	Spider: Tan. Large segments. Long segmented legs.
37	Spider: Tan. Abdomen and thorax similarly sized. Abdomen pinched at end.
38	Beetle: Red stripes on head. Long armor.
39	Spider: Brown. Large abdomen. Pinchlike section at end of abdomen.
40	Beetle: Black. Large abdomen. Small point at end of abdomen.
41	Spider: Large, pink abdomen. Long legs.
42	Beetle: Black with brown spots.
43	Spider: Pink. Large abdomen. Pinched section at end of abdomen.
44	Insect: Tan. Winged. Long legs and antennae. Small abdomen.

45	Spider: Brown. Long abdomen. Small segments.
46	Insect: Brown. Winged. Long, pointed abdomen. Hairy body.
47	Spider: Transparent. Miniscule segments.
48	Spider: Brown. Long, thick legs.
49	Insect: Brown. Long legs and antennae. Brown and white striped abdomen.
50	Cricket
51	Spider: Tan. Small abdomen. Long thin legs.
52	Ant: Black.
53	Beetle: Light green armor.
54	Spider: Tan. Long legs. Miniscule segments.
55	Ant: Yellow.
56	Insect: Black and yellow stripes. Thin stripes. Tan stripe down abdomen. Long legs.
57	Insect: Beige. Segmented. Many disproportionately small legs.
58	Insect: Black. Winged. Long legs and abdomen.
59	Ant: Yellow. Dark black stripes.
60	Insect: Black with white stripes. Two wings. Long legs and body.
61	Beetle: Red.
62	Beetle: Black. Wings extend beyond abdomen.
63	Insect: Brown and tan segments. Fore legs only.
64	Insect: Tan with black bands. Thin. Disproportionately long body.
65	Isopod: Armored.
66	Insect: Red. Long abdomen.
67	Insect: Black. Small segments. Long abdomn.
68	Beetle: Green. Armored.
69	Spider: Tan. Long torso.
70	Spider: Black. Red stripe down two segments.
71	Caterpillar: Black and orange.
72	Inchworm: White. Legs at front and back.
73	Insect: Black with white stripes. Winged. Abdomen thins towards end.
74	Ant: Black. Large segments.
75	Centipede: Pink.
76	Insect: Alternately black and white bands. Winged. Long abdomen.
77	Beetle: Black. Large nose. Rotund thorax.
78	Beetle: Black with gold bands. Armored.
79	Grasshopper: Thick bodied.
80	Insect: Black. Long abdomen. White section near end of abdomen.
81	Bumblebee
82	Cocoon: Black.
83	Centipede: Pink. Long antennae.
84	Spider: Tan. Long thin legs. Miniscule segments.
85	Beetle: Striped abdomen.
86	Beetle: Green. Shiny exoskeleton.
87	Cocoon: Tan.
88	Insect: Black with white stripes. Wasp like abdomen.
89	Insect: Black. Winged. Long abdomen.

Figure 1: Illustrates the native ranges of the genetic parents of *L. x bella*: *L. tartarica* and *L. morrowii*. Image courtesy of Barnes 1974.

Figure 2: Illustrates the approximate range of *L. x bella* in the North America. Image courtesy of Barnes 1974.

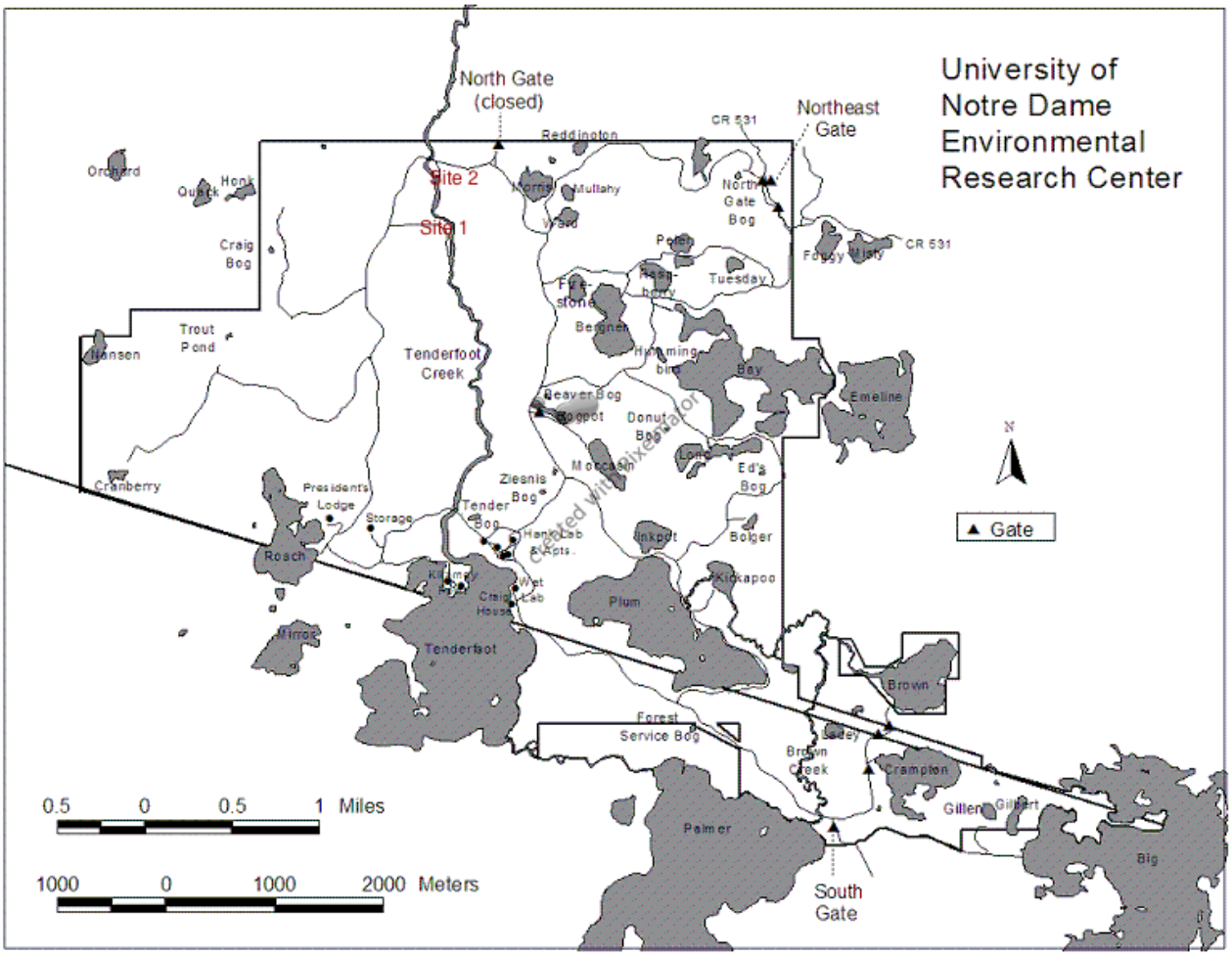

Figure 3: Illustrates the sites examined during the course of this study.

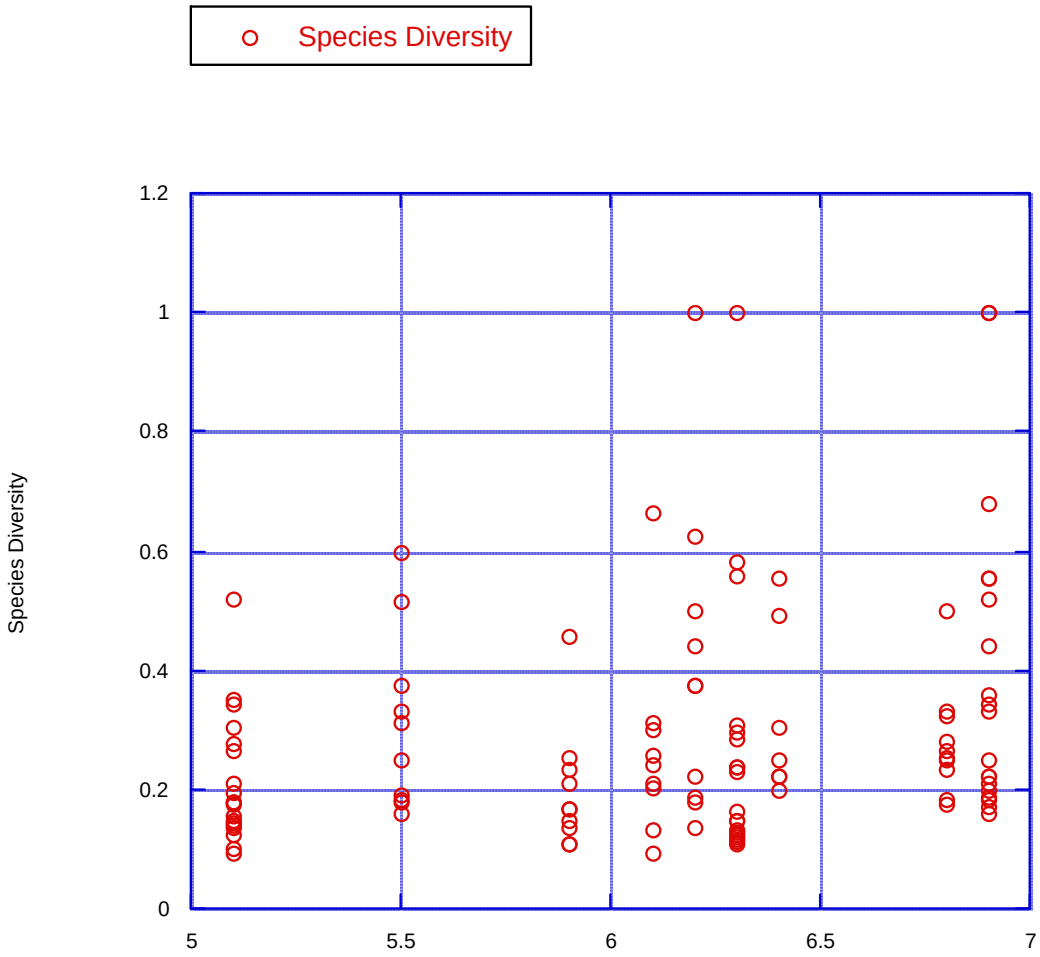

Figure 4: Illustrates the subtle but statistically significant relationship between pH and species diversity.

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