A Comparison of the Effectiveness of Pitfall Traps and Winkler Litter Samples for Characterization of Terrestrial Ant (Formicidae) Communities in Temperate Savannas

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Abstract

Relatively few studies in temperate environments have compared pitfall traps and Winkler litter samples, two of the most commonly used ant (Formicidae) sampling protocols. Most of the comparative work has been performed in tropical and subtropical environments. Temperate studies have primarily taken place in forested environments. Our study focuses on the relative efficiency of these two methods in temperate oak savannas, the major ecotone connecting grasslands and deciduous forest in the Midwest. These environments are often maintained by fire and mechanical brush removal, which tends to decrease the amount of available leaf litter. We sampled 21 sites, varying in age since restoration from un-restored to 22 years of restoration activities in McHenry Co. Illinois. Each site was sampled with 30 pitfall traps and five Winkler litter samples. A total of 38 species in 17 genera in 5 subfamilies were captured and identified. Pitfall traps accounted for 37 of the species, while Winkler litter samples only captured 23 species, and only one species specific to that method. We conclude that in northern temperate savannas, pitfall traps were more effective and more efficient at characterizing the epigeic ant community than Winkler litter samples.

Ants (Formicidae) are one of the most common and abundant terrestrial animals on the planet, occupying every environment except for those permanently covered in ice (Hölldobler and Wilson 1990). Ants forage at all trophic levels and often serve as ecosystem engineers, by transporting soil nutrients and acting as mutualists with a variety of arthropods and plants (Hölldobler and Wilson 1990). In addition, many species are successful colonizers of disturbed habitats and respond to environmental change quickly, which often leads to their considerable economic impact as both agricultural and urban pests (Hölldobler and Wilson 1990). Because ants are functionally important and common in most environments, they are increasingly used as bioindicators for ecosystem health (Majer et al. 2007). Using ant communities to study ecosystem health has many advantages because ants are easy to collect, have high species richness, a good taxonomic base, and stationary nesting habits, so they can be sampled over time (Agosti et al. 2000). Ant communities or individuals species have been successfully identified as indicators of environmental stress and disturbance in a variety of tropical environments (Hoffmann and Andersen 2003, Majer et al. 2007); however, little work has been done in temperate environments (but see Kaspari and Majer 2000, Bestelmeyer and Wiens 2001, Fagan et al. 2010, Orlofske et al. 2010). While ant communities may be relatively easy to sample, different techniques are used based on environment and experimental design.

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Common sampling techniques for terrestrial ants include pitfall traps, leaf litter sifting, baiting, and hand collecting (Agosti et al. 2000). The most successful and efficient technique often depends on the research question and the environment in which it is being used (Bestelmeyer et al. 2000). In addition, different protocols and methods can be employed with each sampling technique (Bestelmeyer et al. 2000). This variety of methodologies and increasing interest in large scale comparative studies has led to the recent push to standardize sampling protocols so that different studies can be compared more directly (Agosti and Alonso 2000, Agosti et al. 2000, Gotelli et al. 2011). The Ants of the Leaf Litter Protocol (ALL) was proposed by Agosti and Alonso (2000) as a general method to allow for the rapid inventory of most terrestrial ant species (≥70%) in different environments by combining the use of pitfall traps and Winkler litter samples. The greatest benefit of the ALL protocol is that by combining pitfall traps with Winkler litter samples, the respective weaknesses of each method are minimized and sampling does not require expertise in ant biology (Agosti and Alonso 2000, Bestelmeyer et al. 2000). One of the greatest advantages of pitfall traps is that they involve multi-day continuous passive sampling, but they can miss wary species and those with small foraging ranges (Bestelmeyer et al. 2000). Winkler litter samples are a single time snapshot of the ants present, which is good for collecting cryptic species with small forging ranges and for capturing trap-wary species, but it tends not to capture large active species (Bestelmeyer et al. 2000). One of the limitations of the proposed ALL protocol is that most of the testing of the protocol's efficiency has taken place in tropical forested environments (Fisher et al. 2000), while relatively little comparative work has been done in temperate environments (but see Martelli et al. 2004, Groc et al. 2007) where using both pitfall trapping and Winkler litter sampling may be redundant rather than complementary.

The simultaneous use of both Winkler litter samples and pitfall traps was initially designed for forested environments (Agosti et al. 2000). In a direct comparison of the efficiency of pitfall and Winkler litter sampling in multiple Amazonian forests, de Souza et al. (2012) found that pitfalls were more efficient (on average 90% of the ant fauna was collected) than Winkler litter samples (on average 26% of the ant fauna was collected), with their combined coverage on average capturing 94% of the ant fauna. Pitfall traps have also been found to be more efficient than Winkler litter samples in the wet-deciduous forests of the Western Ghats (Sabu and Shiju 2010). Conversely, Winkler litter samples have captured more ant species and different species than pitfall traps in Madagascar tropical forests, Brazilian Atlantic forests, and Amazonian forests (Fisher et al. 2000). In more open tropical savanna environments, the differences between pitfall traps and Winkler litter samples are also mixed. Lopes and Vasconcelos (2008) found that Winkler litter samples outperformed pitfall traps in more covered environments, but as the canopy thinned, pitfalls performed better in the Brazilian Cerrado. Parr and Chown (2001) found pitfall traps to perform better in the South African savanna, and van Ingen et al. (2008) were unable to successfully use Winkler litter samples in Australian savannas due to lack of litter. In tropical grasslands in Madagascar, Winkler litter samples outperformed pitfall traps (Fisher and Robertson 2002). While there is variation in the relative importance of Winkler litter samples versus pitfall traps in the tropics, in almost all studies Winkler litter samples collected an important component to the ant community that is missed by pitfall traps.

Far less comparative work on the relative merits of pitfall traps and Winkler litter samples have been performed in temperate environments. In temperate forest environments, pitfall traps captured more species than litter collections using Berlese funnels in upland Florida (King and Porter 2005), and outperformed Winkler litter samples in the Smokey Mountains of Tennessee and North Carolina (Lessard et al. 2007), New York (Ellison et al. 2007), Canada (Higgins and Lindgren 2012), and in Austrian mountains and floodplains (Tista and Fiedler 2011). Conversely, pitfall traps captured fewer species than litter collections using Berlese funnels in Florida hardwood hummocks (King and Porter 2005), and captured fewer species than using Winkler litter samples in Tennessee (Martelli et al. 2004), urban forest fragments in Ohio (Ivanov and Keiper 2009), and pine and oak forests in France (Groc et al. 2007). In the two temperate studies of open environments such as savannas and grasslands, both of which have taken place in Europe, pitfall traps were found to capture more species than Winkler litter samples, but in some cases the species collected by each method were complementary (Groc et al. 2007) while in others Winkler litter samples were mostly redundant compared to pitfall traps (Tista and Fiedler 2011).

In North America, oak savannas are the major ecotone connecting grasslands and deciduous forest in the Midwest (Peterson and Reich 2001, Cavender-Bares and Reich 2012). These savannas are fire maintained systems characterized by 25-50% discontinuous cover of shrubs and trees (Peterson and Reich 2001). Oak savannas have been in decline caused by a combination of fire suppression, agricultural and grazing practices, and changing climate during the Holocene (Abrams 1992). Restoration of Oak savannas usually includes the removal of invasive brush and trees by cutting and wood chipping followed by a return to a regular fire cycle (Nielsen et al. 2003, Brudvig and Asbjornsen 2007). Repeated burning can have a negative effect on arboreal and cryptic leaf litter ants, caused by a decline in the amount of leaf litter and potential nesting sites (Siemann et al. 1997, Houdeshell et al. 2011). It remains uncertain how the success of Winkler litter sampling will compare to pitfall trapping in temperate savannas regularly subjected to burning. Therefore, our goal is to help determine the most appropriate sampling technique for quantitative studies of ant communities in temperate savanna systems.

Methods

Study Design. Ant diversity was surveyed at 21 sites between June and July, 2012 using two methods of collection. The sites, located throughout McHenry County Illinois in savannah environments, varied in age from sites that have been under restoration for more than 20 years to sites that were unrestored and were undergoing succession to young forests (Fig. 1). To compare the effectiveness of pitfall traps and Winkler litter samples, we classified the study sites into four different age categories: unrestored, under restoration for 0-3 years, under restoration for 7-15 years, and under restoration for 20-22 years.

Ants were sampled using two methods at each site. First, we placed 30 50-mL centrifuge tube (27 mm diameter) pitfall traps at each site. Pitfall traps were placed in a 5 × 6 grid with 15 m spacing between each trap. Each centrifuge tube was filled with 25-mL of propylene glycol with a drop of fragrance-free soap. Pitfalls were buried flush with the soil surface, and left capped, in place, at each site for at least 48 hours to allow vegetation to recover from the disturbance. All pitfall traps were opened for 120 hours between 2-3 June 2012 and then collected between 9-10 June 2012 during a span of dry weather. Trap contents were collected, brought back to the lab and fixed in 95% Ethanol. Second, litter-dwelling ants were extracted from five 1-m^2 leaf litter samples using mini-Winkler traps (Bestelmeyer et al. 2000). Litter samples were collected in the shape of an "X" with one sample taken from each corner of the pitfall grid and one from the center of the grid. All litter samples were collected between 19 June and 20 July 2012 between 9 am and 4 pm during peak surface ant activity and at least 24 hours after any form of precipitation. Litter was chopped and sieved in the field and then suspended in the lab for 72 hours. All specimens were preserved in 95% ethanol.

Ants were sorted and identified to species using keys found in *The Ants of Ohio* (Coovert 2005) and *A Field Guide to the Ants of New England* (Ellison et al. 2012). Representative voucher specimens are deposited at the Chicago Field Museum of Natural History.

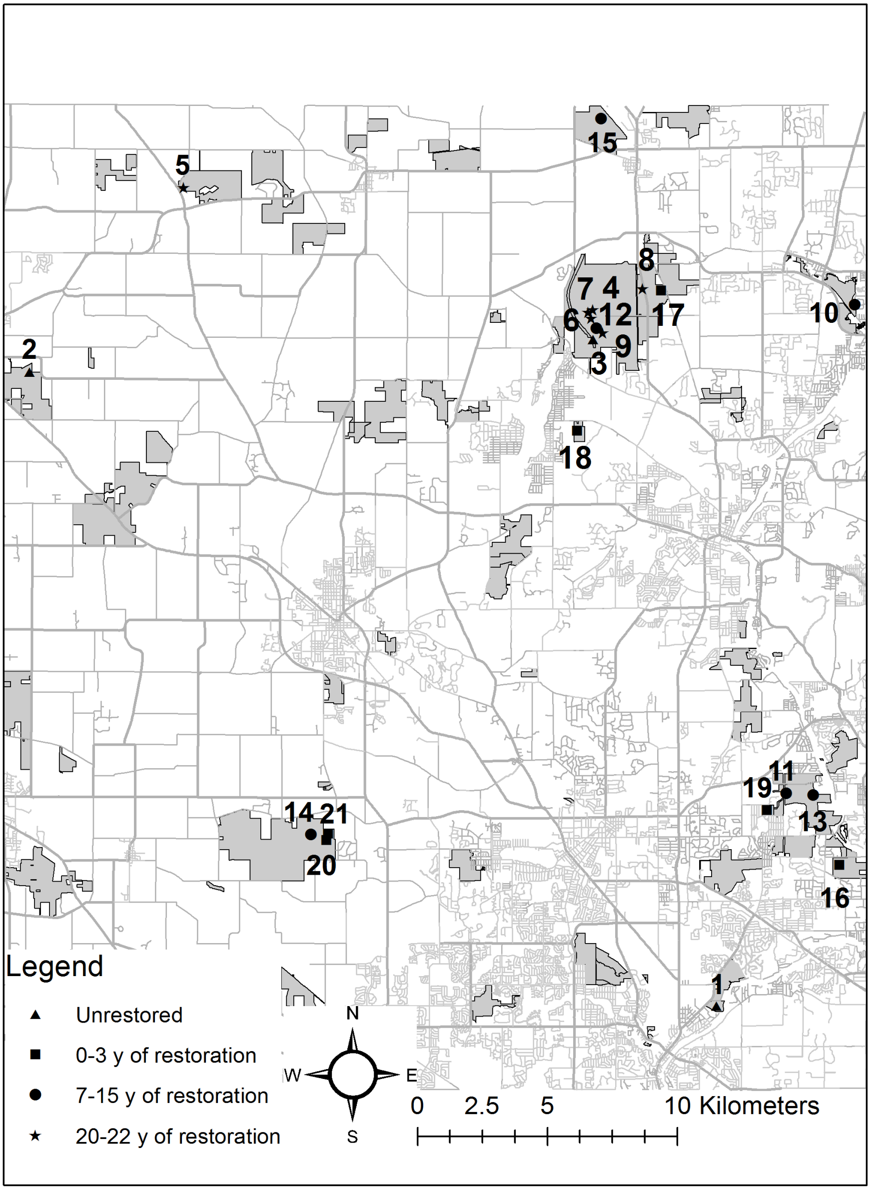

Figure 1. Location of the 21 study sites in McHenry County, Illinois. Grey areas represent land owned by the McHenry County Conservation District, and symbols show the location of study sites.

Data Analyses. All analyses are based on ant workers. Reproductives were not included because the origin of reproductives can be difficult to track (Fisher 1998). With respect to comparisons of ant species richness, individual ant workers in a sample are not the desired metric, while the number of colonies is (Ellison et al. 2007). Since pitfall traps and Winkler litter samples are unable to distinguish the number of colonies in a site, our statistical comparisons use the number of incidences rather than the number of workers. Incidences are the number of traps in which a species occurred, regardless of abundance in a given trap (Ellison et al. 2007). We then used three different statistical approaches to compare the trapping methods for each restoration classification. First, we constructed sample based rarefaction species accumulation curves. Second, we calculated the asymptotic species richness for sample-based data using the Chao2 index. Third, we evaluated the similarity of ant faunas between the two sampling methods using Chao's abundance-based Jaccard Index. All statistics were conducted in EstimateS version 9.1 after being randomized 1000 times (Colwell 2013).

Rarefaction curves were initially created based on the number of species captured in each sample. The curves were then rescaled to the same x-axis for the number of incidences (Gotelli and Colwell 2001) because of large differences in the number of traps placed per site with each method (5 Winkler litter samples or 30 pitfall traps) (Ellison et al. 2007). For calculation of the rarefaction curves and sample based Chao2 index of asymptotic species richness, we pooled data from all of the sites for each restoration category. We used Chao's abundance-based Jaccard Index to look at the similarity of ant communities because the comparison of rarefaction curves and estimates of species richness could yield the same values even if different collection methods capture none of the same species (Ellison et al. 2007). To calculate Chao's abundance-based Jaccard Index, we used the incidences from all sites combined for each trapping method and computed 1,000 random bootstrap samples to calculate the 95% Confidence Intervals. Values for Chao's abundance-based Jaccard Index range from 0 – 1, with a value of 0 indicating no shared species between the two trapping methods while a value of 1 indicates that all species are shared. If the Confidence Intervals encompass 1.0, we cannot reject the null hypothesis that the two collections methods share the same composition of species, as is expected by chance.

We created rank-abundance diagrams based on ant worker incidence to directly compare the ant fauna captured by pitfall traps and Winkler litter samples summed across all sites. We tested for differences in species rank abundances between the two trapping methods using the Wilcoxon Sign-Rank test using JMP version 8.0 (SAS Institute, Cary, North Carolina, USA).

Results

Overall 6,086 individual ant workers representing 38 species in 17 genera in 5 subfamilies were captured in our combined pitfall traps and Winkler litter samples and are listed with their authority and subfamily names in Table 1. Captured individuals were spread evenly between pitfall traps (3,004 workers representing 1,298 incidences) and Winkler litter samples (3,082 workers representing 277 incidences). Overall, pitfall traps captured 37 species (mean ± SE, 2.3 ± 0.07) with two species only occurring once (*Formica glacialis* and *Stenamma impar*). Winkler litter captured 23 species (mean ± SE, 2.6 ± 0.2) with four species occurring once (*F. montana, M. detritinodis, M. punctiventris,* and *Prenolepis imparis*). Pitfall traps captured more species than Winkler litter samples regardless of the age of restoration of the site; un-restored (pitfall/Winkler) = 19/13, 0-3 years under restoration = 17/12, 7-15 years under restoration = 30/20, and 20-22 years under restoration = 34/15 (Table 1). Out of 560 pitfall traps, 88% captured ant workers, while out of 105 Winkler litter samples, 83%

Table 1: Ant species (Hymenoptera: Formicidae) collected in each restoration category by each sampling method (P = Pitfall trap, W = Winkler sample, B = both collection methods).

	All sites	**Unrestored**	**0-3 y of restoration**	**7-15 y of restoration**	**20-22 y of restoration**
Amblypopninae					
Stigmatomma pallipes (Haldeman, 1844)	W			W	
Dolichoderinae					
Tapinoma sessile (Say, 1836)	P	P			P
Formicinae					
Brachymyrmex depilis (Emery, 1893)	B	B	B	B	B
Camponotus pennsylvanicus (De Geer, 1773)	B	P	B	B	B
Formica dolosa (Buren, 1944)	P	P			
Formica fossaceps (Buren, 1942)	P				P
Formica glacialis (Wheeler, 1908)	P				P
Formica incerta (Buren, 1944)	P	P			P
Formica lasioides (Emery, 1893)	P			P	P
Formica montana (Wheeler, 1910)	B			P	B
Formica neogagates (Viereck, 1903)	P			P	P
Formica pallidefulva (Latreille, 1802)	B	B	B	B	P

Table 1: Continued.

	All sites	Unrestored	0-3 y of restoration	7-15 y of restoration	20-22 y of restoration
Formica rubicunda (Emery, 1893)	P			P	
Formica subsericea (Say, 1836)	B	B	P	B	B
Lasius alienus (Foerster, 1850)	B	B	B	B	B
Lasius claviger (Roger, 1862)	P			P	P
Lasius flavus (Fabricius, 1782)	B	B	W	B	B
Lasius neoniger (Emery, 1893)	P	P		P	P
Lasius umbratus (Nylander, 1846)	P			P	P
Nylanderia parvula (Mayr, 1870)	B	P		B	P
Prenolepis imparis (Say, 1836)	B	P	P	B	P
Myrmecinae					
Aphaenogaster picea (Wheeler, 1908)	B	B	B	B	B
Aphaenogaster tennesseensis (Mayr, 1862)	P		P	P	P
Crematogaster cerasi (Fitch, 1855)	B		B	B	B
Myrmecina americana (Emery, 1895)	B	W	B	B	B
Myrmica n.sp. AF-eva	B	P		B	P
Myrmica n.sp. AF-smi	B	B	B	B	B

Table 1: Continued.

	All sites	Unrestored	0-3 y of restoration	7-15 y of restoration	20-22 y of restoration
Myrmica detritinodis (Emery, 1921)	B	P		W	P
Myrmica fracticornis (Forel, 1901)	P	P		P	P
Myrmica pinetorium (Wheeler, 1905)	B	P	P	B	B
Myrmica punctiventris (Roger, 1863)	B	W	P	P	P
Solenopsis molesta (Say, 1836)	P			P	
Stenamma brevicorne (Mayr, 1886)	B	B	B	B	B
Stenamma impar (Forel, 1901)	B	W			P
Temnothorax curvispinosus (Mayr, 1866)	B	W	B	B	B
Temnothorax schaumii (Roger, 1863)	P		P	P	P
Tetramorium caespitum (Linnaeus, 1758)	B			B	B
Ponerinae					
Ponera pennsylvanicus (Buckley, 1866)	B	W	B	B	B
Total species	38	24	18	32	34
Pitfall species	37	19	17	30	34
Winkler species	23	13	12	20	15

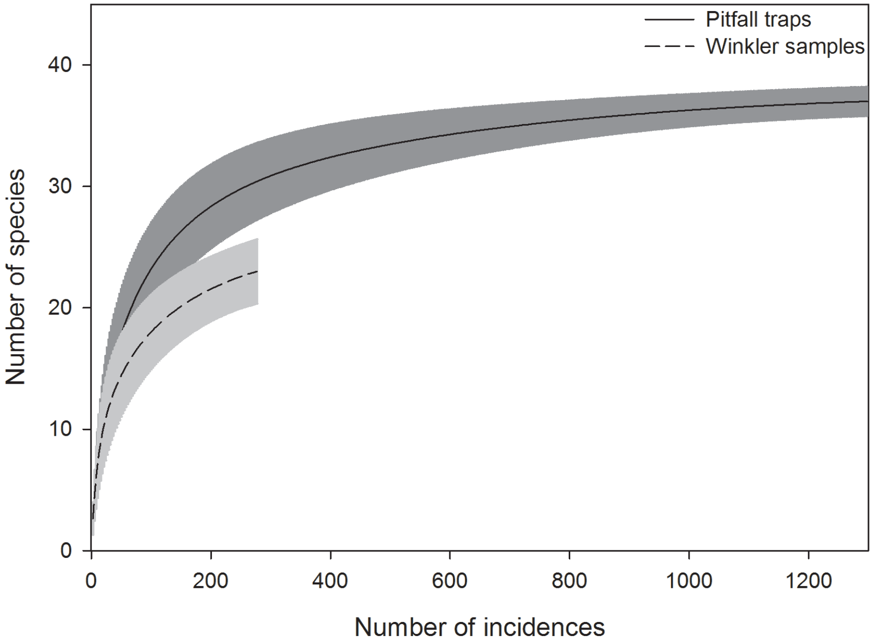

Figure 2. Trap-level rarefaction curves for all 21 sites sampled with pitfall traps (560 traps, solid line) and Winkler litter samples (105 traps, dashed line) with 95% confidence intervals. Curves are corrected for the number of incidences in the samples.

collected ant workers. The mean number of ants captured per pitfall trap was lower than that for Winkler litter samples (mean ± SE, 5.4 ± 0.3 and 29.3 ± 6.1 respectively).

Comparisons of species accumulation curves for all 21 sites combined demonstrated that pitfall traps captured significantly more species than Winkler litter samples (Fig.2). When the data were analyzed based on the age of restoration, differences between pitfall traps and Winkler litter samples were only significant for unrestored sites (Fig. 3A). For sites that have been under restoration for less than 3 years and 7-15 years, Winkler litter samples accumulated species slightly more rapidly, but results were not statistically significant, as determined by overlap of 95% confidence intervals (Fig. 3B and C). In sites that had been under restoration for 20-22 year pitfall traps accumulated species more rapidly than Winkler litter samples, but not significantly so, based on overlap of 95% confidence intervals (Fig. 3D). Total estimated species richness was significantly greater for pitfall traps (37 species) than for Winkler litter samples (24 species) (Fig. 4).

Pitfall traps and Winkler litter samples appear to collect different subsets of species based on the age of restoration of the savannah (Fig. 5). The 95% confidence intervals for Chao's abundance-based Jaccard Index of similarity between the two sampling methods included 1.0 (100% similarity) for sites that had been under restoration (Fig. 5). While the 95% confidence intervals did not overlap 1.0 (100% similarity) for sites that had not been restored, suggesting that the trapping methods were collecting different species.

The rank abundances of species differed significantly (Wilcoxon sign-rank statistic = -318.0, $P < 0.0001$) between pitfall traps and Winkler litter samples

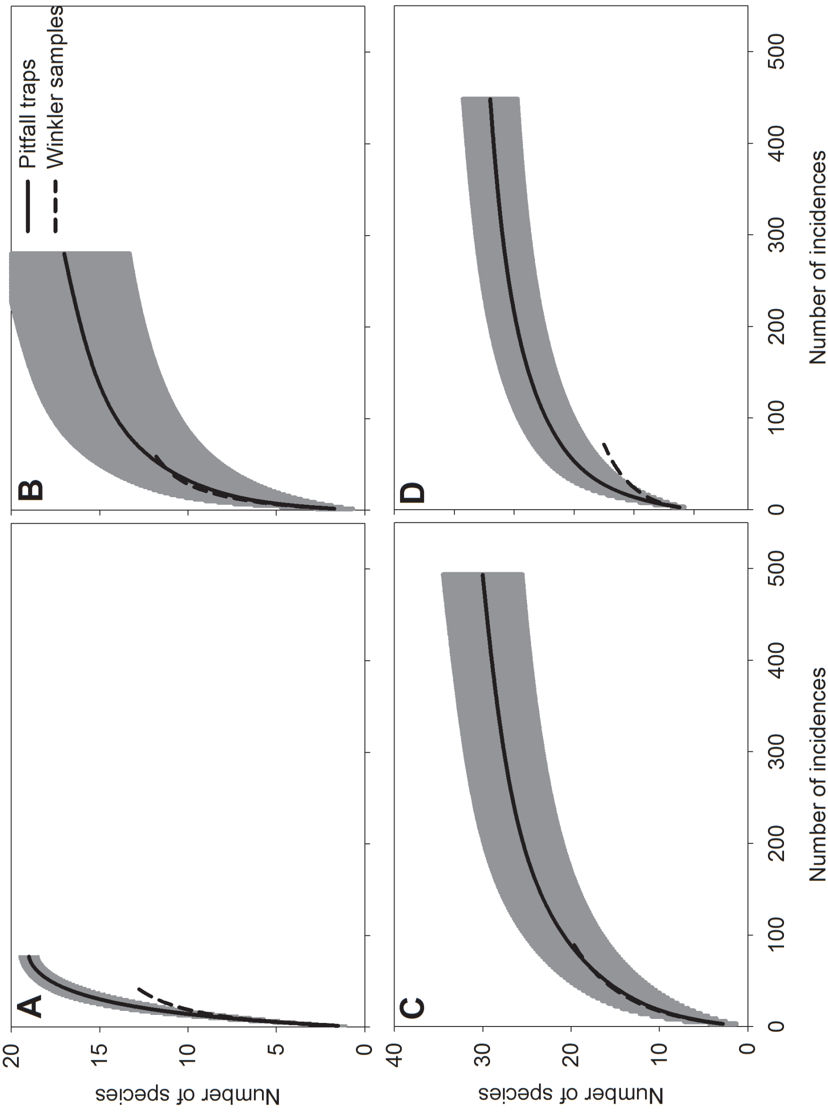

Figure 3. Rarefaction curves comparing pitfall trapping (solid lines) to Winkler litter sampling (dashed line) in different stages of savannah restoration. **(A)** Rarefaction curves for sites that are unrestored. **(B)** Rarefaction curves for sites that have undergone restoration in the past 3 years. **(C)** Rarefaction curves for sites that have been under restoration for 7-15 years. **(D)** Rarefaction curves for sites that have been under restoration for 20-22 years. For clarity, the 95% confidence intervals (gray areas) are shown only for the pitfall traps. Widths of confidence intervals are similar for Winkler litter samples.

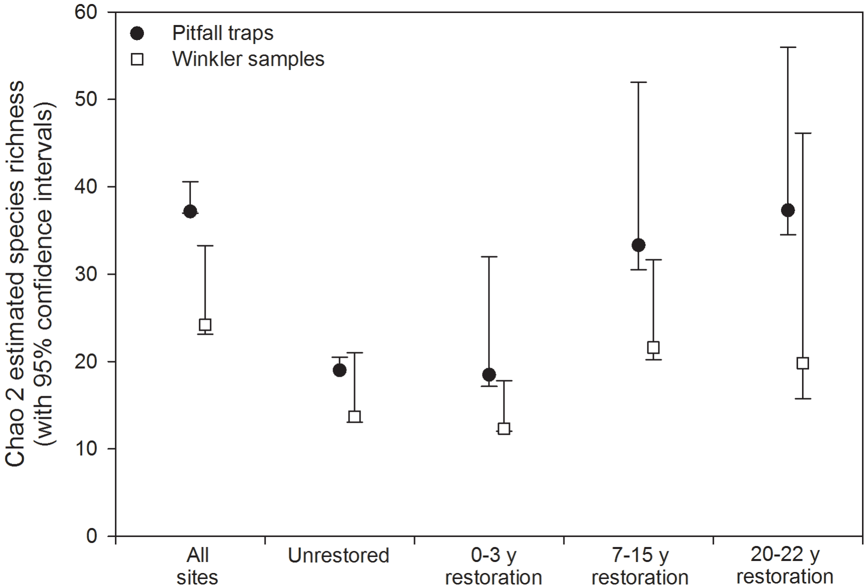

Figure 4. Chao 2 asymptotic estimates of species richness comparing pitfall traps (filled circles) and Winkler litter samples (open squares) at sites with different amounts of time since restoration. Error bars represent 95% confidence intervals.

(Fig. 6). The five most common ant species in pitfall traps (*Lasius alienus, Aphaenogaster picea, Camponotus pennsylvanicus, Myrmica AF-smi, Stenamma brevicorne*) account for 56% of all ant incidences, while the five most common species in Winkler litter samples (*L. alienus, Ponera pennsylvanicus, Temnothorax curvispinosus, Brachymyrmex depilis, Myrmica AF-smi*) account for 63.5% of all ant incidences. The most common species in both pitfall traps and Winkler litter samples was *Lasius alienus* (16.7% and 19.1% respectively). The second most common species in pitfall traps, *Aphaenogaster picea* (10.7%), was only the 7th most common in Winkler litter samples (6.1%). *Ponera pennsylvanica* was the second most common ant species in Winkler litter samples (17%) but only the 27th most common in pitfall traps (0.7%).

Discussion

Our results demonstrate that in northern temperate savanna systems, pitfall traps were more effective and more efficient at characterizing the epigeic ant community than Winkler litter samples. Pitfall traps captured more species overall than Winkler litter samples, even when correcting for sampling intensity (Fig. 2). Pitfall traps also collected at least as many species as Winkler litter samples at different ages of restoration and more species in unrestored sites (Fig. 3). Increased species richness from pitfall sampling occurred despite the fact that individual pitfall traps captured 5x fewer ants than Winkler litter samples. Of the 129 species of ants found in Illinois (antweb.org), oak forest savanna sites have previously been found to contain from 46 species (Talbot 1934) to 42 species (Gregg 1944). Our asymptotic estimate of species richness from pitfall traps of 37 species was much closer to the number of species observed by Talbot (1934) and Gregg (1944) than the asymptotic estimated species richness for Winkler litter samples of 24 species (Fig. 4).

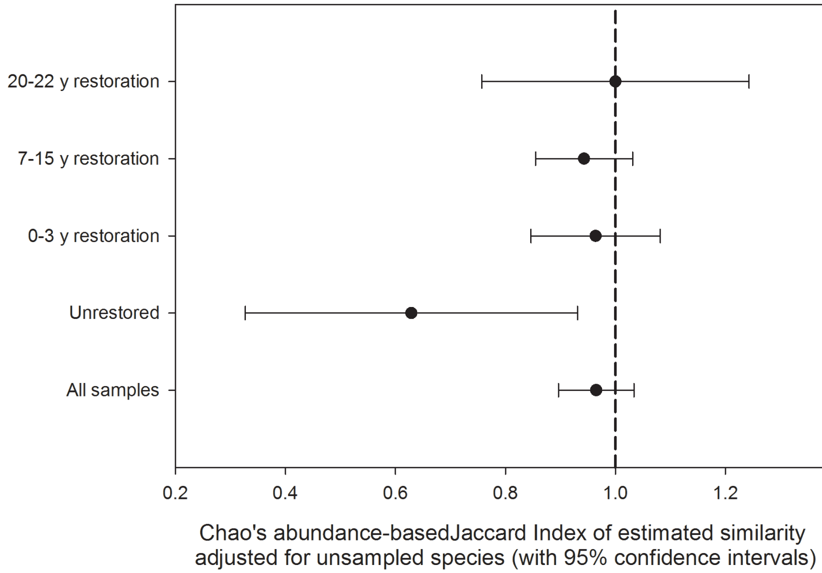

Figure 5. Similarity in species composition captured by pitfall traps and Winkler litter samples among sites of different age since restoration, adjusted for unsampled species (with 95% confidence intervals).

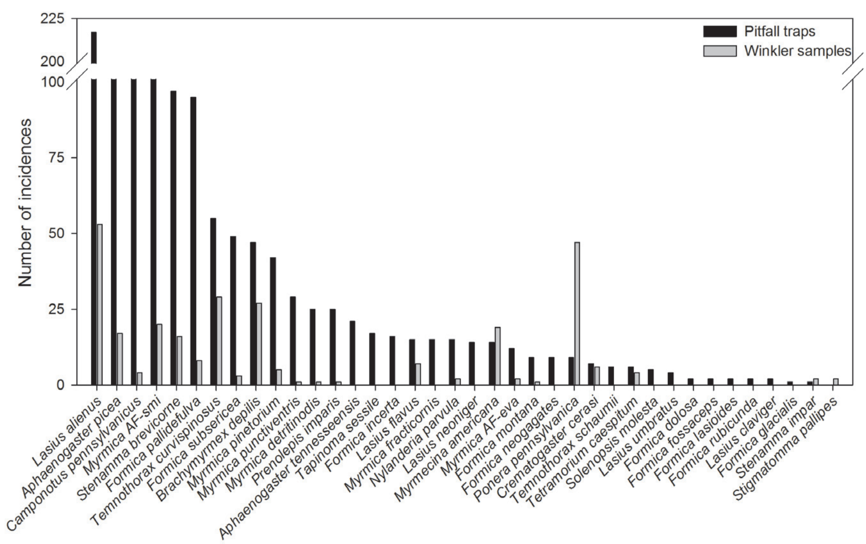

Figure 6. Abundance of ant species from pitfall traps and Winkler litter samples. The species are ordered by their abundance in pitfall traps.

Pitfall traps and Winkler litter samples were initially designed to be used in a complementary manner in the ALL protocol due to the non-overlapping and additive nature of the species they captured (Agosti and Alonso 2000). In our study, the two methods were not complementary. In fact, Winkler litter samples mostly captured a subset of the community trapped by pitfall traps while adding just one species that was not found in pitfall traps (Table 1, Fig. 6). In savanna sites that had been under restoration for more than 20 years and hence the best and most mature examples of a savanna community, pitfall traps captured 34 species while Winkler litter samples only captured 15 species, all of which were also captured by pitfall traps (Table 1, Fig. 5). Winkler litter samples frequently failed to capture many of the larger, more active ants captured in pitfall traps. For instance, Winkler litter samples only captured 3 of the 10 *Formica* species and failed to capture the large active *Aphaenogaster tennesseensis* (Table 1). Also, the large species of *Aphaenogaster, Camponotus, Formica,* and *Myrmica* were represented by relatively few incidences in Winkler litter samples, compared to pitfall traps (Fig. 6). This pattern of reduced capture success of large ants is commonly observed in studies comparing methodologies in tropical forests (Olson 1991, de Souza et al. 2012), sub-tropical savannas (Parr and Chown 2001, Lopes and Vasconcelos 2008), and temperate forests (Ellison et al. 2007). There are several possible reasons for this discrepancy. Regardless of habitat, hand collection of litter and the relatively instantaneous nature of litter collection is likely to exclude large active ants, while small ants that tend to have smaller ranges and move slowly (Kaspari and Weiser 1999) are, therefore, more likely to be captured by litter sampling (Parr and Chown 2001). In addition, Winkler litter samples may not have captured species complementary to those captured in pitfall traps because there are fewer litter specialist species in temperate environments (Lynch 1981, Kaspari et al. 2000). Finally, effectiveness of Winkler litter samples may have been compromised by the relative lack of litter in our savannas, resulting from repeated burning and brush removal during the restoration process (Nielsen et al. 2003, Brudvig and Asbjornsen 2007). Houdeshell et al. (2011) suggest that restoration practices do not affect the frequency of litter nesting ants, but may influence the community composition. Visually, the amount of leaf litter was greatest in sites that had not been restored. Early in restoration, the ground was covered in woodchips from brush clearing, and sites that had been under restoration for longer periods had more grass litter than tree leaf litter. But, grass litter might not be particularly useful for ant nesting, given that there are no true endemic prairie ants in North America (Trager 1998).

Two of the drawbacks of Winkler litter samples are that collections must be processed immediately in the field and they represent an instantaneous sample of a relatively small sample locality. Parr and Chown (2001) in subtropical savannas and de Souza et al. (2012) in tropical savannas demonstrated that collecting and processing Winkler litter samples was more time consuming than pitfall trap samples. One useful measure of effort is the average number of individual ants collected per sample. For instance, in our study there were on average 5× more ants to be identified per Winkler litter sample, representing a far greater time commitment. One possible explanation for the reduced species richness captured in Winkler litter samples is that we only collected 1/6 the number of samples at each site, compared to pitfall traps. This seems like an unlikely explanation given the shape and general overlap of the incidence corrected species accumulation curves (Fig. 3). It does not appear that adding more Winkler litter samples would have significantly increased collections of new species because 95% confidence intervals for Chao 2 estimated species richness are relatively small compared to those for pitfall traps at all restorations ages except for 20-22 years (Fig. 4). This is not surprising, given the relatively low levels of ant species turnover among sites in temperate environments (Lynch 1981, Lessard et al. 2007). Two potential ways to improve sampling efficiencies in temperate savanna environments that lack many litter specialist species would be to conduct Winkler litter sampling at different times of day to sample across the full spectrum of ant community activity, and to conduct "maxi-Winkler" samples. This method consists of collecting litter from many sites in a plot selected based on "expert" knowledge of high quality ant environments rather than the standardized 1m^2 approach used in this study. The maxi-Winkler method is useful for general survey work, but makes quantitative comparisons between trapping methods and localities difficult.

In conclusion, in the common ecotonal environment of the temperate savanna, using the standard sampling protocol of Winkler litter samples as advised in the ALL protocol (Agosti and Alonso 2000) appears to capture fewer species of ants and more ant workers per sample than standardized pitfall trap sampling. In addition, Winkler litter samples are not complementary in terms of species captured by pitfall traps. A number of studies (e.g., Ellison et al. 2007, Groc et al. 2007) have tried to identify the most efficient method of sampling terrestrial ants in temperate environments with two goals in mind; first and foremost, to effectively characterize the ant fauna of a region, and secondly to allow for comparisons between studies. Protocols like ALL are useful in creating a standardized guide for capturing the majority of ants in a region and comparison of ant faunas between different studies (Dunn et al. 2007, 2009). But, it is not necessary to follow all aspects of a protocol or order to compare studies, as long as standardized sampling methods are used. Different studies, that have sufficiently sampled their environments, can still be compared by accounting for differential sampling effort. Studies conducted with only pitfall traps appear to capture most of the species in temperate environments and the results of these studies are readily comparable to those studies in more heterogeneous and diverse environments that require the full ALL protocol.

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